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Linkage Disequilibrium and Physical Mapping of X-Linked Juvenile Retinoschisis

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Summary

X-linked juvenile retinoschisis (RS) is a recessively inherited disorder resulting in poor visual acuity. Affected males typically show retinal degeneration and intraretinal splitting. The prevalence of RS is 1:15,000–1:30,000. Elsewhere we have mapped the RS gene between the markers DXS43 and DXS274 in Xp22.1-p22.2. To narrow the RS region, we analyzed 31 Finnish RS families with the markers DXS418, DXS999, DXS7161, and DXS365 and a new polymorphic microsatellite marker, HYAT1. Multipoint linkage analysis allowed us to localize the RS gene between the markers DXS418 and DXS7161 (LOD score = 31.3). We have covered this region with nine YAC clones. On the basis of the sizes of the YACs, sequence-tagged site (STS) content mapping, and restriction mapping, the physical distance between DXS418 and DXS7161 is ~0.9 Mb. A total of five potential CpG islands could be identified. For haplotype analysis, eight additional Finnish RS families were analyzed with the markers DXS1195, DXS418, HYAT1, DXS999, DXS7161, and DXS365. On the basis of the linkage-disequilibrium data that were derived from the genetically isolated Finnish population, the critical region for RS could be narrowed to 0.2–0.3 cM, between the markers DXS418 and HYAT1.

Introduction

X-linked juvenile retinoschisis (RS; MIM 312700 [McKusick 1994]) is a recessively inherited disorder resulting in poor visual acuity. Because of the progressive nature of the disease, retinal structures and functions gradually deteriorate. Electrodiagnostic findings have suggested that the primary defect might be in Müller cells (Condon et al. 1986). Although Arden et al. (1988) have reported a study in which abnormal rod-cone interaction was demonstrated in carriers, there are no definitive ophthalmoscopic findings for the carrier state. Carrier females are unaffected, and clinical detection of the carrier state is not definitive on the basis of present methods.

Data allowing the prevalence of RS to be determined are scarce. In Finland the prevalence of RS is >1:17,000 (de la Chapelle et al. 1994), and in northern France the corresponding number is 1:28,000 (Puech et al. 1991). It tentatively has been suggested that the worldwide prevalence of RS is 1:15,000–1:30,000 or even higher (de la Chapelle et al. 1994).

A number of linkage studies have localized the RS gene to the distal short arm of the X chromosome (Wieacker et al. 1983; Alitalo et al. 1987, 1988, 1991; Dahl et al. 1988; Gellert et al. 1988; Sieving et al. 1990; Kaplan et al. 1991; Oudet et al. 1992; Bergen et al. 1994; Weber et al. 1995). At present, the nearest flanking markers with which obligate recombination events have been identified are DXS418 on the distal side and DXS999–DXS7161 on the proximal side (Dumur et al. 1995; Pawar et al. 1995; Trump et al. 1996; Van de Vosse et al. 1996; present paper). Three YAC contigs spanning the RS region have been published elsewhere (Alitalo et al. 1995; Ferrero et al. 1995; Van de Vosse et al. 1996). Using new YAC clones and clones from a 6-Mb YAC contig described elsewhere (Alitalo et al. 1995), we now have constructed a restriction map of the DXS418–DXS7161 region, which suggests that the length of the RS critical region is ~900 kb.

It has been shown that linkage disequilibrium can provide a powerful tool for high-resolution genetic mapping of genes in isolated populations. The method has been applied to the mapping of several disease genes in the Finnish population (Hästbacka et al. 1992; Lehesjoki et al. 1993; Sulisalo et al. 1994; Tahvanainen et al. 1995; Höglund et al. 1995), and it has decisively guided the actual isolation of genes (Hästbacka et al. 1994; Pennaccio et al. 1996). Because a finer localization of the RS gene is desirable to facilitate the positional cloning of the gene, we now have isolated new microsatellite markers from the DXS418-DXS7161 region and, in linkage-

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disequilibrium analyses, have used the distribution of their alleles to further narrow the RS candidate region.

Subjects and Methods

Subjects

A total of 31 Finnish RS families from southern and northern Finland were chosen for linkage studies (Alitalo et al. 1991). The pedigrees of these families have been published elsewhere (Alitalo 1990). Data from an additional eight RS families were included in the haplotype analysis. Altogether, 277 individuals, including 101 affected patients, were analyzed. DNAs from 100 normal men and 50 normal women from western and northern Finland (Alitalo et al. 1991) were used to obtain allele frequencies and heterozygosity values for the new markers.

Isolation of New CA Repeats

The cosmids ICRFc104E2112, ICRFc104L0845, and ICRFc104K1942 were obtained by hybridizing the ICRF Reference Library cosmid filters (Lehrach et al. 1990) with the YAC end fragments 6717L, 3847L, and 1774L, respectively. After digestion and electrophoresis, the cosmid DNA was transferred to nylon membranes (Hybond N; Amersham). The filters were hybridized with a [α-^{32}P]dCTP-labeled poly-CA probe (Pharmacia) by use of standard protocols. Positive cosmids were digested with *Sau*3AI and were subcloned into pBluescript-vector. CA-positive subclones were then sequenced by use of an automatic sequencer (373A; Applied Biosystems). Primers for the microsatellite markers were chosen either manually or by the PRIMER program (version 0.5; Whitehead Institute for Biomedical Research). All new STSs are listed in table 1. The sequences have been deposited in GenBank (accession numbers U82599–U82606).

