

Clinical Genetic Analysis of Retinitis Pigmentosa in Indian Population

Subhabrata Chakrabarti^a, Virinder Kaur Sarhadi^a, Daljit Singh^b, Indu Ravijit Singh^b and
Jai Rup Singh^{a*}

^aCentre for Genetic Disorders, Guru Nanak Dev University, Amritsar, India; ^bDr. Daljit Singh Eye Hospital,
Amritsar, India

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ABSTRACT Four hundred families affected with Retinitis Pigmentosa (RP), from 16 different states of India were analysed to ascertain segregation pattern of RP and to study the association of clinical variables like "age of onset" and "rate of progression" with the different clinical subtypes of RP. Families were categorized on the basis of clinical types and inheritance pattern. Segregation analysis, based on the maximum likelihood estimate, was carried out on 426 sibships from 400 families. 75% of the cases were autosomal recessive (AR), 10% autosomal dominant (AD) and 1% X-linked. In the remaining 14%, the inheritance pattern was not clear. Segregation analysis of the sibship data showed good agreement with respect to the AR cases, with a segregation value of 0.23. Penetrance was relatively low (42%) in AD cases. Segregation analysis of AR cases, with the inclusion of simplex cases, indicated that these cases might be having an underlying recessive mode of inheritance. High frequency of recessive cases could be attributed to inbreeding and consanguinity in families. Statistically significant differences with respect to "age of onset" and "rate of progression" were observed among different clinical types of RP, which could be of importance in counseling.

INTRODUCTION

Retinitis Pigmentosa (RP) is a group of inherited disorders characterized by progressive degeneration of the retina. This term, first coined by Donders (1855), comprises a variety of pigmentary retinopathies with progressive photoreceptor damage. The common symptoms are nyctalopia, progressive loss of peripheral vision with difficulty in mid and peripheral visual fields, bone-spicule pigmentation and a reduced or extinguished electroretinogram. RP is marked by great clinical heterogeneity in terms of age of

onset, rate of progression and severity. Its incidence ranges from 1 in 2000 to 1 in 7000 (Ammann et al. 1965; Merin et al. 1974; Boughman et al. 1980). Apart from its sporadic occurrence, RP is known to be inherited as autosomal dominant (AD), autosomal recessive (AR) and X-linked recessive (XLR). The paucity of extensive genetic data on RP from India, especially from the northern and eastern part, motivated us to undertake this study to determine the frequency of various forms of RP. The segregation analysis, based on the maximum likelihood estimate (MLE), was performed to confirm the modes of inheritance and penetrance for each type.

MATERIALS AND METHODS

400 families affected with RP, diagnosed at the Dr. Daljit Singh Eye Hospital, Amritsar, India, during 1990 to 2000, and having 953 affected individuals, were investigated. The clinical examination included direct and indirect ophthalmoscopy, slit lamp examination, fundus testing through scanning laser ophthalmoscopy and fluorescein angiography. Bone spicule pigmentation on the retina and fundus characteristics were also checked along with the refraction and visual acuity. Patients with only bilateral involvement were included in the study.

The patients were from 16 different states of India. The detailed family history and pedigree following Bennett et al. (1995), were recorded for each case. On the basis of clinical symptoms the cases were classified (Jimenez-Sierra et al. 1989) into three categories, viz., a) Classical RP (Typical RP only); b) RP variants (including syndromes associated with RP; Table 2); c) RP with other eye disorders (cataract, high myopia, nystagmus).

Corresponding Author: *Prof Dr Jai Rup Singh, Coordinator, Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar- 143005, India, *Phone:* +91-183-258802 to 09, Extn. 3277, *Fax:* +91-183-258863, 258820, *E-mail:* jairup@vsnl.com & jairup@hotmail.com

The cases exhibiting a positive family history were classified as Autosomal dominant (AD), Autosomal recessive (AR), or X-linked (XL) on the basis of inheritance pattern. Where more than one individual was affected but inheritance of the disease did not follow a clear Mendelian pattern, these were labelled as Undetermined (UND). Cases with only one affected individual without any positive family history were termed as Simplex (SIM).

