Original articles

Severe manifestions in carrier females in X linked retinitis pigmentosa

Eric Souied, Bertrand Segues, Imad Ghazi, Jean-Michel Rozet, Sophie Chatelin, Sylvie Gerber, Isabelle Perrault, Antoinette Michel-Awad, Marie-Louise Briard, Ghislaine Plessis, Jean-Louis Dufier, Arnold Munnich, Josseline Kaplan

Abstract

Retinitis pigmentosa (RP) is a group of progressive hereditary disorders of the retina in which various modes of inheritance have been described. Here, we report on X linked RP in nine families with constant and severe expression in carrier females. In our series, however, the phenotype was milder and delayed in carrier females compared to hemizygous males. This form of X linked RP could be regarded therefore as partially dominant. The disease gene maps to chromosome Xp2.1 in the genetic interval encompassing the RP3 locus (Zmax=13.71 at the DXS1110 locus). Single strand conformation polymorphism and direct sequence analysis of the retinitis pigmentosa GTPase regulator (RPGR) gene, which accounts for RP3, failed to detect any mutation in our families. Future advances in the identification of X linked RP genes will hopefully help to elucidate the molecular basis of this X linked dominant RP.

(*J Med Genet* 1997;34:793–797)

Keywords: X linked retinitis pigmentosa; carrier females

Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U-393, Hôpital des Enfants-Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France
E Souied
B Segues
J-M Rozet
S Chatelin
S Gerber
I Perrault
M-L Briard
A Munnich
J Kaplan

Service d'Ophtalmologie, Hôpital Laënnec, 75007 Paris, France
I Ghazi
A Michel-Awad
J-L Dufier

Laboratoire de Cytogénétique, Centre Hospitalier Régional Clémenceau, 14033 Caen Cedex, France
G Plessis

Correspondence to:
Dr Munnich.

Received 17 January 1996
Revised version accepted for publication 21 May 1997

Retinitis pigmentosa (RP) is a group of progressive hereditary disorders of the retina causing night blindness, reduction of the visual field with gradual involvement of the central vision, total blindness, and degeneration of the retina. Various modes of inheritance have been described in RP including autosomal dominant, autosomal recessive, and X linked recessive.[1] We have previously found two different clinical profiles in X linked RP. The first clinical form ascribed to RP2 has an early onset with severe myopia (mean age of onset (1 SD) 3 (0.5) years). The second form starts later with night blindness with mild or no myopia (mean age of onset (1 SD) 10.6 (4) years). This form has been ascribed to RP3 by linkage analysis in informative families.[2] Carrier females have normal vision, normal fundus, sectorial or peripheral pigmentary deposits, and a metallic sheen reflex.[3–5] They occasionally display a mild amplitude reduction on electroretinogram (ERG).[6 7] Here, we report on X linked RP in nine families with consistent expression of the disease in carrier heterozygotes and provide evidence of linkage of this form to chromosome Xp2.1 at (or close to) the RP3 locus. However, no mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene were found in our series.[8]

Patients

In the last seven years, we have recruited nine unrelated RP families with no male to male but consistent male to female transmission, suggesting X linked inheritance of the trait (fig 1). Both affected males and carrier females consistently met the diagnostic criteria for RP laid down by the International Symposium of Ophthalmology, namely (1) bilateral involvement, (2) concentric depression of the visual field, (3) severe scotopic involvement in ERG resulting from alteration of rods or no ERG response, and (4) progressive loss of photoreceptor function. Yet, the disease in affected males started later and had a milder course than in X linked recessive RP. Indeed, the onset of the disease in males was delayed to the second decade and the central vision was preserved until 40-45 years of age. Heterozygous females developed severe RP symptoms as well, namely night blindness, concentric reduction of visual field, generalised pigmentary degeneration at the fundus, and extinction of ERG (fig 2). In heterozygotes, however, the disease had an even later onset and a milder clinical course compared to hemizygous males. In addition, a certain degree of intrafamilial variability was observed among carrier females and an apparent phenomenon of anticipation was noted.