DNA Marker Typing

Published primer sequences and PCR conditions were used for the markers DXS418, DXS999, DXS7161, and DXS365 (Browne et al. 1992; Weissenbach et al. 1992; Van de Vosse et al. 1993; J. Weissenbach, personal communication). Primer sequences for the markers HYAT1 and HYAT2 are shown in table 1. PCR reactions and electrophoresis were performed by use of standard protocols. The gels were visualized by use of a modified silver-staining protocol (Bassam et al. 1991).

Linkage Analysis

Pairwise and multilocus linkage analyses were performed by use of the FASTLINK 2.2 version of the LINKAGE package of computer programs (Lathrop et al. 1984; Cottingham et al. 1993). Pairwise LOD scores (Z) between RS and the markers were calculated by use of the MLINK and LODSCORE programs. The program options were set for a recessive sex-linked trait, and all results were obtained under the assumption of complete penetrance in individuals hemizygous for the disease allele. The gene frequency of the disease was estimated to be .001 in those rural districts of Finland where our patients originated, and the mutation frequency was estimated to be .000001. These parameters were chosen so that any error in the analysis would be on the conservative side. Our mutation-rate choice was similar to those used in other Finnish disease-heritage analyses (Sulisalo et al. 1994; Höglund et al. 1995). The previously published microsatellite allele frequencies were used (Kidd et al. 1989; Browne et al. 1992; Fasman et al. 1994). The allele frequencies of HYAT1 and HYAT2 were those obtained from Finnish blood donors (table 2). Multipoint analysis was performed by use of LINKMAP. We utilized a fixed order for marker loci (Nelson et al. 1995) and our own data.

Haplotype Analysis

Haplotype analysis was performed with the markers DXS43, DXS1195, DXS418, HYAT1, DXS999, DXS7161, DXS365, and DXS274. All markers were dinucleotide repeats, except for the earlier-analyzed RFLPs, DXS43 and DXS274 (Alitalo et al. 1988, 1991). The haplotypes for the disease-bearing chromosomes, as well as for the healthy chromosomes, were derived from inspection of the family data.

Linkage-Disequilibrium Analysis

Linkage-disequilibrium tests are commonly applied under the hypothesis of a major founding disease haplotype in the disease chromosomes. It was known, however, that the birthplaces of Finnish RS carrier females form two distinct groups, one western and one northern, when placed on the map of Finland (shown in the report by Alitalo [1990]). Thus, the two groups of patients have been treated separately in all the analyses.

Three different methods were applied, to fully exploit the potential of linkage-disequilibrium analysis in disease-gene mapping. First, Fisher's exact test with Bonferroni correction for multiple alleles was used to record possible linkage disequilibrium; that is, the nonrandom distribution of alleles of the markers DXS43, DXS1195, DXS418, HYAT1, DXS999, DXS7161, DXS365, and DXS274 in disease haplotypes was compared with that in the normal haplotypes.

Second, those loci in which the allele distribution in disease chromosomes deviated significantly from that of normal chromosomes in the first test were subjected to further analyses. The level of linkage disequilibrium was used as a measure of the genetic distance between each

Table 1

STSs Used in Present Study

Locus	Marker	PCR Primers[a] (5′ to 3′)	Product Size (bp)
DXS 9911	HYAT1	CCCGATTTCCTCTAAAACAGT / GCTATATATCTGCTATGCAGTGG	160–186
DXS 9912	HYAT2	GTGCATGTGGTGTTGCAGG / AACTCAACATGAGCCCAGAG	188–194
	HYAT3	AGCACATGCACGCATGTTAC / ATTAGTCCAGGGCAATTGGC	241
	6717R	GGACCCCTTCCTGGTAACAT / CTGGGACCCTGCATTTTCTA	143
	6717L	ATTCAGGACTCTTATGCCC / CTACTCTGTATCTCTGAGG	157
	3847R	TTCCTCTGCACCTCACTGC / TAACACGAACCAACAAATCACC	202
	3847L	CACACTGTTTCCCAGAGCAC / CATCCAGGCAGCATGATTAA	192
	08931R	CCTGCCCCAGTTTATTGAG / CATGGGTGTATTTATGTTCAGACTAG	111

[a] A 55°C annealing temperature was used in all PCR reactions, except for marker HYAT2 (63°C).

polymorphic marker and the RS locus, by use of the algorithm published elsewhere (Hästbacka et al. 1992; Lehesjoki et al. 1993). The generation number and recombination fraction (θ) in this algorithm refer to those meioses in which recombination has been possible; that is, only meioses in females are counted. The relationship between the generation number that was used in calculations (reported in table 3 and fig. 1) and true time interval in generations since the beginning of expansion of the founder mutation depends both on mutation rate in males and on selection against males who have RS; these factors are not known with certainty. When interpreting the outcome of the algorithm, we used the estimation that, in every second generation, males have transmitted the trait, and thus the real-time interval in generations is approximately two times the generation number used.

Third, we performed a multipoint linkage-disequilibrium test with a recently introduced algorithm, which can combine the information of several markers—in our case seven—into a comprehensive assay for the probability of the disease-gene locus being located at each map point (Terwilliger 1995). The results are given as Z values that, in both outlook and statistical interpretation, closely resemble multipoint linkage analysis (fig. 2).

