Am J Hum Genet 30:406–424, 1978

Hereditary Retinoblastoma: Delayed Mutation or Host Resistance?

EI MATSUNAGA[1]

INTRODUCTION

It is generally accepted that there are two forms of retinoblastoma, hereditary and nonhereditary [1]. The nonhereditary form, which comprises the majority of sporadic unilateral cases [2–6], is thought to represent about 60% of all retinoblastomas [6, 7]. The remaining 40% are hereditary cases, inherited from (1) a parent who survived bilateral or unilateral disease; (2) an unaffected parent carrying the gene; or (3) due to a new dominant mutation in the gonads of a healthy parent.

While penetrance of the gene for retinoblastoma has been estimated to be 80%–95% [7–9], a number of pedigrees in the literature show multiple occurrences in distant collateral relatives connected through unaffected carriers. Macklin [10] reported that the degree of penetrance may be as low as 20%. However, since penetrance is very high after the appearance of cases in a branch of a family, several workers [5, 7, 11, 12] assumed that the germinal mutation arises, in some instances as a result of delayed mutation. This concept was originally suggested by Auerbach [13] for a type of dominantly inherited split-hand in one pedigree where the pattern of inheritance was similar to that found in *Drosophila* after treatment with certain chemical mutagens.

We have reported, using data from 29 families with two or more affected, that penetrance and expressivity of the gene in children increased with increasing expressivity in the parent [14]. For example, if the parent was bilaterally affected, the proportion of affected children was close to .5, and the disease was almost always bilateral; if the parent was an unaffected carrier, the proportion decreased to .27 and 33% were affected bilaterally. Admittedly, the standard errors of these estimates were large because of the small sample, but this finding suggests that either inherited host resistance or suppressor genes at other loci play a significant role in the manifestation of the major gene for retinoblastoma, or there are multiple alleles with different penetrance at the retinoblastoma locus. The latter is, however, ruled out by the variability of penetrance in different sibships in the same family. Thus, *both penetrance and expressivity are determined by the same mechanism which involves genetic and environmental factors*. We may call this the host resistance model.

Received October 14, 1977; revised March 3, 1978.

This work (contribution no. 1175 from the National Institute of Genetics) was supported by grants from the Ministry of Education and Ministry of Health and Welfare.

[1] Department of Human Genetics, National Institute of Genetics, 411 Mishima, Shizuoka-Ken, Japan.

Delayed mutation presupposes a premutational change of the major gene, whereas host resistance is concerned with changes in the organism caused by genetic and environmental factors other than the major gene. Both mechanisms can cause variability in penetrance and are not mutually exclusive. It is important to examine which model is more compatible with various observations concerning hereditary retinoblastoma. Delayed mutation is generally thought to be specifically the result of chemical mutagens in experimental organisms; evidence for it in man would imply chemically induced mutation by such a substance as might be contained in nutrients or as a product of metabolism [13]. This paper will show by analyzing the literature that host resistance explains variation in the expression of the retinoblastoma locus and that there is little need to postulate delayed mutation.

QUESTIONS TO BE ANSWERED

Answers to the following questions may serve to distinguish between the mechanism of delayed mutation and host resistance.

Is the Gene for Bilateral Retinoblastoma Fully Penetrant?

According to the delayed mutation model, bilateral retinoblastoma is due to a completely mutated allele produced either by immediate or delayed mutation. If other evidence indicates that a gene is incompletely penetrant, delayed mutation is not usually detectable [13].

Does the Segregation Ratio in the Offspring of Unaffected Carriers Vary?

The delayed mutation model presumes a *labile* premutation. By its very nature, a premutation may revert to the normal allele or by delayed mutation, give rise to a stable abnormal allele [13]. Carriers of a labile premutation thus could be gonadic mosaics of normal phenotype. If this were the case with the retinoblastoma locus, the proportion of affected among the offspring of an unaffected carrier would vary from sibship to sibship, depending on the proportion of gonadal cells with the mutated allele. This argument would apply also to the offspring of a unilaterally affected individual, if some of the familial unilateral cases were, as believed by Hermann [12, 15], due to delayed somatic mutation in the carrier of a premutated allele.

Is There a Regular Pattern to the Distribution of Unaffected and Affected (Unilateral and Bilateral) among Carriers of the Gene for Retinoblastoma?

If, as Knudson suggests [7, 16], the occurrence of a second (somatic) mutation that causes the retinal tumor were a random event, independent of host resistance, the distribution of the three phenotypes would follow a Poisson distribution. If, however, the number of tumors were determined by genetic and environmental factors, the distribution would follow a pattern expected from the multifactorial model with two thresholds [17]. On the other hand, when unaffected carriers and unilateral cases were gonadic mosaics, there would be no regular pattern to the phenotype distribution among their offspring.

Can Multiple Occurrences of Retinoblastoma among Distant Collateral Relatives be Consistently Explained by the Host Resistance Model? If not, is There any Alternative other than Delayed Mutation?

As shown in the Japanese data [14], the degree of penetrance of the gene for retinoblastoma will be increased if it is transmitted from an unaffected carrier through a unilateral case to a bilateral case. This may explain pedigrees where retinoblastoma appears in two or more not distantly related members and then shows high penetrance in subsequent generations. Whether this argument can be applied to cases of multiple occurrences among distant collateral relatives is unknown.