The nine families consistent with X linked dominant RP included 97 subjects: 28 affected males, 34 affected females, 24 healthy children (14 males, 10 females) born to an affected mother, and 10 subjects (three males, seven females) whose status was ambiguous and was therefore regarded as unknown (fig 1). All family members underwent extensive investigations including careful questioning, anterior segment and fundus examinations, visual

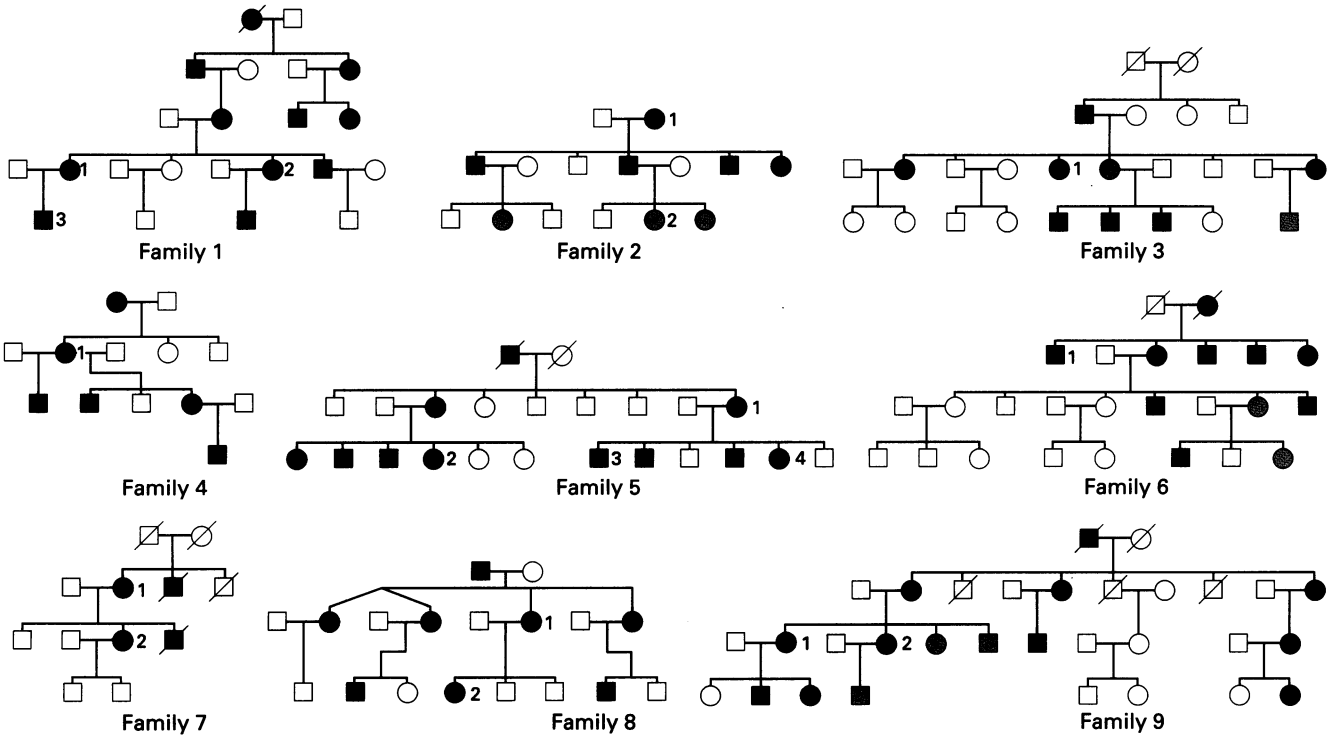

Figure 1 Pedigrees of X linked dominant RP families.

acuity measurement, colour vision study using pseudo-isochromatic plates and Farnsworth-Munsell 15 hue or 100 hue tests, Goldman perimetry, and electroretinography using contact lens electrodes and topical anaesthesia. The frosted lenses provide Ganzfeld stimulation. Rod and cone ERGs are easily distinguished by their wave forms, which vary according to whether rods only (scotopic), rods and cones (mesotopic), or cones only (photopic) are activated. We performed, successively, dim flash and dark adaptation, bright flash and dark adaptation, and bright flash and light adaptation in order to record scotopic, mesotopic, and photopic ERGs, respectively. Table 1 summarises the main clinical features of a subset of symptomatic family members for which a full data set was available.

