Contribution to carrier detection and genetic counselling in X linked retinoschisis

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Abstract
X linked retinoschisis (RS) is a vitreoretinal disease resulting from microcystic degeneration of the macula associated with peripheral lesions. The disease gene has already been assigned to the distal short arm of the X chromosome (Xp22.2) by linkage studies. In order to contribute both to a better localisation of the RS locus and to genetic counselling in RS families, we have carried out a clinical and genetic analysis in seven pedigrees. We show, first, that in contrast with previous reports, heterozygote carriers frequently express the disease, and display peripheral retinal alterations similar to those found in affected males. Second, while distal markers *DXS16*, *DXS207*, and *DXS43* are closely linked to the disease locus, a high level of recombination events was found with centromeric markers, namely *DXS274*, *DXS41*, and *DXS164*. These findings must be taken into account for both carrier detection and prenatal diagnosis in X linked RS.

X linked retinoschisis (RS) is a vitreoretinal disease owing to a microcystic degeneration of the macula. This disorder causes intraretinal splitting (schisis) and peripheral lesions, resulting in the detachment of the retina, with vitreous disturbances.[1] RS causes a static or slowly progressive impairment of vision usually discovered during the first decade, sometimes earlier and sometimes much later, depending on the size and location of the lesions. When the schisis is limited to the peripheral retina, reasonably good central vision can be conserved for several decades. When it is located within the macula, impairment of vision is severe and major evidence of progression of the disease is the observation of an increase in the size of the retinoschisis bulla.