

Cryptic von Hippel-Lindau disease: germline mutations in patients with haemangioblastoma only

F J Hes, S McKee, M J B Taphoorn, P Rehal, R B van der Luijt, R McMahon, J J van der Smagt, D Dow, R A Zewald, J Whittaker, C J M Lips, F MacDonald, P L Pearson, E R Maher

Department of
Medical Genetics,
University Medical
Centre, Utrecht, The
Netherlands

F J Hes
R B van der Luijt
R A Zewald
P L Pearson

Department of
Internal Medicine,
University Medical
Centre, Utrecht, The
Netherlands

F J Hes
C J M Lips

West Midlands
Regional Genetics
Service, Birmingham
Women's Hospital,
Edgbaston,
Birmingham B15 2TG,
UK

S McKee
P Rehal
F MacDonald
E R Maher

Department of
Neurology, University
Medical Centre,
Utrecht, The
Netherlands
M J B Taphoorn

East Anglian Genetics
Service,
Addenbrooke's
Hospital, Cambridge,
UK

R McMahon
D Dow
J Whittaker

Clinical Genetics
Centre Leiden, PO
Box 9600, 2300 RC
Leiden, The
Netherlands
J J van der Smagt

Section of Medical and
Molecular Genetics,
Department of
Paediatrics and Child
Health, University of
Birmingham,
Birmingham, UK
E R Maher

Correspondence to:
Professor Maher,
E.R.Maher@bham.ac.uk

Revised version received
1 May 2000
Accepted for publication
26 September 2000

Abstract

Objectives—Central nervous system haemangioblastoma (HAB) is a major feature of von Hippel-Lindau (VHL) disease, and it is estimated that about 30% of HAB patients have VHL disease. Consequently, it is widely recommended that sporadic HAB patients are screened for clinical and radiological features of VHL disease because of the risk of multiple tumours. We investigated the frequency of VHL germline mutations in patients with HAB only with no clinical or radiological evidence of VHL disease to define the role of molecular genetic analysis in the management of such patients.

Methods—Eighty four patients with a single HAB (23 Dutch, 61 UK) and four with multiple HAB (two Dutch, two UK) were studied by direct sequencing of the coding region and quantitative Southern blotting.

Results—A VHL germline mutation was found in three of 69 (4.3%) single HAB patients aged 50 years or less (three of 84 (3.6%) total single HAB patients). A germline VHL mutation was detected in a 44 year old woman with a solitary cerebellar HAB, as well as in four clinically unaffected close relatives, and in two single HAB cases presenting at the ages of 29 and 36 years. Germline VHL mutations were detected in two of four cases with multiple HAB.

Conclusions—Early detection of VHL disease is important to reduce morbidity and mortality and therefore we recommend that, in addition to conventional clinical and radiological investigations, VHL gene mutation analysis should be offered to all HAB patients younger than 50 years. HAB patients aged >50 years will have a lower a priori risk of VHL disease and further data are required to evaluate the role of routine molecular genetic investigations in late onset HAB cases. The failure to detect germline VHL mutations in some patients with multiple HAB may indicate the presence of somatic mosaicism or additional HAB susceptibility genes.

(*J Med Genet* 2000;37:939-943)

Keywords: haemangioblastoma; von Hippel-Lindau disease; VHL; germline mutation

Haemangioblastomas (HAB) are non-metastasising tumours of the central nervous system and account for about 2% of all intracranial tumours.¹ HAB arise preferentially in the cerebellum (~75%), medulla, and spinal cord (~25%); HAB in the cerebrum are rare.³ HAB are regarded as benign on their histopathological characteristics and do not normally invade the surrounding brain. However, complications may arise because of the tendency of HAB to form expanding cysts, leading to raised or even life threatening intracranial pressure.² They are composed predominantly of vascular and stromal cells.^{2,4} The frequent presence of haemorrhages and cysts means the tumours vary in morphological appearance.² Four types of HAB can be recognised macroscopically: 5% are cysts, 60% predominantly cystic, 26% predominantly solid, and 9% solid.⁵