Methods

For each subject, a 20 ml EDTA blood sample was collected and DNA was prepared from lymphocyte pellets by SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris EDTA resuspension. For restriction fragment length polymorphism (RFLP) analysis, genomic DNA was digested, then separated by gel electrophoresis, transferred onto nylon membranes (Zetabind, Flo Cuno), and hybridised to probes in the hybridisation solution overnight at 65°C. Membranes were washed twice at room temperature in 2 × SSEP and 0.1% SDS, then washed at 65°C in 1 × SSEP and 0.1% SDS. Autoradiographs were obtained by exposure to Kodak X/OMAT films for one to four days at −80°C. For genotyping using the polymerase chain reaction (PCR) technique, hypervariable microsatellites were used. Genomic DNA (200 ng) was amplified using *Taq* polymerase (0.5 U, Amersham), 20 µmol/l of each deoxynucleotide, and 20 µmol/l primers in 1.5 mmol/l $MgCl_2$ (20 µl). Amplification conditions were 95°C for 10 minutes followed by 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final extension of 10 minutes at 72°C. Amplified DNA (1 µl) was mixed with formamide (2 µl) and loading buffer (xylene cyanol/bromophenol blue/glycerol), electrophoresed at 1400 volts for two hours in 6% denaturing polyacrylamide gels, and transferred onto charged nylon membranes (Hybond N+, Amersham). Membranes were hybridised with a [^{32}P]dCTP (CA)^{12} oligonucleotide labelled with terminal deoxynucleotide transferase (Boehringer Mannheim). Hybridisation was carried out for 90 minutes at 42°C in 0.13 mol/l sodium phosphate buffer, pH 7.2, 0.25 mol/l NaCl, 7% SDS, and 10% polyethyleneglycol. Blots were washed for five minutes at room temperature in 2 × SSC, 0.1% SDS and autoradiographed. Linkage analysis was performed using the MLINK and LINKMAP options of version 5.1 of the LINKAGE program.[9]

To test the pattern of X inactivation in obligate carriers, we used the correlation between X inactivation and methylation of the *Hpa*II and *Hha*I sites at the human androgen receptor locus.[10]

In order to screen for RPGR gene mutations, we used a combination of single stand conformation polymorphism (SSCP) and direct sequence analysis of each exon. The 19 exons and their flanking intronic sequences (30 bp) were analysed. DNA (100 ng) from peripheral blood leucocytes was amplified using 0.5 µmol/l of each primer and 0.1 µl of [α^{33}P]dCTP (10 mCi/ml) in a 25 µl amplification mixture containing 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, and 0.5 units of *Taq* polymerase.[8]

Amplified DNA (6 µl) was mixed with an equal volume of formamide loading dye (95% formamide, 20 mmol/l EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). The samples (5 µl) were denatured for 10 minutes at 95°C, loaded onto a polyacrylamide gel (20 × 45 × 0.04 cm Hydrolink MDE, Bioprobe), and electrophoresed at room temperature for at least 18 hours (4 W) in a 0.6 × TBE running buffer. Gels were transferred onto Whatman papers, dried, and autoradiographed with Kodak X-OMAT films for 24 hours. Exons that displayed abnormal SSCP patterns were sequenced on an automatic DNA sequencer. The PCR fragments were excised from a low melting point gel (GTG NuSieve). Direct sequencing was performed on single strand DNA, produced by asymmetrical PCR using the amplification primers as sequencing primers.

