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A genetic analysis of retinitis pigmentosa

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SUMMARY Genetic analysis of 457 patients with retinitis pigmentosa (RP) included categorisation of families by recognised mendelian pattern of inheritance and formal segregation analysis of all informative sibships. Of the 368 probands a surprisingly high 18% (68) had significant congenital loss of hearing and were diagnosed as having Usher syndrome. The RP probands were categorised as: 21·7% autosomal dominant, 9·0% X-linked, 16·0% autosomal recessive, 3·3% genetic type uncertain, and 50·0% simplex. Segregation analysis reflected this high proportion of simplex cases, accounting for reduced penetrance in dominant families; only 20% remain classified as sporadic (possibly nongenetic). In the matings between normal persons estimates of the segregation ratio also indicate lower values than expected. Unlike in RP sibships, segregation in the Usher syndrome is consistent with the hypothesis of recessive inheritance. Therefore RP with significant hearing loss segregates as expected, while even if a proband is classified as a dominant or recessive the recurrence risk for the RP phenotype may be below mendelian expectation.

Genetic heterogeneity in hereditary retinal dystrophies such as retinitis pigmentosa (RP) has been well established by family studies,[1 2] by the recognition of several genetic syndromes of which RP is a feature,[2 3] and by animal experiments.[4 5] The distribution of RP cases into genetic categories has been described for several populations, with varying results.[6–13] Comparisons must be made carefully and conclusions about population differences cautiously drawn, since ascertainment biases have not been accounted for in most studies.[12]

Beyond distribution of cases into genetic categories by mendelian laws formal genetic analysis can be undertaken to account for ascertainment probability. Previous segregation analyses of retinitis pigmentosa have shown that mendelian hypotheses may not appropriately account for the excess of simplex cases.[12 14] From these analyses it becomes apparent that other genetic and/or environmental factors are possibly contributing to the unexpectedly low proportion of affected persons in families. An example might be the families with dominant inheritance reported by Berson and colleagues[15] that show reduced penetrance.

The combination of congenital hearing loss and retinitis pigmentosa is referred to as the Usher syndrome.[16] Genetic heterogeneity in Usher syndrome has also been indicated by clinical studies[17 18] as well as suggested by family studies.[19] The Usher syndrome population has not been carefully examined to document the segregation ratio or the presence of excess simplex cases which would suggest further heterogeneity. The proportion of cases in clinical RP populations that have a form of Usher syndrome has not been clearly established.

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Material and methods

PATIENTS

The clinical population consisted of 457 patients diagnosed as having retinitis pigmentosa from the University of Illinois Eye and Ear Infirmary, Retinitis Pigmentosa Clinical Research Center. Of these, 368 cases were considered as probands (or index cases). Diagnostic examination included best corrected visual acuity; slit-lamp biomicroscopic evaluation of the cornea, anterior chamber, lens, and vitreous; and examination of the retina by both direct and indirect ophthalmoscopy. Intraocular pressure by applanation tonometry was also obtained. Most patients had the visual-field measured on a Goldmann perimeter and dark-adaptation testing by a Goldmann-Weekers dark adaptometer, as well as electro-retinography (ERG) and fluorescein angiography.

On funduscopic examination the majority of

patients showed varying degrees of typical bone-spicule pigment clumping and migration. All patients recognised some degree of nyctalopia, and abnormal rod function was found on ERG. In the majority of cases the ERGs also showed abnormal cone function.

Of the 457 patients 19% (87) were recognised as having Usher syndrome because of a congenital neurosensory hearing impairment that varied from moderate to profound. Of the 368 probands 68 (18%) were diagnosed as Usher syndrome patients.

