

Refinement of the chromosomal position of the X linked juvenile retinoschisis gene

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Abstract

Linkage analysis was carried out in seven X linked juvenile retinoschisis (XLRS) families using four DNA probes and four CA repeat polymorphisms from the Xp22 region. Close linkage was observed between the XLRS locus and DXS207 ($\theta_{\max}=0.04$, $Z_{\max}=3.71$), DXS999 ($\theta_{\max}=0.00$, $Z_{\max}=4.59$), DXS365 ($\theta_{\max}=0.07$, $Z_{\max}=2.22$), and DXS451 ($\theta_{\max}=0.05$, $Z_{\max}=3.26$). The analysis of recombination breakpoints and multipoint linkage analysis suggests the order Xpter-DXS16-(DXS43, DXS207)-RS-DXS365-(DXS451, DXS41)-Xcen, thereby refining the position of the XLRS locus to an interval of approximately 3-4 cM. These results improve the feasibility of diagnosis in XLRS considerably, since carriers of this disease cannot be identified clinically.

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X linked juvenile retinoschisis (XLRS) is a hereditary vitreoretinal degeneration leading to an increasingly severe visual handicap after the fourth decade of life. The condition is characterised by microcystic maculopathy, peripheral retinal lesions, and vitreous body alterations. The vast majority of carriers do not show any ocular abnormality.^{1,2} A number of carriers may be detected by abnormal rod-cone interactions.³

The gene (RS) for XLRS has been localised to the distal part of the human X chromosome. Two point linkage analysis showed close linkage between RS and the XG blood group, and between RS and several markers from the Xp22 region, including DXS9, DXS41, and DXS43.⁴⁻⁷ Multipoint linkage analyses suggested the order Xpter-(DXS9, (DXS16-DXS207-DXS43))-RS-DXS274-DXS41-DXS92-Xcen.⁸⁻¹¹

The genetic fine mapping of the RS locus has been hampered by the scarcity of highly polymorphic markers from the Xp22.2-p22.1 region. Recently, these markers have become available.¹² Here we present two point and multipoint linkage data for XLRS using both conventional DNA markers and CA repeat polymorphisms from the XLRS gene region.

Materials and methods

SUBJECTS

Members of all families but one (P 24130) were examined by MvS at the Netherlands Ophthalmic Research Institute. Members of family P 24130 were examined by A J G L Pinck-

ers at the Ophthalmic Department of the University of Nijmegen. The clinical studies included seven families (fig 1). X linked inheritance was obvious in all pedigrees and all families comprised at least two or more patients. Follow up studies of several patients were performed at the Netherlands Ophthalmic Research Institute.

Clinical diagnosis was based on complete ophthalmological examination. ERGs were performed in order to confirm the diagnosis only in those cases in which ocular examination by itself was not conclusive. The typical negative type ERG was observed in all cases. In pedigree 24130, healthy male III.1 underwent ophthalmic examination, but he refused examination by ERG to confirm his healthy status. The severity of the visual handicap was variable in all patients, as is common in RS. Patients had visual acuity in the better eye ranging from counting fingers at 3 m to almost 20/20 (age range 4-74 years).

LINKAGE ANALYSIS

Two families were studied previously with conventional DNA probes (P9157 and P2207)¹¹ and here that analysis was extended with four CA repeats. Five additional families were screened with four DNA probes and four CA repeat polymorphisms from the Xp22.2 region.

SOUTHERN ANALYSIS

DNA and PCR analysis were carried out as described previously.^{13,14} Details of the probes and primers used are given in Davies *et al*,¹⁵ Weissenbach *et al*,¹² and The Human Genome Database.

STATISTICS

Persons over the age of 20 were included in the analysis. Penetrance values for obligatory carriers were set to 0.00. A gene frequency of 0.0002 was used. Lod and location scores were calculated using the computer programs MLINK and LINKMAP (LINKAGE version 5.03).¹⁶ Multipoint linkage analysis which required long calculation times was carried out on the VAX VMS 6000 computer of the University Hospital, Amsterdam. Acceptable calculation times were obtained with the analysis of two or three markers with multiple alleles plus the disease locus. Since DXS207 and DXS365 were fully informative in almost all meioses, these loci were considered to yield full information. Simultaneous analysis of additional