[2] While female carriers never have any macular alterations, we have observed that they often display more or less severe peripheral lesions. It is of the highest importance to look for these peripheral anomalies in females belonging to an X linked retinoschisis family, since these alterations indicate carrier status and must be taken into account in genetic counselling according to the Bayesian method.[3]
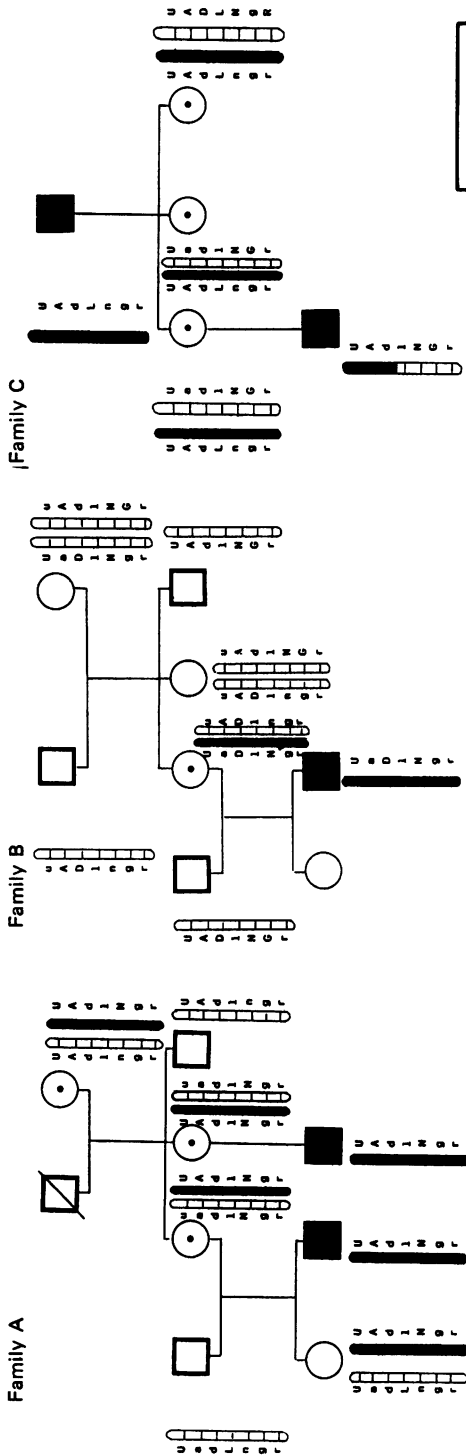
In order to contribute both to localisation of the RS locus and to carrier detection in RS families, we have analysed seven pedigrees using polymorphic DNA markers linked to the disease gene on the distal short arm of the X chromosome (Xp22.2).[4]
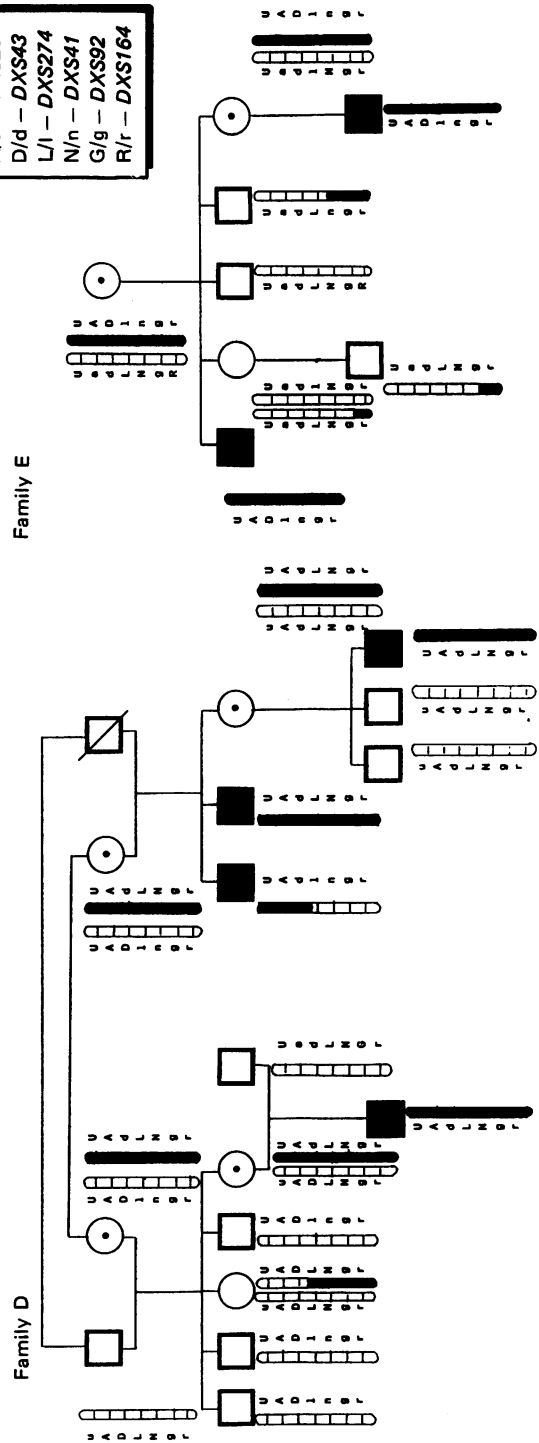
Patients and methods
Seven unrelated families were studied, including 22 affected males, 17 obligate carriers, 12 possible carriers, and 19 healthy boys. A total of 22 members belonging to six families underwent careful ophthalmological examination for RS by the same ophthalmologist (15 affected males, five obligate carriers, two possible carriers). For the other seven affected males and for most healthy boys, written ophthalmological reports were required.
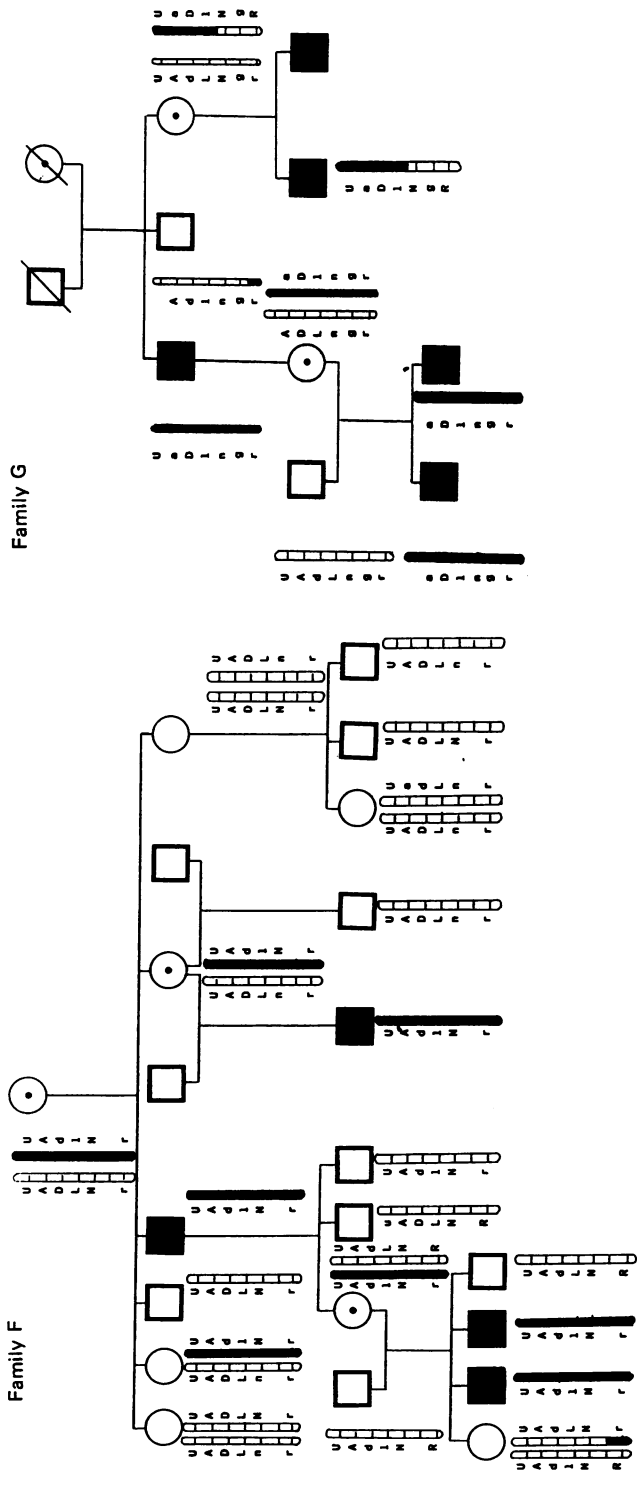
Blood samples were obtained from 74 subjects from the seven families studied (20 affected males, 17 obligate carriers, 11 possible carriers, 19 healthy males, and seven husbands and wives), corresponding to 57 meiotic events.

