Efficient DNA carrier detection in X linked juvenile retinoschisis

Arthur A B Bergen, Jacoline B ten Brink, Mary J van Schooneveld

Abstract

Juvenile retinoschisis is a rare, X linked hereditary vitreoretinal degeneration. Female carriers of the disease do not develop any ocular abnormalities. Therefore, carrier detection by DNA analysis is extremely useful for these females. In order to evaluate the usefulness of a new class of DNA markers for carrier detection in X linked juvenile retinoschisis, DNA carrier detection or carrier exclusion was carried out in four possible carriers for X linked juvenile retinoschisis. The use of these highly polymorphic CA repeats, closely linked to the RS gene, greatly enhances both the reliability and feasibility of carrier detection in X linked juvenile retinoschisis.

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X linked juvenile retinoschisis (XLRS) is a hereditary vitreoretinal degeneration with a frequency of 1:10 000. Although the clinical expression of the disease is variable, male patients usually develop an increasingly severe

visual impairment after the fourth decade of life. The condition is characterised by microcystic maculopathy, peripheral retinal lesions, and vitreous body alterations. The vast majority of female carriers do not reveal any ocular abnormality.^{1,2} Some carriers may exhibit abnormal rod-cone interactions.³

The gene (RS) for XLRS was assigned to band Xp22.2 of the human X chromosome by linkage analysis.⁴⁻⁷ Multipoint linkage analyses established the gene marker order Xpter-(DXS9,(DXS16-DXS207-DXS43))-RS-DXS274-DXS41-DXS92-Xcen.⁸⁻¹² Carrier detection has previously been carried out with conventional DNA markers from Xp22.2. However, the efficiency of carrier detection in XLRS has been hampered by the limited availability and low polymorphism information content of these markers. Furthermore, the reliability of DNA diagnosis was often insufficient because of the large genetic distances between RS and flanking markers. Bergen *et al*¹³ applied several CA repeat polymorphisms in an XLRS linkage study which yielded the order Xpter - (DXS9, (DXS16 - DXS207 - DXS43)) - RS - ((DXS443 - DXS365 - DXS451), DXS274) - DXS41 - DXS92 - Xcen. Here, we describe the efficiency of these new highly polymorphic Xp22.2 markers in carrier detection of X linked juvenile retinoschisis.

Materials and methods

SUBJECTS

The clinical studies included two families (Figs 1 and 2). X linked inheritance was obvious in both pedigrees. Clinical diagnosis was based on complete ophthalmic examination. ERGs were performed in two cases, in which the typical negative-type ERG was observed (for P 22.337, III-5 and for P 23.153, IV-1). The severity of the visual handicap was variable, as is common in XLRS. Six patients had visual acuity in the better eye from 20/180 to almost 20/20 (range age 16-51 years); in one 4 year old (P23.153), the visual acuity was not known.

SOUTHERN AND PCR ANALYSIS

DNA and PCR analyses were carried out as described previously.^{14,15} Details of the probes and primers used are given elsewhere,^{16,17} and the Human Genome Database. In the diagnostic assays, only those probes and primers were used which are generally and easily available to all investigators through ATTC (12301 Parklawn Drive,

The Netherlands
Ophthalmic Research
Institute, PO Box
12141, Amsterdam, the
Netherlands
A A B Bergen
J B ten Brink
M J van Schooneveld

Correspondence to:
Dr A A B Bergen, The
Netherlands Ophthalmic
Research Institute, PO Box
12141, 1100 AC
Amsterdam, the
Netherlands.

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Figure 1 DNA carrier detection in XLRS family P 22.337.

Figure 2 DNA carrier detection in XLRs family P 23.153.

Rockville, Maryland 20852, USA) and the Human Genome Database (hosted at Johns Hopkins University, 2024 East Monument Street, Baltimore, Maryland, 21205-2100 USA). Confidence limits of the 95% level for genetic distances were calculated according to Conneally *et al.*¹⁸

Results

A prior linkage study in XLRs suggest the gene marker order Xpter-(DXS9,(DXS16-DXS207-DXS43))-RS-((DXS443-DXS365-DXS451),DXS274)-DXS41-DXS92-Xcen.¹³ In that study, no recombination was found between RS and DXS999 ($z_{\max}=4.59$). According to the consensus genetic map of the human X chromosome, DXS999 is located between DXS207 and DXS443, which is the obligate RS chromosomal interval.