The standard treatment is complete microsurgical removal,⁵⁻⁷ aided if necessary by preoperative embolisation to reduce the tumour's vascularity.⁸ Stereotactic radiosurgery shrinks or stops the growth of small or medium sized HAB.^{7,9,10} Adjoining cysts, however, do not respond to radiosurgery and require later and sometimes repeated evacuation.⁷

HAB may occur in sporadic form or as a manifestation of von Hippel-Lindau (VHL) disease.¹¹ VHL disease is an autosomal dominant tumour syndrome with an estimated birth incidence of approximately 1/36 000.¹² The disease is characterised by a predisposition to bilateral and multifocal tumours. The most common tumours in VHL disease are HAB in the central nervous system and retina, clear cell carcinoma in the kidney, pheochromocytoma in the adrenal gland, endolymphatic sac tumours in the inner ear, as well as cysts in the kidney, pancreas, and epididymis. In the presence of a positive family history, a diagnosis of VHL disease can be made by the identification of a single retinal or cerebellar HAB, renal cell carcinoma, pheochromocytoma, or multiple pancreatic cysts in an at risk subject.^{13,14} In isolated cases of VHL disease, two or more HABs, or a single HAB in association with a visceral manifestation are required.¹⁴

The basis of familial inheritance of VHL disease is a germline mutation in the VHL tumour suppressor gene, first identified in 1993 and located in chromosome region 3p25.¹⁵ In both VHL disease and sporadic HAB, allelic losses and mutations of the VHL tumour suppressor gene affecting stromal cells have been found,

suggesting that stromal cells represent the neoplastic component of a HAB.^{4,16} In addition, it was shown that vascular endothelial growth factor (VEGF) is upregulated in stromal cells as a consequence of mutations in the *VHL* gene.¹⁷

VHL disease shows variable expression, age dependent penetrance, and a low but consistent new mutation rate.^{12,18} The diagnosis of VHL disease should be considered in all patients with a HAB, as early recognition of a predisposition to develop further HAB and other tumours (for example, renal cell carcinoma) may reduce morbidity and mortality. The diagnosis of "new mutation" VHL cases is frequently delayed because at least two typical manifestations are required, whereas molecular genetic diagnosis of VHL disease offers the potential to detect subclinical cases of VHL disease in sporadic patients with a single HAB. VHL disease shows complex genotype-phenotype correlations. Most VHL gene mutations predispose to HAB, but specific missense mutations may cause high or low risks for renal cell carcinoma or pheochromocytoma.¹⁹⁻²³ In addition, rare missense mutations may produce a pheochromocytoma only phenotype.²⁴⁻²⁶ This suggests that specific *VHL* gene mutations might cause a HAB only phenotype.

To investigate the genetic epidemiology of HAB in the central nervous system, we performed an international multicentre study of patients with single HAB and multiple HAB without evidence of VHL disease (that is, HAB only). As the mean age of VHL patients with HAB is significantly younger than that for sporadic cases, 29 v 48 years²⁷ (or 33.5 v 43.6 years in Richard *et al*²⁸), we directed our study towards younger patients with single HAB as these present the most difficult diagnostic problems in clinical practice.

Patients and methods

PATIENTS

We investigated two groups of patients with HAB only in the central nervous system. Group 1 consisted of 61 UK and 23 Dutch HAB patients with a single HAB. These cases were ascertained with the help of neurosurgeons, neurologists, internists, and clinical geneticists. In addition to DNA analysis, all patients underwent clinical examinations for detection of VHL associated tumours (ophthalmological examination and abdominal sonography or MRI) with negative findings. Group 2 consisted of four patients with multiple HABs, but no other evidence of VHL disease (that is, absence of further VHL related tumours) on clinical screening and radiological screening. All patients had histopathologically confirmed HAB at operation.