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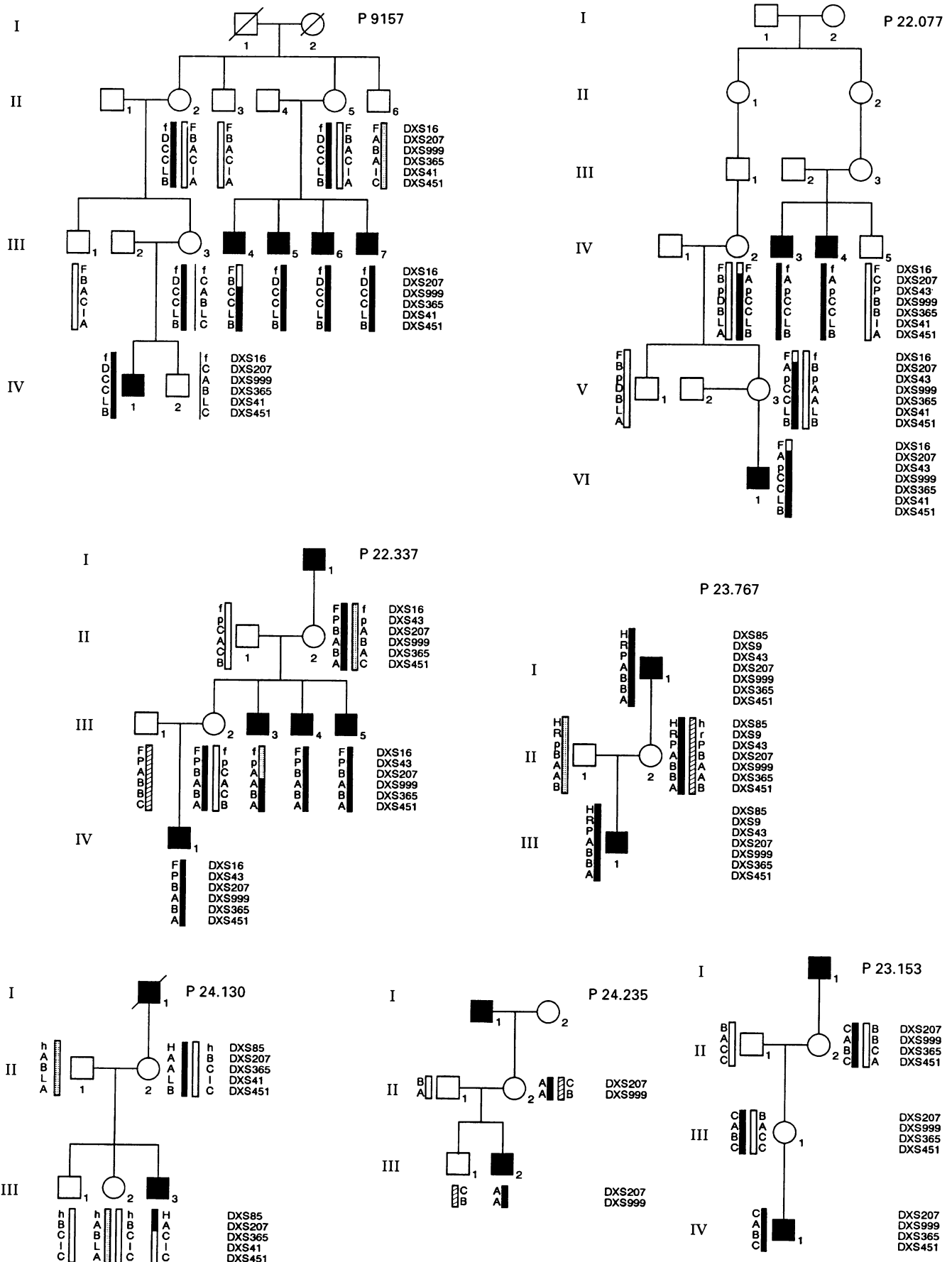


Figure 1 Linkage analysis in seven X linked juvenile retinoschisis families.

Table 1 Two point linkage result between RS and flanking loci

Locus	θ_{max}	Z_{max}	θ						
			0-00	0-01	0-05	0-10	0-20	0-30	
DXS9	0-00	0-70	0-70	0-69	0-67	0-64	0-54	0-41	
DXS16	0-12	0-58	—∞	—0-8	0-29	0-56	0-45	0-08	
DXS207	0-04	3-71	—∞	3-40	3-70	3-50	2-76	1-90	
DXS43	0-00	1-23	1-23	1-20	1-11	0-99	0-74	0-48	
DXS999	0-00	4-59	4-59	4-51	4-17	3-73	2-80	1-81	
DXS365	0-07	2-22	—∞	1-72	2-19	2-18	1-81	1-24	
DXS41	0-07	2-08	—∞	1-59	2-06	2-05	1-71	1-12	
DXS451	0-05	3-26	—∞	2-91	3-26	3-11	2-49	1-69	

Table 2 Multipoint linkage results

Order (Dis = 1, DXS207 = 2, DXS999 = 3, DXS365 = 4)	Relative likelihood
1-2-3-4	3.4×10^{-2}
2-1-3-4	1-0
2-3-1-4	0.5×10^{-1}
2-3-4-1	8.0×10^{-2}

loci was not considered useful, since these loci are only informative in some of the meioses studied with DXS207 and DXS365. Confidence limits were defined according to Conneally *et al.*¹⁷

Results

KEY RECOMBINANTS

It has previously been established that RS is localised between the Xp loci DXS43 and DXS41. Since the current consensus genetic map of Xp22.2 is defined by the order (Xpter-) DXS207-DXS43-DXS999-DXS365-DXS274-DXS41-DXS451(-Xcen) all these loci are presumably closely linked to RS.