Table 1 RS and flanking DNA markers

Locus flanking RS		Marker type	$\theta_{\max}$	$z_{\max}$	$\theta_{95\%}$	Het
Distal	Proximal					
DXS85		Probe	0.20	6.27	0.18	0.48
DXS9		Probe	0.11	4.17	0.24	0.24
DXS16		Probe	0.06	12.6	0.17	0.17
DXS207		CA _n	0.05	5.25	0.21	0.85
DXS43		Probe	0.02	13.9	0.06	0.50
DXS999	DXS999	CA _n	0.00	4.59	0.12	0.75
	DXS443	CA _n	—	—	0.15	0.60
	DXS274	Probe	0.05	9.92	0.13	0.38
	DXS365	CA _n	0.07	2.22	0.15	0.82
	DXS41	Probe	0.07	2.08	0.15	0.49
	DXS451	CA _n	0.05	3.26	0.23	0.80
	DXS28	Probe	—	—	0.20	0.28
	DXS67	Probe	—	—	0.20	0.15

$\theta_{\max}$ refers to the recombination distance between RS corresponding with a maximum likelihood of $z_{\max}$. $\theta_{95\%}$ denotes the 95% genetic confidence distance between RS and a flanking locus. The latter values are used in individual risk calculations. Het refers to the calculated or observed heterozygosity of that particular locus. Note the high heterozygosity values of CA repeat polymorphisms. Data are obtained from refs 8, 9, 11 and 12. —=data not significant. $\theta_{95\%}$ for these loci are obtained from the position of that locus on the consensus genetic map of the X chromosome.

Consequently, DXS999 is very closely linked to RS and may be used as an additional locus in DNA diagnosis of XLRs.

FAMILY P 22.337

In pedigree P22.337 (Fig 1) the possible carriers III-6, IV-2, and IV-3 were interested to learn about their carrier status. No ocular abnormalities were detected in all three females. DNA diagnosis was carried out with conventional flanking marker analysis.¹⁹ The loci DXS9, DXS28, DXS41, DXS67, DXS85 (corresponding to conventional DNA probes) yielded non-informative results (not shown). The loci DXS16 and DXS43 yielded informative results (Fig 1). However, both loci are located on a chromosomal segment distal to the RS gene. Thus, DNA diagnosis could not be carried out with sufficient reliability because of a lack of useful polymorphic markers proximal to RS. Reliable DNA diagnosis became possible by using CA repeat polymorphisms (DXS207, DXS999, DXS443, DXS365, and DXS451), all of which yielded informative results (Fig 1). In the pedigree, the XLRs disease phenotype cosegregates, in general, with the alleles F (DXS16)-P (DXS43)-B (DXS207)-A (DXS999)-A (DXS443)-B (DXS365)-A (DXS451) which can be determined from the haplotypes from patients III-4, III-5, and IV-1. Only patient III-3, with the haplotype f-p-A-A-A-B-A, is recombinant for DXS16, DXS43, and DXS207. Assuming no double recombination, female III-6 inherited an X chromosome from her father, which, for the RS gene region, is characterised by the alleles f-p-C-A-C-C-B. Thus, her maternal X chromosome can be identified by the alleles f-p-A-B-B-A-C. Since the X chromosome carrying the mutated RS gene in this pedigree can be identified by the alleles F-P-B-A-A-B-A, III-6 is probably not a carrier for this disease entity. The probability for which she is a carrier is 0% plus the chance of the occurrence of a double recombinant between the RS gene and flanking markers DXS207 and DXS443. Without taking the segregation of DXS999 into account, the latter risk would equal the maximum probability of a single recombinant between DXS207 and RS (0.21; see Table 1) times the maximum probability of a single recombinant between DXS443 and RS (0.15). Thus, the chance for a double recombinant to occur would be 3.15%. Since DXS999 is located somewhere between DXS207 and DXS443, and the analysis of the DXS999 segregation pattern also suggests that female III-6 is not a carrier, her risk for still being a carrier is actually much less (<0.1%).

