Improved genetic mapping of X linked retinoschisis

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Abstract

X linked retinoschisis (RS) causes poor vision in affected males owing to radial cystic changes at the macula. Genetic linkage analysis was carried out in 16 British families with X linked retinoschisis using markers from the Xp22 region. Linkage was confirmed between the RS locus and the markers DXS207 (lod score, $Z_{\max}$ = 17.9 at recombination fraction θ = 0.03; confidence interval for θ = 0.007-0.09), DXS1053 ($Z_{\max}$ = 18.0 at θ = 0.01, CI = 0.001-0.06), DXS43 ($Z_{\max}$ = 12.9 at θ = 0.03, CI = 0.004-0.09), DXS1195 ($Z_{\max}$ = 6.4 at θ = 0.00), DXS418 ($Z_{\max}$ = 8.2 at θ = 0.00), DXS999 ($Z_{\max}$ = 21.2 at θ = 0.01, CI = 0.001-0.05), DXS443 ($Z_{\max}$ = 14.2 at θ = 0.03, CI = 0.004-0.09), DXS365 ($Z_{\max}$ = 24.5 at θ = 0.008, CI = 0.001-0.04). Key recombinants placed RS between DXS43 distally and DXS999 proximally. Multi-point linkage analysis gave odds of 344:1 in favour of this location for RS and supported the map Xpter-(DXS207, DXS1053)-DXS43-1 cM-RS-1 cM-DXS999-DXS443-DXS365-DXS1052-Xcen.

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Key words: retinoschisis; X chromosome; microsatellites.

X linked retinoschisis (RS) (McKusick No 312700¹) is said to be the commonest cause of juvenile macular degeneration in males.² Patients present with squint and nystagmus in infancy or poor vision in later childhood. Sight threatening complications, such as vitreous haemorrhage and retinal detachment, occur in up to 20% of affected boys,^{3,4} while adult patients may experience deteriorating vision in their fourth and fifth decades owing to macular degeneration.⁵ The disease is fully penetrant in males with variable expression. Foveal schisis is said to occur in all patients² and over 50% have peripheral retinoschisis.³ These changes may become less specific in older patients.

The biological abnormality underlying RS is unknown but histological⁶ and electrophysiological⁷ findings suggest a defect in the Müller cell. Obligate carriers are clinically normal⁸ with the possible exception of abnormalities shown by complex psychophysical tests of retinal function.⁹ Identification of the genetic defect would be helpful in diagnosing carrier status as well as understanding the pathogenesis of RS.

Early genetic linkage studies showed linkage of RS to the Xg blood group^{10,11} on the distal

short arm of the X chromosome.¹² Subsequent studies using restriction fragment length polymorphisms (RFLP) localised RS to a 7 cM region flanked by (DXS207, DXS43) distally and DXS274 and DXS41 proximally.¹³⁻¹⁸ Several new microsatellite markers spanning the RS region have recently been identified¹⁹⁻²¹ and RS has been shown to lie between (DXS43, DXS207) distally and DXS365 proximally.²²

In this study we report linkage analysis in 16 British families using the microsatellite markers DXS207, DXS1053, DXS43, DXS1195, DXS418, DXS999, DXS443, DXS365, and DXS1052.

Patients and methods

FAMILIES

Sixteen families affected by X linked retinoschisis were examined by one ophthalmologist (NDLG). All available family members underwent a full ophthalmic examination including best corrected visual acuity, ocular movements, colour vision (Ishihara and City University plates), slit lamp examination, and ophthalmoscopy with dilated pupils.

The criteria for the diagnosis of X linked retinoschisis were: (1) typical foveal schisis and a reduced b wave on the ERG, occurring in at least one affected male member from each family, (2) X linked pattern of inheritance with affected males in more than one generation and transmission through unaffected females. Males were diagnosed as affected if they had either typical foveal schisis or a history of bilateral visual impairment since childhood associated with macular changes. Obligate carriers were diagnosed on the basis of having an affected father, or having an affected son plus an affected brother or other maternal male relative.

A total of 208 subjects were examined including 60 affected males, 46 obligate carriers, and 44 females of unknown status; 176 patients provided blood or mouthwash samples for DNA extraction.

TYPING OF MICROSATELLITE MARKERS

DNA was extracted using standard methods.²³ The microsatellite markers DXS207,¹⁹ DXS1053,²⁴ DXS43,²⁵ DXS999,²⁶ DXS443,²⁷ DXS365,²⁷ and DXS1052²¹ were analysed by polymerase chain reaction (PCR) in all 16 families. Each 10 µl reaction mix contained 100-200 ng of genomic DNA, 5-10 pmol of primer, 0.1 pmol of ³²P end labelled primer, 0.25 U of *Taq* DNA polymerase, and

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Table 1 Results of two point linkage analysis in RS

Locus	Lod score Z at recombination fraction θ							Zmax	θ max	Confidence interval for θ
	0	0.001	0.01	0.05	0.1	0.2	0.3			
DXS207	— ∞	14.6	17.2	17.8	16.6	13.1	9.1	17.9	0.03	0.007–0.09
DXS1053	— ∞	17.3	18.0	17.3	15.7	12.0	7.9	18.0	0.01	0.001–0.06
DXS43	— ∞	10.9	12.6	12.8	11.8	9.0	5.9	12.9	0.03	0.004–0.09
DXS418	8.2	8.1	8.0	7.5	6.7	5.1	3.4	8.2	0.00	0.00–0.07
DXS1195	6.4	6.4	6.3	5.8	5.3	4.1	2.8	6.4	0.00	0.00–0.09
DXS999	— ∞	20.6	21.2	20.3	18.4	14.2	9.6	21.2	0.01	0.001–0.05
DXS443	— ∞	12.2	13.9	14.0	13.0	10.2	6.9	14.2	0.03	0.004–0.09
DXS365	— ∞	24.0	24.6	23.4	21.2	16.4	11.0	24.6	0.008	0.001–0.04