Several interesting recombinants between RS and these markers were found (fig 1). In family P 9157, assuming the fewest number of recombinants, the disease locus is located on the chromosome identified by the alleles f (DXS16)- D (DXS207)- C (DXS999)- C (DXS365)- L (DXS41)- B (DXS451). Patient III-4, however, has the alleles F-B-C-C-L-B, and he is therefore recombinant for DXS16 and DXS207. Thus, the recombination places RS proximal to both DXS16 and DXS207.

A similar observation was made in pedigree P 22.337 (fig 1). Generally, in this pedigree, the disease phenotype cosegregates with the

alleles F (DXS16)-P (DXS43)-B (DXS207)-A (DXS999)-B (DXS365)-A (DXS451). Patient III-3, with the haplotype f-p-A-A-B-A, is recombinant for DXS16, DXS43, and DXS207. The analysis of the recombination event confirms that RS is located proximal to DXS16 and DXS207, and also centromeric to DXS43.

In family P 24.130 (fig 1) the phase of the markers is determined by the male III-1 and female III-2. Female III-2 inherited the X chromosome characterised by h (DXS85)-A (DXS207)-B (DXS365)-L (DXS41)-A (DXS451) from her father (DXS43 and DXS999 yielded uninformative results). Hence, her other X chromosomal alleles (h-B-C-l-C) are of maternal origin. Apparently, healthy male III-1 inherited the similar chromosome (h-B-C-l-C) from the mother. Without recombination, one would expect male patient III-3 to inherit the other X chromosome from the mother (H-A-A-L-B). However, since the observed haplotype in III-3 is H-A-C-l-C, the patient is most likely recombinant for DXS365, DXS41, and DXS451. Consequently, the latter recombination event places RS distal to these loci. The analysis of recombination breakpoints suggests the order Xpter-DXS16-(DXS207, DXS43)-(DXS999, (RS-(DXS365, DXS41, DXS451)))-Xcen.

TWO POINT LINKAGE ANALYSIS

Two point linkage results for RS and eight loci are presented in table 1. Close linkage between RS and several regional conventional DNA probes was confirmed. Close linkage without recombination was found between RS and the CA repeat corresponding to the DXS999 locus ($Z_{max}=4.59$). In addition, significant close linkage was observed between RS and the loci DXS207 ($\theta_{max}=0.04$, $Z_{max}=3.71$), DXS365 ($\theta_{max}=0.07$, $Z_{max}=2.22$), and DXS451 ($\theta_{max}=0.05$, $Z_{max}=3.26$).

MULTIPOINT LINKAGE ANALYSIS

Several analyses with various combinations of markers and RS were performed (see Materials and methods). Three point linkage analysis with DXS207, DXS365, and RS yielded the most interesting results, which are presented in table 2 and fig 2. Accordingly, the RS gene is most likely located between DXS207 and DXS365.

Discussion

So far, linkage studies in RS have only been carried out with conventional DNA probes. In general, the usefulness of these studies was limited because of the low PIC values of the linked probes and the large genetic distances found between RS and flanking markers.¹¹ CA repeat polymorphisms from Xp22.2 have only recently become available.¹² We have studied seven XLRs families with both DNA probes and CA repeats from Xp22.2. Combining our results with those published, we suggest the order Xpter-(DXS9, (DXS16-(DXS43, DXS207))-RS-(DXS365-(DXS451, DXS41), DXS274)-Xcen.

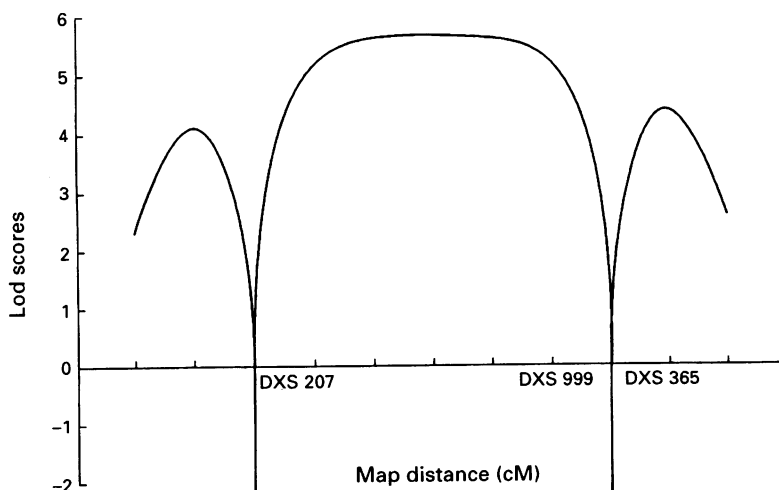


Figure 2 Statistical evaluation of the XLRs segregation data.

No recombination was found between RS and DXS999 ($Z_{\max}=4.59$) which suggests that these loci are very closely linked. This result agrees well with the consensus genetic map of Xp22, which places DXS999 between DXS207 and DXS365, which is the putative RS region. Unfortunately, DXS999 was not informative in family P 24130 in which patient III:1 (fig 1) is recombinant for DXS365 and other loci proximally adjacent to RS. The data presented here refine the genetic map around the RS gene. Furthermore, our linkage data between RS and highly informative flanking markers improve the feasibility and reliability of DNA diagnosis for this disorder considerably.

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