METHODS OF ANALYSIS

Patients were classified genetically on the basis of family history criteria as described previously.[11] Direct vertical transmission reported in 3 generations permitted classification as an autosomal dominant case. Pedigrees with X-linked recessive inheritance contained multiple affected males, and/or affected male(s) and female carrier(s) as ascertained by funduscopic examination and ERG testing. Patients included in the autosomal recessive group had reportedly normal parents and multiple affected siblings or documented parental consanguinity. In the 10 cases of uncertain inheritance multiple family members were affected, but a specific mode of inheritance could not be determined. Simplex cases were defined as patients with no other family history or consanguinity recognised in the pedigree.

Segregation analysis was performed on nuclear family units according to the method first described by Morton[20] by means of the computer program SEGRAN. Estimation of 3 population parameters is achieved in this series of analyses: π, the ascertainment probability; p, the segregation ratio or recurrence risk in the families in specific mating type categories; and x, the proportion of sporadic cases, which is estimated by the excess of simplex cases beyond probabilistic prediction.

To perform segregation analysis proband sibships are first divided by reported parental mating type (AFFECTED×NORMAL, or NORMAL×NORMAL). After estimation of π from the data, the Mendelian ratios expected for each group are first tested as null hypotheses. Estimates of p and x are calculated using a maximum likelihood algorithm. From these calculations estimates are obtained for the segregation ratio or recurrence risk, and an estimate of the excess of simplex cases is obtained in the N×N group after appropriately adjusting for family size and mendelian probabilities. A mathematical estimate of penetrance, the proportion of individuals with the genotype actually manifesting the phenotype, is also calculated.

Table 1 *Distribution of cases of RP by genetic type*

Classification	*Proband*		*Patient*	
	No.	*%*	*No.*	*%*
Autosomal dominant	65	21·7	105	28·4
Autosomal recessive	48	16·0	60	16·2
X-linked	27	9·0	44	11·9
Uncertain	10	3·3	11	3·0
Simplex	150	50·0	150	40·5
Total	300	100·0	370	100·0

Results

DISTRIBUTION OF GENETIC CASES

In determining the distribution of cases by genetic classification, only patients personally examined by one of us (G.F.) were included. The distribution of probands and patients examined is summarised in Table 1. Of the 370 patients 105 (28·4%) were shown to be autosomal dominant cases, while a smaller proportion (21·7%) of the probands fell into this category. The proportion of autosomal recessive cases was approximately the same for both groups, with 16·2% (60) of the patients and 16·0% (48) of the probands fitting these criteria. Almost 12% of the patients or 9% of the probands had pedigrees or family member examinations that confirmed X-linked recessive inheritance. Only 3% of the cases came from multiplex families (more than one family member affected) in which the genetic type could not be determined with certainty. The highest proportion of cases were simplex or isolated. These 150 cases represented 50% of the probands and 40·5% of the patients in this clinic population.

Table 2 illustrates the proportionate distributions with the inclusion of patients with Usher syndrome. The Usher syndrome cases accounted for approximately 19% of the RP patient population at this clinical centre, a surprisingly high proportion considering the low incidence of Usher syndrome. Of the 147 patients with known recessive forms of RP 59% had Usher syndrome, while 68 of the 116 recessive probands or 57% had Usher syndrome. The narrow definition used for inclusion in the autosomal recessive category does not include genetic cases of

Table 2 *Distribution of cases by genetic type including Usher syndrome*

Classification	*Proband*		*Patient*	
	No.	*%*	*No.*	*%*
Autosomal dominant	65	17·7	105	23·0
Autosomal recessive	48	13·0	60	13·1
X-linked	27	7·3	44	9·7
Uncertain	10	2·7	11	2·4
Usher syndrome	68	18·5	87	19·0
Simplex	150	40·8	150	32·8
Total	368	100·0	457	100·0

RP that are by chance simplex, while any patient with Usher syndrome is considered to have a recessive form of RP in this calculation. If all simplex cases are assumed to be autosomal recessive, the Usher syndrome still accounted for 29·2% of the recessive patients or 25·5% of the probands.

