Original articles

Von Hippel-Lindau disease: a genetic study

E R Maher, L Iselius, J R W Yates, M Littler, C Benjamin, R Harris, J Sampson, A Williams, M A Ferguson-Smith, N Morton

Abstract

Genetic aspects of von Hippel-Lindau (VHL) disease were studied in familial and isolated cases. Complex segregation analysis with pointers was performed in 38 kindreds with two or more affected members. Dominant inheritance with almost complete penetrance in the highest age classes (0·96 at 51 to 60 and 0·99 at 61 to 70 years) was confirmed and there was no evidence of heterogeneity between families ascertained through complete and incomplete selection. The point prevalence of heterozygotes in East Anglia was 1·89/100 000 (1/53 000) persons with an estimated birth incidence of 2·73/100 000 (1/36 000) live births. Reproductive fitness was 0·83. Direct and indirect estimates of the mutation rate were 4·4 (95% CI 0·9 to 7·9)× 10^{-6}/gene/generation and 2·32×10^{-6}/gene/generation respectively. There was no significant association between parental age or birth order and new mutations for VHL disease.

Von Hippel-Lindau disease (McKusick 19330) is an inherited cancer syndrome with variable expression. The most frequent complications are haemangioblastomas of the retina and central nervous system, renal cell carcinoma, phaeochromocytoma, and renal, pancreatic, and epididymal cysts.[1–3] Following early descriptions of familial retinal angioma by Collins,[4] von Hippel,[5 6] and others, Lindau[7] recognised the association between retinal angiomas and cerebellar haemangioblastoma and described the development of hypernephroma in 1927. More than 700 patients with VHL disease have been reported[2 3] and the clinical features have been well defined. Nevertheless, some of the genetic aspects of VHL disease have received little attention. Dominant inheritance was suggested by Møller,[8] and several large families in which VHL disease segregated as a dominant trait through five[2 9] and six[10] generations have since been described. However, Shokeir[11] proposed autosomal recessive inheritance in one family. We report complex segregation analysis and estimates of the prevalence, fitness, mutation rate, and parental age effect in VHL disease.

Cambridge University Department of Pathology, Cambridge.
E R Maher, J R W Yates, M A Ferguson-Smith

CRC Research Group in Genetic Epidemiology, University of Southampton, Southampton.
L Iselius, M Littler, N Morton

Department of Medical Genetics, University of Manchester, Manchester.
C Benjamin, R Harris

Institute of Medical Genetics for Wales, University of Wales College of Medicine, Cardiff.
J Sampson, A Williams

Correspondence to Dr Maher, Department of Medical Genetics, Level 1, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

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Patients and methods

PATIENT DETAILS

Patients with VHL disease from 66 kindreds containing 236 affected subjects and 194 unaffected relatives at 50% prior risk were analysed. Twelve kindreds contained only one affected person. Most patients were included in previous clinical studies of VHL disease.[3 12] Genetic linkage studies in 14 families have been described elsewhere.[13] Patients were ascertained by contacting specialists in genetics, nephrology, neurology, neurosurgery, ophthalmology, and urology from Great Britain and Ireland. Details of families with multiple affected members and of isolated cases were requested. VHL disease was diagnosed by conventional criteria[1]: for isolated cases two or more haemangioblastomas, or a single haemangioblastoma in association with a visceral manifestation are required. When there is a family history of retinal or CNS haemangioblastoma, only one haemangioblastoma or visceral complication is required to make the diagnosis of VHL disease. Details of the clinical status and age at diagnosis were confirmed from patient interviews, hospital notes, and necropsy records.

SEGREGATION ANALYSIS OF FAMILIAL CASES

Thirty-eight pedigrees containing 194 patients for whom detailed age at onset information was available

were submitted for complex segregation analysis with multiple pointers.[14] Each of these pedigrees contained two or more affected patients. Pedigrees containing a single affected person were not analysed because the diagnosis of VHL disease in such patients can only be made when two complications have developed. Ascertainment of cases of VHL disease is therefore biased towards familial cases who can be diagnosed after a single manifestation. By limiting segregation analysis to familial cases the ascertainment bias against isolated cases was circumvented. Age at diagnosis was taken as the first manifestation and current age was taken to be at death, or if alive, at the time of ascertainment. The following types of nuclear families were distinguished.[14] (1) Index sibships including a proband as a child with an additional affected sib causing the family to be ascertained (truncate selection of the sibs of the index case who is coded as a child's pointer (1A)). (2) Index sibships including a proband as a child with an affected parent causing the family to be ascertained (single selection). (3) Children of probands with an affected child causing the family to be ascertained (truncate selection). (4) Children of collateral cases (complete selection with closest affected relative as pointer).

