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Characterisation of a highly polymorphic microsatellite at the *DXS207* locus: confirmation of very close linkage to the retinoschisis disease gene

Claudine Oudet, Chantal Weber, Josseline Kaplan, Bertrand Segues, Marie-Françoise Croquette, Elisabeth Ollagnon Roman, André Hanauer

Abstract

Juvenile retinoschisis (RS) is an X linked recessive vitreoretinal disorder for which the basic molecular defect is unknown. The gene for RS has been previously localised by linkage analysis to Xp22.1-p22.2 and the locus order Xpter-*DXS16*-(*DXS43*, *DXS207*)-*RS*-*DXS274*-*DXS41*-Xcen established. To improve the resolution of the genetic map in the RS region, we have isolated a highly polymorphic microsatellite at *DXS207*, which displays at least nine alleles with a heterozygosity of 0·83. Using this microsatellite and four other Xp22.1-p22.2 marker loci, *DXS16*, *DXS43*, *DXS274*, and *DXS41*, we performed pairwise and multilocus linkage analysis in 14 kindreds with RS. The microsatellite was also typed in the CEPH (Centre d'Etude du Polymorphisme Humain) reference families. Tight linkage was found between *RS* and *DXS207* ($Z(\theta) = 14{\cdot}32$ at $\theta = 0{\cdot}0$), *RS* and *DXS43* ($Z(\theta) = 8{\cdot}10$ at $\theta = 0{\cdot}0$), and *DXS207* and *DXS43* ($Z(\theta) = 40{\cdot}31$ at $\theta = 0{\cdot}0$). Our linkage results combined with data previously reported suggest that the *DXS207*-*DXS43* cluster is located less than 2 cM telomeric to the RS locus. The microsatellite reported here will be a very useful marker for further linkage studies with retinoschisis as well as with other diseases in this region of the X chromosome.

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Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, INSERM Unité 184, Institut de Chimie Biologique, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg, France.
C Oudet
C Weber
A Hanauer

INSERM Unité 12, Clinique de Génétique Médicale, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75743 Paris, France.
J Kaplan
B Segues

Centre de Génétique, Hôpital Saint Antoine, 329 Boulevard Victor Hugo, 59019 Lille, France.
M F Croquette

Service de Génétique, Hôtel-Dieu, 1 place de l'Hôpital, 69288 Lyon, France.
E Ollagnon Roman

Correspondence to
Dr Hanauer.

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X linked juvenile retinoschisis (RS) is a recessive disorder of the sensory retina resulting in progressive visual impairment. It is the most common cause of macular disease in children and adolescents.[1] Affected male patients show microcystic degeneration of the retina leading to intraretinal splitting, mainly in the nerve fibre layer. The disease is characterised by marked interfamilial, and even intrafamilial, phenotypic variation. While foveal retinoschisis is present in almost all patients, there is wide variability of expression of the peripheral retinal features. Vision of female carriers is usually normal, but they frequently show peripheral retinal alterations similar to those found in affected males.[2] Although an abnormality in the Müllerian cells has been suggested,[3] the basic defect in RS remains unknown. The RS gene has been mapped to Xp22.1-p22.2, between *DXS274* on the proximal side and *DXS207* and *DXS43* on the distal side, closely linked to the two latter markers.[2 4–8] This localisation was further supported by data also showing close linkage between *RS* and two additional Xp22.1-p22.2 polymorphic loci, *DXS197* and *DXS43*.[4 5 8 9] The latter markers are closely linked to *DXS207* and *DXS43*.[10 11] Despite the variability in the clinical expression of the disease, no evidence for genetic heterogeneity for RS was found. These results have already rendered genetic prediction feasible in many RS families.[2] A more precise mapping of the RS locus is a prerequisite for positional cloning. Accuracy of genetic mapping, including identification of recombinants which provide crucial information for orienting the gene search, was limited by the lack of highly informative markers in the region of the RS gene. We have identified a highly informative microsatellite marker at *DXS207*. Our linkage results suggest that this marker is located less than 2 cM telomeric to the RS locus.