Table 2

Allele Sizes and Frequencies of Two New Microsatellite Markers

Allele	Size (bp)	Frequency[a]
HYAT1, heterozygosity .74 (35/47):		
1	160	.01
2	170	.01
3	174	.02
4	176	.03
5	178	.35
6	180	.40
7	182	.12
8	184	.04
9	186	.02
CEPH133202	180, 178	
HYAT2, heterozygosity .10 (5/50):		
1	188	.05
2	190	.02
3	192	.92
4	194	.01
CEPH133202	192, 188	

[a] Estimated from 100 X chromosomes from Finland.

Restriction Map of the DXS418-DXS7161 Region

A total of six individual YAC clones from a previously published YAC contig (Alitalo et al. 1995), covering the region DXS418–DXS7161, were restriction mapped. In addition to these clones, we obtained two new YACs, yWDX6717 (240 kb) and yWDX3847 (190 kb), from the St. Louis library (Kere et al. 1992; Nagaraja et al. 1994) and one YAC, CEPHy904E08931 (170 kb), from the ICRF (Albertsen et al. 1990). These YACs were positive for the STSs 1774L, 0922R, and 0922L. The YAC end fragments 1774L and 0922R flanked a region that, in our previous YAC contig (Alitalo et al. 1995), was

Table 3

Significance of Allelic Association, Allelic Excess, and Point Estimates for Distance of Each Marker from RS Gene

Marker	Significance	Allelic Excess	Distance Estimates for Female Generation Number of (cM)		
			10	20	30
DXS43	<.05	.61	4.71	2.33	1.53
DXS1195	<.001	.82	1.92	.91	.58
DXS418	<.001	.92	.74	.32	.18
HYAT1	Not significant	.5	6.66	3.34	2.20
DXS999	<.001	.68	3.64	1.78	1.16
DXS7161	<.001	.83	1.69	.80	.50
DXS365	<.01	.61	4.67	2.31	1.51

covered with only one highly unstable YAC, yhCEPH939H9. YAC DNA extraction, pulsed-field electrophoresis, and restriction mapping were performed as described elsewhere (Alitalo et al. 1995).

Generation of New STSs from YAC Ends

End fragments of the new YACs were isolated by use of ligation-mediated PCR as described elsewhere (Kere et al. 1992; Alitalo et al. 1995). Isolated end fragments were purified with the Wizard DNA purification system (Promega) and were sequenced to get new STSs.

Results

New Microsatellite Markers

Dinucleotide markers HYAT1 ($[CA]_{24}$), HYAT2 ($[CA]_8G[CA]_{13}$), and HYAT3 ($[CA]_8$) were isolated from

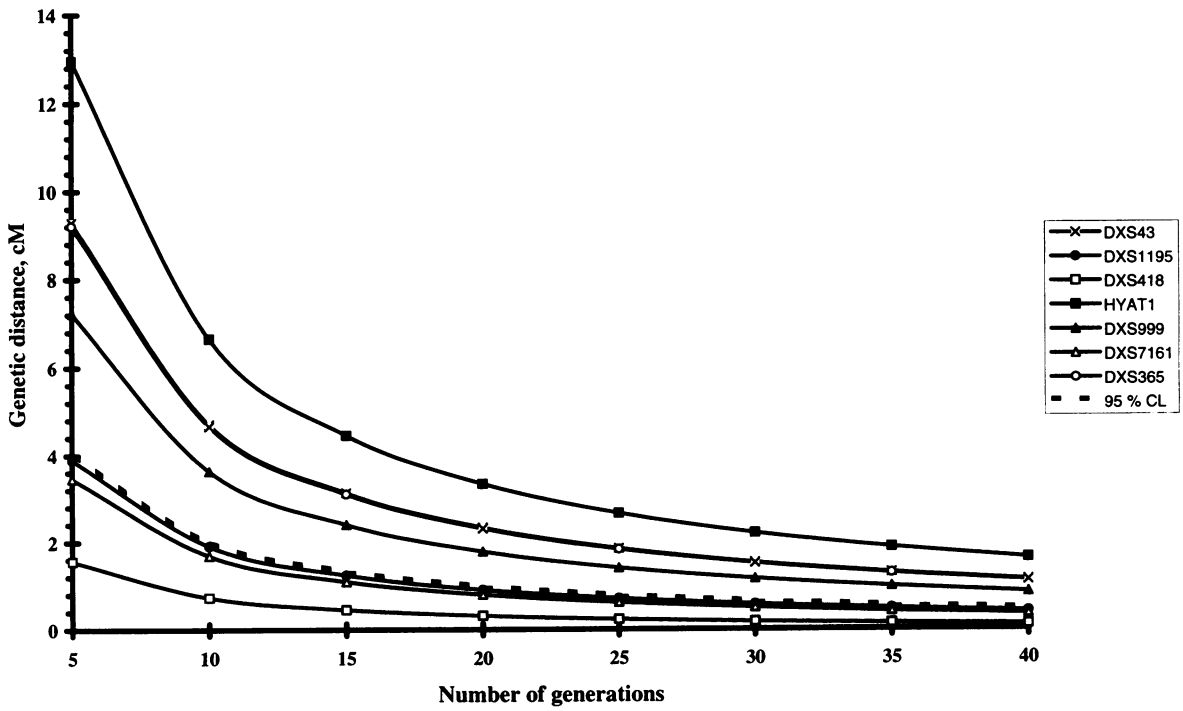

Figure 1 Estimates of genetic distances between RS and seven marker loci. Estimates are shown as a function of number of (female) generations. Calculations were performed by use of a method based on the Luria-Delbrück principle, as described in the text. The 95% confidence interval, based on sampling error for P_{excess}, is shown for locus DXS418.