The "age of onset" was termed as "early" if the onset of symptoms like nyctalopia in the proband, was before 20 years of age, and "late" if it was after 20 years. The rate of progression of RP at the time of clinical evaluation, was classified as "rapid", when deterioration, since the onset of RP symptoms, had occurred in weeks or months and "slow", if it involved years (Jimenez-Sierra et al. 1989). It was termed as "stationary", when there was no significant deterioration since onset. The contingency χ^2 test was performed to ascertain the statistical significance.

Segregation Analysis: Segregation analysis, following the method of Morton (1959), was undertaken on sibships with 2 or more affected individuals. Proband sibships were grouped with respect to the parental mating type i.e., Affected x Normal for AD, and Normal x Normal for AR cases. Families having multiple sibships, were further subdivided into nuclear families for segregation analysis. Based on the ascertainment probability (π), Mendelian ratios expected for each of the parental mating type were tested as Null hypotheses under an ideal segregation value (p). AR cases were ascertained with and without the inclusion of SIM cases (x) (Morton 1982). The segregation ratio (θ) and its variance $v(\theta)$ for AR mating was calculated under a model of incomplete selection. The maximum likelihood estimate (\bar{p}) was calculated both for the AR and AD categories (Morton 1982). The χ^2 for each mating type was estimated for the acceptance or rejection of the Null hypothesis with respect to the parental mating type. Penetrance was calculated following Boughman et al. (1983).

RESULTS

Out of 400 families investigated, 215 (53.7%) showed positive family history while 185

(46.3%) were of SIM type. Amongst the familial cases, the highest proportion was of AR (28.2%), followed by AD (10.2%) and XL (1.0%). In 14.3% of the cases, the inheritance pattern was not clear. Consanguinity among the first degree relatives was seen in 32 families (8.5%). However, most of the non-consanguineous families were found to have married within their own caste groups. A higher ratio of affected males (670/953) as compared to females (283/953) was observed (2.3:1).

Segregation Analysis

The ascertainment probability (π), was estimated to be 0.36 which is ideal under the model of multiple incomplete ascertainment ($0 < \pi < 1$). Null hypothesis for the "Affected x Normal" matings (55 matings), was tested under a model of AD inheritance ($p=0.5$; $x=0.0$). It gave a χ^2 value of 10.12 rejecting the Null hypothesis. From a maximum likelihood estimate (MLE) of $\bar{p}=0.21$, penetrance was estimated to be only 42%, which is very low for dominant cases (Table 1).

Null hypothesis for "Normal x Normal" mating category (166 matings) was tested after excluding the SIM cases for the AR model ($p=0.25$; $x=0.39$). A value of ' θ ' = 0.22 and MLE = 0.15 was obtained for this category that rejected the hypothesis ($\chi^2=5.325$). However, pooling of the SIM sibships with the recessive ones (under a model of $p=0.25$; $x=0.0$), accepted the Null hypothesis ($\chi^2=2.705$). The value of ' θ ' was 0.23, which is close to the ideal value of 0.25 for AR cases. Therefore, for all further analyses the SIM cases were pooled with the AR cases. Upon

Table 1: Segregation analysis of 426 sibships from 400 RP families

Mating type and hypothesis	No. of sibships	χ^2	MLE (\bar{p})
NxN $H_0:p=0.25$; $x=0.39$	166 †	5.325*	0.15
NxN $H_0:p=0.25$; $x=0.0$	371 ††	2.705**	0.19
AxN $H_0:p=0.5$; $x=0.0$	55	10.120*	0.21

*Significant ($P < 0.05$) N: Normal; A: Affected

**Not Significant ($P > 0.05$)

† AR excluding SIM cases

†† AR including SIM cases

Table 2: Distribution of clinical phenotypes of RP and their inheritance patterns