Each subject was assigned to one of eight age related liability classes. The risk attributed to the jth liability class was $R_j=(I_j-M_{j-1})/(1-M_{j-1})$ where I_j is the cumulative incidence to the midpoint of j and M_{j-1} is the cumulative specific mortality to the end of the preceding class. The I_j values were estimated from the age at onset data in the material and M_j values from the survival curve using the actuarial method.[3] It was assumed that the prevalence of VHL disease in the highest age group was 1/100 000. A total of 610 subjects from 114 nuclear families were submitted to segregation analysis using the computer program POINTER.[14]

Prevalence, birth frequency, mutation rates, and parental age and birth order effects were studied by standard methods.[15] The prevalence of heterozygotes was determined in East Anglia (population 2 034 400) as ascertainment was most likely to be complete in this region. There were 24 affected patients (six of whom were isolated cases) and 32 relatives at 50% prior risk from 18 kindreds in East Anglia. Of these, 16 affected patients (five isolated cases) and 11 at risk relatives were aged 25 to 49 years.

The risk of a person with an affected parent being an asymptomatic heterozygote was calculated according to the eight age related liability classes defined for the segregation analysis. The prevalence and birth frequency of heterozygotes was calculated in a similar manner for those aged 25 to 49 years as the ascertainment of heterozygotes was likely to be most accurate in this age group. Below the age of 25 most isolated cases will not have been diagnosed and after 50 years specific mortality from VHL disease will reduce the heterozygote prevalence (see Results section).

The mutation rate (μ) was calculated by direct and indirect methods. For the direct method an affected person was considered to represent a new mutation if there was no other relevant family history and both parents were alive or had died from an unrelated cause aged >50 years. The indirect method of calculating the mutation rate requires the birth frequency (I) and reproductive fitness (F) to be known such that $\mu=0{\cdot}5\times I\times(1-F)$. The reproductive fitness (F) was calculated by comparing the number of children/100 reproductive years born to affected subjects and their normal sibs.[15 16] The birth frequency of VHL disease is not known but was estimated by determining in East Anglia the number of affected patients (both alive and dead) and likely number of heterozygotes among at risk relatives, born between 1940 and 1964, as a proportion of the population in this age group. Evidence for a parental age effect on mutagenesis was sought by comparing the parental ages at the birth of patients thought to represent new mutations to that expected for the general population. From 1938 onwards data for mean maternal age corrected for the number of previous children is available from the Registrar General. Before 1938 estimates for mean maternal age are available but are not corrected for parity. Paternal age data is only provided from 1962 onwards and we estimated the expected paternal age for births before 1962 by the method of Bundey *et al.*[16] Evidence for an association between birth order effect and new mutations for VHL disease was tested for by the method of Haldane and Smith.[17]

Results

FAMILY DATA AND SEGREGATION ANALYSIS

The calculated risks of having VHL for each age related liability class (R_j) are given in table 1. In the older age groups the risks are decreasing because of the specific mortality at earlier ages. Only one obligate heterozygote (aged 36 years, both parent and child affected) had no evidence of VHL disease after ophthalmological and systemic screening (cranial CT scan, abdominal ultrasound, 24 hour urinary VMAs). In four nuclear families two affected children had been born to parents with no history of VHL disease aged >45 years, but none of the parents had undergone screening to detect asymptomatic lesions. In one kindred, initially ascertained as three separate families, VHL disease had been transmitted through six generations. This kindred was originally described by Nicol[9] who observed that no affected patient had shown retinal involvement. However, since then, a further 11 family members have been affected, five of whom have retinal angiomatosis.

The results of the segregation analysis are given in table 2. There was strong evidence for a dominant

Table 1 Liability classes and risks for von Hippel-Lindau disease.

Class	Age	*Cumulative incidence** I_i	Cumulative mortality† M_i	*Risk of having VHL* ($\times 10^{-5}$) R_i
1	0–10	0·02	0·00	0·02
2	11–20	0·19	0·03	0·19
3	21–30	0·52	0·12	0·49
4	31–40	0·78	0·19	0·66
5	41–50	0·91	0·55	0·72
6	51–60	0·96	0·70	0·41
7	61–70	0·99	0·91	0·29
8	70+	1	1	0·09

*To midpoint of interval. †To end of interval.