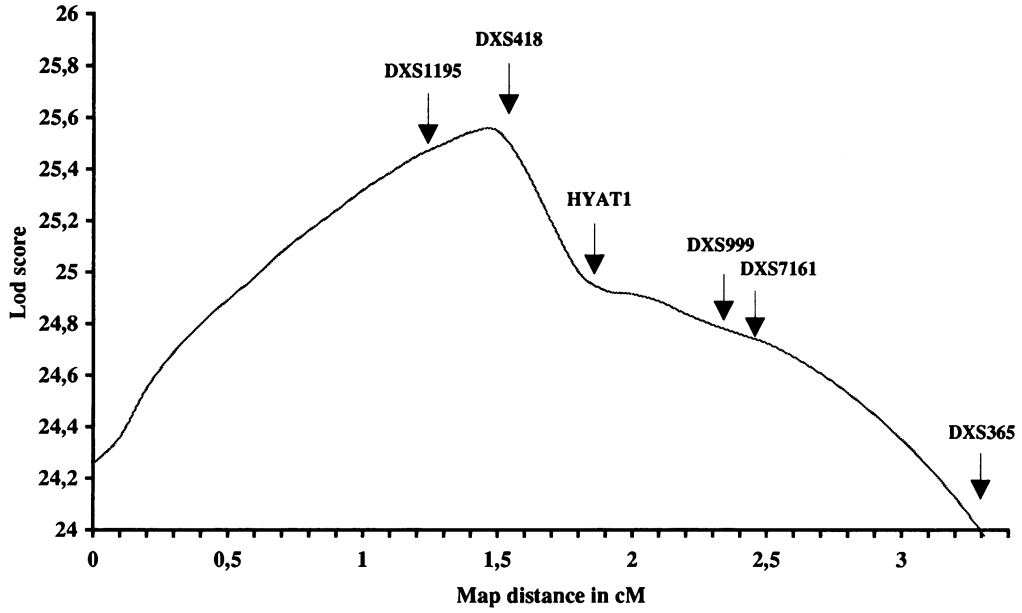

Figure 2 Multipoint linkage-disequilibrium analysis. Locus DXS43 was chosen as the starting point (0). The telomere is to the left.

the cosmids of the DXS418-DXS7161 region. The allele sizes and frequencies for markers HYAT1 and HYAT2 are shown in table 2. HYAT1 proved to be a very informative marker, with a heterozygosity value of .74—in contrast to both HYAT2, which was not informative in our families, and HYAT3, which was not polymorphic.

Genetic Mapping of RS

Linkage analysis.—Results of the two-point linkage analysis are summarized in table 4. No recombinations were observed between RS and markers HYAT 1 (Z_{max} = 13.35 at θ = .00) and DXS999 (Z_{max} = 17.48 at θ = .00). The closest flanking markers that showed recombinations with RS were DXS418 on the distal side and DXS7161 on the proximal side.

Table 4

Z_{max} for Linkage between RS and Seven Polymorphic X-Chromosome Markers in 31 Families

Locus	Z_{max}	θ_{max}	95% Confidence Limits[a]
DXS43	13.90	.015	.00–.08
DXS418	24.78	.016	.002–.06
HYAT1	13.35	.00	.00–.06
DXS999	17.48	.00	.00–.06
DXS7161	14.20	.014	.00–.07
DXS365	9.70	.022	.00–.11
DXS274	7.99	.056	.00–.16

[a] Based on the ±1-Z method.

Multilocus linkage analyses were used to determine the most likely position of RS with respect to a fixed map of markers. The multipoint linkage analysis (LINKMAP) was performed with RS and the markers DXS43-DXS418-HYAT1-DXS999-DXS7161-DXS274-DXS365, in that order and with distances of 1.6 cM, 0.3 cM, 0.5 cM, 0.1 cM, 1 cM, and 1 cM. This order is based on our physical map and on the consensus physical map of the human X chromosome (Nelson et al. 1995). Because four of the markers mapped within a region of 900 kb, it was not possible to obtain reliable genetic distances by use of the available linkage data. We therefore used physical distances, assuming that 1 Mb = 1 cM in this region. Our previous studies support this estimation (Alitalo et al. 1995), and, if the whole X chromosome is taken into consideration, 1 Mb will, on average, be 1.2 cM (Nelson et al. 1995). The results of the eight-point analysis are shown in figure 3. The LINKMAP analysis gave a peak multipoint Z of 31.3 for the RS locus being located between DXS418 and DXS7161. The odds for this location were 10,000 times greater than the odds favoring any other marker interval.

Haplotype analysis.—The distribution of alleles at marker loci DXS43, DXS1195, DXS418, HYAT1, DXS999, DXS7161, DXS365, and DXS274 among the RS-carrying chromosomes and normal chromosomes is shown in table 5. If only the extensively studied markers DXS1195–DXS418–HYAT1–DXS999–DXS7161–DXS365 are taken into consideration, 12 different haplotypes can be seen on the disease-bearing chromosomes. The most common haplotype found in the northern fam-