MATERIALS

The present study was based on 261 pedigrees with two or more cases of retinoblastoma taken from eleven different sources [2, 3, 10, 14, 18–24]. The 109 compiled by Kaelin [18] cover almost all pedigrees published up to 1951. Since most of the pedigrees were ascertained by single selection, segregation analysis assumes this. Accordingly, the following three series were excluded: seven pedigrees in Switzerland [19] and 21 in Holland [3], both ascertained apparently by a nation-wide survey (with no indication of probands) over the last 30 and 100 years, respectively; and seven pedigrees presented as "some special examples" [23]. For the analysis of the offspring of bilateral cases, data [2, 3, 5, 14, 21, 23, 25] obtained by follow-up investigations of the survivors were used. Informative pedigrees used in this study are listed in the Appendix (tables 1A and 2A).

RESULTS

Segregation Ratio

There were 67 parents affected with bilateral retinoblastoma who had 57 affected of 116 children, a segregation ratio of .491 ± .046 (table 1).

TABLE 1

DISTRIBUTION OF OFFSPRING FROM A BILATERALLY AFFECTED PARENT

REFERENCE	NO. AFFECTED PARENTS	NO. OFFSPRING	
		Total	Affected (%)
Complete Selection:			
[2]	2	3	0
[3]*	7	22	12
[21]	5	5	2
[25]	10	14	8
[5]*	8	9	5
[23]	5	8	2
[14]	4	10	5
Single Selection:			
[18]	10	18	10
[10]	1	2	0
[21]	11	19	11
[14]	2	4	2
[24]	2	2	0
Total	67	116	57 (49.14)

* Excluding probands in the case of single selection.

TABLE 2

DISTRIBUTION OF SIBSHIPS WITH A UNILATERALLY AFFECTED PARENT

No. Affected Sibs		Sibship Size, Excluding Probands						Total
		1	2	3	4	5	6	
0	Observed	9	4	2	...	...	...	15
	Expected	11.6	4.7	0.8	0.5	0.1	0.1	17.8
1	Observed	11	8	1	2	1	...	23
	Expected	8.4	6.8	1.7	1.3	0.5	0.3	19.0
2	Observed	...	2	1	2	1	1	7
	Expected	...	2.5	1.2	1.4	0.7	0.6	6.4
3	Observed	...	...	...	...	...	...	0
	Expected	...	...	0.3	0.7	0.5	0.6	2.1
4	Observed	...	...	...	...	...	1	1
	Expected	...	...	...	0.1	0.2	0.3	0.6
5–6	Observed	...	...	...	...			0
	Expected	...	...	...	...	0.0	0.1	0.1
Total	Observed	20	14	4	4	2	2	46
	Expected	20.0	14.0	4.0	4.0	2.0	2.0	46.0

Although this estimate is consistent with a fully penetrant dominant gene, we asked whether a bilaterally affected parent *can* have an unaffected carrier child. This occurred in one pedigree (no. 717, A. C.) [21]: the proband was a bilaterally affected girl, with an unaffected father and a bilaterally affected paternal grandmother.

Table 2 gives the distribution of 46 sibships with a unilaterally affected parent. There were 41 affected out of a total of 98 sibs, a segregation ratio of .418 ± .050. There were six instances where it was certain that the gene in the affected parent was inherited from an unaffected carrier (see table 1A in Appendix). According to Herrmann [12, 15], such an occurrence is explained by delayed mitotic mutation. If this were the case, the segregation ratio of .418 must be taken as an average of different ratios. The segregation ratio among children of these six unilaterally affected parents is 2/11, whereas the ratio among children of the remaining 40 parents is 39/87. Although the difference is not significant ($P = .083$), the low segregation ratio in the former group seems to favor the delayed mutation model. The difference in mean sibship size (1.83 vs. 2.18) is not significant ($P > .3$), but it suggests that the parents of the former group may have been more eager to limit childbearing after the birth of the proband with the resulting lower segregation ratio.

The 46 unilaterally affected parents may contain more than six in whom the gene was inherited from an unaffected carrier. Therefore, the validity of the Hermann hypothesis can be tested by comparing the observed distribution of the sibships of a given size with the binomial $n_s\ (.418 + .582)^s$, where n_s is the number of sibships of size s. Table 2 shows an agreement between the observed and the expected distribution for varying numbers of affected sibs. The Herrmann hypothesis predicts too many sibships, except for those of $s = 1$, with no affected sibs; however, this was not found.

Table 3 shows the distribution of sibships with two or more affected, whose parents

TABLE 3

DISTRIBUTION OF SIBSHIPS WITH TWO OR MORE AFFECTED, PARENTS UNAFFECTED

No. Affected		No. Sibships with Size s												Total
		3	4	5	6	7	8	9	10	11	12	14	16	
2	Observed	8	6	4	6	2	2	...	...	1	2	...	...	31
	Expected	9.8	5.9	6.8	5.0	1.6	1.2	0.8	0.3	0.4	0.2	0.1	0.0	32.1
3	Observed	4	2	5	6	...	1	1	1	2	...	...	...	22
	Expected	2.2	2.7	4.6	4.5	1.8	1.7	1.2	0.5	0.9	0.4	0.1	0.1	20.7
4	Observed	...	1	4	...	3	2	1	1	...	...	...	...	12
	Expected	...	0.4	1.4	2.0	1.1	1.3	1.1	0.6	1.1	0.5	0.2	0.2	9.9
5	Observed	...	...	...	...	...	...	1	...	1	...	1	...	3
	Expected	...	...	0.2	0.5	0.4	0.6	0.7	0.4	0.9	0.5	0.2	0.2	4.6
6	Observed	...	...	...	...	...	...	1	...	...	...	...	...	1
	Expected	...	...	...	0.0	0.1	0.2	0.2	0.2	0.5	0.3	0.2	0.2	1.9
7–9	Observed	...	...	...	...	...	...	...	...	...	...	...	...	0
	Expected	...	...	...	...	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.3	0.8
10	Observed	...	...	...	...	...	...	...	...	...	...	...	1	1
	Expected	...	...	...	...	...	...	...	0.0	0.0	0.0	0.0	0.0	0.0
Total Sibships (n_s)	Observed	12	9	13	12	5	5	4	2	4	2	1	1	70 (N)
	Expected	12.0	9.0	13.0	12.0	5.0	5.0	4.0	2.0	4.0	2.1	0.9	1.0	70.0
Total Affected		28	22	39	30	16	15	18	7	13	4	5	10	207 (R)

