Short reports

Linkage mapping of a large Colombian family segregating for X linked retinoschisis: refinement of the chromosomal location

Barkur S Shastri, James F Hejtmancik, Alvaro Rodriguez, Francisco Rodriguez, Marta L Tamayo

Abstract

Juvenile X linked retinoschisis (RS) is a bilateral vitreoretinal dystrophy that develops early in life. Previous linkage studies have localised the RS gene to Xp22.1-p22.3 between DXS207 and AFM 291Wf5, which represents a genetic distance of approximately 3.7 cM. In an effort to facilitate the eventual cloning of the RS gene, we have analysed a large Colombian family, using 10 microsatellite markers that have been mapped to the region Xp22.1-p22.3. A total of 93 members, including 19 affected and eight unaffected males, two affected females, and six obligate carrier females were analysed. Close linkage was observed between the disease locus and DXS999 ($Z_{\max}=2.27$, $\theta_{\max}=0.05$), DXS987 ($Z_{\max}=2.61$, $\theta_{\max}=0.1$), DXS443 ($Z_{\max}=4.23$, $\theta_{\max}=0.1$), and DXS274 ($Z_{\max}=3.49$, $\theta_{\max}=0.05$) markers. Recombination with the RS locus was found for all marker loci except DXS197, DXS43, and DXS1195. These results place the RS locus within an interval of approximately 2 cM between the flanking markers DXS1053 and DXS999, approximately 1.7 cM closer than the previously reported boundary. The results also further confirm the lack of genetic heterogeneity of RS.

(*J Med Genet* 1997;34:504-506)

Keywords: retinoschisis; X linked; linkage

Juvenile retinoschisis (RS) is a recessively inherited X linked eye disorder which results in poor visual acuity. The disorder develops early in life and is characterised by variable acuity, vitreous veil, cartwheel, splitting of nerve fibre layer, and vitreous degeneration.¹⁻³ It has a high degree of penetrance and most affected subjects are males. The majority of female carriers have normal vision and cannot be identified by clinical signs alone,⁴ although a small proportion of heterozygous females can be detected by abnormal rod-cone interactions.^{5,6} The progression of the disease is from mild to

moderately severe and can, if complicated by retinal detachment and vitreous haemorrhage, ultimately lead to blindness. Although foveal schisis is the most common abnormality, there is a wide phenotypic variation and the condition often appears without any family history. The biochemical defects underlying RS are unknown, but histopathological¹ and electrophysiological studies⁷ have suggested the involvement of Muller cells.

Previous linkage studies have localised the RS gene to the short arm of the X chromosome with close linkage to the markers DXS207 and DXS274.⁸⁻¹⁰ We previously reported¹¹ linkage studies on four families of different geographical origins using microsatellite markers; no evidence for locus heterogeneity was found for RS. To facilitate the eventual cloning of the RS gene we have studied a new Colombian family using 10 microsatellite markers located close to the RS gene.

Members of all families were given complete ophthalmological examinations by two of us (A and F Rodriguez). We collected 20 ml venous blood from several affected and unaffected subjects and high molecular weight DNA was extracted. All oligonucleotide primers were synthesised commercially with the primer sequences obtained from published articles. Genomic DNA from several affected and unaffected family members was amplified by the polymerase chain reaction (PCR). The reaction conditions were 30 cycles of 1.5 minutes at 94°C, one minute at 60°C, two minutes at 72°C in a buffer containing 100 ng genomic DNA, thermostable *Taq* polymerase (2-4 units), 50 μ mol/l each of four deoxynucleotides, 1 μ Ci of α -³²P dATP, 10 pmol each of the primer, 1.5 mmol/l MgCl₂, and 10 mmol/l tris-HCl, pH 8.3. The PCR products were separated by electrophoresis on a 9% acrylamide gel and visualised by autoradiography. Two point linkage analysis was carried out using the LINKAGE program package.¹² For all analyses, penetrance used for the disease allele is 0.999 and the gene frequency is 0.0002.¹³

Eye Research Institute,
Oakland University,
422 Dodge Hall,
Rochester, MI
48309-4401, USA
B S Shastri

National Eye Institute,
Bethesda, MD 20892,
USA
J F Hejtmancik

Fundacion
Oftalmologica
Nacional, Bogota,
Colombia
A Rodriguez
F Rodriguez

Instituto de Genetica
Humana Universidad
Javeriana, Bogota,
Colombia
M L Tamayo

Correspondence to:
Dr Shastri.