Table 2 Details of individual recombinants in families with RS. Markers showing recombination with the disease are denoted by X, non-recombinant markers by O, and non-informative markers by n. The dashed line indicates regions of probable exclusion of RS

Family	Subject	Sex	Status	DXS 207	DXS 1053	DXS 43	DXS 1195	DXS 418	DXS 999	DXS 443	DXS 365	DXS 1052
6228	II.2	M	A	X-----X	O	O	O	O	n	O	O	O
6165	III.4	M	A	X-----n-----X	O	O	O	O	n	n	O	
6229	II.1	F	OC	X-----n-----X	n	O	n	n	n	n	O	
6656	III.2	M	A	n	n	n	O	O	X-----X-----X	X		n
7577	III.3	M	A	O	n	O	O	O	n	X-----X	X	
6228	II.5	M	A	O	O	O	O	O	n	O	O	X

A = affected, OC = obligate carrier.

0.25 mmol/l of each dNTP in buffer containing 1.5 mmol/l MgCl_2 , 50 mmol/l KCl, 10 mmol/l Tris, and 0.01% gelatin (2 mmol/l MgCl_2 buffer was used in the case of DXS43). The reaction mixes were subjected to 30 cycles at 94°C for one minute, annealing (56°C for DXS999 and DXS43, 57°C for DXS207, 58°C for DXS443, DXS365, and DXS1052, 63°C for DXS1053) for one minute, and 72°C for one minute. PCR products were separated by electrophoresis on 4% or 6% denaturing polyacrylamide gels and alleles were visualised by autoradiography. The markers DXS1195²⁴ and DXS418²⁸ were analysed in the key recombinants only.

LINKAGE ANALYSIS

Genetic linkage analysis was performed using the computer program LIPED²⁹ for two point analysis and the LINKMAP option of the LINKAGE program³⁰ for multipoint analysis. The frequency of the retinosis gene was set at 0.0001. Confidence intervals were taken as the values of the recombination fraction at a lod score one unit below the maximum. For multipoint analyses the fixed map was (DXS207, DXS1053)–0.1 cM–DXS43–2 cM–DXS999–1 cM–DXS443–2 cM–DXS365 taken from published data.^{31 32}

Results

TWO POINT LINKAGE ANALYSIS

Table 1 shows the results of two point analysis, which confirmed close linkage of all markers to RS.

ANALYSIS OF INDIVIDUAL RECOMBINANTS

In pedigree 6228 (fig 1), affected male II.2 was recombinant for DXS207 and DXS1053 having inherited the opposite alleles to his three affected brothers. DXS999 was uninformative and DXS43, DXS443, DXS365, and

DXS1052 were non-recombinant. His affected grandson IV.1 has inherited the same recombinant haplotype. These data map DXS43 and RS proximal to DXS207 and DXS1053. In the same pedigree, affected male II.5 was recombinant for DXS1052, mapping RS distal to this marker.

In pedigree 6229, II.1 was an obligate carrier (having an affected brother and two affected sons) and was recombinant for DXS207 and DXS43. DXS1053, DXS999, and DXS443 were uninformative, but DXS365 was non-recombinant. This recombinant was inherited by her two affected sons, III.1 and III.2, and therefore maps RS proximal to DXS43.

In pedigree 6656, affected male III.2 was recombinant for DXS999, DXS443, DXS365, and DXS1052 having inherited different alleles to his affected brothers. DXS207, DXS1053, and DXS43 were uninformative. This result maps RS distal to DXS999.

Taken together these recombinants identify DXS999 as the proximal and DXS43 as the distal flanking markers to RS. DXS1195 and DXS418 map to the same interval³² and were typed in the key recombinants (table 2) but were either non-recombinant or uninformative.

MULTIPOINT LINKAGE ANALYSIS

In the LINKMAP analysis the maximum location score was 125 with RS located at a point midway between DXS43 and DXS999. This position was favoured by odds of 344:1 compared to a location between DXS999 and DXS443 and by odds of 10⁴:1 compared to a location between DXS43 and DXS1053.

Discussion

Using the microsatellite markers DXS207, DXS43, DXS1053, DXS999, DXS443, and DXS365 we have confirmed close linkage to RS. A recombinant in pedigree 6229 mapped RS proximal to DXS43. In pedigree 6656 a

Pedigree 6228

Pedigree 6656

Pedigree 6229

Figure 1 Pedigrees 1, 2, and 3. Affected males are shaded and obligate carrier females are indicated by a central dot. The order of the markers for each haplotype is shown in the key.

key recombinant mapped RS distal to DXS999. Together these data place RS between DXS43 distally and DXS999 proximally, a genetic distance of 2 cM. Multipoint analysis supported the map Xpter-(DXS207, DXS1053)-DXS43-1 cM-RS-1 cM-DXS999-1 cM-DXS443-2 cM-DXS365-Xcen. We have narrowed the genetic interval containing the RS locus from 5 cM to

approximately 2 cM. This corresponds to a physical distance estimated at 2.5 Mb.³³ The data presented represent a significant contribution to the effort to localise and ultimately clone the gene for RS. In addition we have identified highly informative microsatellite markers which can be used for accurate carrier detection.

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