For restriction fragment length polymorphism (RFLP) analysis, DNA was extracted from lymphocyte pellets by cell lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension.[5] DNA (5 µg) was cleaved with the restriction enzymes *Bgl*II, *Xba*I, *Pvu*II, *Msp*I, *Pst*I, *Taq*I, and *Bam*HI under appropriate buffer and temperature conditions according to

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U/u — DXS16
A/a — DXS207
D/d — DXS43
L/l — DXS274
N/n — DXS41
G/g — DXS92
R/r — DXS164

Family C

Family B

Family A

Family E

Family D

Pedigrees and RFLP haplotypes in X linked RS.

Table 1 X chromosome markers used.

Locus	Probe	Location	Enzyme	Polymorphism		Reference
				Length (kb)	Frequency	
DXS16	pXUT23	Xp22.3–p22.2	BglII	17.5/12.5	0.84/0.16	Bakker et al, 1985
DXS207	pPA4B	Xp22	XbaI	12/9.5, 2.5	0.52/0.48	Ahrens and Kruse, 1986
DXS43	pD 2	Xp22.2	PvuII	6.6/6.0	0.29/0.71	Aldridge et al, 1984
DXS274	CRI–L1391	Xp22.2–p22.1	MspI	10.5/8.6	?	Donis-Keller et al, 1987
DXS41	p99.6	Xp22.1	PstI	22/13	0.71/0.29	Aldridge et al, 1984
DXS92	pXG–16	Xp22.1	TaqI	3.8/7.1, 3.5	0.50/0.40/0.10	Sefiani et al, 1987
DXS164	pERT87–15	Xp21.2	BamHI	9.4/7.1, 2.3	0.38/0.62	Monaco et al, 1987

the manufacturer's recommendations. The fragments were separated by horizontal gel electrophoresis in Tris-acetate EDTA buffer and transferred onto a nylon membrane (Zetabind, Flo Cuno) using the method of Southern.[6] Probes were radiolabelled by nick translation to a high specific activity with ^{32}P–dCTP (Amersham).[7] After hybridisation, the filters were washed and exposed to KODAK X/OMAT films with intensifying screens. The probes and some of their relevant characteristics are listed in table 1. Linkage analysis was carried out using the LINKAGE program[8] version 4·8 on an IBM PC AT.

Results
Among the five obligate carriers examined, none had macular alterations but four had peripheral lesions. These peripheral lesions of the retina ranged from mild greyish-white spots or dendrite-like areas to a more severe 'palisade' aspect, with possible retinal breaks, corresponding to aberrant zones of under-developed capillaries. One obligate carrier also displayed a true peripheral schisis in the lower temporal part of her retina, which required laser photocoagulation. Two additional obligate carriers required cryocoagulation in childhood for peripheral lesions of the retina but no information regarding these lesions could be obtained. Of the two possible carriers, one had dendrite-like alterations in the peripheral retina with a true unilateral schisis in the lower temporal periphery.

Genetics studies were carried out with seven polymorphic probes on the distal short arm of the X chromosome and informative meiotic events were obtained for all probes but one (DXS92). Strong

evidence of linkage to the RS locus was found with three flanking markers, namely DXS207, DXS43, and DXS274 ($\hat{Z}>3$). Interestingly, no recombination events were observed with the three polymorphic markers distal to the disease locus (DXS43, DXS207, and DXS16). In contrast, five recombination events were found with three proximal markers, namely DXS274, DXS41, and DXS164. The segregation of seven polymorphic markers in our families is shown in the figure and table 2 gives the results of the two point linkage study in these pedigrees.

Finally, the present data suggest that the closest marker centromeric to the disease locus is not DXS41, as previously reported,[9–11] but rather DXS274 ($\theta=0·08$ and 0·18 for DXS274 and DXS41 respectively, at $\hat{Z}=3·28$ and 1·16 respectively) (table 2). It appears therefore that DXS43 and DXS274 are the closest flanking markers in this disease and are at present the most suitable probes for both carrier detection and prenatal diagnosis in RS.