SEGREGATION ANALYSIS

Formal genetic analysis permits testing of hypotheses regarding the segregation ratio for each mating type category, the presence of sporadic cases, and estimation of the penetrance of the gene(s), while correction for ascertainment bias is accounted for. Table 3 summarises the results of segregation analysis on the 227 informative RP sibships. For a family to be considered informative the phenotypes of siblings and parents must be known, and the sibship must consist of 2 or more, so that segregation could have occurred.

The ascertainment probability, π, was estimated to be less than 0·01, which is not unusual for this type of clinic population. The method of population ascertainment approaches single selection ($\pi \to 0$), since almost every family was ascertained through a single proband, and then subsequently family members were tested.

In the offspring of AFFECTED×NORMAL matings the hypothesis of dominant inheritance was tested ($p=0·5$), and it was assumed that there were no sporadic cases ($x=0·0$). When the sibships of all 40 probands were included in this analysis, the hypothesis of $p=0·5$ was rejected ($\chi^2=142·9$), and the maximum likelihood estimate of the segregation ratio was 0·16. When only those sibships with the proband over 30 were included, the dominant hypothesis fitted much better ($\chi^2=29·1$), but the segregation ratio was still significantly lower than 0·5 with the maximum likelihood estimate of $\hat{p}=0·2$.

In the NORMAL×NORMAL mating category the hypothesis of $p=0·25$ and $x=0·0$ was tested. Both autosomal recessive and X-linked cases would fit this hypothesis if sex of offspring is ignored and carrier female phenotypes are considered 'normal' rather than 'affected.' When all 187 informative sibships were included. the hypothesis that all cases were genetic ($x=0·0$) and segregating at $p=0·25$ was resoundingly rejected ($\chi^2=181·2$). When maximum likelihood estimates of the parameters p and x were simultaneously calculated, it was found that $\hat{p}=0·25$ and $\hat{x}=0·59$. The limitation of the proband population to 30 years or older did not make a dramatic difference in the fit of the model ($\chi^2=143·4$), and the estimates of $p(\hat{p}=0·22)$ and $x(\hat{x}=0·58)$ were not altered.

When only multiplex sibships were analysed, the hypothesis of all genetic cases segregating at $p=0·25$ was acceptable ($\chi^2=1·97$). Because x could be assumed to be 0·0, only p was estimated and found to be 0·19, much less than the predicted 0·25.

A substantial proportion of the cases in this clinical centre population were diagnosed as having Usher syndrome. Of the 368 probands 68 or 18% were diagnosed as Usher syndrome. Of the 457 patients 87 or 19% had the diagnosis. Of the 245 informative sibships of the NORMAL×NORMAL mating category, 58 or 23·7% fell into the Usher group. The results of the segregation analysis performed on the 58 informative Usher syndrome sibships are shown in Table 4. Of the 58 sibships 34 were simplex and 24 multiplex. The ascertainment probability, π, was estimated to be 0·57, indicating that several sibships were ascertained through multiple probands independently or simultaneously. The model of all cases being genetic and segregating as recessives fitted very well ($\chi^2=2·3$), with a maximum likelihood estimate of $\hat{p}=0·21$.

Table 3 *Segregation analysis of informative RP sibships. $\pi \simeq 0·01$*

Mating type and hypothesis tested	*No. of sibships*	χ^2	$\hat{p}$	$\hat{x}$
AFFECTED×NORMAL				
$p=0·5, x=0·0$				
All probands	40	142·90	0·16	—
Probands ≥ 30 yr	29	29·10	0·20	—
NORMAL×NORMAL				
$p=0·25, x=0·0$				
All probands	187	181·20	0·23	0·59
Probands ≥ 30 yr	123	143·40	0·22	0·58
Multiplex (recessive)	35	1·97	0·19	—

Discussion

The criteria used for classification of cases are reasonably rigorous and provide conservative estimates of the proportions of genetic types in this population. The cases of uncertain genetic type consisted of a few pedigrees showing either 2-generation inheritance with no male-to-male transmission or multiplex sibships with only males affected.