were unaffected. The segregation ratio, p, and its variance, V_p, may be estimated as follows [26]:

$$\frac{R - N}{p} = \sum_s \frac{(s-1)n_s}{1 - q^{s-1}},$$

$$\frac{1}{V_p} = \frac{R - N}{p^2 q} - \frac{1}{q^2} \sum_s \frac{(s-1)^2 n_s q^{s-1}}{(1 - q^{s-1})^2}$$

where $q = 1 - p$, N is the total number of sibships, and R the total number of affected. Figures for n_s, N and R are given in table 3. Putting them into the above equations, p is estimated by iteration at $.312 \pm .028$.

Using $p = .312$, expected sibship distribution for varying numbers of affected shows excellent agreement with that observed. In the delayed mutation model, unaffected carriers presumably comprise gonadic mosaics, and one would expect an excess of sibships with two affected sibs only. This was not observed.

Distribution of Phenotypes among Children Inheriting the Retinoblastoma Gene

Table 4 summarizes the distribution of unilateral and bilateral cases among affected children by expressivity in the carrier parent. There were 54.1% bilaterally affected when the parent was unaffected, 76.2% when the parent was unilaterally affected, and 89.8% when the parent was bilaterally affected. Thus, expressivity in the affected children increases with increasing expressivity in the parent.

Using the estimates for the segregation ratio and the proportion of bilaterally affected children, the frequencies of the three phenotypes among the children who inherited the gene for retinoblastoma were computed (table 5). It is now possible to answer our third question.

Let the observed frequency of unaffected and unilaterally and bilaterally affected among the gene carriers be c, u, and b, respectively, where $c + u + b = 1$. If tumors are distributed according to a Poisson distribution, the expectation for c, u, and b would be e^{-m}, $2e^{-m/2}(1 - e^{-m/2})$, and $(1 - e^{-m/2})^2$, respectively, where m is the mean number of tumors formed per individual. It follows that $u^2 = 4cb$. Contrary to this expectation: for the children with unaffected carrier parents, $u^2 = .078$ and $4cb = .517$; for those with unilaterally affected parents, $u^2 = .040$ and $4cb = .410$; and for those with bilaterally affected parents, $u^2 = .010$ and $4cb = .070$. Thus the assumption of a Poisson distribution must be rejected.

If the distribution of the three phenotypes follows the multifactorial model with two thresholds [17], then assuming a normal distribution of liability in the sense of Falconer [27] or of resistance (which seems a more pertinent term in this case) to the manifestation of the gene for retinoblastoma, the distance between the thresholds is expected to be constant for different groups of children provided the underlying scale is the same. Let T_1 be the threshold beyond which neither eye is affected, and T_2 be the other one below which both eyes are affected. For the children with unaffected carrier parents, the deviation of T_1 from the population mean in terms of the standard deviation unit is .305, corresponding to the proportion of unaffected carriers (38%), and that of T_2 is $-.412$ corresponding to the proportion of bilaterally affected (34%), giving .717

TABLE 4

DISTRIBUTION OF UNILATERAL AND BILATERAL CASES AMONG AFFECTED CHILDREN

REFERENCE	No. SIBSHIPS	No. AFFECTED	
		Unilateral	Bilateral (%)
A. Both parents unaffected, having two or more children			
[18]	43	60	56 (48.3)
[2]	2	1	6
[10]	8	9	18 (66.7)
[3]	8	6	16 (72.7)
[21]	15	11	23 (67.7)
[14]	18	24	12 (33.3)
[24]	2	2	2
Total	96	113	133 (54.1)
B. One parent affected unilaterally			
[18]	27	12	33 (73.3)
[2]	1	1	1
[20]	3	0	3
[10]	1	0	1
[3]	12	5	13 (72.2)
[21]	13	5	25 (83.3)
[14]	12	7	10 (58.8)
[24]	7	0	10 (100.0)
Total	76	30	96 (76.2)
C. One parent affected bilaterally			
[18]	20	5	25 (83.3)
[10]	1	0	1
[3]	6	2	11 (84.6)
[21]	13	1	21 (95.5)
[25]	7	1	7
[14]	8	0	10 (100.0)
[24]	4	0	4
Total	59	9	79 (89.8)

for $T_1 - T_2$. For the children with unilaterally affected parents, $T_1 - T_2$ is .636, and for those with bilaterally affected parents, it is .879; in the latter group, however, the distance is not precisely estimated because of the low incidence (2%) of unaffected carriers. We do not know to what extent the variance differs among the three groups of children, so the estimates for $T_1 - T_2$ are not strictly comparable. The standard deviation is not likely to differ greatly, and the distributions of the three phenotypes are probably in accord with the multifactorial model with two thresholds.