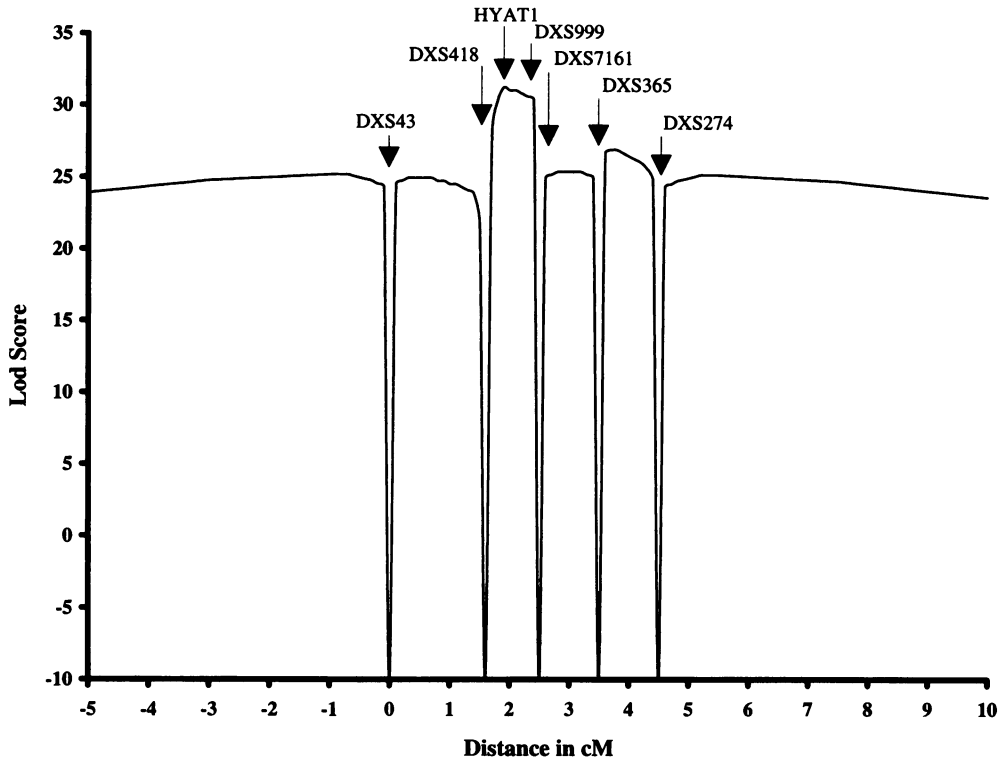

Figure 3 Results of an eight-point linkage analysis of 31 Finnish RS families (by LINKMAP). Locus DXS43 was chosen as the starting point (0), and the other loci were positioned from it according to the distances as described in the text. The centromere is to the right.

ilies (58%; 2-12-5-1-7-5) differs totally from the most common haplotype in the western families (78%; 1-8-6-3-5-4). None of the normal chromosomes carried these haplotypes. This finding strongly supports the hypothesis that there are at least two different mutations responsible for RS in Finland.

The 2.1-Mb DXS1195-DXS7161 region was conserved in all the northern families, but in the western families six different variant haplotypes could be seen. Of those six haplotypes, only two RS chromosomes carried, at DXS418, an allele other than 8. Even these two haplotypes, 1-7-5-7-2 and 2-9-6-9-2, share either the common allele 1 at DXS1195 or allele 6 at HYAT1, with the ancestral haplotype 1-8-6-3-5. The atypical haplotypes represent either different RS mutations, ancestral recombinations, or, perhaps more likely, mutations at the DXS418 locus. DXS418 seems to be a highly mutable microsatellite marker, since we have been able to observe three actual mutations in our RS families, both in RS and in non-RS X-chromosome segregation. The alleles are numbered according to their repeat lengths, and the observed changes represent shifts in length of −2 and +2 bp; there are two 8→7 mutations and one 8→9 mutation (data not shown). In conclusion, the haplotype data suggest that the RS gene is located close to the most conserved locus—namely, DXS418. A total of 93% of all the RS chromosomes carried allele 8 at this locus, whereas only 15% of the normal X chromosomes had allele 8 (P_{excess} = .92).

Linkage-disequilibrium mapping of RS.—The distribution of alleles on RS chromosomes of the western patients was significantly nonrandom at each tested marker locus, except for HYAT1 (table 3). Because the haplotype analysis of the northern patients showed a conserved haplotype that encompasses the whole DXS1195-DXS7161 region, northern data were not used in further analysis.

To calculate point estimates for genetic distances, we used the analytic method based on Luria-Delbrück theory. Point estimates for the distances between markers and the disease gene, plotted against the number of generations, are depicted in figure 1. Estimated distances for generation values 10, 20, and 30 are also shown in table 3. RS appears to lie closest to the marker DXS418, with an estimated distance of 0.2–0.3 cM. The generation number used refers to female meiosis only, not to the real-time interval measured in generations. We estimate that the mutation was introduced into the population ≥40–50 generations ago (for more details, see Discussion). If it is assumed that there is

Table 5

Haplotypes Associated with RS at Seven Marker Loci—and Alleles found in RS Chromosomes of Western Patients, Compared with Alleles in Normal X Chromosomes

A. Haplotypes associated with RS at Seven Marker Loci

Distance between Markers (kb)	Marker	Western RS Families (n = 30) Haplotype[a]										Northern RS Families (n = 9) Haplotype[a]			
1,000	DXS43	2	1	2	ND	2	ND	ND	1	1	1	1	ND	ND	1
200	DXS1195	1	1	1	1	1	1	1	3	1	2	2	2	2	2
300	DXS418	8	8	8	8	8	8	8	8	7	9	12	12	12	12
500	HYAT1	6	6	6	6	6	4	5	7	5	6	5	5	5	5
100	DXS999	3	3	3	3	3	3	5	1	7	9	1	1	1	1
1,000	DXS7161	5	5	5	5	5	5	6	5	2	2	7	7	7	7
1,000	DXS365	4	4	4	4	3	4	6	6	ND	1	5	5	6	4
	DXS274	1	1	2	ND	1	ND	ND	1	1	1	1	ND	ND	ND
	No. of Familes	15	1	1	1	2	4	1	3	1	1	6	1	1	1