Received 28 August 1996
Revised version accepted for
publication 3 December
1996

Figure 1 Segregation pattern of 10 microsatellite markers that have been mapped to the region Xp22.1-p22.3. Recombination with the RS locus was found for all marker loci except DXS197, DXS43, and DXS1195.

The pedigree used for the linkage analysis contains 27 affected males, four affected females, and 23 obligate carriers with five consanguineous and several multiple marriages (family is not shown). A total of 93 members, including 19 affected and eight unaffected males, two affected females, and six obligate carrier females were analysed. The segregation of PCR alleles identified with 10 microsatellite markers is shown below these subjects (fig 1; the rest of the subjects are not shown in the figure). Obligate recombinations between retinoschisis and DXS987 are seen in three unaffected subjects: IV.14, V.1, and V.4. DXS207 and DXS1053 also show obligate recombination with retinoschisis in two of these unaffected subjects, V.1 and V.4. Markers DXS197, DXS43, and DXS1195 show a single allele for

all people tested. In spite of this, they show small positive lod scores owing to the preponderance of affected subjects inheriting this allele from mothers whose DNA was not available for testing. DXS999 shows an obligate recombination with retinoschisis in affected subject IV.23. DXS443 shows obligate recombination in unaffected subjects IV.14 and V.4, while DXS365 shows obligate recombination in V.4 and DXS274 shows an obligate recombination event in IV.14. Both affected females, IV.8 and V.8, are the offspring of affected fathers, one in a consanguineous mating. Both are homozygous for all markers throughout the region, consistent with homozygosity for the retinoschisis mutation.

Results of two point linkage analysis for the disease locus and the 10 markers are presented in table 1. Four markers gave significant lod scores. A lod score of 2.27 at recombination fraction 0.05 was obtained with the marker DXS999, while DXS443 yielded a value of 4.23 at a recombination fraction of 0.10. DXS274 gave a maximum lod score of 3.49 at a recombination fraction of 0.05 and DXS987 gave a maximum lod score of 2.61 at a recombination fraction of 0.10. Recombination with the RS locus was found for all marker loci except DXS197, DXS43, and DXS1195. Based on the recombination events observed and taking into account the most recent CEPH

Table 1 Pairwise linkage results

Marker	Lod score at recombination fraction θ								Z_{max}	θ_{max}
	0	0.01	0.05	0.1	0.2	0.3	0.4			
DXS987	-2.08	0.56	2.17	2.61	2.51	1.91	1.04	2.67	0.13	
DXS207	-1.37	0.37	1.43	1.71	1.61	1.19	0.63	1.73	0.126	
DXS1053	-2.62	-0.84	0.27	0.61	0.71	0.56	0.31	0.72	0.17	
DXS197	0.76	0.74	0.66	0.56	0.38	0.22	0.10	0.76	0	
DXS43	1.35	1.32	1.20	1.04	0.72	0.44	0.19	1.35	0	
DXS1195	1.13	1.10	0.98	0.85	0.58	0.35	0.15	1.13	0	
DXS999	1.03	1.86	2.27	2.26	1.90	1.37	0.74	2.29	0.07	
DXS443	1.35	3.32	4.21	4.23	3.58	2.57	1.33	4.28	0.07	
DXS365	0.26	1.25	1.74	1.79	1.54	1.09	0.56	1.79	0.10	
DXS274	2.17	3.13	3.49	3.37	2.76	1.94	1.01	3.49	0.05	

family data and map distance,^{14 15} the most likely location for RS is (DXS987, DXS207, DXS1053, DXS43, DXS1195)-RS-DXS999-DXS443-DXS365-DXS274. Since the genetic distance between markers DXS207 and DXS999 has been estimated as 2 cM,¹⁴ these results place the RS locus to within an interval of approximately 2 cM, about 1.7 cM closer than the previously reported boundary of the candidate region for the disease gene.^{9 10}

RS belongs to the still poorly defined group of vitreoretinal dystrophies with no known cure. The disease locus has been previously mapped to the short arm of the X chromosome.¹⁴⁻¹⁶ In the present study we have reduced the position of the RS locus to an interval of approximately 2 cM, about 1.7 cM closer than the previous boundary. Identification of recombinant events between RS and the markers which were studied places the RS gene between markers DXS1053 and DXS999. These results improve the feasibility of cloning the RS gene. In addition, the closely linked flanking markers (DXS443 is most informative in this family and detected homozygosity in two affected females) will be of significant value in the molecular genetic diagnosis of the RS carriers and homozygotes, since carriers of this disease cannot be identified by clinical means alone.¹⁷ Results of the study will also help in counselling patients regarding their genetic risks for development of the disorder. The data presented above also support the proposition that RS is genetically a homogeneous disease. Future studies on cloning and characterisation of a candidate gene may provide valuable insight into the mechanisms underlying the pathogenesis of RS.