AGE AT DIAGNOSIS

Between 1973 and 1996 a total of 182 HAB patients were reported to the National Dutch Pathological Archive (Palga). Fig 1 shows the age at diagnosis of Palga patients as well as patients studied. Details of age distribution of a previous population based cohort of UK HAB patients have been reported previously.²⁷

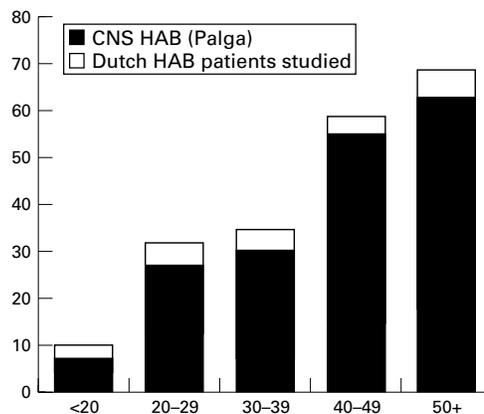


Figure 1 Number of patients and age at diagnosis for 182 patients with haemangioblastoma (HAB) in the central nervous system (CNS) reported in the National Dutch Pathological Archive (Palga) between 1973 and 1996 and 26 Dutch patients in this study with a single HAB between 1996 and 1999.

In 1996, guidelines were distributed via the Dutch newsletter for neurologists on screening all patients with a HAB for a VHL germline mutation. The Dutch patients in the present study were referred for DNA diagnosis from 1996 to 1999, and the mean age at diagnosis was 37.5 years (range 14-71 years). Compared to unselected cases, the age distribution in the UK as well as in the Dutch cases in this study was biased towards an earlier age at onset.

DNA ANALYSIS

High molecular weight DNA of the probands was isolated from peripheral blood according to established procedures. Exons 1, 2, and 3 of the *VHL* gene and their immediately flanking sequences were amplified using the polymerase chain reaction.²⁹ The flanking sequences included 90 nucleotides upstream of the start codon (the first nucleotide of the coding region is 214) and 45 nucleotides downstream of the stop codon. The nucleotides are numbered according to Latif *et al*⁵ (Genbank accession number L15409). PCR products were purified and subjected to sequence analysis using either an ABI automated sequencer or the dideoxy chain termination reaction with a pUC sequencing kit (Boehringer Mannheim, Mannheim, Germany), using γ -³⁵S dATP (600 Ci/mmol). The amplification primers were used as primers in the sequencing reactions. In the UK cases, intragenic mutations were also sought by single strand conformation polymorphism (SSCP).

Screening for genetic rearrangements and deletions was performed by Southern blot analysis, or a novel PCR based deletion assay (Dow *et al*, in preparation). In Southern blot analysis, DNA was digested with *EcoRI* alone or with *EcoRI* and *AseI* double digest. After gel electrophoresis and transfer to Hybond-N filters, the genomic DNA was hybridised with the *VHL* g7-cDNA probe,¹⁵ according to the manufacturer's instruction. Quantitative Southern blotting was performed by hybridising genomic DNA with the *VHL* g7-cDNA probe and with a beta globin probe, to detect deletions encompassing the entire VHL gene.³⁰

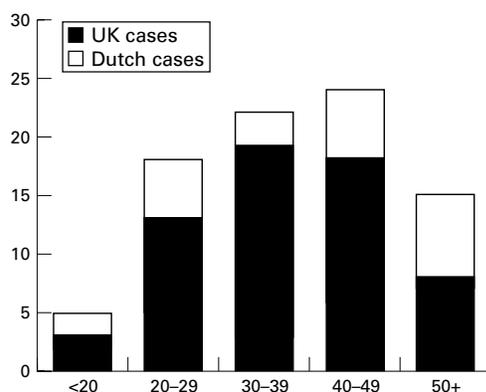


Figure 2 Distribution of single HAB patients (dark = UK, light = Dutch) by age at diagnosis.

Results

SINGLE HAB

Eighty four cases with single HAB in the central nervous system (23 Dutch, 61 UK) were investigated. The age distribution of these cases is shown in fig 2. *VHL* germline mutations were identified in three cases (table 1). The incidence of a *VHL* germline mutation in single HAB cases younger than 30 years was 4.3% (1/23), 30-39 years was 4.5% (1/22), 40-49 years was 4.2% (1/24), and 50 years or older was 0% (0/15).