It is important to compare the genetic proportions in the proband and patient groups. Comparisons show no dramatic differences between the distribution of cases in the 2 groups, but the trends are obvious. The largest differences are in the dominant and simplex proportions, not because these are the largest groups

Table 4 *Segregation analysis of 62 informative sibships with Usher syndrome. $\pi=0·57$*

Mating type and hypothesis tested	*No. of sibships*	χ^2	$\hat{p}$	$\hat{x}$
NORMAL×NORMAL				
H_0: $p=0·25, x=0·0$	58	2·30	0·28	0·16
H_1: $p=0·25, \hat{x}=0·0$	58	1·20	0·21	—

but because these categories are most sensitive to possible biases in sampling. Since there is only one case in each simplex family, no chance exists for multiple ascertainment, while there is ample opportunity for ascertaining more than one case in dominant families. These potential biases must be accounted for appropriately in any formal genetic analysis of patient populations.

Comparisons of the distribution of genetic proportions with other population studies are shown in Table 5. It is only with caution that conclusions about population differences can be drawn from these studies representing 5 separate countries. It is most interesting that this study from the USA closely parallels the averages for all studies, as does the Finnish study of Voipio *et al.*[7]

The classification of cases by genetic type requires the making of very strict assumptions about mendelian inheritance patterns and forces the creation of the separate category for simplex cases. The proportion of these simplex cases that can be accounted for as being 'chance isolated' but genetic may be estimated by formal genetic analysis. The proportion that cannot be accounted for by probability is termed 'sporadic.' These cases may be the result of new mutation or environmental phenocopies. If retinitis pigmentosa can also be caused by multifactorial inheritance, either polygenic and/or environmental factors being important, then this proportion of sporadic cases will be inflated. Results of the segregation analysis clearly show that mendelian segregation of major recessive genes accounts for only a small proportion of the simplex cases. Other genetic and/or environmental factors must be invoked to account for the reduced segregation ratios in both the dominant and recessive groups as well as the high proportion of 'sporadic' cases in the second mating category.

The maximum likelihood estimate of the segregation ratio in the AFFECTED×NORMAL matings was p=0·16. As these families were ascertained through an affected child, the expectation was that the cases would segregate as dominants. This estimate of p=0·16 reflects a very low penetrance estimate of 32% (0·16÷0·5=0·32). Because some children in these families have not yet passed into the age of risk for developing RP symptoms, the families in which the child proband was less than 30 were removed and the remaining 29 families reanalysed. The estimate for p was still a low 0·20, corresponding to a penetrance of only 40%.

The genetic and/or environmental causes for this reduced segregation ratio are not evident from the data analysed thus far in this population. More complete family data, including ERGs on all siblings and parents, would be required to ascertain patients with subclinical disease. This would permit us to discern if the altered segregation ratio of our dominant cases was related to the presence of several asymptomatic 'carriers' of the dominant gene.

In the NORMAL×NORMAL mating category the significant excess of simplex cases suggested that the mendelian recessive model would not fit. Simultaneous maximum likelihood estimation of parameters p and x provided a model of nearly 60% 'sporadic' cases, with the remainder segregating as recessives. Truncation of the proband population below 30 years of age did not change these estimates. The multiplex families, presumed genetic, were also segregating at p=0·19 rather than the hypothesised 0·25, although the recessive model was acceptable. Because this sample is small and includes children of all ages, some alteration in the segregation ratio would be expected.