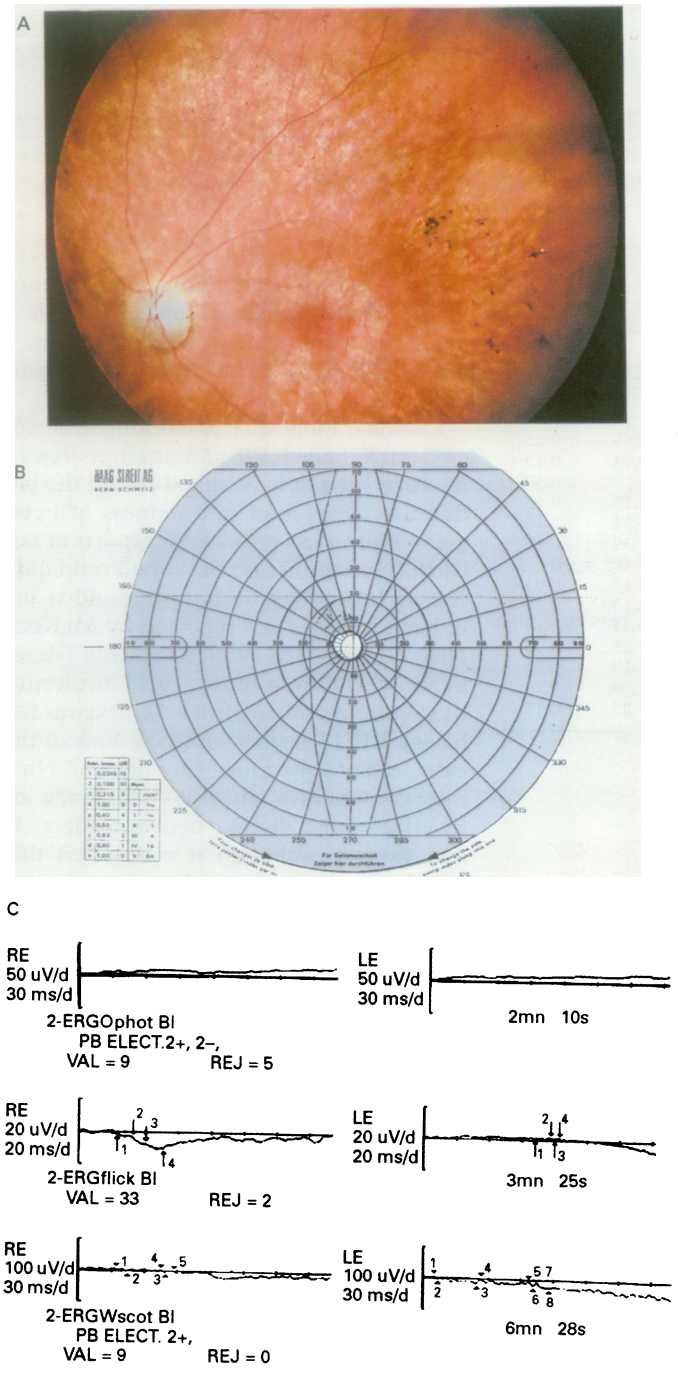

Figure 2 Fundus aspect, visual field, and electroretinogram of affected subjects. (A) Fundus shows attenuated blood vessels, waxy pale disc, and pigment deposits (patient 2, family 1). (B) Visual field shows tubular vision (patient 1, family 1). (C) Electroretinogram shows undetectable response (patient 1, family 1).

Results

Significant pairwise lod scores for linkage of the disease gene to polymorphic markers of the X chromosome were obtained at loci DXS1110, OTC, MAOA, and DXS1003 (table 2). The highest lod score values were observed at loci DXS1110 and OTC which are known to flank the RP3 gene (Zmax=9.10 at θ=0 and Zmax=8.17 at θ=0.03 for loci DXS1110 and OTC respectively).

The location score method was used to estimate the position of the disease locus. In this procedure, the map of the marker loci is fixed and the position of the disease locus is varied throughout the map. The order pter DXS164 $_{0.05}$ DXS84 $_{0.05}$ DXS1110 $_{0.05}$ OTC $_{0.04}$ MAOA $_{0.03}$ DXS1003 $_{0.05}$ DXS1039 cen has been established previously by the Fifth International Workshop on the X chromosome.[11] Intermarker recombination estimates were calculated with the combined CEPH and disease pedigrees. The maximum likelihood estimate for location of the disease gene was over the DXS1110 locus (Z=13.71 at θ=0, fig 3). Haplotype analyses supplemented the multipoint analysis by providing direct evidence of recombination events (not shown, available on request). The positioning of recombinant events by this method was consistent with the placement of the disease gene over locus DXS1110 and excluded the genetic interval encompassing the RP2 gene in our series. No preferential pattern of X inactivation was observed in the obligate carriers tested. The methylation pattern obtained was consistent with a random inactivation of the X chromosome in affected females (not shown).