Category	RP-Type	AR	AD	XL	UND	Total
Classical RP	Typical RP	168	30	4	27	229
		(73.4%)	(13.1%)	(1.7%)	(11.8%)	(57.3%)
RP variants	RP with HMD	56	3	-	10	69
		(81.2%)	(4.3%)		(14.5%)	(17.3%)
RP with associated eye disorders	RP (Others)**	40	5	-	-	45
		(88.9%)	(11.1%)			(11.2%)
	RP with Cataract	16	2	-	10	28
		(57.1%)	(7.2%)		(35.7%)	(7.0%)
RP with associated eye disorders	RP with High Myopia	11	-	-	7	18
		(61.1%)			(38.9%)	(4.5%)
	RP with Nystagmus	7	1	-	3	11
		(63.6%)	(9.1%)		(27.3%)	(2.7%)
Total		298	41	4	57	400
		(74.5%)	(10.2%)	(1.0%)	(14.3%)	(100.0%)

**Others include RP (central) 3.0%; RP (sine pigmento) 1.5%; RP (punctate albescens) 1.8%; Laurence-Moon-Bardet-Biedl (LMBB) syndrome 0.7%; Usher syndrome 1.2%; RPE dystrophy 1.8%; RP (Drusen maculopathy) 0.5%; RP (optic atrophy) 0.7%.

pooling, the AR cases constituted 74.5% of the total cases. As there were only 1% XL cases, segregation analysis was not performed on these.

Clinically, the largest category was of classical RP with 229 cases (57.3%), of these 168 cases (73.4%) were of AR type. 114 cases (28.5%) were RP variants of which 69 cases (17.3%) were of RP with heredomacular degeneration (HMD). The remaining 45 cases (11.2%) consisted of RP variants other than RP with HMD and were collectively termed as RP (Others) for further analyses. In 57 cases (14.2%) RP was associated with other eye disorders. In all the types of RP the AR cases were more prevalent (Table 2).

Association Studies

The results of association between the "age of onset" and clinical types are shown in table 3. Most of the clinical types exhibited "early" onset (81%). "Late" onset was seen in 19.2% cases of classical RP and in 30.4% RP with HMD. The χ^2 test indicated significant ($p < 0.05$) differences in the "early" and "late" onset categories. The "rate of progression" was found to be "rapid" in most of the cases (60.2%), followed by "slow" (30.3%) and "stationary" (9.5%). The differences between various categories were significant ($p < 0.05$) (Table 4).

DISCUSSION

Almost 15% of our cases did not show any

clear inheritance pattern. Slightly lower frequencies of 8.0% (Kar et al. 1995) and 8.4% (Vinchurkar et al. 1996) of the UND cases have been reported in earlier Indian studies. However, a higher frequency of UND cases (48%) has been reported in studies where UND cases were pooled with the SIM cases (Greenberg et al. 1993).

46.3% of our cases were of SIM type. The inclusion of these cases in the segregation analysis of AR cases gave a segregation ratio of $\theta = 0.23$, which approached an ideal value of 0.25,

Table 3: Association between "age of onset" and "clinical types" of RP

Category	RP-Type	Early	Late
Classical RP	Typical RP	185	44
		(80.8%)	(19.2%)
RP variants	RP with HMD	48	21
		(69.6%)	(30.4%)
RP with associated eye disorders	RP (Others)**	39	2
		(95.1%)	(4.9%)
	RP with Cataract	25	3
		(89.3%)	(10.7%)
RP with associated eye disorders	RP with High Myopia	15	2
		(88.2%)	(11.8%)
	RP with Nystagmus	7	3
		(70.0%)	(30.0%)
Total		394	75
		(100.0%)	(19.0%)

χ^2 cal= 13.78; df. 5; $p < 0.05$ [χ^2 tab=11.07; df. 5; $p > 0.05$]
 **Others include RP (central), RP (sine pigmento), RP (punctate albescens), LMBB syndrome, Usher syndrome, RPE dystrophy, RP (Drusen maculopathy), RP (optic atrophy)