Patients and methods

PATIENTS

Fourteen unrelated families with X linked recessive retinoschisis were analysed, seven of which have been used in a previous linkage study reported by Kaplan *et al*.[2] The diagnosis was, in each case, confirmed by careful ophthalmological examination. DNA analysis was performed on 181 subjects including 39 affected males.

MOLECULAR STUDIES

The procedures for genomic DNA extraction, restriction enzyme digestion, gel electrophoresis, Southern blotting, and hybridisation to radioactively labelled probes have been described elsewhere.[12]

PCR amplification of the *DXS207* microsatellite was carried out in 25 µl with 3 pmol of end labelled primer, 3 pmol of the same unlabelled primer, and 6 pmol of a second unlabelled primer, 200 mmol/l each dNTP, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8·2), 1·5 mmol/l $MgCl_2$, 100 ng of genomic DNA, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer/Cetus). Samples were overlaid

Table 1 The dinucleotide repeat and flanking sequence. The priming sequences are underlined.

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5'-CCCCTCCGGTTCACTCCACATTCTGCCAT
CAGAGTCATTTCTCCGAGACACAGATCTCG
CAGATCTGGTAATTTCACACACACACACAC
ACACACACACACACACACACACACACACAC
ACACACACACACCCTCCTCCTCAAGGGCTG
TCAATTTTATACAGATAAACATGGGTTTAA
AGGCTTTCCCTGATC-3'
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Table 2 Allele frequencies for the dinucleotide repeat at DXS207.

Allele (size in bp)	Allele frequency (96 chromosomes)
A1 (115)	0·035
A2 (117)	0·12
A3 (119)	0·12
A4 (121)	0·10
A5 (123)	0·24
A6 (125)	0·19
A7 (127)	0·12
A8 (129)	0·017
A11 (137)	0·035

with mineral oil and processed through one step of denaturation (94°C for five minutes), followed by 25 cycles of denaturation (94°C for one minute), annealing (55°C for one minute), and elongation (67°C for two minutes), and a last step of elongation (seven minutes at 67°C). One µl of each reaction was mixed with 8 µl of 90% formamide blue and 3 µl of this mix were loaded on a 8% polyacrylamide-50% urea sequencing gel. Gels were run at 1400 volts for four hours and subsequently exposed to Kodak X-AR films for a few hours.

GENETIC LINKAGE ANALYSIS

In addition to the microsatellite polymorphism, six RFLPs, including pXUT23 and pSE 3.2L (*DXS16*), pD2 (*DXS43*), pBA4B (*DXS207*), CRI-L1371 (*DXS274*), and p99.6 (*DXS41*) were used in the present study. The probes and conditions are described in Biancalana *et al.*[11] Pairwise and multilocus linkage analyses were performed using the LINKAGE program package (version 4·8).[13] RS was assumed to have complete penetrance, and the allele frequency of RS was taken to be 10^{-5}. The genotypic data of CEPH (Centre d'Etude du Polymorphisme Humain) families were extracted from the CEPH database version 5.

Results and discussion

We tested for the presence of a (CA) repeat, a 35 kb cosmid clone obtained by screening an X chromosome specific library[14] with probe pBA4B at *DXS207*. Hybridisation of an *Eco*RI restriction digest to a ^{32}P labelled poly-(dA.dC).poly(dT.dG) probe showed a strongly hybridising fragment. We directly subcloned a *Sau*3A digest of the cosmid into a Bluescript vector, and screening of these subclones with poly(dA.dC).poly(dG.dT) resulted in a positive clone which was sequenced. A (CA)27 repeat was identified and two oligonucleotide primers were synthestised, flanking a region of 117 bp including the (CA)27 repeat. The repeat and flanking sequence is shown in table 1. Genomic DNA from a set of CEPH family mothers was tested for polymorphisms by PCR amplification of the repeat using the flanking primers, one of which was ^{32}P labelled. Nine alleles ranging in size from 115 to 137 bp were identified (table 2). The alleles differ from adjacent ones by one dinucleotide pair, but so far no allele has been detected with product sizes of 131, 133, and 135 bp. From observed allele frequencies a heterozygosity of 0·83 was predicted. The combined heterozygosity at *DXS207*, using the RFLP detected with pBA4B and the new microsatellite, was 90% in 48 unrelated females.