SSCP and sequence analysis of the RPGR gene in the probands showed two base changes resulting in the substitution of an arginine for a lysine and an isoleucine for a valine at positions 425 and 431 respectively. These base changes were also observed in 2/100 controls and were regarded therefore as rare polymorphisms. No mutations were detected in the other exons of the RPGR gene.

Discussion

Here, we report on X linked RP in nine families with constant and severe expression of the disease in carrier females. In these RP families, the onset of the disease was delayed to the second decade and the central vision was preserved until 40-45 years. This clinical profile is therefore different from RP2, which has a very early onset (mean age of onset (1 SD) 3.5 (0.5) years[2]). In addition, while expression of X linked recessive RP in carrier females is usually absent or limited to metallic sheen (tapetal-like reflex) or peripheral pigmentary deposits in the fundus, or both, both sexes were affected in our

Table 1 Phenotypes of affected members in X linked dominant RP families

Family/ subject	Age (y)/ sex	Age at onset (y)	Corrected visual acuity		Spherical equivalent, D		Cataract	Fundus aspect			Visual field		ERG
			OD	OS	OD	OS		BCP	ABV	WPD	OD	OS	
1/1	46/F	18	5/400	20/200	+1	+1	+	+++	+++	+++	TV	TV	NR
1/2	38/F	20	20/50	20/100	−4	−3.5	+++ removed	+	++	++	TV	RS	NR
1/3	21/M	20	20/30	20/50	−8	−10	−	+	+	+	PAD	PAD	RA++
2/1	58/F	15	20/25	20/25	−1	−1	+	+++	+++	+++	RS	RS	NR
3/1	44/F	30	20/30	20/25	−1	−0.5	−	+	++	++	PAD	RS	NR
4/1	63/F	61	20/50	5/400	+1.5	+1	−	+	++	++	CC	CC	NR
5/1	50/F	?	20/200	20/50	−15	−15	+	+++	+++	+++	TV	RS	RA+++
5/2	10/F	?	20/200	20/100	−23	−20	−	0	+	+	CC	CC	RA++
5/3	19/M	13	20/50	20/50	−6.5	−7	+++	0	+	+	RS	TV	NR
5/4	4/F	?	20/20	20/20	−7	−8.5	−	0	0	0	?	?	RA++
6/1	36/F	12	20/200	20/400	+1	+1	+	+++	+++	+++	TV	TV	?
7/1	58/F	15	20/50	20/50	−1.50	−1.75	+	+++	++	+	RS	PAD	?
7/2	36/F	12	20/100	20/200	+3	+3.75	+	+++	+++	++	TV	TV	NR
8/1	43/F	40	30/50	20/30	+1	+1	−	+	++	+	PAD	PAD	RA+++
8/2	16/F	8	20/50	20/60	−3	−2	−	++	++	++	RS	RS	?
9/1	40/F	8	20/100	20/200	−9.75	−9	−	+	++	+	RS	RS	NR
9/2	39/F	5	20/100	20/100	−3.50	−3.75	−	+	++	+	TV	TV	NR

OD=right eye, OS=left eye, BCP=bone corpuscular pigment, ABV=attenuated blood vessels, WPD=waxy pale disc, CC=concentric constriction, RS=ring scotoma, PAD=partial annular defect, TV=tubular vision, ERG=electroretinogram, RA=reduced amplitude, NR=non-recordable.

Table 2 Pairwise linkage between the disease gene and 14 polymorphic loci on chromosome Xp

	Recombination fraction							
	0	0.01	0.05	0.10	0.20	0.30	Zmax	θ
DXS41	−∞	−3.93	−0.78	−0.34	0.98	0.88	0.99	0.25
DXS269	−∞	−0.05	0.65	0.88	0.92	0.75	0.94	0.15
DXS270	−∞	−2.94	−1.51	−0.89	−0.33	−0.09	0	0.50
DXS164	−∞	0.61	1.78	2.10	2.01	1.52	2.13	0.15
DXS206	−∞	1.24	1.78	1.84	1.56	1.06	1.84	0.10
DXS84	−∞	1.94	2.39	2.36	1.94	1.32	2.39	0.05
DXS1110	9.10	8.85	8.29	7.45	5.64	3.69	9.10	0
OTC	−∞	8.00	8.06	7.51	5.93	4.04	8.17	0.03
DXS7	−∞	0.85	1.94	2.15	1.92	1.37	2.15	0.10
MAOA	−∞	−0.67	2.31	3.14	3.19	2.48	3.31	0.15
DXS1003	−∞	−3.81	1.14	2.71	3.28	2.65	3.28	0.20
DXS1039	−∞	−4.78	−0.95	0.37	1.14	1.08	1.17	0.25
DXS255	−∞	−6.91	−1.66	0.26	1.53	1.59	1.66	0.25
DXS14	−∞	−0.92	1.46	2.11	2.15	1.59	2.25	0.15

