CLASSEUR genetique 3501

Hum Genet (1995) 96:79–82

ORIGINAL INVESTIGATION

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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

Received: 9 January 1995

Abstract The gene involved in juvenile retinoschisis (RS) has previously been localized, by genetic linkage analyses, to Xp22.1–p22.2, between DXS274 and DXS43/DXS207; it is closely linked to the latter markers. From our recent data, this interval represents a genetic distance of approximately 10 cM. In the present study, we have studied 14 French families with X-linked juvenile RS by using four CA polymorphisms that are closely linked to the RS locus and that have recently been included in an Xp22.1–p22.2 high-resolution map. Complete cosegregation with the disease locus was observed for three of them, DXS207, DXS418, and DXS999, which further confirms the locus homogeneity for RS and the close linkage to this region. One recombinant was found with the most proximal marker, AFM291wf5, thereby defining this marker as the new proximal boundary of the candidate region for RS. Under the assumption that DXS207 and DXS43 constitute the distal boundary, the present study further reduces the region containing the disease gene to a interval of 3–4 cM. The results reported here should facilitate the eventual cloning of the RS gene.

Introduction

Juvenile retinoschisis (RS, MIM 312700) is an X-linked recessive vitreoretinal disorder resulting in progressive visual impairment. Ocular manifestations of the disease in male patients may include microcystic macular degeneration, peripheral retinal lesions, and vitreous body alterations. Although foveal lesion is found in almost all affected male patients, there is a wide phenotypic variability and expression of the peripheral retinal features.

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A small proportion of heterozygous females may be clinically identified by abnormal rod-cone interactions (Arden et al. 1988) or ocular examination (Kaplan et al. 1991). The majority of carriers, however, have normal vision with no retinal abnormalities. The cause of this pathology is unknown and no strong hypothesis has emerged with respect to the biochemical defect. The gene responsible for RS has been previously mapped by linkage studies to Xp22.1–p22.2; it is flanked proximally by DXS274 and distally by two tightly linked markers, DXS207 and DXS43, and is closely linked to the latter cluster (Sieving et al. 1990; Kaplan et al. 1991; Alitalo et al. 1991a). Based on a restriction fragment length polymorphism map established by Alitalo et al. (1991b), the distance between the flanking markers was estimated to be approximately 7 cM. Our more recent data using a highly polymorphic CA-repeat at DXS207 combined with data previously reported supported the localization of the disease gene at 1–2 cM centromeric to the DXS207/DXS43 loci, whereas DXS274, the proximal flanking marker, showed a recombination rate of 8% with RS (Oudet et al. 1992). No evidence for locus heterogeneity has been found for RS among the different sets of families so far studied.

Further reduction of the RS candidate region depends on the availability of new polymorphic markers in this relatively empty region of Xp22.1–p22.2. To address this need, we have recently assigned five GENETHON markers to the DXS43/DXS207–DXS274 interval and have constructed a multipoint map based on Centre d'Etude du Polymorphisme Humain families. The most likely locus order and map distances (in cM) are: Xpter-DXS16-(3.4) – (DXS207, DXS43, DXS1053) – (2.0) – (DXS999, DXS257) – (1.7) – AFM291wf5 – (1.4) – DXS443 – (2.0) – (DXS1229, DXS365) – (2.1) – (DXS1052, DXS274, DXS41) – Xcent (7).

The dinucleotide repeats AFM291wf5 and that at DXS999 are good candidates for being close proximal flanking markers for RS because of their positions on the map. In the present study, these two markers were used to perform a linkage analysis in 14 new RS families. The microsatellite polymorphisms at DXS207, a known distal

Table 1 Two-point lod scores between RS and four closely linked CA-repeat markers

Marker loci	Recombination fraction							
	0.00	0.01	0.05	0.10	0.20	0.30	θmax	Z(θmax)
DXS207[a]	9.70	9.51	8.77	7.82	5.86	3.84	0.00	9.70
DXS207[b]	−∞	31.47	29.70	26.89	20.31	13.07	0.005	31.60
DXS418	8.30	8.12	7.43	6.54	4.75	2.99	0.00	8.30
DXS999	6.87	6.73	6.18	5.46	3.99	2.51	0.00	6.87
AFM291wf5	−∞	5.78	5.90	5.45	4.19	2.78	0.03	5.97

[a] Data from the present study
[b] Data from the present study that have been combined with data from Alitalo et al. (1991a) and Oudet et al. (1992)

flanking marker, and that at DXS418, recently assigned to the (DXS43/DXS207)-DXS999 interval using the meiotic breakpoint panel described by Biancalana et al. (1994; unpublished results), were also included in the study. A crossover event observed in one family enabled us to define AFM291wf5 as the new closest proximal flanking marker for RS and to narrow down the candidate region for RS to an interval of 3–4 cM.