Details of the three germline mutations identified were as follows. (1) A C to T transition at nucleotide 454 was detected in a 44 year old woman (D24) with a single cerebellar HAB (fig 3). This missense mutation leads to a change of proline to serine at codon 81 (P81S) in the VHL protein. DNA analysis of other close relatives showed that four clinically unaffected first and second degree relatives (aged 17-77 years) were also carriers of a P81S germline mutation. (2) A 7 bp frameshift mutation (del 582 GACACAC) was detected in a 29 year old patient (B1) with a single cerebellar HAB with no family history and no evidence of VHL disease on clinical and radiological screening. However, she subsequently developed pancreatic cysts at 34 years of age. (3) A large germline deletion was identified by Southern blot analysis in a 36 year old woman (B2) with a single cerebellar HAB but no other features of VHL disease.

Two patients with a single HAB developed some additional features of VHL disease during the study: one patient (B1) with a VHL gene mutation (del 582 GACACAC) developed pancreatic cysts (see above) and one patient (B3) developed renal cell carcinoma

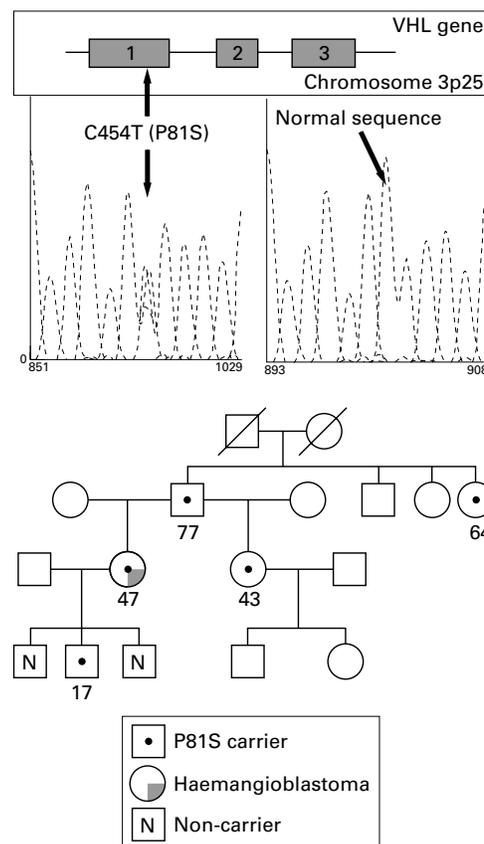


Figure 3 Pedigree of patient D24, showing four clinically unaffected relatives and their ages with the *VHL* germline mutation P81S.

aged 44 years following a cerebellar HAB at the age of 40 years, but no VHL mutation was identified.

MULTIPLE HABs

Four patients with multiple HABs (two Dutch and two UK) and without additional VHL related tumours were analysed for VHL germline mutations. Deletions were detected in two patients, (1) a male (B5) with one cerebellar and one medullary HAB at the age of 40 years, and (2) a male (B6) with multiple spinal HABs at the age of 44 years. Germline mutations were not identified in the two Dutch patients (D13 and D32) with both cerebellar and spinal HABs (aged 44 and 66 years).

Discussion

We found that the overall risk for finding a VHL germline mutation in a population of 84 patients with a single HAB in the central nerv-

Table 1 Summary of results

Patient	Age	Clinical features		Family history	Mutation	Previously reported
		HAB	Other			
D24	44	Solitary cerebellar	—	—	P81S	21, 30, 35
B1	29	Solitary cerebellar	Subsequent pancreatic cysts	—	del 582 GACACAC	No
B2	36	Solitary cerebellar	—	—	Deletion	NA
B5	40	Cerebellar & medullary	—	—	Deletion	NA
B6	44	Multiple spinal	—	—	Deletion	NA

Patient: patient's ID; Age: age at diagnosis, in years; HAB: type and origin of haemangioblastoma; Other: further VHL associated manifestations after clinical screening; Family history: further VHL associated manifestations in family members of proband; Mutation: VHL germline mutation; Previously reported: whether the mutation has been published (references); NA: not applicable.