Table 2 Segregation analysis of von Hippel-Lindau disease families.

Model	−2lnL+C	H	q	t	d
Sporadic	1724·39	(0)	(0)	—	—
Polygenic	−200·04	1·00	(0)	—	—
Recessive	−173·91	(0)	0·00099	10·8	(0)
Dominant	−323·12	(0)	0·0000007	7·0	(1)

H=heritability, q=gene frequency, t=displacement, d=dominance.

major gene (χ^2_3=123.12, p<0·001) giving virtually complete penetrance for the highest risk class. The likelihood curve for higher values of t is very flat and mapping on lower values of t shows that a t value as low as 5·13 is still compatible with the data (taking the t value for a likelihood 3·84 less than the maximum value of 323·12). This gives a gene frequency (q) of 0·000003 compared with 0·0000007 for the maximum likelihood estimate. These values of 2q bracket the incidence estimated below. Because of specific mortality, the segregation analysis will underestimate gene frequency.

There was no significant heterogeneity between families ascertained through complete and incomplete selection (χ^2_2=5·54).

PREVALENCE AND INCIDENCE OF VHL DISEASE

On the study day (1 July 1990) there were 24 affected patients (16 index cases) and 32 relatives at 50% prior risk alive in East Anglia. Analysis of the at risk relatives using age at onset data (see above) showed that there were likely to be 9·4 heterozygotes among them. Isolated cases of VHL disease are unlikely to be diagnosed aged less than 25 years as they can only be recognised when two complications have occurred. The prevalence of isolated cases of VHL disease aged 25 to 49 was calculated (n=5) and from this it was estimated that there are 5·1 isolated heterozygotes aged under 25 years in East Anglia who have not yet been diagnosed. Therefore the most likely number of heterozygotes for VHL disease in East Anglia is 38·5 giving a minimum heterozygote prevalence of 1·89 per 100 000 or 1 per 53 000 persons.

The birth incidence for VHL disease in East Anglia was calculated by determining the point prevalence of heterozygotes aged 25 to 49 years and then adding one patient with familial VHL disease born after 1 July 1940 but who had died before the study date. This was then divided by the total number of persons in East Anglia aged 25 to 49 years, giving a minimum birth incidence of 2·73/100 000 or 1/36 000 live births.

MUTATION RATE

Estimation of mutation rate by the direct method

In East Anglia six patients (five of whom were index cases) aged between 25 and 50 years were considered to be new mutations, a population frequency of 1·03/100 000. The mutation rate per gene by the direct method was 4·4 (SD 1·8)$\times 10^{-6}$ with 95% confidence interval of 0·9 to 7·9$\times 10^{-6}$.

Reproductive fitness

Patients with VHL disease had a mean 8·1 children/100 reproductive years compared to 9·79 children/100 reproductive years for their normal sibs. The reproductive fitness for patients with VHL disease is therefore 0·83.

Estimation of mutation rate by the indirect method

The reproductive fitness was combined with the estimated birth frequency for VHL disease (see above) and a mutation rate of 2·32$\times 10^{-6}$ per gene calculated by the indirect method. This is within the

Table 3 Birth order data for 13 cases of VHL disease.

Sibship size	Birth order						Total
	1	2	3	4	5	6	
2	8	2	—	—	—	—	10
3	2	1	—	—	—	—	3
4	—	—	—	—	—	—	0
5	—	—	1	—	—	—	1
6	—	—	—	1	—	—	1
Total	10	3	1	1	0	0	15

Observed 6A=177. Theoretical 6A=150. Standard error 6A=19·05. z=(177–150)/19·05=1·42, p=0·16.

95% confidence interval for the mutation rate calculated by the direct method.

PARENTAL AGE AND BIRTH ORDER FOR NEW MUTATIONS

Twenty-two patients were considered to be new mutations according to the criteria defined previously. The mean (SD) paternal age at birth for these patients was 30·5 (4·8) years compared to an expected 29·1 years. Mean (SD) maternal age was 26·5 (3·8) years compared to an expected 27·2 years. The mean (SD) difference between observed and expected paternal and maternal ages for each patient were 1·34 (4·33) (t=1·13, p=0·27) and −0·7 (4·34) (t=0·66, p=0·52), respectively. Thus, there was no evidence of an association between advanced parental age and new mutations for VHL disease. Seven out of 22 cases classified as new mutations were their parents' only child. The birth order data for the remaining 15 patients are shown in table 3. The data were analysed by the method of Haldane and Smith[17] and no significant birth order effect was detected (p=0·16).