Materials and methods

A total of 105 members belonging to 14 families and including 34 affected males and 31 obligate carrier females were investigated. Most of the families originate from the north of France, and none has previously been used for linkage analysis. Detailed family histories and pedigree information were obtained through interviews with family members. No common ancestry between the different families is known. Members were ascertained and examined by the same ophthalmologist (B.P.). The diagnosis of juvenile RS was based on complete ophthalmological examination (including indirect and biomicroscopic ophthalmoscopy after cycloplegia) and electroretinography. For 15 females with a 25% or 50% risk of being carriers, the carrier status could not be determined by ophthamological examination, and thus, they were included as having an unknown carrier status. The clinical aspects of the present study will be published elsewhere (B. Puech, in preparation).

Blood samples were drawn and used for DNA extraction following standard procedures. Primers and polymerase chain reaction conditions for DXS987, DXS207, DXS418, DXS999, AFM291wf5, and DXS443 were as previously reported (Schaefer et al. 1993; Biancalana et al. 1994). Pairwise linkage analyses were carried out using the computer program MLINK from the LINKAGE package 5.01 (Lathrop et al. 1985).

Results

Each family was informative for at least two of the four markers tested. The most distal marker, the CA-repeat at DXS207, and the most proximal, AFM291wf5, were each informative in at least 10 pedigrees, whereas eight families were fully informative for both markers. Two-point lod scores obtained for linkage between the RS trait and the four CA-repeats are presented in Table 1. As expected, peak lod score values and corresponding recombination frequencies confirmed close linkage of the disease gene and these markers. RS showed complete cosegregation with DXS207, DXS418, and DXS999, with lod scores of 9.70, 8.30, and 6.87, respectively (Table 1). Analysis with the most proximal marker, AFM291wf5, revealed a crossover event with the disease locus, giving a maximum lod score of 5.97 at a recombination fraction of 0.03. The recombination occurred in a 22-year-old male patient (III-3) belonging to a three-generation pedigree (family L-17, Fig. 1). This family, in addition to the recombinant individual (III-3), includes three other affected males (III-1, III-4 and III-5). Careful examination by one of us (B.P.) confirmed the diagnosis of RS in all four males. The three distal marker loci, DXS207, DXS418, and DXS999, which are also completely informative in family L-17, cosegregate with RS in individual III-3 (Fig. 1). To confirm the recombination event on the centromeric side with a second marker locus, the family members were subsequently analyzed with the CA-repeat polymorphism at DXS443, mapping 2 cM proximal to AFM291wf5 (Biancalana et al. 1994). Patient III-3 was also found to be recombinant between RS and DXS443. Figure 1 shows the segregation pattern of the five repeat marker loci in family L-17. The order of the loci in each haplotype follows the order previously established (Biancalana et al. 1994).

Families not informative for the CA-repeat at DXS207 were further inspected for evidence of recombination on the RS distal side by typing the CA-repeat marker DXS987, which maps approximately 3 cM distal to DXS43/DXS207 (unpublished results; Gyapay et al. 1994). Three additional families were informative at this locus, but no recombination was detected (lod score not calculated).

Discussion

Fourteen French RS families have been typed with highly informative microsatellite markers that are closely linked to the RS region. All of the families were fully informative for at least two markers, and no evidence of unlinked families was found. Thus, our results further confirm the apparent lack of genetic heterogeneity of RS.

The two-point data substantially extend our previous report of close linkage between RS and the DXS207 locus. When the data for DXS207 are combined with those previously reported (Oudet et al. 1992), the maximum lod score between RS and DXS207 becomes 31.60 at $\theta = 0.005$. As marker loci DXS207 and DXS43 are tightby linked both genetically (Oudet et al. 1992), with no recombination between them being so far reported, and physically (Trivier et al. 1994; Alitalo et al. 1994), we have also combined the present data for DXS207 with those previously reported for DXS43 (Oudet et al. 1992). A combined lod score of 65.00 at $\theta = 0.01$ was obtained