Heritability of Host Resistance to the Retinoblastoma Gene

We have seen that the three phenotypes of the children are apparently determined by quantitative differences in host resistance to the retinoblastoma gene. To estimate the

TABLE 5

DISTRIBUTION OF UNAFFECTED AND UNILATERALLY AND BILATERALLY AFFECTED AMONG CHILDREN WHO INHERITED THE GENE FOR RETINOBLASTOMA ACCORDING TO THE EXPRESSIVITY OF CARRIER PARENTS

EXPRESSIVITY OF CARRIER PARENT	ESTIMATED SEGREGATION RATIO	DISTRIBUTION OF CHILDREN WHO INHERITED THE GENE		
		Unaffected Carrier	Unilateral	Bilateral
Unaffected	0.31	0.38	0.28	0.34
Unilateral	0.42	0.16	0.20	0.64
Bilateral	0.49	0.02	0.10	0.88

heritability of this character, we must know the distribution of the three phenotypes in the population from which the parents were selected. Ignoring the few cases inherited from survivors, the parents are classified into two groups, one arising from a new mutation (group 1) and the other inherited from an unaffected carrier (group 2). Table 6 represents the respective frequencies in the two groups, partitioned in the following way. First, it is assumed that the values for c, u, and b are at equilibrium by the balancing force of selection and mutation, as was the case prior to the modern ophthalmologic care. Then, unaffected carrier parents whose frequency is c will distribute the gene to their unaffected, unilaterally, and bilaterally affected children according to the proportion of .38, .28 and .34, respectively (table 5). Subtracting these from c, u, and b will yield the respective portions due to recurrent mutation.

The values for c, u, and b can be determined if we knew, from an unselected sample obtained by a population survey, the proportion of bilateral cases belonging to group 2 and the ratio of hereditary unilateral to bilateral cases, excluding those inherited from the survivors. Only few data are available for this purpose. Falls and Neel [8] found, in a survey in Michigan over 1938–1951, 24 sporadic bilateral cases and one familial bilateral case with unaffected parents, five familial cases with affected parents, and 42 sporadic unilateral cases. In the Böhringer series [19] in Switzerland, there were 21 sporadic bilateral and 74 sporadic unilateral cases, in addition to five familial cases with affected parents. In our survey in Hokkaido [28] over 1945–1957, we found 22 sporadic bilateral cases, one familial bilateral case with unaffected parents, and 46 sporadic unilateral cases; this is very similar to the findings in

TABLE 6

PARTITION OF THREE PHENOTYPES AMONG UNSELECTED SAMPLE OF CARRIERS OF GENE FOR RETINOBLASTOMA BY ORIGIN, NOT INCLUDING CASES INHERITED FROM AFFECTED PARENTS

GROUP	ORIGIN	PHENOTYPE			TOTAL
		Unaffected	Unilateral	Bilateral	
1	Recurrent mutation	$0.62c$	$u - 0.28c$	$b - 0.34c$	$u + b$
2	From unaffected carrier parents	$0.38c$	$0.28c$	$0.34c$	c
Total	...	c	u	b	$u + b + c$

Michigan. However, the proportion of bilateral cases coming from group 2 must be considered higher than 1/25 or 1/23, because sporadic bilateral cases may contain chance isolated cases. Taking into account the distribution of sibship size and using the segregation ratio of .31, the proportion of bilateral cases inherited from unaffected carrier parents was estimated at about 8%. On the other hand, we estimated, from the result of follow-up investigations of sporadic unilateral cases, the proportion of the hereditary form at about 5% [6], so that the ratio of hereditary unilateral to bilateral cases is about 1:10. Based on these results we have the following equations: $c + u + b = 1$; $.34c/b = .08$; and $u/b = .1$. Table 7 gives the respective proportions of the three phenotypes in the two groups. Thus, for group 1, which may be regarded as a general population having received a new mutation, approximately 13% do not manifest retinoblastoma.

The unaffected carrier parents (tables 3 and 4) may be considered a selected sample taken from groups 1 and 2 with the ratio of .62: .38, respectively. The frequency of unaffected carriers in the population from which they were selected is calculated therefore, by weighting the corresponding frequencies in the two groups with the above ratio, as 16%, whereas in their children this was increased to 38% (table 5). Thus the heritability, h^2, of the host resistance may be estimated, taking into account the reduction in the variance of the children, by the following formula [17]:

$$h^2 = 2\,\frac{x_p - x_R\sqrt{1 - (x_p^2 - x_R^2)\,(1 - x_p/a)}}{a + x_R^2\,(a - x_p)},$$

where x_p and x_R are the deviations from the mean for the parental and children's populations, respectively, of the threshold beyond which retinoblastoma is not manifested, and a is the mean resistance of the unaffected carrier parents. Putting $x_p = .994$, $a = 1.521$, and $x_R = .305$, h^2 is estimated at 94%.

The heritability can be estimated also from the frequency of bilaterally affected among the children who received the gene from bilaterally affected parents, if we knew the corresponding frequency in the parental population. The overwhelming majority of our bilaterally affected parents (tables 1 and 4) are considered due to new mutations, but they include several cases inherited from survivors as well as several from unaffected carriers. Since the effects of including these two groups should cancel out, we may treat our parents as if they were all in group 1 with the frequency of

TABLE 7

ESTIMATED PROPORTION OF THREE PHENOTYPES IN AN UNSELECTED SAMPLE OF CARRIERS OF GENE FOR RETINOBLASTOMA, NOT INCLUDING CASES INHERITED FROM AFFECTED PARENTS

GROUP	PHENOTYPE			TOTAL
	Unaffected	Unilateral	Bilateral	
1	0.109 (13%)	0.026 (3%)	0.689 (84%)	0.824 (100%)
2	0.067	0.049	0.060	0.176
Total	0.176	0.075	0.749	1.000

TABLE 8

PREDICTED CHANGE IN DEGREE OF PENETRANCE OF GENE FOR RETINOBLASTOMA AMONG OFFSPRING OF UNAFFECTED CARRIERS BY SUCCESSIVE SELECTION, WITH 90% HERITABILITY OF HOST RESISTANCE

OFFSPRING GENERATION*	SEGREGATION RATIO			PENETRANCE
	Normal	Affected	Unaffected Carrier	
1	0.5	0.335	0.165	0.67 (π_1)
2	0.5	0.24	0.26	0.48 (π_2)
3	0.5	0.17	0.33	0.34 (π_3)
4	0.5	0.12	0.38	0.24 (π_4)
5	0.5	0.09	0.41	0.18 (π_5)

* The founder is assumed to be a carrier arising from mutation.

bilaterally affected being 84%. Since this proportion increased to 88% in their children (table 5), h^2 was estimated, as above, at 92%, and the two estimates agree well.