Discussion

The results of complex segregation analysis of VHL disease were clearly compatible with a single dominant gene giving almost complete penetrance by the age of 60 years. Despite the marked variability in expression in VHL disease and interfamilial differences in predisposition to phaeochromocytoma,[2 3] there was no evidence for heterogeneity in the segregation analysis. A locus for VHL disease has been mapped to the short arm of chromosome 3 and family linkage studies have shown no evidence of genetic heterogeneity.[13 18] Segregation analysis among 220 members of a large Hawaiian family by Go *et al*[19] (the same kindred has been reported subsequently by Lamiell *et al*[2]) was also consistent with a single autosomal dominant gene. Although Shokeir[11] suggested recessive inheritance in one family, dominant inheritance with incomplete penetrance would also explain his observations. The penetrance of VHL disease is clearly age related and although most patients have symptomatic complications by the age of 60 we have detected evidence of VHL disease in an asymptomatic parent with three affected children only after careful ophthalmological and radiological screening.[3]

The prevalence of heterozygotes for VHL disease in East Anglia (1/54 000) is higher than that in some previous reports. VHL disease is often not diagnosed unless at risk relatives and patients with apparently isolated retinal angioma or cerebellar haemangioblastoma are carefully screened for other manifestations. The proportion of patients with VHL disease among all patients with cerebellar haemangioblastoma has been variously estimated at 0%,[20] 3·6%,[21] 9·2%,[22] 14·5%,[23] 23%,[24] and 40%.[13] Although Piotrowski and Röhrborn[25] found a prevalence of 1 in 230 000 in northern Germany, Neumann[26] (personal communication) found the prevalence of affected patients in south western Germany to be 1 per 40 000 persons, which is closer to that found by us in East Anglia. We believe that these higher prevalence rates result from more complete ascertainment. Although variations in the prevalence of a rare disorder between areas might result from the presence of a single huge kindred in one region, no single family contributed more than 17% of the total number of heterozygotes in East Anglia. Furthermore the frequency of isolated patients with VHL disease in East Anglia was considerably greater than that reported elsewhere.[25]

VHL disease may cause death or severe handicap in early adulthood and it might be anticipated that reproductive fitness would be reduced in this disorder. We found a reproductive fitness of 0·83 but are not aware of any other estimates. The difficulty in recognising new mutations for VHL disease in the absence of other affected relatives has been discussed. The mutation rate for VHL disease was not significantly different when calculated directly or estimated by the indirect method. The direct estimate ($4{\cdot}4\times10^{-6}$/gene) is of the same order as those estimated for other dominant disorders such as retinoblastoma ($6–7\times10^{-6}$)[27] and Apert's syndrome (3×10^{-6}),[28] but considerably less than that for von Recklinghausen neurofibromatosis ($3{\cdot}1–10{\cdot}4\times10^{-5}$)[29] or tuberous sclerosis ($2{\cdot}5\times10^{-5}$)/haploid genome/generation).[30] Because some new mutations with a single manifestation and no affected relatives cannot be recognised, there will be a tendency to underestimate the mutation rate for VHL disease. Nevertheless, our estimate is much higher than that of Röhrborn *et al*[31] who calculated a mutation rate of $1{\cdot}8\times10^{-7}$, the lowest reported for a classical dominant condition defined by a specific phenotype.[32] For such a low mutation rate to be correct then reproductive fitness should not be significantly reduced and the incidence of VHL disease should be much lower than we found. It seems likely that Röhrborn *et al*[31] underestimated the mutation rate because of underascertainment of isolated cases of VHL disease. We could not find any

association between birth order or parental age and the occurrence of new mutations. Although larger numbers of patients would be needed to detect a small but statistically significant association, it is clear that VHL disease does not resemble Apert's syndrome or achondroplasia for which there is a clear association between advanced paternal age and new mutations.[33] It has been shown recently that there is a predisposition for new mutations to arise in the paternal germline in retinoblastoma and von Recklinghausen neurofibromatosis,[34] two dominantly inherited conditions for which no paternal age effect is apparent. Studies to determine whether a similar situation occurs in VHL disease are in progress.

Detailed information on the data used for the segregation analysis can be provided to interested persons by the first author.

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