ous system and no further features of VHL disease at the time of diagnosis was approximately 4%. In clinical studies of sporadic patients with a HAB it was suggested that a substantial proportion of HAB could be associated with VHL disease upon more detailed examination, that is, 23% to 34.3% were found to be affected with VHL disease.^{6, 28} Molecular genetic analysis of the VHL gene had indicated that sporadic patients with a HAB may have a risk of a VHL gene germline mutation of approximately 10%.^{31, 32} In our larger study, we found a lower detection rate despite using more sensitive methods of VHL gene analysis, but this is presumably related to the very careful clinical and radiological screening performed before entry into our study. The identification of a VHL germline mutation has important implications for the risk of further tumours and for the risk of VHL disease in relatives, so the identification of a VHL mutation in a significant proportion of single HAB patients despite conventional clinical and radiological investigation is important.

Statistical analysis of the age at onset of HAB in VHL disease and non-VHL cases (based on clinical criteria) is consistent with a one and two hit tumourigenesis model as predicted by the Knudson hypothesis.²⁷ Mean age at diagnosis of cerebellar HAB in VHL disease is younger than in sporadic cases (29 years versus 48 years, respectively) so we anticipated a higher incidence of unsuspected VHL gene mutations in early onset cases. In addition, older patients with VHL gene mutations would be more likely to manifest other evidence of VHL disease and so be excluded from our study. Thus, although our results were broadly compatible with this hypothesis, the identification of germline VHL gene mutations in two of a group of 46 patients aged 30 to 49 years with a single HAB and no clinical or radiological features of VHL disease suggests that molecular genetic analysis should be used in all single HAB patients younger than 50 years. For older onset patients, the frequency of germline mutations is likely to be less (see above) and larger research based studies are required to define whether molecular genetic analysis should be performed when clinical and radiological screening has shown no evidence of VHL disease.

A major strength of the present study was the use of recently developed techniques to detect large germline deletions. Before the introduction of these techniques, there was a *VHL* germline mutation detection rate (with Southern blotting and sequencing of the coding region) of approximately 80% in known VHL families.²² However, methods to detect large deletions (for example, quantitative Southern blotting) have significantly increased the detection rate, reaching 100% in proven familial VHL disease.³⁰ Therefore a conservative estimate of the mutation detection sensitivity of the strategy used in this study would be in the order of 95%. Although other studies of sporadic patients with a HAB have used less sensitive techniques,³¹⁻³³ it is of interest that Oberstrass *et al*³¹ detected a germline mutation

in two of 20 patients (aged 18 and 40 years) with HAB of the central nervous system (although no data were available about a possible family history of VHL disease). Decker *et al*³³ reported a 29 year old patient with recurrent spinal HAB, a negative family history of VHL disease, and a de novo frameshift *VHL* gene exon 2 mutation. However, this case was not comparable to those in our study because a renal mass and pancreatic and renal cysts were detected on clinical screening. In a series of 18 sporadic patients with a HAB, Olschwang *et al*³² found a missense mutation in two patients (42 and 56 years of age) without clinical investigations showing any evidence of VHL disease. The latter case would only have been detected by also screening single HAB patients aged over 50 years for a VHL gene mutation.

Although specific missense VHL mutations may cause a pheochromocytoma only phenotype,²⁴⁻²⁶ we did not find unequivocal evidence for *VHL* gene mutations that would predispose to a HAB only phenotype. However, the *VHL* germline mutation (P81S) that was found in a 44 year old woman with a solitary HAB and in four clinically unaffected family members was associated with an unusually low penetrance within this family. To reduce the possibility of a genetic polymorphism, 50 non-VHL patients were sequenced. Sequence analysis of exon 1 showed that all persons were homozygous for nucleotide 545C (data not shown). Phenotypic expression in VHL disease is influenced by allelic heterogeneity, stochastic events, and genetic modifiers.³⁴ The P81S mutation has been reported four times previously: (1) in an isolated German patient with a full blown VHL tumour spectrum (cerebellar and spinal HAB, renal cell carcinoma, and renal, pancreatic, and epididymal cysts); (2) in a 34 year old American patient with HAB only; (3) in a 35 year old American patient with retinal haemangioblastoma and islet cell tumour of the pancreas (the father is the only other relative with a VHL related tumour and had a pheochromocytoma); and (4) in an isolated Japanese patient with multiple HABs and a renal cell carcinoma.^{21, 30, 35} These findings suggest that P81S mutation carriers in the family are also at risk of renal cell carcinoma. Moreover, only one of the P81S carriers had affected family members, which may imply that this missense mutation has a low penetrance.