B. Alleles in RS Chromosomes of Western Patients, Compared with Alleles in Normal X Chromosomes

No. of Normal Chromosomes Analyzed	Marker	Allele (%[b])	Allele (%[b])	Allele (%[b])	Allele (%[b])	Allele (%[b])
38	DXS43	1 (25/74)	2 (75/26)			
27	DXS1195	1 (87/22)	2 (3/27)	3 (10/52)		
61	DXS418	7 (3/18)	8 (94/15)	9 (3/21)		
64	HYAT1	4 (13/6)	5 (7/36)	6 (70/42)	7 (10/11)	
63	DXS999	1 (10/25)	3 (80/38)	5 (3.33/5)	7 (3.33/27)	9 (3.33/0)
64	DXS7161	2 (7/25)	5 (90/47)	6 (3/17)		
34	DXS365	2 (3/3)	3 (7/26)	4 (76/24)	6 (14/18)	
36	DXS274	1 (96/58)	2 (4/42)			

[a] The region retaining the common haplotype is boxed.
[b] % of alleles in western RS chromosomes/% of alleles in normal chromosomes.

no selection pressure, this corresponds to a (female) generation number of ~20–25.

The results of multipoint linkage-disequilibrium analysis are shown in figure 2. The highest Z value, 25.55, was obtained at 0.0009 cM distal to DXS418, with a 99.9% confidence interval ($Z_{max} \pm 3$ Z units) that spans the whole 3.3-cM DXS43-DXS365 region.

Physical Map of the RS Region

Since our goal is to map and clone the RS gene, it is important to estimate distances between the flanking markers and to have a dense STS coverage over the RS region. The ~1-Mb YAC contig, extending from DXS418 to DXS7161, is depicted in figure 4. Twenty STSs were mapped to this region. The new STSs are given in table 1. Confirmation of the overlaps between the YACs was obtained by probe-content mapping and restriction mapping. Although a minimum of four nonchimeric YACs covers the RS region, the restriction map was constructed by use of nine YACs. Our data suggest that the distances between the markers DXS418-HYAT1-DXS999-DXS7161 are, in order, ~300 kb, ~500 kb, and ~50 kb, and thus the distance between the flanking markers DXS418 and DXS7161 is ~850 kb.

Enzymes that frequently cut in CpG islands were used in order to determine the likely gene density in the region. A total of five CpG islands were identified, under the assumption that the presence of at least three rare-cutter sites is indicative of a putative CpG island. The restriction sites were found in at least two YACs, whenever two YACs were overlapping. The mapping data showed no discrepancies between the different YACs analyzed.

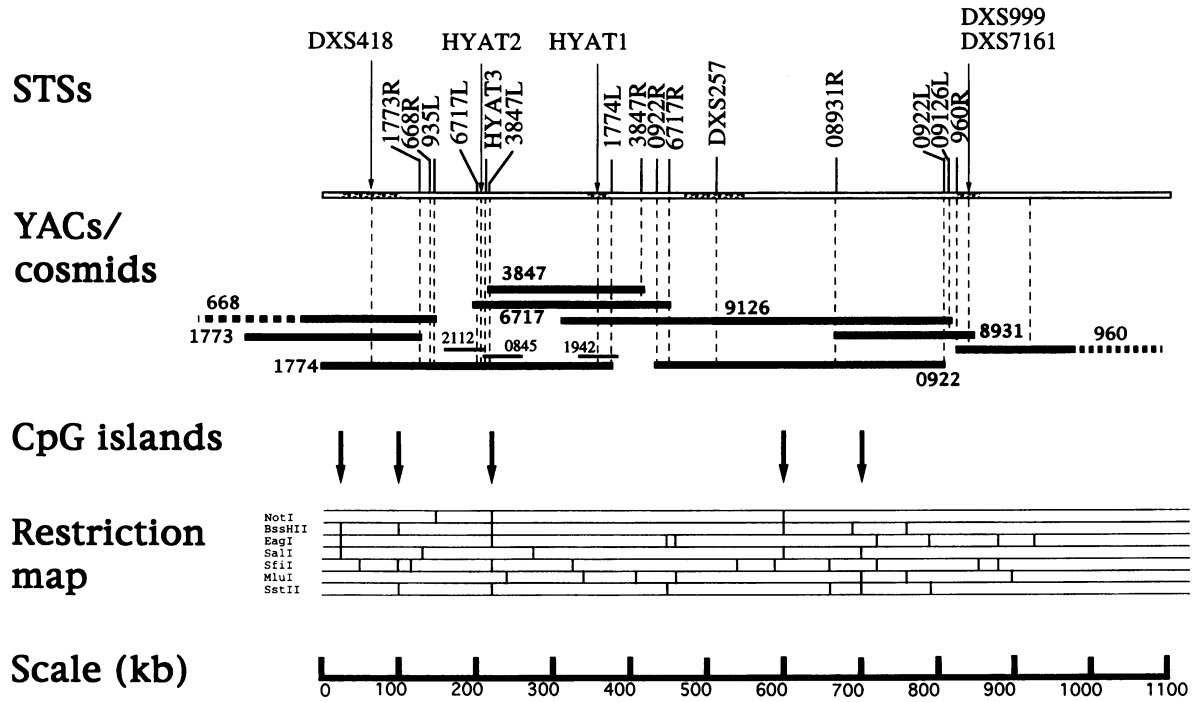

Figure 4 Restriction map of the DXS418-DXS7161 region. The STSs were defined by PCR and/or hybridization (table 1; Alitalo et al 1995). Potential CpG islands are indicated by arrows. The gray regions of the YAC clones indicate areas that are not X specific.

