Statistical analysis of the two stage mutation model in von Hippel-Lindau disease, and in sporadic cerebellar haemangioblastoma and renal cell carcinoma

E R Maher, J R W Yates, M A Ferguson-Smith

Abstract

Analysis of the age incidence curves for unilateral and bilateral retinoblastoma led Knudson to propose that hereditary tumours may arise by a single event and sporadic tumours by a two stage mutation process. It has been suggested recently that sporadic renal cell carcinoma may arise from a two stage mutation process. We analysed the age incidence curves for symptomatic renal cell carcinoma (n=26) and cerebellar haemangioblastoma (n=68) in 109 patients with von Hippel-Lindau (VHL) disease, and compared them to 104 patients with sporadic renal cell carcinoma and 43 patients with sporadic cerebellar haemangioblastoma. The age incidence curves for renal cell carcinoma and cerebellar haemangioblastoma in VHL disease were compatible with a single mutation model, whereas the age incidence curves for sporadic renal cell carcinoma and cerebellar haemangioblastoma suggested a two stage mutation process. These data are compatible with the VHL gene functioning as a recessive tumour suppressor gene. Sporadic cerebellar haemangioblastoma and some renal cell carcinoma may arise from somatic mutations inactivating both alleles at the VHL locus.

Knudson suggested that human cancers could arise by a two stage mutation process. The first mutation could arise in either germline or somatic tissue, but the second event was always in the latter. Statistical analysis of the age incidence of unilateral retinoblastoma tumours (most of which are sporadic) showed a pattern expected if two independent somatic mutations were necessary for a tumour to develop. In contrast, bilateral retinoblastoma tumours (all of which are inherited) behaved as if only one somatic mutation was necessary, and it was suggested that the first mutation is an inherited germline event. The second mutation is somatic and may occur by a variety of mechanisms including chromosome loss (with or without reduplication), deletion, mitotic recombination, gene conversion, or point mutation. The retinoblastoma gene thus functions as a recessive ‘tumour suppressor gene’ and this model has become a paradigm for the study of inherited cancer syndromes and their sporadic counterparts.

Statistical analysis of age at diagnosis rates for inherited and sporadic cases of Wilms’ tumour, neuroblastoma and phaeochromocytoma, and medullary cell carcinoma gave similar results to those in retinoblastoma. Recently, Erlandssen et al. analysed the age incidence of renal cell carcinoma and found evidence that sporadic tumours arise from a two stage mutation process whereas inherited renal cell carcinomas require only a single somatic mutation as in inherited retinoblastoma cases. Von Hippel-Lindau (VHL) disease is an autosomal dominant disorder with a predisposition to develop a variety of tumours, most frequently haemangioblastoma of the retina, cerebellum, and spinal cord, renal cell carcinoma, and phaeochromocytoma. Although renal cell carcinoma has been said to occur in up to 40% of VHL patients, it appears that most affected subjects will develop renal cell carcinoma if they live long enough. Patients with VHL disease were not included in the study by Erlandssen et al. We have therefore analysed the age at diagnosis of cerebellar haemangioblastoma and renal cell carcinoma in VHL disease, and compared it to that of sporadic tumours to determine if there is statistical evidence for a two hit mutation process occurring in these VHL disease tumours and their sporadic counterparts.

Patients and methods

Patients with VHL disease were ascertained for the purpose of molecular genetic studies by contacting...
medical specialists throughout Great Britain. Clinical details were obtained on 109 patients (eight sporadic, 101 familial from 37 kindreds), of whom 68 had a cerebellar haemangioblastoma and 34 a renal cell carcinoma. All patients with cerebellar haemangioblastoma were symptomatic at diagnosis. However, eight cases of renal cell carcinoma were diagnosed in asymptomatic subjects and these were excluded from the analysis as they would bias towards an earlier age at diagnosis in VHL disease patients. Control patients with sporadic (non-familial) tumours were ascertained from records of the Department of Histopathology at Addenbrooke's Hospital. Forty-three patients with sporadic cerebellar haemangioblastoma were diagnosed between 1977 and 1989, and 104 patients with renal cell carcinoma (clear cell or adenocarcinoma) were diagnosed from 1980 to 1988.

Statistical analysis of tumour age incidence was performed according to the method of Knudson. The proportion of patients not yet diagnosed was plotted against age (t). For a single stage mutation process, as seen in bilateral retinoblastoma, a first order equation of the form \( \log S = a - kt \) is expected. For a two hit mutation process as occurs in unilateral (sporadic) retinoblastomas a second order equation in the form \( \log S = a - kt^2 \) is expected. For each tumour type the data were analysed to determine if a linear relationship (first order) or curvilinear relationship between log S and t was apparent. Linear regression lines were calculated according to the least squares method. Unpaired t testing and \( \chi^2 \) test (with Yates's correction) were applied as necessary. Statistical significance was taken at the 5% level.

Results

CEREBELLAR HAEMANGIOBLASTOMA

The age distribution of cerebellar haemangioblastomas is shown in fig 1. Mean age at diagnosis in VHL disease was significantly younger than in sporadic cases (29·0 (SD 10·1) versus 47·7 (SD 14·9) years, \( t = 7·24, p<0·001 \)). The relationship between proportion of cases not yet diagnosed (S) and age for VHL disease tumours followed a single order equation (fig 2). In contrast, for sporadic cases the data fitted a second order equation.