Multiple Occurrences among Collateral Relatives

Having estimated the heritability of host resistance, one can predict the change in the degree of penetrance (π) of the gene for retinoblastoma among offspring of unaffected carriers by successive selection. Although heritability will decline in the course of repeated selection, experiments have shown that the response is usually maintained with little change over 5, 10, or more generations [29]. Table 8 shows the predicted change up to the fifth generation, together with the change in the segregation ratio; in this computation, it is assumed that heritability is 90% and the founder is a carrier arising by mutation. Using the predicted value for penetrance in the offspring of an unaffected carrier, the expected occurrence of retinoblastoma in two collateral relatives was calculated (table 9).

In the literature, there are 33 pedigrees with two or more affected collateral relatives who were apparently connected through unaffected carriers (table 2A in Appendix). For each pedigree, the relationship between two affected members, defined by the following rule, was identified. Counting from a common ancestor who is presumably an unaffected carrier, the first case is defined. If the pedigree contained two affected members only, their relationship is readily identified. If there are more affected, the one who is the closest to the first case but who belongs to a different sibship is defined as the second case, and his/her relationship with the first case was identified. As controls, sibships with two or more affected sibs but with no known case in the collateral relatives were surveyed; in addition to 57 listed in table 1A in the Appendix, there were 17 with only two affected sibs.

Table 10 represents the distribution of different pedigrees according to the degree of relationship between two affected members defined by the criteria mentioned above. The expected distribution was computed using the figures shown in table 9, assuming that each type of pedigree will be ascertained and reported in the literature with equal probability. It is evident that the observed distribution agrees well with expectation if two affected members are only related up to first cousins once removed. The observed

TABLE 9

EXPECTED PROPORTION OF OCCURRENCE OF RETINOBLASTOMA IN TWO COLLATERAL RELATIVES AMONG DESCENDANTS OF AN UNAFFECTED CARRIER, ASSUMING NO MUTATION

Relationship of Affected	Probability of Occurrence		Expected Proportion
Sibs	$\pi_1^2 =$	.4489	.7075
Uncle (aunt) and nephew (niece)	$\pi_1 (1 - \pi_1) \pi_2 =$	.1061	.1672
Granduncle and nephew (niece)	$\pi_1 (1 - \pi_1) (1 - \pi_2) \pi_3 =$	.0391	.0616
First cousins	$(1 - \pi_1)^2 \pi_2^2 =$	.0251	.0396
First cousins once removed	$(1 - \pi_1)^2 (1 - \pi_2) \pi_2 \pi_3 =$	.0092	.0145
Second cousins	$(1 - \pi_1)^2 (1 - \pi_2)^2 \pi_3^2 =$	.0034	.0054
Second cousins once removed	$(1 - \pi_1)^2 (1 - \pi_2)^2 (1 - \pi_3) \pi_3 \pi_4 =$	.0016	.0025
Third cousins	$(1 - \pi_1)^2 (1 - \pi_2)^2 (1 - \pi_3)^2 \pi_4^2 =$	.0007	.0011
Third cousins once removed	$(1 - \pi_1)^2 (1 - \pi_2)^2 (1 - \pi_3)^2 (1 - \pi_4) \pi_4 \pi_5 =$	.0004	.0006
Total	...	.6345	1.0000

TABLE 10

DISTRIBUTION OF PEDIGREES SHOWING MULTIPLE OCCURRENCE OF RETINOBLASTOMA AMONG COLLATERAL RELATIVES WHO ARE CONNECTED THROUGH UNAFFECTED CARRIERS

AFFECTED RELATIVES	NO. PEDIGREES				
	TOTAL NO. AFFECTED		TOTAL		
	2	3 and more	Observed	Expected	Observed/Expected
Sibs	43	31	74	75.69	0.98
Uncle (aunt) and nephew (niece)	...	15	15	17.89	0.84
Granduncle and nephew (niece)	1	1	2	6.59	0.30
First cousins	4	3	7	4.22	1.66
First cousins once removed	2	1	3	1.56	1.9
Second cousins	2	1	3	0.58	5.2
Second cousins once removed	1	...	1	0.27	3.7
Third cousins	1	...	1	0.13	7.7
Third cousins once removed	1	...	1	0.07	14.3
Total	55	52	107	107.00	1.00

number of pedigrees with granduncle and nephew (niece) affected appeared smaller, whereas the number with first cousins affected was slightly larger than expected; however, such discrepancies may well be accounted for by differences in ascertainment probability.

From the ratio of observed to expected, there are a small number of pedigrees in excess of expected with more distantly related members affected; however, of the six pedigrees, only one contained affected members other than second cousins, whereas in each of the other five with second cousins or more remote relatives affected, there were no other members affected. Therefore the occurrence of retinoblastoma in the two distantly related members might be a coincidence.