Discussion

This paper presents the results of genetic and physical mapping of RS. Both informative recombination events in the 31 Finnish RS families and multipoint linkage analysis localized the RS gene between the markers DXS418 and DXS7161, confirming previous reports (Dumur et al. 1995; Trump et al. 1996; Van de Vosse et al. 1996). A complete YAC contig covering the RS candidate region was constructed, and 20 STSs were mapped to this interval. Restriction mapping of the YACs was performed to refine the estimates of distance between the markers of the DXS418-DXS7161 region. Another feature of the 1-Mb restriction map was to reveal CpG islands. Consonant with the idea that high GC level is indicative of high gene content (Bernardi 1989), combined with the information that ~45% of all genes may be associated with CpG islands (Larsen et al. 1992), the five putative CpG islands found in the 1-Mb region suggest that the segment is probably gene poor. Because the ~900-kb DXS418-DXS7161 region is still a fairly large region, when one is thinking about effective cloning attempts, we isolated two new microsatellite markers from the interval. One useful microsatellite (HYAT1) with nine alleles was obtained from a cosmid positive for the YAC end fragment 1774L. No recombinations were found between HYAT1 and RS, and the allele results proved to be useful in haplotype and linkage-disequilibrium analysis.

Linkage-disequilibrium mapping has been a useful tool in the mapping of genetic diseases. Because the method exploits recombinations that have occurred during the entire history of the population studied, it partially avoids the typical restrictions of traditional linkage analysis. Isolated populations, such as the Finns, are ideal for linkage-disequilibrium studies. Finland's present population of ~5 million is believed to originate largely from a small founder population whose expansion began 2,000–2,500 years ago (Nevanlinna 1972; Norio 1981; de la Chapelle 1993). Perhaps more important, Satakunta county in western Finland, where 70% of the Finnish RS patients originate, is known to have had an already exceptionally stable inhabitation during the Stone Age 3000–1600 B.C. (Pertola 1977). The accumulation of RS in Satakunta is highest in the rural district of Noormarkku, where 1.2% of the females are RS carriers (Eriksson et al. 1972). It is known that a small number of people already were living in the eastern parts of Noormarkku in 4000–1400 B.C., but the permanent inhabitation most probably was established in ~600 A.D. (Peevo 1977). On the basis of the historical information, we have estimated that the RS mutation in western Finland has existed for ≥40–50 generations, thus allowing recom-

binations to narrow considerably the region of strongest linkage disequilibrium. The northern part of Finland, where the rest of the RS patients originate, was inhabited only 500 years ago. Most of the markers analyzed in this study map within 1 Mb, yet the most common haplotype of the western patients differs totally from the northern haplotype, strongly favoring the hypothesis of two independent RS mutations in Finland. Recombination events alone cannot explain these findings. It can be assumed that the potential northern RS mutation is younger, or was introduced into the population later, than the western mutation.

The dichotomy seen in grandparental birthplaces and disease-chromosome haplotypes of RS patients could have complicated our linkage-disequilibrium analyses. The division of RS patients into two groups was clear, except for two patients who might represent either independent RS mutations or simply mutations at marker loci. The potential problem was resolved after analysis of the haplotypes of the northern patients. The distribution of alleles on RS chromosomes of northern patients was nonrandom for the markers DXS418, DXS999, DXS365, and DXS274. The allelic excess values were 1.0 for all the markers except DXS365 (.78), which made it impossible to estimate distances between RS and the markers studied (data not shown). In view of the fact that the whole DXS1195-DXS7161 region was conserved in all the northern patients, the results were not used in further analysis, and the distance estimations were based on the results obtained from the western patients only.

The distribution of alleles of the western patients was significantly nonrandom at each tested marker locus, except for HYAT1. Luria-Delbrück analysis was used successfully to estimate the distances between RS and the other markers. On the basis of this analysis, the RS gene is located 200–300 kb from DXS418, provided that the generation number is 40–50 and 1 cM = 1 Mb. We note that the uncertainties about both the number of generations since founding and the relation between physical and genetic distances must be taken seriously when one is interpreting the results for single polymorphic markers. On the other hand, the distance estimation correlates well with our linkage and haplotype data, which suggested that the RS gene is located proximal to DXS418 and distal to HYAT1, a distance of ~300 kb. The multipoint linkage-disequilibrium analysis is in agreement with this location; however, the confidence interval was large.

The chromosomal area in which RS most likely is located is presently narrowed almost beyond the practical limits of genetic studies, and future research will be performed by physical means. Our present studies are directed toward the isolation of genes in the 300-kb RS region.