RENAI CELL CARCINOMA

The age distributions of renal cell carcinoma are shown in fig 3. The mean age at diagnosis of renal cell carcinoma in VHL patients with symptomatic renal cell carcinoma was significantly younger than those with sporadic tumours (44·8 (SD 9·6) versus 61·8 (SD 10·8) years, \( t = 7·3, p<0·001 \)). An excess of males was apparent in sporadic cases (72 males, 32 females), but not in VHL disease patients (13 males, 13 females).

Figure 1  Age distribution of cerebellar haemangioblastoma in sporadic cases and in patients with VHL disease.

Figure 2  Age incidence of cerebellar haemangioblastoma in VHL disease and in sporadic cases. For sporadic cases the data fitted a second order equation \( (\log_{10} S = 0·23 - 2·78 \times 10^{-4} t^2, r = 0·92) \) better than a first order equation. For VHL disease a first order equation was fitted \( (\log_{10} S = 0·69 - 0·04 t, r = 0·99) \).

Figure 3  Age distribution of renal cell carcinoma in sporadic cases and in patients with VHL disease.
However, this difference in sex distribution between
the two groups was not statistically significant
(χ²=2.6, p>0.1). Analysis of age incidence for VHL
disease and sporadic renal cell carcinoma cases gave
similar results to those seen for cerebellar haemangioblastoma. The relationship between log S (proportion
of cases not yet diagnosed) and t (age) was best
described by a first order equation for VHL disease
tumours and a second order equation for sporadic
tumours (fig 4). The linear regression equation for
renal cell carcinoma in VHL disease (log₁₀ S=1.56–
0.04t) was similar to that obtained by Erlandssen et al⁶
for familial renal cell carcinoma (log₁₀ S=1.23–0.03t).

Discussion
We found that both cerebellar haemangioblastoma
and renal cell carcinoma occurring in VHL disease
followed a single stage (somatic) mutation model
originally described for bilateral retinoblastoma
tumours by Knudson.¹ Both sporadic cerebellar
haemangioblastoma and renal cell carcinoma fitted
a two stage mutation model. Erlandssen et al⁶ (using
data from published studies) also fitted a two stage
mutation model curve to the age incidence data for
sporadic renal cell carcinoma. In Knudson’s analyses
of childhood retinoblastoma¹ and Wilms’ tumour²
the equations for the first and second order relationships
(log S=a–kt and log S=a–kt²) were constrained so
that a=0. In contrast, we and others⁵ ⁶ who studied
tumours which occur in adults found that a>0. The
mathematical justification for this is clear, as a linear
relationship between log S and t could not be fitted if
a was constrained to equal 0. However, there is no
primary biological reason why a should equal 0 for
adult tumours. The value of a may be determined by
the age dependent pattern of growth and differentiation
for the particular cell type from which the tumour
arises. For renal tumours there is no clinical overlap
between the age at presentation of Wilms’ tumour and
renal cell carcinoma. For Wilms’ tumour the opportunity
for the second mutation only seems to occur in
childhood. For renal cell carcinoma the opportunity
for the second mutation may only occur in adulthood
and it seems reasonable that the value of a should
be different for the two tumours.

Between 30 and 40% of all cerebellar haemangioblastomas occur in patients with VHL disease,¹² ¹³
which is similar to Knudson’s¹ ¹³ estimates that 35 to
40% of retinoblastoma cases and 38% of Wilms’
tumours were inherited. The gene for VHL disease
has been mapped to the short arm of chromosome 3
by genetic linkage analysis.¹⁴ Cerebellar haemangioblastomas in VHL disease patients show loss of
heterozygosity for DNA markers on chromosome 3p.¹⁵
In these cases the loss of heterozygosity is on the
chromosome with the wild type allele, which is
compatible with the retinoblastoma model of
culminogenesis occurring in VHL disease. It is
anticipated that sporadic cerebellar haemangioblastoma will also show loss of heterozygosity on 3p,
but this has not yet been demonstrated.

The incidence of VHL disease is approximately 1
per 100 000. Renal cell carcinoma accounts for up to
3% of human cancers so that only a small proportion
of all renal cancers are caused by VHL disease. Both
sporadic renal cell carcinoma and tumours from VHL
disease patients show loss of heterozygosity for
chromosome 3p markers,¹⁵⁻¹⁸ and cytogenetically
visible deletions have also been reported.¹⁷ ¹⁹ ²⁰
Although these observations may seem compatible
with the retinoblastoma model of tumourigenesis
where sporadic renal cell carcinoma would result from
somatic mutation of both alleles of the VHL disease
gene, there is evidence that genes at loci other than
the VHL gene are involved in the pathogenesis of
sporadic renal cell carcinoma. Inherited renal cell
carcinomas not caused by VHL disease have been
associated with translocations involving the short arm
of chromosome 3,²¹ ²² A constitutional t(3;8)
reciprocal translocation was associated with an 80%
risk of renal cell carcinoma up to the age of 60 in one
family.²¹ The translocation breakpoint was at
3p14.2.²³ The most likely location for the VHL locus is
telomeric to this at 3p25–p26.²⁴ There are other
elements of familial renal cell carcinoma not associ-
ated with VHL disease or chromosomal abnormalities.²⁵ Current data would seem to suggest that at
least two loci on the short arm of chromosome 3 may
be involved in the pathogenesis of renal cell carcinorna,
one locus being proximal, and another the locus for
VHL disease, which appears to map to the distal part
of 3p.

Our data present statistical evidence for VHL
disease being caused by an inherited mutation of a
'tumour suppressor gene' of the retinoblastoma type. Sporadic cerebellar haemangioblastoma and some sporadic renal cell carcinoma may result from somatic mutations involving both alleles of the VHL disease gene. Further molecular genetic studies will help to confirm or refute this hypothesis.

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