The cause or causes of this high proportion of apparent sporadic cases cannot be clearly defined at present, though it is tempting to suggest that some cases are of nonrecessive (i.e., multifactorial) etiology or environmental phenocopies. An additional source of simplex cases that can be accounted for mathematically is patients with dominant disease with parents who did not manifest the phenotype—a 'new mutation' so far as phenotype is concerned. Thus, as with the dominant cases, examination of all parents, including ERG testing, would be necessary to help clarify the possible number of 'simplex' cases that in fact

Table 5 *Percentage* of genetic types in families with retinitis pigmentosa*

Genetic category	*Amman* et al.[6]	*Jay*[9]	*Voipio* et al.[7]	*Panteleeva*[8]	*Bird*[10]	*Fishman*[11]	*Present study*	*Average*
Autosomal dominant	9·0	39·0	19·5	12·7	25·5	19·0	21·7	21·0
Autosomal recessive	90·0	15·0	37·0	27·9	13·0	19·0	16·0	33·0
X-linked recessive	1·0	25·0	4·5	1·1	21·5	8·0	9·0	10·0
Undetermined genetic type	—	—	—	—	—	2·0	3·3	0·5
Isolated (sporadic)	—	21·0	39·0	58·3†	43·0	52·0	50·0	35·0

*Refers to percentage in families
†Calculated as 100% minus 41·7% in our interpretation of data.

Table 6 *Genetic distribution of probands adjusted for reduced penetrance of the dominant gene(s)*

	All 227 sibships from segregation analysis				*152 sibships from segregation analysis with proband >30*			
	Before adjustment		*After adjustment*		*Before adjustment*		*After adjustment*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Dominant	40	17·6	100	44·0	29	19·1	72	47·4
Recessive	57	25·1	57	25·1	52	34·2	52	34·2
X-linked	20	8·8	20	8·8				
Sporadic	110	48·5	50	22·0	71	46·7	28	18·4

represent autosomal dominant pedigrees with interfamilial variability in expressivity and/or penetrance.

Table 6 summarises the changes in genetic percentages if the reduced penetrance of the dominant gene(s) is taken into account. Because almost all parents of probands are over 30, the penetrance value of 40% obtained from the segregation analysis was used. As shown in the table, a dramatic increase in the proportion of dominant cases can be seen in the >30 age group as well as the whole population, with the adjusted distribution of cases having more than 40% categorised as dominant. This shift results in approximately 20% of the probands remaining in the sporadic category.

Therefore in order to confirm such mathematical suggestions careful clinical evaluation of the simplex cases should be undertaken. At least some dominant cases have specific clinical findings, such as sector pigment deposition and characteristic ERG changes. The presence of probands in the simplex group showing such changes would lend support to our working hypothesis that a substantial proportion of simplex or sporadic cases may actually be the offspring of a parent without signs of the disease or a parent with minimal signs and/or symptoms that had not been ascertained.

The analysis of the entire clinical population of 368 probands, including the Usher syndrome patients, led to the surprisingly high estimate that almost 20% of the cases and 57% of the known recessives had profound or severe enough hearing impairment to be diagnosed as having Usher syndrome. We have no reason to suspect specific ascertainment bias in obtaining Usher cases, though it may be more likely for an individual with a double sensory handicap to seek medical advice at a specialist centre. We did find that multiple ascertainment of families was more common in this group. Although this high proportion may reflect some medical bias, there is now clinically confirmed evidence of a very high proportion of RP cases with significant congenital hearing deficits. The national survey showed nearly 30% of the probands reported hearing loss in addition to the 2% with diagnosed Usher syndrome.[12]

Segregation analysis of the Usher sibships showed that the recessive model fits the population. No test of genetic heterogeneity among recessive genotypes is possible in this type of analysis. It is evident from the different results of segregation analysis that congenital hearing impairment appears to be a genetic marker; that is, if a proband has a congenital neurosensory hearing impairment, he belongs to a population of recessive genotypes.