We have applied this strongly informative polymorphism to linkage analysis in retinoschisis by typing DNA from 14 RS kindreds, seven of which have previously been used in the linkage study reported by Kaplan *et al.*[2] In addition, six RFLP markers spanning the Xp22.1-p22.2 region were analysed, including pXUT23 and PSE3.2L (*DXS16*), pD2 (*DXS43*), pBA4B (*DXS207*), CRI-L1371 (*DXS274*), and p99.6 (*DXS41*). The results of the two point linkage analysis, which are given in table 3, were obtained using the program MLINK and include the data concerning the seven RS families reported by Kaplan *et al.*[2] Analysis at locus *DXS207* was found to be fully informative in the 14 families and no recombination event was observed with *RS*. The maximum lod score for *RS-DXS207* was 14·32 at a recombination fraction of 0·00 (95% confidence interval of $\theta = 0{\cdot}00$-$0{\cdot}03$). Additionally, no recombination was evident in our families between *DXS43* and *RS* ($Z(\theta) = 8{\cdot}10$, $\theta = 0{\cdot}00$). Thus, these data confirm and extend previous findings of close linkage between *RS* and both *DXS207* and *DXS43*.[5,6,8] When our data for *DXS207* and *DXS43* are combined with the data published by these authors, the maximum lod scores between *RS* and *DXS207* and between *RS* and *DXS43* become $Z(\theta) = 21{\cdot}96$ at $\theta = 0{\cdot}01$ and $Z(\theta) = 33{\cdot}40$ at $\theta = 0{\cdot}02$ respectively. The confidence intervals

Table 3 Pairwise lod scores for linkage between RS and five Xp22.1-p22.2 marker loci.

Marker loci	Recombination fraction						θmax	Z(θmax)
	0·00	0·01	0·05	0·10	0·20	0·30		
DXS16	−0·53	1·92	2·36	2·33	1·94	1·38	0·07	2·38
DXS43	8·10	7·93	7·26	6·41	4·71	2·99	0·00	8·10*
	−∞	33·31	32·55	29·79	22·83	14·88	0·02	33·40†
DXS207	14·32	14·06	12·98	11·58	8·63	5·51	0·00	14·32*
	−∞	21·96	20·93	19·07	14·45	9·23	0·01	21·96‡
DXS274	−∞	0·56	2·80	3·29	3·00	2·11	0·12	3·31
DXS41	−∞	−1·97	0·48	1·23	1·46	1·12	0·18	1·47

* Data from the present study. †Data from the present study that have been combined with data from Alitalo *et al*,[5] Dahl *et al*,[6] and Sieving *et al.*[8] ‡Data from the present study that have been combined with data from Alitalo *et al.*[5]

Table 4 Pairwise lod scores for linkage between DXS207 and four other Xp22.1-p22.2 marker loci in the combined CEPH panel of reference families and 14 RS pedigrees.

Locus v locus	Recombination fraction						θmax	Z(θmax)
	0·00	0·01	0·05	0·10	0·20	0·30		
DXS207-DXS43	40·31	39·17	37·10	33·66	26·10	17·62	0·00	40·31
DXS207-DXS274	−∞	18·37	24·75	25·10	21·34	15·07	0·08	25·29
DXS207-DXS41	−∞	6·07	12·19	13·86	12·36	8·85	0·11	13·87
DXS207-DXS16	−∞	30·53	30·29	28·18	22·29	15·25	0·02	30·86

obtained from the pooled data for the *RS-DXS207* and the *RS-DXS43* linkage, which are important for diagnosis, were 0·005–0·05 and 0·005–0·06 respectively. At least two crossover events were observed with *DXS274* ($Z(\theta)=3{\cdot}31$ at $\theta=0{\cdot}12$) which confirm looser linkage with the closest proximal flanking marker. Combination of our data for *DXS274* and those of Alitalo *et al*[7] yields a lod score of 12·20 at $\theta=0{\cdot}08$.