Similar calculations can be made for the risk estimate of females IV-2 and IV-3 (Fig 1). Only, in this case, DXS999 is not informative for the mother (III-2). Based on the segregation patterns of DXS207 and DXS443 it can be calculated that both females must be carriers with a probability greater than 96.9% ($1 - (0.21 \times 0.15)$). Additional flanking markers are used to check the segregation

patterns in order to avoid DNA diagnostic pitfalls such as non-paternity or experimental errors.

FAMILY P 23.153

In family P 23.153 the possible carrier III-2 wished for more information about her genetic status. Ophthalmic examination revealed no abnormalities.

Initially, DNA analysis was carried out with conventional DNA probes corresponding to the loci DXS9, DXS16, DXS28, DXS41, DXS43, DXS85, all of which yielded uninformative results (not shown). Recently, the newly available CA repeat polymorphisms were applied. All loci yielded informative results (Fig 2).

The phase of the X chromosomal markers apparently associated with the XLRS phenotype is suggested by the haplotype of patient IV-1 (C (DXS207)-A (DXS999)-C (DXS443)-B (DXS365)-C (DXS451)). The phase of the markers is confirmed after closer inspection of the genotype of obligate carrier III-1. Since III-1 inherited the alleles B-A-D-C-C from II-1, the other, disease associated maternal alleles are indeed represented by C-A-C-B-C.

Thus, the disease associated chromosome with the grandmother (II-2) is also identified by the alleles C-A-C-B-C, unless a recombination in the Xp22.2 region has occurred with the segregation of chromosomes from II-2 to III-1 (a priori this is not known). However, the phase of the markers in II-2 is confirmed by the allele patterns in II-3 and III-2. Although it is a priori not known if III-2 is a carrier, she did inherit the haplotype C-C-A-A-B from II-3. Thus, her other alleles (B-B-C-C-A) must be from maternal origin, which confirms the phase assignment in II-2.

Further analysis of the genotype of III-2 reveals that she inherited the maternal X chromosome B-B-C-C-A, which is not associated with the disease locus. Therefore, it is highly unlikely that she is a carrier for XLRS unless a double recombination has occurred between RS flanking markers. Based on the segregation patterns of the fully informative RS flanking loci DXS207 and DXS365, the chance for a double recombinant would be 0.21 times 0.15. Thus, the risk of III-2 being a carrier is less than 3.15%. Additional loci (DXS443, DXS999, and DXS451) can be used to confirm the segregation patterns and to improve the risk estimate. Taking the segregation of these additional markers into account the actual carrier risk estimate for III-2 is less than 0.1%.

Conclusions

Previously, the possibilities of conventional DNA carrier detection in XLRS were limited because the limited availability of key probes, the low polymorphism information content (PIC) values of the linked DNA probes and the large genetic distances found between RS and flanking markers.^{11 12} Theoretically, only a

maximum of 73% of all possible RS carriers could be identified by DNA analysis with a reliability of at least 93.6%.¹²

Recently, CA repeat polymorphisms from Xp22.2 have become available.¹² Formal proof of linkage between these markers and RS was presented by Bergen *et al.*,¹³ thereby rendering these loci useful for DNA diagnosis. At present, at least four conventional DNA probes and one CA repeat polymorphism distally adjacent to RS are both readily available and useful for DNA diagnosis in XLRS (Table 1). Centromeric to RS, two DNA probes and three CA repeat polymorphisms can be used (Table 1). These markers yield a heterozygote frequency of more than 98.7% on either side of the RS locus for at least one linked marker. Theoretically, DNA diagnosis can now be carried out for approximately 98% of all possible XLRS carriers with a reliability of at least 94.5%, using at least two markers flanking RS on either side. However, these figures may be an overestimation since, in individual families, the possibility of DNA diagnosis may be hampered by a lack of key family members, non-paternity, and other diagnostic pitfalls. In general, the use of highly informative CA repeat markers for DNA diagnosis in XLRS improves the feasibility and reliability of DNA diagnosis for this disorder considerably.

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