Note added in proof

While this paper was in press, an article by George *et al* (*J Med Genet* 1996;33:919-22) reported that RS is located between DXS43 and DXS999, which is consistent with the present report.

We thank the members of the Colombian family who donated blood samples for this study. We would also like to thank Drs F J Giblin and J Bernal for critically reading the manuscript. Our

appreciation also to G Keyeux, C Duran, C Rodas and R Gomez for laboratory work and other members from Fundacion Oftalmologica Nacional and Instituto de Genetica Humana in Bogota, Colombia for their valuable field work. The above work was supported in part by a grant from the Retinopathy of Prematurity Foundation (ROPARD), Beaumont Hospital Research Institute (RI-96-05) and a core grant for vision research from National Eye Institute (EY05230); in Colombia by a grant from Colciencias (cod-6207-04-322-95).

- 1 Condon GP, Brownstein S, Wang NS, Kearns AF, Ewing CC. Congenital hereditary retinoschisis. *Arch Ophthalmol* 1986;104:576-83.
- 2 George NDL, Yates JRW, Moore AT. Clinical features in affected males with X linked retinoschisis. *Arch Ophthalmol* 1996;114:274-80.
- 3 Geiser EP, Falls HF. Hereditary retinoschisis. *Am J Ophthalmol* 1961;51:1193-200.
- 4 Ewing CC, Cullen AP. Fluorescein angiography in X-chromosomal maculopathy with retinoschisis. *Can J Ophthalmol* 1972;7:19-28.
- 5 Arden GB, Gorin MB, Polkinghorne PJ, Jay M, Bird AC. Detection of the carrier state of X-linked retinoschisis. *Am J Ophthalmol* 1988;105:590-5.
- 6 Kaplan J, Pelet A, Hentati H, *et al*. Contribution to carrier detection and genetic counseling in X linked retinoschisis. *J Med Genet* 1991;28:383-8.
- 7 Peachey NS, Fishman GA, Derlacki DJ, Brigell MIG. Psychophysical and electroretinographic findings in X-linked juvenile retinoschisis. *Arch Ophthalmol* 1987;105:513-16.
- 8 Bergen AAB, Brink JB, Bleeker-Wagemakers LM, Schooneveld MJ. Refinement of the chromosomal position of the X linked juvenile retinoschisis. *J Med Genet* 1994;31:972-5.
- 9 Dumur V, Trivier E, Puech B, *et al*. Genetic analysis of new French X-linked juvenile retinoschisis kindreds using microsatellite markers closely linked to the RS locus: further narrowing of the RS candidate region. *Hum Genet* 1995;96:79-82.
- 10 Pawar H, Bingham EL, Lunetta KL, *et al*. Refined genetic mapping of juvenile retinoschisis. *Hum Hered* 1995;45:206-10.
- 11 Shastry BS, Hejtmanick FJ, Margherio RT, Trese MT. Linkage mapping of new X-linked juvenile retinoschisis kindreds using microsatellite markers. *Biochem Biophys Res Commun* 1996;220:824-7.
- 12 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-6.
- 13 Alitalo T, Forsius H, Karna J, *et al*. Linkage relationships and gene order around the locus for X-linked retinoschisis. *Am J Hum Genet* 1988;43:476-83.
- 14 Biancalana V, Trivier E, Weber C, *et al*. Construction of a high resolution linkage map for Xp22.1-p22.2 and refinement of the genetic localization of the Coffin-Lowry syndrome gene. *Genomics* 1994;22:617-25.
- 15 Alitalo T, Francis F, Kere J, Lehrach H, Schlessinger D, Willard HF. A 6 Mb YAC contig in Xp22.1-Xp22.2 spanning the DXS69E, XE59, GLRA2, PIGA, GRPR, CALB3 and PHKA2 genes. *Genomics* 1995;25:691-700.
- 16 Oudet C, Weber C, Kaplan J, *et al*. Characterization of a highly polymorphic microsatellite at the DXS207 locus: confirmation of very close linkage to the retinoschisis disease gene. *J Med Genet* 1993;30:300-3.
- 17 Dahl N, Pettersson U. Use of linked DNA probes for carrier detection and diagnosis of X-linked juvenile retinoschisis. *Arch Ophthalmol* 1988;106:1414-16.