Table 4: Association between “rate of progression” and “clinical types” of RP

Category	RP-Type	Rapid	Slow	Stationary
Classical RP	Typical RP	135 (61.4%)	79 (35.9%)	6 (2.7%)
RP variants	RP with HMD	35 (50.7%)	21 (30.4%)	13 (18.9%)
	RP (Others)**	27 (65.8%)	12 (29.3%)	2 (4.9%)
RP with associated eye disorders	RP with Cataract	20 (71.4%)	4 (14.3%)	4 (14.3%)
	RP with High Myopia	13 (72.2%)	-	5 (27.8%)
	RP with Nystagmus	4 (36.4%)	-	7 (63.6%)
Total	389 (100.0%)	234 (60.2%)	118 (30.3%)	37 (9.5%)

χ^2 cal=75.80; df. 10; $p < 0.05$ [χ^2 tab=18.30; df. 10 ; $p > 0.05$]

**Others include RP (central), RP (sine pigmento), RP (punctate albescens), LMBB syndrome, Usher syndrome, RPE dystrophy, RP (Drusen maculopathy), RP (optic atrophy)

indicating that the SIM cases might be having underlying recessive mode of inheritance. Thus, with their inclusion, the AR type formed the largest group (74.5%). Similar studies that classified SIM cases as recessives, have respectively reported 90% (Ammann et al. 1965) and 84% (Boughman et al. 1980) of their cases to be of AR type. High frequency of AR cases in our sample can also be attributed to inbreeding and consanguinity. Other studies on Indian populations, have reported 36% (Kar et al. 1995) and 27.7% (Vinchurkar et al. 1996) of their cases as AR. However, they did not combine the SIM cases with AR cases and the segregation analysis had been undertaken only in the former study.

The low frequency of AD cases (10.2%) observed in our study, is similar to the other Indian reports (Kar et al. 1995; Vinchurkar et al. 1996) and also to most of other studies (Ammann et al. 1965, Boughman et al. 1980; Haim 1993). However some studies have reported more than 20% of their cases to be of AD type (Boughman and Fishman 1983; Greenberg et al. 1993). Segregation analysis of the “Affected x Normal” sibships showed low penetrance (42%) for AD cases. Low penetrance of 60% and 32% in the AD cases has also been reported by Boughman et al. (1980) and Boughman and Fishman (1983) respectively. Apart from the genetic and environmental causes, these studies have suggested that variable age of onset might be a significant factor for reduced penetrance in ADRP.

Clinically, most of the cases comprised of classical RP. Amongst the RP variants, RP with HMD was most frequent. Molecular genetic studies of macular dystrophies and its subtypes (Clarke et al. 2000) have shown that same gene may be responsible for the causation of RP and also for RP associated with macular degeneration. We observed a relatively low frequency (0.7-3.0%) of other RP variants as compared to 18% cases of Usher syndrome reported in the RP cases of USA (Boughman and Fishman 1983). Cataract was observed to be more frequently (7.0%) associated with RP followed by high myopia (4.5%) and nystagmus (2.7%). This finding differs from the earlier reports that observed 75% of RP cases to be associated with myopia (Sieving and Fishman 1978), 53% with cataract (Fishman et al. 1985) and 20% with nystagmus (Foxman et al. 1985). The higher frequency of nystagmus (Foxman et al. 1985) reported is only for juvenile and early onset RP. In our study also 70% of the RP cases with nystagmus were from the “early onset” group.

Among the RP variants, except in cases of RP with HMD the “late onset” or “stationary” forms were not observed. The late age of onset has been reported to be a common phenomenon in various macular dystrophies (Zhang et al. 1996). The observation of low frequency of “stationary” forms in classical RP cases (2.7%) as well as in other RP categories is in agreement with the fact that RP exhibits progressive

degeneration of retinal cells. The presence of statistically significant differences among the variables of "age of onset" and "rate of progression" could be of importance in counseling.

RP exhibits significant phenotypic and genetic heterogeneity and its exact causal mechanism is not known. Till date 30 loci have been mapped for RP on different chromosomes including a mitochondrial mutation (RetNet 1996-2001). To understand the mechanisms for the causation of RP, we are currently evaluating at molecular level some large informative families exhibiting different clinical forms of RP.

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