The probability of the independent occurrence of a second case among any collateral relatives is equal to the incidence of retinoblastoma in the population, which is somewhat less than 1:20,000 infants [9]. Although this figure may appear small, the probability will be increased rapidly as one investigates thoroughly more and more remote relatives. Let the mean number of children born per couple be s and the survival rate, v, then the mean number of second cousins, second cousins once removed, third cousins, and third cousins once removed will be, on both paternal and maternal sides of an individual, $4(sv - 1)s^2v$, $4(sv - 1)s^3v^2$, $8(sv - 1)s^3v^2$ and $8(sv - 1)s^4v^3$, respectively. If one assumes $s = 4$ and $v = 0.8$, which approximates the observed data in many developed countries until the current generations, the respective numbers of these more remote relatives will be 112, 360, 720, and 2306; the probability of the independent occurrence of a second case can never be ignored. The probability of finding the second case depends upon the degree of thoroughness in carrying out the investigation. Macklin [10] investigated over 4,000 relatives of one particular kindred. If one could investigate 100 different families as thoroughly as she did, one would probably find several families with two distant relatives affected with retinoblastoma by chance. In fact, of the five pedigrees with two affected only among more distantly related members, four are from the Macklin series [10] (table 2A in the Appendix).

The above interpretation suggests that one should find a few pedigrees with two *unrelated* members affected. There are two such instances in the literature: no. 307 [21] and no. 421 [24].

The reported pedigrees with two or more collateral relatives affected could be explained either by the host resistance model or by coincidence. Two pedigrees with an unusual pattern of inheritance deserve special remarks; one is no. 60 and no. 63 in the Macklin series [10], and the other is a pedigree [19] not listed in table 2A in the Appendix because of insufficient documentation. Both pedigrees contain sibships with affected members, which could be traced through 4–6 generations to two apparently separated lines, and consanguineous marriages are involved. In the Macklin pedigree containing four sibships with affected members, two were the product of such marriages, and in the other two, consanguineous marriages had occurred not only in the grandparents but also in the great-grandparents. The Böhringer pedigree involved only one instance of consanguineous marriages in the ancestors, but it was noted that such marriages were relatively frequent in the region investigated. Although Macklin discussed the possibility of a recessive gene running in that family, it might be explained more reasonably by the known inbreeding effect in polygenic inheritance [30]. Since the underlying factors for the host resistance to retinoblastoma can be considered polygenic, consanguineous marriages would produce, as a result of increased homozygosity, those children who are more susceptible as well as more resistant to the tumor formation.

DISCUSSION

The results presented above demonstrated that there is little need to postulate delayed mutation at the retinoblastoma locus. Segregation data covering 2 generations give no indication that unaffected carriers or unilaterally affected parents carry a labile premutation in their gonads. The reported pedigrees with two or more collateral relatives affected could be explained by either the host resistance model or coincidence. However, it is impossible to exclude delayed mutation by conventional methods of formal genetics.

We have found further evidence for the host resistance model. With bilaterally affected parents, the segregation ratio was close to expectation for a fully penetrant dominant gene, although other pedigree data indicate that the gene is not fully penetrant. This is consistent with the cases of bilateral disease with spontaneous regression [31, 32], and also with the discordant pair of identical twins [33]. The report [14] that penetrance and expressivity in children varied consistently with the degree of expressivity in the carrier parents has been confirmed. The distribution of unaffected and unilaterally and bilaterally affected among the gene carriers was consistent with the multifactorial model with two thresholds, and not with a Poisson distribution pattern of tumors. It is possible that the variation in the number of tumors is determined by host resistance, while second (somatic) mutation may be a random event of the Poisson type. Heritability of host resistance was estimated, from two sets of independent data, at about 90%.

Estimates of heritability for human characters is often too high because of the inclusion of environmental variance common to relatives. This is particularly true

when the estimate is based on full sibs or when the character in question is concerned with liability to a disease with late onset. In retinoblastoma, the error is probably not large because of its early onset with its principal causal agent not an exogenous one but a mutant gene, and because the estimation is based on parents and offspring. The high value for h^2 leaves little room for dominance or environmental variances. A recent survey of twins with retinoblastoma [34] supports a high degree of heritability: three of 17 pairs of monozygotic and presumed monozygotic twins were discordant. One of these pairs was unilateral and thus probably nonhereditary; in a second, the unaffected twin died at 3 months; and the last pair is cited above [33].

Table 7 shows that in the general population (group 1) approximately 13% of those who received a new mutation from a healthy parent do not manifest retinoblastoma. This high frequency of resistant individuals suggests that the suppressors are not specific to retinoblastoma. Considering a mutation rate of 10^{-5} [2, 3, 5, 6] at this locus, it is unlikely that suppressors could have accumulated by the selecting force of that particular gene. The three phenotypes of gene carriers are viewed as pleiotropic effects of polymorphic genes concerned with the growth of tumor cells.

Significant associations of HLA antigens with retinoblastoma have been reported [35], and cell-mediated immune response was thought to play a role in the host resistance [36]. The host resistance model suggests that nonhereditary retinoblastoma, due to somatic mutations [2, 7], occurs in the most susceptible group of the general population, whereas unaffected carriers including spontaneously regressed cases are the most resistant group. Therefore, a genetic marker correlated with host resistance to retinoblastoma would be found with decreasing frequency in unaffected carriers, hereditary unilateral cases, general population, bilateral and nonhereditary cases. The detection of such a marker would be maximized by comparing unaffected carriers with nonhereditary cases.

SUMMARY

Evidence is presented from the literature that there is little need to postulate delayed mutation for the retinoblastoma locus. Both penetrance and expressivity in the gene carrier can be defined as a variable determined by genetic and environmental factors, not by a Poisson distribution of tumors formed. Of individuals who received a new mutation from a healthy parent, approximately 13% do not manifest retinoblastoma, and the heritability of the host resistance is estimated at about 90%. The nonhereditary form of retinoblastoma may occur in the most susceptible group of the population.