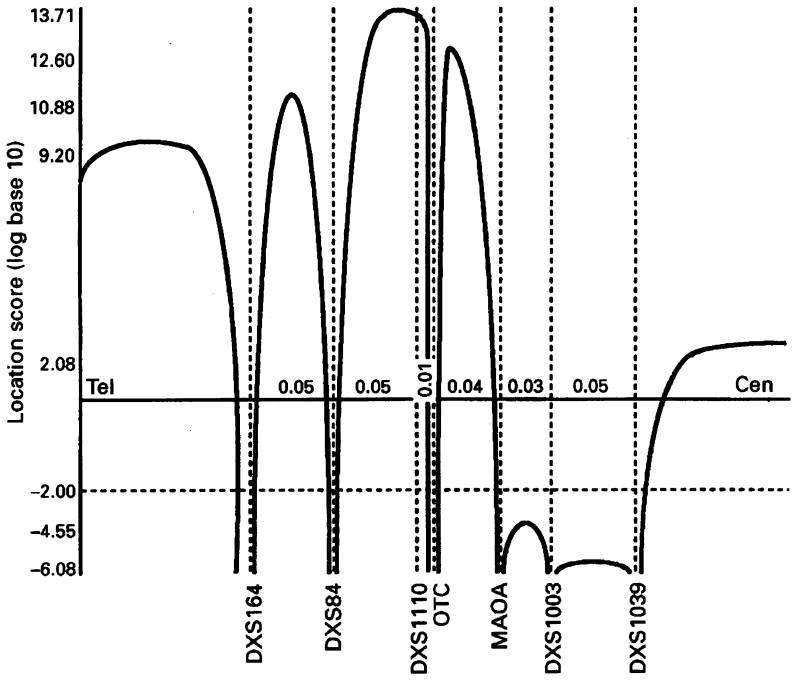

Figure 3 Support for location of an X linked dominant RP gene with respect to seven highly polymorphic DNA markers of chromosome Xp. Likelihood estimates are given as log base 10.

series. Yet, since expression of the disease was milder and delayed in carrier females compared to hemizygous males, this form of X linked RP should be regarded as partially dominant.

Dominant inheritance has long been considered in X linked RP and attempts have been made to subdivide X linked RP on the basis of different degrees of affectedness of heterozygotes. Only a few of the early reports of families with X linked disease described retinal degeneration in females,[12 13] but profound visual loss in heterozygotes was reported by McKenzie in New Zealand.[14] In 1959, Kobayashi[15] described a family with even more severe involvement in the female members, five heterozygous females having RP, 11 having some stigmata of the disease, and at least four being normal. No accurate comparative analysis was made of the severity of the disease between affected men and women, although it was stated that the females were more mildly affected than the males. From this information, it was concluded that there were three separate X linked conditions, X linked recessive, X linked intermediate, and X linked dominant RP.

The mapping of an X linked dominant RP gene to the interval encompassing RP3 poses the question of whether one is dealing with a different clinical phenotype at the RP3 locus or with a distinct locus mapping close to RP3. In addition, assuming that the RP3 locus is involved, one may be dealing with either allelic heterogeneity at this locus or a skewed pattern of chromosome X inactivation, as already noted at the RP2 locus.[16] Although no general conclusion can be drawn from X chromosome inactivation in circulating leucocytes, our results do not support a skewed inactivation of the X chromosome as the cause of the disease in carrier females. This negative result along with the clinical differences of X linked recessive RP favour either allelic heterogeneity at the RP3 locus or involvement of a distinct locus mapping close to RP3. In keeping with this, the absence of abnormal SSCP patterns in

9/9 probands tends to exclude RPGR as the disease causing gene in this form of X linked dominant RP.