The assignment of a case to a genetic category goes beyond being an academic exercise, since giving advice on the risk of recurrence is based on just such a categorisation process. Our results confirm the proportions of known genetic types of RP as described in a smaller population from this laboratory,[11] and they also clearly suggest that mendelian segregation criteria are not fulfilled by these data because of the presence of reduced penetrance for the autosomal dominant form(s) and excess of simplex cases in this population. It can be concluded from the analysis of this current clinical population that, even if a proband is classified as a dominant or recessive, the recurrence risk may be well below the mendelian expectation, and that, on the other hand, asymptomatic relatives may also have an RP gene. Although these data appear to complicate the counselling picture, such information must be taken into account before appropriate advice can be given.

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References

1 Bell J. Retinitis pigmentosa and allied disorders. In: Pearson K, ed. *Treasury of human inheritance.* London: Cambridge University Press, 1922: **1:** 1–28.
2 McKusick VA. *Mendelian inheritance in man: catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes.* 5th ed. Baltimore: Johns Hopkins University Press, 1978.
3 Botermans CHG. Primary pigmentary degeneration and its association with neurological diseases. In: Vinken PJ, Bruyn GW, eds. *Neuroretinal degenerations: handbook of clinical neurology.* New York: American Elsevier, 1972: **13:** 148–379.
4 Aguirre G, Farber D, Lolley R, Fletcher RT, Chader GJ. Rod-cone dysplasia in Irish setters: a defect in cyclic GMP metabolism in visual cells. *Science* 1978; **201:** 1133.
5 LaVail MM. Analysis of neurological mutants with inherited retinal degeneration. Friedenwald lecture. *Invest Ophthalmol Visual Sci* 1981; **21:** 638–57.
6 Ammann F, Klein D, Franceschetti A. Genetic and epidemiological investigation on pigmentary degeneration of the retina and allied disorders in Switzerland. *J Neurol Sci* 1965; **2:** 183–96.
7 Voipio H, Gripenberg V, Raitta C, Horsmanheims A. Retinitis pigmentosa; a preliminary report. *Hereditas* 1964; **52:** 247.
8 Panteleeva DA. The hereditary tapetoretinal degenerations. *Vestn Oftalmol* 1969; **1:** 53–6.

9 Jay B. Hereditary aspects of pigmentary retinopathy. *Trans Ophthalmol Soc UK* 1972; **92:** 173–8.
10 Bird AC. X-linked retinitis pigmentosa. *Br J Ophthalmol* 1975; **59:** 177–97.
11 Fishman GA. Retinitis pigmentosa: genetic percentages. *Arch Ophthalmol* 1978; **96:** 822–6.
12 Boughman JA, Conneally PM, Nance WE. Population genetic studies of retinitis pigmentosa. *Am J Hum Genet* 1980; **32:** 223–35.
13 Hu DN. Genetic aspects of retinitis pigmentosa in China. *Am J Med Genet* 1982; **12:** 51–6.
14 Massof RW, Finkelstein D, Boughman JA. Genetic analysis of subgroups within simplex and multiplex retinitis pigmentosa. In: *Proceedings of the international symposium of genetics and ophthalmology*. New York: Liss, in press.
15 Berson EL, Gouras P, Gunkel RD, Myrianthopoulos NC. Dominant retintis pigmentosa with reduced penetrance. *Arch Ophthalmol* 1969; **81:** 226–34.
16 Usher CH. On the inheritance of retinitis pigmentosa with notes of cases. *R Lond Ophthalmol Hosp Rep* 1914; 130.
17 Merin S, Abraham FA, Auerbach E. Usher's and Hallgren's syndromes. *Acta Genet Med Gemellol (Roma)* 1974; **23:** 49–55.
18 Fishman GA. Usher's syndrome: visual loss and variations in clinical expressivity. *Perspect Ophthalmol* 1979; **3:** 97–103.
19 Davenport SL, O'Nuallain S, Omenn GS, Wilkus RJ. Usher syndrome in four hard-of-hearing siblings. *Pediatrics* 1978; **62:** 578–83.
20 Morton NE. Genetic tests under incomplete ascertainment. *Am J Hum Genet* 1959; **11:** 1–16.