ACKNOWLEDGMENTS

I thank both referees for valuable comments on the manuscript, and Miss Mieko Matsumoto for her assistance with this study.

REFERENCES

1. François J, Matton MTh, Bie SDe, Tanaka Y, Vandenbulcke D: Genesis and genetics of retinoblastoma. *Ophthalmologica* 170:405–425, 1975
2. Vogel F: Neue Untersuchungen zur Genetik des Retinoblastoma (Glioma retinae). *Z Menschl Vererb-u Konstitut* 34:205–236, 1957

3. SCHAPPERT-KIMMIJSER J, HEMMES GD, NIJLAND R: The heredity of retinoblastoma. *Ophthalmologica* 151:197–213, 1966
4. NIELSEN M, GOLDSCHMIDT E: Retinoblastoma among offspring of adult survivors in Denmark. *Acta Ophthalmol (Kbh)* 46:736–741, 1968
5. BRIARD-GUILLEMOT ML, BONAÏTI-PELLIÉ C, FEINGOLD J, FRÉZAL J: Étude génétique du retinoblastoma. *Humangenetik* 24:271–284, 1974
6. MATSUNAGA E, OGYU H: Retinoblastoma in Japan: follow-up survey of sporadic cases. *Jpn J Ophthalmol* 20:266–282, 1976
7. KNUDSON AG JR: Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68:820–823, 1971
8. FALLS HF, NEEL JV: Genetics of retinoblastoma. *Arch Ophthalmol* 46:367–389, 1951
9. VOGEL F: Genetic prognosis in retinoblastoma, in *Modern Trends in Ophthalmology*, edited by SORSBY A, London, Butterworth, 1967, pp 34–42
10. MACKLIN MT: A study of retinoblastoma in Ohio. *Am J Hum Genet* 12:1–43, 1960
11. NEEL JV: Mutations in the human population, in *Methodology in Human Genetics*, edited by BURDETTE WJ, San Francisco, Holden-Day, 1962, pp 203–226
12. HERRMANN J: Delayed mutation model: carotid body tumors and retinoblastoma, in *Genetics of Human Cancer*, edited by MULVIHILL JJ, MILLER RW, FRAUMENI JF JR, New York, Raven Press, 1977, pp 417–438
13. AUERBACH CH: A possible case of delayed mutation in man. *Ann Hum Genet* 20:266–269, 1956
14. MATSUNAGA E: Hereditary retinoblastoma: penetrance, expressivity and age of onset. *Humangenetik* 33:1–15, 1976
15. HERRMANN J: Delayed mutation as a cause of retinoblastoma: application to genetic counseling. *Birth Defects: Orig Art Ser* 12(1):79–90, 1976
16. KNUDSON AG JR: Mutation and childhood cancer: a probabilistic model for the incidence of retinoblastoma. *Proc Natl Acad Sci USA* 72:5116–5120, 1975
17. REICH T, JAMES JW, MORRIS CA: The use of multiple thresholds in determining the mode of transmission of semi-continuous traits. *Ann Hum Genet* 36:163–184, 1972
18. KAELIN A: Statistische Prüf- und Schätzverfahren für die relative Häufigkeit von Merkmalsträgern in Geschwisterreihen bei einem der Auslese unterworfenen Material mit Anwendung auf das Retinagliom. *Arch Klaus Stift Vererbungsforsch* 30:263–485, 1955
19. BÖHRINGER HR: Statistik, Klinik und Genetik der Schweizerischen Retinoblastomfälle (1924–1954). *Arch Klaus Stift Vererbungsforsch* 31:1–16, 1956
20. TUCKER DP, STEINBERG AG, COGAN DG: Frequency of genetic transmission of sporadic retinoblastoma. *Arch Ophthalmol* 57:532–535, 1957
21. ELLSWORTH RA: The practical management of retinoblastoma. *Trans Am Ophthalmol Soc* 67:462–534, 1969
22. CZEIZEL A, GÁRDONYI J: Retinoblastoma in Hungary, 1960–1968. *Humangenetik* 22:153–158, 1974
23. GORDON H: Family studies in retinoblastoma. *Birth Defects: Orig Art Ser* 10(10):185–190, 1974
24. MINODA K: National registration of retinoblastoma children in 1975. *Acta Soc Ophthalmol Jpn* 80:1648–1657, 1976
25. SORSBY A: Bilateral retinoblastoma: a dominantly inherited affection. *Br Med J* 2:580–583, 1972
26. HALDANE JBS: A test for homogeneity of records of familial abnormalities. *Ann Eugen (Lond)* 14:339–341, 1949
27. FALCONER DS: The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Ann Hum Genet* 29:51–76, 1965
28. MATSUNAGA E, OGYU H: Genetic study of retinoblastoma in a Japanese population. *Jpn J Hum Genet* 4:156, 1959
29. FALCONER DS: *Introduction to Quantitative Genetics*. New York, Ronald Press, 1960, p 191