Hitherto, no pedigrees similar to those reported here have been published and no genetic analysis in X linked retinal dystrophies with severely affected carriers has been reported so far, except for the recent mapping of a gene for X linked dominant cone rod dystrophy to chromosome Xp22.11-p22.13.[17] In this study, we report on the mapping of an X linked RP gene, consistently causing severe manifestations in carrier females, to chromosome Xp2.1 in the genetic interval encompassing the RP3 locus (DXS1110-OTC) and find no evidence of involvement of RPGR in this form of RP.[8 18] The future identification of X linked RP genes and the characterisation of disease causing mutations will hopefully help to elucidate the molecular basis of this X linked dominant form of RP.

We thank Monique Dailhat and Gisèle Gal for their help in preparing this manuscript. This study has been suported by Association Française contre les Myopathies (AFM) and Association Française Retinitis Pigmentosa (AFRP).

1 Kaplan J, Bonneau D, Frézal J, Munnich A, Dufier JL. Clinical and genetic heterogeneity in retinitis pigmentosa. *Hum Genet* 1990;**85**:635-42.
2 Kaplan J, Pelet A, Martin C, *et al.* Phenotype-genotype correlations in X linked retinitis pigmentosa. *J Med Genet* 1992;**29**:615-23.
3 Bird AC. X-linked retinitis pigmentosa. *Br J Ophthalmol* 1975;**59**:177-99.
4 Fishman A, Weinberg AW, McMahon TT. X-linked recessive retinitis pigmentosa: clinical characteristics of carriers. *Arch Ophthalmol* 1986;**104**:1329-35.
5 Musarella MA, Anson-Cartwright L, Burghes A, Worton RG, Lesko JG, Nussbaum RL. Linkage analysis of a large Latin-American family with X-linked retinitis pigmentosa and metallic sheen in the heterozygote carrier. *Genomics* 1989;**4**:601-5.
6 Arden GB, Carter RM, Hogg CR. A modified ERG technique and the results obtained in X-linked retinitis pigmentosa. *Br J Ophthalmol* 1983;**67**:419-30.
7 Berson EL, Rosen JB, Simonoff EA. Electroretinographic testing as an aid in detection of carriers of X-chromosome-linked retinitis pigmentosa. *Am J Ophthalmol* 1979;**87**:460-8.
8 Meindl A, Dry K, Herrmann K, *et al.* A gene RPGR with homology to the RCC1 guanine nucleotide exchange factor in mutated in X-linked retinitis pigmentosa RP3. *Nat Genet* 1996;**13**:35-42.
9 Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985;**37**:482-98.
10 Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HpaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 1992;**51**:1229-39.
11 Willard H, Cremers F, Mandel JL, Monaco AP, Nelson D, Schlessinger D. Report of the fifth international workshop on human X chromosome mapping *Cytogenet Cell Genet* 1994;**67**:296-328.
12 McQuarrie MD. Two pedigrees of hereditary blindness in man. *J Genet* 1935;**30**:147-53.
13 Janssen O. *Zur erbbiologie der retinitis pigmentosa.* Inaugural dissertation, Munster in Westfalen, 1938.
14 McKenzie DS. The inheritance of retinitis pigmentosa in one family. *Trans Ophthalmol Soc New Zealand* 1951;**5**:79-82.
15 Kobayashi F. Genetic study on the retinitis pigmentosa. *Acta Soc Ophthalmol Jpn* 1959;**63**:3839-59.
16 Friedrich U, Warburg M, Jorgensen AL. X-inactivation pattern in carriers of X-linked retinis pigmentosa: a variable means of prognostic evaluation? *Hum Genet* 1993;**92**:359-63.
17 McGuire RE, Sullivan LS, Blanton SH, Church MW, Heckenlively JR, Daiger SP. X-linked dominant cone-rod degeneration: linkage mapping of a new locus retinitis pigmentosa (RP15) to Xp22.13-p22.11. *Am J Hum Genet* 1995;**57**:84-94.
18 Swaroop A, Buraczynska M, Wu W, *et al.* Genetic and molecular studies of X-linked retinitis pigmentosa. *VII International Symposium on Retinal Degeneration, Sendai, Japan*, 1996.