Interestingly, we did not find VHL germline mutations in two of the four patients with multiple HABs. As the presence of two or more retinal or cerebellar HAB satisfies the strict diagnostic criteria for VHL disease,^{12, 14} this was an unexpected finding in the light of the high sensitivity of the mutation detection methods used, and could perhaps indicate additional HAB susceptibility gene(s). Alternative explanations would include a mutation in part of the VHL gene not analysed (for example, regulatory domain) or somatic mosaicism. In this context it is interesting that the two multiple HAB patients with germline mutations had the earliest age at onset and the patients with later onset may be mosaic and so have a milder phe-

notype or represent phenocopies (independent mutation events giving rise to various HAB could also be expected by chance). Although mosaicism has so far been described infrequently in VHL disease,³⁶ it seems likely that it will be relatively common, as in neurofibromatosis type 2.³⁷

We have shown that VHL gene mutation analysis facilitates the management of sporadic patients with HAB and recommend that it should be performed in all patients younger than 50 years with a single HAB even if there is no other clinical or radiological evidence of VHL disease. Although the detection rate in older patients should be lower, we suggest that such patients need to be studied on a research basis, using the latest mutation detection strategies to define cost-benefit consequences for molecular genetic analysis of this group of patients.

F J Hes received grants from Zorg Onderzoek Nederland and the Janivo Foundation. The VHL g7-cDNA probe was kindly provided by I Kuzmin, Frederick, MD, USA. The beta globin probe was kindly provided by C Stolle, Philadelphia, PA, USA. We thank Dr H P H Neumann, Freiburg, Germany, Dr C Stolle, Philadelphia, PA, USA, Dr G Glenn, Rockville, MD, USA, and Dr Masahiro Yao, Yokohama, Japan for clinical data on P81S carriers.