30. NEWCOMBE HB: Panel discussion of the session on epidemiologic studies. *Second International Conference on Congenital Malformations*, New York, International Medical Congress, 1964, pp 345–349
31. BONIUK M, GIRARD LJ: Spontaneous regression of bilateral retinoblastoma. *Trans Am Acad Ophthalmol Otolaryngol* 73:194–198, 1969
32. MORRIS WE, LAPIANA FG: Spontaneous regression of bilateral multifocal retinoblastoma with preservation of normal visual acuity. *Ann Ophthalmol* 6:1192–1194, 1974
33. KANTER YC, HARRIS JE: Retinoblastoma occurring in one of a pair of identical twins. *Arch Ophthalmol* 72:783–787, 1964
34. WARBURG M: Retinoblastoma, in *Genetic and Metabolic Eye Disease*, edited by GOLDBERG MF, Boston, Little, Brown, 1974, pp 447–461
35. BERTRAMS J, SCHILDBERG P, HÖPPING W, BÖHME U, ALBERT E: HL-A antigens in retinoblastoma. *Tissue Antigens* 3:78–89, 1973
36. CHAR DH, ELLSWORTH R, RABSON AS, ALBERT DM, HERBERMAN RB: Cell-mediated immunity to a retinoblastoma tissue culture line in patients with retinoblastoma. *Am J Ophthalmol* 78:5–11, 1974

(Appendix follows)

APPENDIX

TABLE 1A

PEDIGREES REPORTED IN THE LITERATURE

Reference and Pedigree No.	Sibship Size	No. Affected Sibs
A. Transmission of retinoblastoma to children from a bilaterally affected parent (excluding proband)		
[18]:		
50	4	3
62*	1	1
66	1	1
77	2	1
79*	1	1
85	1	0
91	3	2
101	1	1
109†	2	0
110	2	0
[10]:		
41†	2	0
[21]:		
124	4	3
131	2	2
307	2	0
313	1	0
449	1	1
515‡	1	1
515‡	1	1
528	1	1
529	1	1
719	1	0
801	4	1
[14]:		
8‡	2	1
8‡	2	1
[24]:		
162	1	0
411	1	0
B. Transmission of retinoblastoma to children from a unilaterally affected parent (excluding proband)		
[18]:		
15	2	1
30	6	2
39	2	2
40	6	4
41	1	1
51	2	1
61	2	1
69	1	0
71	1	1
72	3	0
93†	1	0
95	1	1
97	1	0
102	2	1
103	1	1
107	3	2
[2]:		
3	4	1
[10]:		
47†	3	0
[21]:		
175	2	1
181	1	1
212	1	1
236	2	0
324	5	1
336	2	1
409*	2	1
420	2	1
425†	2	0
507	4	1
575	3	1
577	1	1
[14]:		
6‡	4	2
6‡	4	2
13†	1	0
14	1	1
15†	2	0
16	2	0
17‡	1	1
17‡	1	1
19	1	0
23†	2	2
25	1	0
26	1	0
[24]:		
14	1	0
72	5	2
136	1	0
230	1	1

TABLE 1A

PEDIGREES REPORTED IN THE LITERATURE

Reference and Pedigree No.	Sibship Size	No. Affected Sibs	Reference and Pedigree No.	Sibship Size	No. Affected Sibs
C. Sibships with two or more affected, parents unaffected (including proband)					
[18]:					
1	7	4	93	9	5
3	5	4	109	4	2
4	11	3	[2]:		
6	6	2	1	10	4
7	8	3	2	6	3
8†	14	5	[10]:		
9	5	2	60†	9	4
10	6	2	77	3	2
11	5	3	115	5	3
12	6	3	[21]:		
14	3	2	153	4	2
17	6	3	330†	7	4
19	5	3	225†	8	2
20†	10	3	288	4	2
21	5	4	415	5	2
22	4	3	425	5	4
23†	16	10	537†	5	3
24	3	2	541	5	2
25	3	2	639	6	2
27†	8	4	655	3	2
35	6	3	717*	5	2
36	11	3	[14]:		
42	11	5	1	6	2
47†	12	2	2	4	2
54	4	3	4†	4	2
55	4	2	5	7	2
57	8	4	7	6	3
57†	5	4	9	7	2
58	8	2	10	3	3
59	11	2	13	9	3
73	6	2	15	6	2
75	3	3	22	6	3
78	9	6	23	5	3
81	3	3	24†	3	2
82†	12	2	[24]:		
83	7	4	276	3	2
86	3	2			
88	4	4			
89	3	3			

* One of the grandparents is affected with retinoblastoma.
† One of the grandparents is an unaffected carrier.
‡ Listed twice because of two probands.

TABLE 2A

MULTIPLE OCCURRENCES OF RETINOBLASTOMA AMONG COLLATERAL RELATIVES APPARENTLY CONNECTED BY UNAFFECTED CARRIERS

Reference and Pedigree No.	No. Sibships with Affected	No. Affected*	Relationship of Affected†
[18]:			
5	2	several	u-n
8	3	9	1½c
16	2	4	u-n
20	2	5	u-n
23	2	11	u-n
27	2	5	u-n
29	2	2	1c
36	2	5	u-n
47	2	3	u-n
57	2	8	u-n
70	2	2	1c
82	2	5	u-n
[10]:			
14	2	2	3½c
40	2	2	gu-n
52	2	2	2c
55, 62	2	2	2½c
60	4	9	gu-n
67	2	2	1c
116	3	3	2c
[3]:			
v.Zw	2	4	u-n
Hs	2	9	u-n
[21]:			
330, 225	3	7	u-n
458	2	2	2c
537	6	9	u-n
612	2	2	1c
792	2	2	1½c
823	2	2	1½c
877	2	2	3c
[22]:	5	8	u-n
[23]:			
7	2	4	1c
[14]:			
3	2	2	1c
4	2	3	u-n
24	3	4	1c

* Offspring of affected members were not included.

† Counting from the common ancestor, the first and the second affected belonging to different sibships were taken. u-n: uncle (aunt) and nephew (niece); gu-n: granduncle and nephew (niece); 1c: first cousins, 1½c: first cousins once removed; 2c: second cousins; 2½c: second cousins once removed; 3c: third cousins; 3½c: third cousins once removed.