Linkage relationships between the *DXS207* marker locus and four Xp22.1-p22.2 marker loci, including *DXS16, DXS43, DXS274*, and *DXS41*, were also further investigated using 36 CEPH pedigrees informative at *DXS207* and the 14 RS pedigrees from the present study. For the CEPH pedigrees, marker data for the RFLPs identified at *DXS16, DXS207, DXS43, DXS274*, and *DXS41* were obtained from the CEPH database. Table 4 gives the results of the pairwise linkage analyses between *DXS207* and the four other Xp22.1-p22.2 marker loci in the combined CEPH and RS pedigrees. They confirm and firmly support the previously established values.[10] The marker loci *DXS207* and *DXS43* showed completely concordant inheritance ($Z(\theta)=40{\cdot}31$ at $\theta=0{\cdot}00$). Previous studies have shown that these two markers are very tightly linked,[10 11] and no recombinations between them have been reported so far.

A multilocus linkage analysis was performed using the LINKMAP program. We used the following map of markers as established by Alitalo *et al*[10]: tel-*DXS16*-1·6-(*DXS43, DXS207*)-8-*DXS274*-3·5-*DXS41*-cen. The distance (*DXS207,DXS43*)-*DXS274* was slightly increased to conform with the data we obtained for linkage between these markers. As no recombination between *DXS207* and *DXS43* was observed, haplotypes for these two markers were constructed and used in multipoint linkage analysis. A maximum multipoint lod score of 16·52 was obtained for *RS* being at a position corresponding to the cluster *DXS207-DXS43* (figure). The one unit support interval (Zmax-1) was about 4 cM around the *DXS207-DXS43* cluster. The absence of recombination between *RS, DXS207*, and *DXS43* in our families did not allow us to infer an order for these loci. However, the few recombinational events detected between *RS* and both the *DXS207* and *DXS43* marker loci by Alitalo *et al*[7] and Sieving *et al*[8] support the localisation of the disease gene on the proximal side of both *DXS207* and *DXS43*. Taken together, the data position the *DXS207* locus, tightly linked to the *DXS43* locus, less than 2 cM telomeric to the disease gene.

Conclusions

The identification of the informative microsatellite polymorphism allowed us to assess the genetic distances in Xp22.2 more accurately and to refine the localisation of the RS gene further. Furthermore, as all 14 families were informative for *DXS207* and as we observed no crossovers, the present study reduces further the possibility of genetic heterogeneity in RS. Future efforts to identify additional flanking and tightly linked markers in the RS subregion will now be facilitated by the availability of this informative polymorphism. This highly polymorphic and rapidly typed marker should greatly improve genetic counselling in RS families. It may also prove useful for mapping and diagnosis of other disease genes assigned to the Xp22.1-p22.2 region, in which informative markers are sparse. These include Coffin-Lowry syndrome (CLS),[11] X linked hypophosphataemic rickets (HYP),[15] Nance-Horan syndrome (NHS),[16] and spondyloepiphyseal dysplasia tarda (SEDL).[17]

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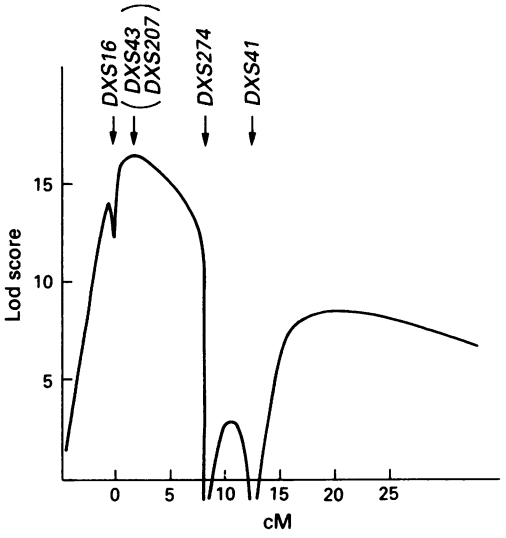

Multilocus Z analysis for the placement of the RS locus with respect to a fixed map of markers as described in the text. The locus DXS16 was arbitrarily placed at 0.0

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