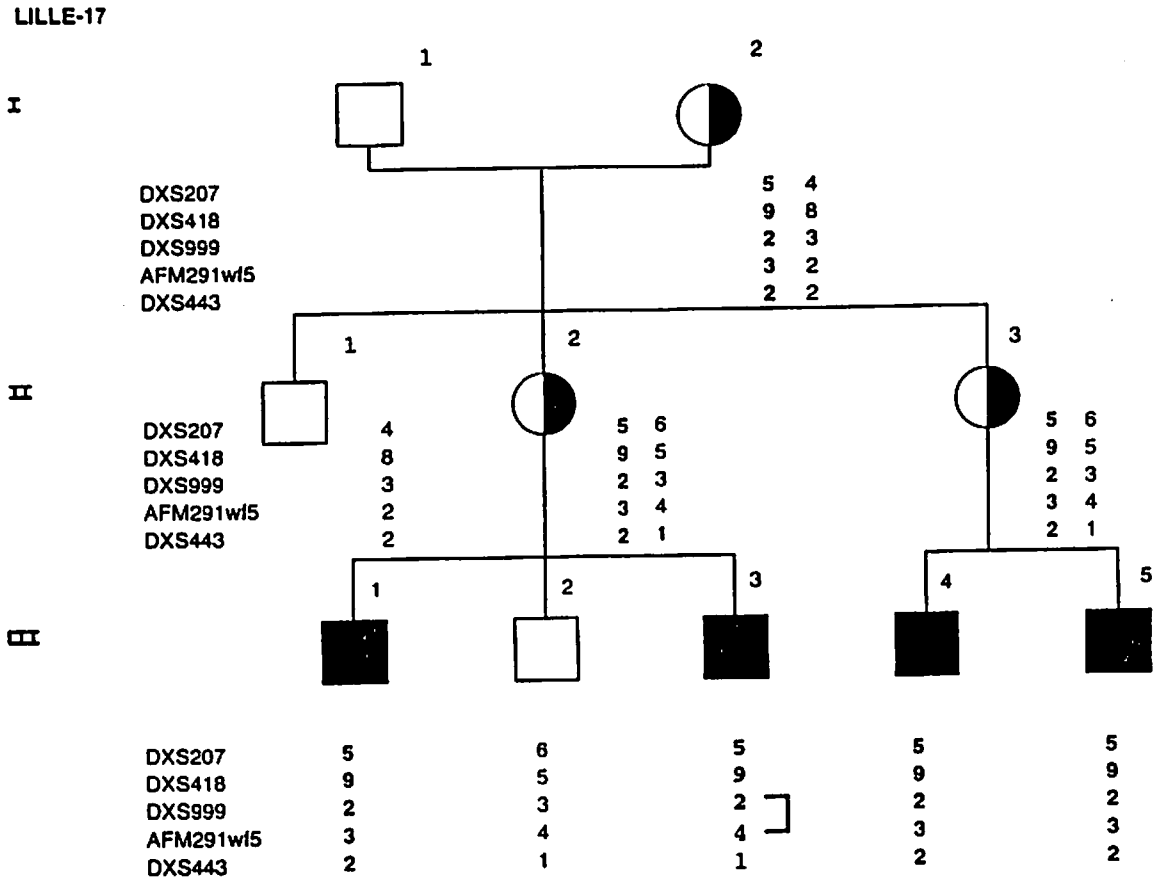

Fig. 1 Pedigree of family L-17 with RS; segregation pattern of five informative microsatellite marker loci closely linked to RS. *Square symbol* Male, *circle* female, *closed square* male RS patient, *half closed circle* obligatory RS carrier

for linkage between the disease locus and the cluster DXS43/DXS207 (maxlod-1 confidence interval: 0.002–0.02). Identification of a recombination event between RS and the most proximal of the markers studied, AFM291wf5, defines this marker as the new proximal boundary of the candidate region for the disease gene. By taking into account the map previously established (Biancalana et al. 1994) and linkage data reported by Alitalo et al. (1991a) and Sieving et al. (1990), our results further reduce the size of the interval likely to contain the disease locus to about 3–4 cM, the distance between DXS207 and AFM291wf5. Preliminary physical mapping data based on yeast artificial chromosomes (YAC) suggest that this interval has a size of approximately 2.5 Mb (Trivier et al. 1994; Alitalo et al. 1994). Two CA-repeat markers, DXS418 and DXS999, map within this interval. Since the combined two-point linkage data support a position of the disease gene 1–2 cM from the cluster DXS43/DXS207, the determination of the relative positions of RS with respect to loci DXS418 and DXS999, which both map within 2 cM of the cluster DXS43/DXS207 (Biancalana et al. 1994), is now critical for positional cloning strategy. Unfortunately, both DXS418 and DXS999 cosegregated completely with RS in the pedigrees from the present study, and in the set of 14 families previously reported (Oudet et al. 1992; Biancalana et al. 1994), and analysis of additional families is necessary.

No obvious candidate genes are known to map to the RS candidate region delineated by the present study. Overlapping YAC clones covering the whole interval have previously been isolated. They should facilitate the construction of a long-range physical map and the isolation of candidate genes for RS.

Acknowledgements We should like to thank all the families whose participation made this study possible. This work was supported by grants to J.L. Mandel and A. Hanauer from Centre Hospitalier Regional et Universitaire de Lille, Commission of the European Communities, Ministère de la Recherche, and the Association Francaise contre les Myopathies (AFM).

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