- Ellison D, Love S, Chimelli L, Harding B, Lowe J, Roberts GW, Vinters HV. Miscellaneous neoplasms: hemangioblastoma. In Hodgson S, ed. *Neuropathology, a reference text of CNS pathology*. Vol 1. London: Mosby, 1998;45.4-7.
- Wizigmann Voos S, Plate KH. Pathology, genetics and cell biology of hemangioblastomas. *Histol Histopathol* 1996;11:1049-61.
- Russell D, Rubenstein LJ. *Pathology of tumours of the central nervous system*. Vol 5. London: Edward Arnold, 1989:639-57.
- Vortmeyer AO, Gnarr JR, Emmert-Buck MR, Katz D, Linehan WM, Oldfield EH, Zhuang Z. von Hippel-Lindau gene deletion detected in the stromal cell component of a cerebellar hemangioblastoma associated with von Hippel-Lindau disease. *Hum Pathol* 1997;28:540-3.
- Richard S, Campello C, Taillandier L, Parker F, Resche F. Hemangioblastoma of the central nervous system in von Hippel-Lindau disease. French VHL Study Group. *J Intern Med* 1998;243:547-53.
- Neumann HP, Eggert HR, Weigel K, Friedburg H, Wiestler OD, Schollmeyer P. Hemangioblastomas of the central nervous system. A 10-year study with special reference to von Hippel-Lindau syndrome. *J Neurosurg* 1989;70:24-30.
- Niemela M, Lim YJ, Soderman M, Jaaskelainen J, Lindquist C. Gamma knife radiosurgery in 11 hemangioblastomas. *J Neurosurg* 1996;85:591-6.
- Tampieri D, Leblanc R, TerBrugge K. Preoperative embolization of brain and spinal hemangioblastomas. *Neurosurgery* 1993;33:502-5.
- Chang SD, Meisel JA, Hancock SL, Martin DP, McManus M, Adler JR Jr. Treatment of hemangioblastomas in von Hippel-Lindau disease with linear accelerator-based radiosurgery. *Neurosurgery* 1998;43:28-34.
- Patrice SJ, Sneed PK, Flickinger JC, Shrieve DC, Pollock BE, Alexander E, Larson DA, Kondziolka DS, Gutin PH, Wara WM, McDermott MW, Lunsford LD, Loeffler JS. Radiosurgery for hemangioblastoma: results of a multi-institutional experience. *Int J Radiat Oncol Biol Phys* 1996;35:493-9.
- Huson SM, Harper PS, Hourihan MD, Cole G, Weeks RD, Compston DA. Cerebellar hemangioblastoma and von Hippel-Lindau disease. *Brain* 1986;109:1297-310.
- Maier ER, Iselius L, Yates JR, Littler M, Benjamin C, Harris R, Sampson J, Williams A, Ferguson Smith MA, Morton N. Von Hippel-Lindau disease: a genetic study. *J Med Genet* 1991;28:443-7.
- Melmon KL, Rosen SW. Lindau's disease: review of the literature and study of a large kindred. *Am J Med* 1964;36:595-617.
- Maier ER, Kaclin WG Jr. Von Hippel-Lindau disease. *Medicine* 1997;76:381-91.
- Latif F, Tory K, Gnarr J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou FW, Li H, Wei MH, Chen F, Glenn GM, Choyke PL, Walther MM, Weng Y, Duan DR, Dean M, Glavac D, Richards FM, Crossey PA, Ferguson Smith MA, Paslier ID, Chumakov I, Cohen D, Chnault AC, Maier ER, Linehan WM, Zbar B, Lerman MI. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993;260:1317-20.
- Lee JY, Dong SM, Park WS, Yoo NJ, Kim CS, Jang JJ, Chi JG, Zbar B, Lubensky IA, Linehan WM, Vortmeyer AO, Zhuang Z. Loss of heterozygosity and somatic mutations of the VHL tumor suppressor gene in sporadic cerebellar hemangioblastomas. *Cancer Res* 1998;58:504-8.
- Stratmann R, Krieg M, Haas R, Plate KH. Putative control of angiogenesis in hemangioblastomas by the von Hippel-Lindau tumor suppressor gene. *J Neuropathol Exp Neurol* 1997;56:1242-52.
- Richards FM, Payne SJ, Zbar B, Affara NA, Ferguson Smith MA, Maier ER. Molecular analysis of de novo germline mutations in the von Hippel-Lindau disease gene. *Hum Mol Genet* 1995;4:2139-43.
- Crossey PA, Richards FM, Foster K, Green JS, Prowse A, Latif F, Lerman MI, Zbar B, Affara NA, Ferguson Smith MA, Maier ER. Identification of intragenic mutations in the von Hippel-Lindau disease tumor suppressor gene and correlation with disease phenotype. *Hum Mol Genet* 1994;3:1303-8.
- Chen F, Slife L, Kishida T, Mulvihill J, Tisherman SE, Zbar B. Genotype-phenotype correlation in von Hippel-Lindau disease: identification of a mutation associated with VHL type 2A. *J Med Genet* 1996;33:716-17.