Discussion
X linked RS is not an easy disease to diagnose and the difficulty in recognising this condition might possibly account for the longstanding debate regarding the incidence of the disease gene. When schisis is present the diagnosis is certain, but RS can be misdiagnosed when no schisis is observed. It is therefore of primary importance to be aware of all ophthalmological features of the disease. Indeed, RS involves both macular and peripheral lesions of the retina, as previously described by many authors.[12–14] Micro-cystic degeneration of the macula is a consistent feature of X linked RS: its typical angiographic

Table 2 Lod scores between RS and six polymorphic DNA markers using linkage.

	0·00	0·05	0·10	0·20	0·30	0·40	Zmax	θmax
DXS16	2·42	2·22	2·01	1·56	1·09	0·57	2·42	0·00
DXS207	4·17	3·76	3·34	2·47	1·57	0·71	4·17	0·00
DXS43	5·91	5·33	4·73	3·46	2·14	0·85	5·91	0·00
DXS274	—	3·19	3·26	2·73	1·83	0·80	3·28	0·08
DXS41	—	0·08	0·86	1·15	0·87	0·39	1·16	0·18
DXS164	—	−5·6	−0·1	0·19	0·24	0·16	0·24	0·28

expression is the so-called 'spoke-wheel' aspect of the macula.[15] The peripheral lesions, present in about 50% of male patients, involve silver-grey, glistening, spotty areas scattered over the retina. In addition, perivascular grey cuffs with veils in the vitreous and greyish-white spots suggestive of a healed chorioretinitis are frequently observed. Vitreous haemorrhage and anterior or posterior vitreous detachment may also be noted along with whitish, dendrite-like structures and other vascular disorders. When associated with macular alterations, these peripheral lesions of the retina are suggestive of X linked RS, even if no schisis is found and if the typical 'spoke-wheel' aspect of the macula has not been recognised. Indeed, these peripheral lesions allow one to rule out other macular degenerations, such as Stargardt's disease which never involves extramacular alterations.[1]

The recognition of peripheral alterations in affected males prompted us to investigate obligate carriers for these particular lesions. In contrast with previous studies,[10 11 16] we were able to show that a proportion of heterozygous females do express the disease: while no macular alterations were found, a careful investigation of their retinas led to the frequent discovery of peripheral alterations (4/5), similar to those found in affected males. The severity of the symptoms differed among carriers, probably owing to random inactivation of the X chromosome. These features must be taken into account especially since none of them had myopia in our series.

Several linkage studies in RS have been previously reported.[4 9–11 16–20] The disease gene has been already assigned to the distal short arm of the X chromosome (Xp22.2) between *DXS43* and *DXS41*. In the study by Dahl *et al*,[11] the peak lod scores using the *DXS43* and *DXS41* markers were 4·98 and 4·08 respectively at a recombination fraction (θ) of zero. Similarly, the study of Alitalo *et al*[10] gave maximum lod scores of 13·87 and 9·6 for *DXS43* and *DXS41* at a recombination fraction of $\hat{\theta}=0·02$ and $\hat{\theta}=0·06$ respectively. Both authors concluded that *DXS43* and *DXS41* are the two closest informative markers for carrier detection. The present study confirms the close proximity of the RS locus to the distal markers *DXS16*, *DXS207*, and *DXS43*. In addition, our study also confirms that the closest marker centromeric to the disease gene is *DXS274*, the most likely gene order in the region being Xpter—*DXS16*–(*DXS207*–*DXS43*)–*RS*–*DXS274*–*DXS41*–*DXS164*–Xcen.[18] In contrast, our study does not support close linkage between the disease gene and the proximal marker *DXS41* since five recombination events were observed in 28 informative meioses ($\hat{Z}=1·16$ at $\theta=0·18$). These findings suggest that the genetic distance between *RS* and the *DXS41* marker is greater than previously described. Alternatively, a hot spot for recombination might exist in this region. In keeping with this, a high rate of crossing over has

been recently reported between the *DXS41* locus and other disease genes of the region, namely those of Duchenne muscular dystrophy[21] and Coffin–Lowry disease.[22] These recombination events must be taken into account for risk calculation and genetic counselling and especially for determination of carrier status and prenatal diagnosis.

In conclusion, the present study provides information useful for genetic counselling in cases of X linked RS. First, we show that, in striking contrast with previous reports, heterozygous carriers frequently express the disease. This feature must be taken into account for risk calculations in affected families. Second, while distal polymorphic markers are closely linked to the disease locus, we believe that the high level of recombination events with the proximal markers represents an important risk of error that must be taken into account in both carrier detection and in prenatal diagnosis.

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