- Zbar B, Kishida T, Chen F, Schmidt L, Maier ER, Richards FM, Crossey PA, Webster AR, Affara NA, Ferguson Smith MA, Brauch H, Glavac D, Neumann HPH, Tisherman S, Mulvihill JJ, Gross DJ, Shuin T, Whaley J, Seizinger B, Kley N, Olschwang S, Boisson C, Richard S, Lips CJM, Linehan WM, Lerman M. Germline mutations in the von Hippel-Lindau disease (VHL) gene in families from North America, Europe and Japan. *Hum Mutat* 1996;8:348-57.
- Maier ER, Webster AR, Richards FM, Green JS, Crossey PA, Payne SJ, Moore AT. Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. *J Med Genet* 1996;33:328-32.
- Brauch H, Kishida T, Glavac D, Chen F, Pausch F, Hofler H, Latif F, Lerman MI, Zbar B, Neumann HP. Von Hippel-Lindau (VHL) disease with pheochromocytoma in the Black Forest region of Germany: evidence for a founder effect. *Hum Genet* 1995;95:551-6.
- Crossey PA, Eng C, Ginalska Malinowska M, Lennard TW, Wheeler DC, Ponder BA, Maier ER. Molecular genetic diagnosis of von Hippel-Lindau disease in familial pheochromocytoma. *J Med Genet* 1995;32:885-6.
- Neumann HP, Eng C, Mulligan LM, Glavac D, Zauner I, Ponder BA, Crossey PA, Maier ER, Brauch H. Consequences of direct genetic testing for germline mutations in the clinical management of families with multiple endocrine neoplasia, type II. *JAMA* 1995;274:1149-51.
- Woodward ER, Eng C, McMahon R, Voutilainen R, Affara NA, Ponder BA, Maier ER. Genetic predisposition to pheochromocytoma: analysis of candidate genes GDNF, RET and VHL. *Hum Mol Genet* 1997;6:1051-6.
- Maier ER, Yates JR, Ferguson Smith MA. Statistical analysis of the two stage mutation model in von Hippel-Lindau disease, and in sporadic cerebellar hemangioblastoma and renal cell carcinoma. *J Med Genet* 1990;27:311-14.
- Richard S, Beigelman C, Gerber S, Van Effenterre R, Gaudric A, Sahel M, Binaghi M, de Kersaint Gilly A, Houtteville JP, Brunon JP, Guerin J, Cornu P, Redondo A, Huth M, Resche F. [Does hemangioblastoma exist outside of von Hippel-Lindau disease?] L'hémangioblastome existe-t-il en dehors de la maladie de von Hippel-Lindau? *Neurochirurgie* 1994;40:145-54.
- Gnarr JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh FM, Lubensky J, Duan DR, Florence C, Pozzatti R, Walther MM, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Isaacs WB, Lerman MI, Zbar B, Linehan WM. Mutations of the VHL tumor suppressor gene in renal carcinoma. *Nat Genet* 1994;7:85-90.
- Stolle C, Glenn G, Zbar B, Humphrey JS, Choyke P, Walther M, Pack S, Hurley K, Andrey C, Klausner R, Linehan WM. Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. *Hum Mutat* 1998;12:417-23.
- Oberstrass J, Reifenberger G, Reifenberger J, Wechsler W, Collins VP. Mutation of the Von Hippel-Lindau tumour suppressor gene in capillary haemangioblastomas of the central nervous system. *J Pathol* 1996;179:151-6.
- Olschwang S, Richard S, Boisson C, Giraud S, Laurent-Puig P, Resche F, Thomas G. Germline mutation profile of the VHL gene in von Hippel-Lindau disease and in sporadic hemangioblastoma. *Hum Mutat* 1998;12:424-30.
- Decker HJ, Neuhaus C, Jauch A, Speicher M, Ried T, Bujard M, Brauch H, Storkel S, Stockle M, Seliger B, Huber C. Detection of a germline mutation and somatic homozygous loss of the von Hippel-Lindau tumor-suppressor gene in a family with a de novo mutation. A combined genetic study, including cytogenetics, PCR/SSCP, FISH, and CGH. *Hum Genet* 1996;97:770-6.
- Webster AR, Richards FM, MacDonald FE, Moore AT, Maier ER. An analysis of phenotypic variation in the familial cancer syndrome von Hippel-Lindau disease: evidence for modifier effects. *Am J Hum Genet* 1998;63:1025-35.
- Japan CRGfV. Germline mutations in the von Hippel-Lindau disease (VHL) gene in Japanese VHL. *Hum Mol Genet* 1995;4:2233-7.
- Sgambati MT, Stolle C, Choyke PL, Walther MM, Zbar B, Linehan WM, Glenn GM. Mosaicism in von Hippel-Lindau disease: lessons from kindreds with germline mutations identified in offspring with mosaic parents. *Am J Hum Genet* 2000;66:84-91.
- Evans DG, Wallace AJ, Wu CL, Trueman L, Ramsden RT, Strachan T. Somatic mosaicism: a common cause of classic disease in tumor-prone syndromes? Lessons from type 2 neurofibromatosis. *Am J Hum Genet* 1998;63:727-36.