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References

Albertsen HM, Abderrahim H, Cann HM, Dausset J, Le Paslier D, Cohen D (1990) Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. Proc Natl Acad Sci USA 87:4256–4260

Alitalo T (1990) Gene mapping of juvenile retinoschisis and Åland island eye disease on the human X chromosome. ISBN 951-45-5610-0. PhD thesis, University of Helsinki, Helsinki

Alitalo T, Forsius H, Kärnä J, Frants RR, Eriksson AW, Wood S, Kruse TA, et al (1988) Linkage relationship and gene order around the locus for X-linked retinoschisis. Am J Hum Genet 43:476–483

Alitalo T, Francis F, Kere J, Lehrach H, Schlessinger D, Willard HF (1995) A 6 Mb YAC contig in Xp22.1-Xp22.2 spanning the DXS69E, XE59, GLRA2, PIGA, GRPR, CALB3 and PHKA2 genes. Genomics 25:691–700

Alitalo T, Kärnä J, Forsius H, de la Chapelle A (1987) X-linked retinoschisis is closely linked to DXS41 and DXS16 but not DXS85. Clin Genet 32:192–195

Alitalo T, Kruse TA, de la Chapelle A (1991) Refined localization of the gene causing X-linked juvenile retinoschisis. Genomics 9:505–510

Arden GB, Gorin MB, Polkinghorne PJ, Jay M, Bird AC (1988) Detection of the carrier state of X-linked retinoschisis. Am J Ophthalmol 105:590–595

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Bergen AAB, ten Brink JB, Bleeker-Wagemakers LM, van Schooneveld MJ (1994) Refinement of the chromosomal position of the X linked juvenile retinoschisis gene. J Med Genet 31:972–975

Bernardi G (1989) The isochore organization of the human genome. Annu Rev Genet 23:637–661

Browne D, Barker D, Litt M (1992) Dinucleotide repeat polymorphisms at the DXS365, DXS443 and DXS451 loci. Hum Mol Genet 1:213

Condon GP, Brownstein S, Wang N-S, Kearns JAF, Ewing CC (1986) Congenital hereditary (juvenile X-linked) retinoschisis: histopathologic and ultrastructural findings in three eyes. Arch Ophthalmol 104:576–583

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53:252–263

Dahl N, Goonewardena P, Chotai J, Anvret M, Pettersson U (1988) DNA linkage analysis of X-linked retinoschisis. Hum Genet 78:228–232

de la Chapelle A (1993) Disease gene mapping in isolated human populations: the example of Finland. J Med Genet 30:857–865

de la Chapelle A, Alitalo T, Forsius H (1994) X-linked juvenile retinoschisis. In: Wright A, Jay B (eds) Molecular genetics of inherited eye disorders, 1st ed. Harwood Academic, Chur, Switzerland, pp 339–357

Dumur V, Trivier E, Puech B, Peugnet F, Zanlonghi X, Hache JC, Hanauer A (1995) 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. Hum Genet 96:79–82

Eriksson AW, Forsius H, Vainio-Mattila B (1972) X-chromosomal recessive retinoschisis. Duodecim 88:43–51

Fasman KH, Cuticchia AJ, Kingsbury DT (1994) The GDB human genome data base anno 1994. Nucleic Acids Res 22: 3462–3469

Ferrero GB, Franco B, Roth EJ, Firulli BA, Borsani G, Delmas-Mata J, Weissenbach J, et al (1995) An integrated physical and genetic map of a 35 Mb region on chromosome Xp22.3-Xp21.3. Hum Mol Genet 4:1821–1827

Gellert G, Petersen J, Krawczak M, Zoll B (1988) Linkage relationship between retinoschisis and four marker loci. Hum Genet 79:382–384

Hästbacka J, de la Chapelle A, Kaitila I, Sistonen P, Weaver A, Lander E (1992) Linkage disequilibrium mapping in isolated founder populations: diastrophic dysplasia in Finland. Nat Genet 2:204–211

Hästbacka J, de la Chapelle A, Mahtani M, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, et al (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78:1073–1087

Höglund P, Sistonen P, Norio R, Holmberg C, Dimberg A, Gustavson K-H, de la Chapelle A, et al (1995) Fine mapping of the congenital chloride diarrhea gene by linkage disequilibrium. Am J Hum Genet 57:95–102

Kaplan J, Pelet A, Hentati H, Jeanpierre M, Briard ML, Journel H, Munnich A, et al (1991) Contribution to carrier detection and genetic counselling in X linked retinoschisis. J Med Genet 28:383–388

Kere J, Nagaraja R, Mumm S, Ciccodicola A, D'Urso M, Schlessinger D (1992) Mapping human chromosomes by walking with sequence-tagged sites from end fragments of yeast artificial chromosome inserts. Genomics 14:241–248

Kidd KK, Bowcock AM, Schmidtke J, Track RK, Ricciuti F, Hutchins G, Bale A, et al (1989) Report of the DNA committee and catalogs of cloned and mapped genes and DNA polymorphisms. Cytogenet Cell Genet 51:622–947

Larin Z, Monaco AP, Lehrach H (1991) Yeast artificial chromosome libraries containing large inserts from mouse and human DNA. Proc Natl Acad Sci USA 88:4123–4127

Larsen F, Gundersen G, Lopez R, Prydz H (1992) CpG islands as gene markers in the human genome. Genomics 13:1095–1107

Lathrop GM, Lalouel JM, Julier C, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lehesjoki A-E, Koskiniemi M, Norio R, Tirrito S, Sistonen P, Lander E, de la Chapelle A (1993) Localization of the EPM1 gene for progressive myoclonus epilepsy on chromosome 21: linkage disequilibrium allows high resolution mapping. Hum Mol Genet 2:1229–1234

Lehrach H, Drmanac R, Hoheisel J, Larin Z, Lennon G, Monaco AP, Nizetic D, et al (1990) Hybridization fingerprinting in genome mapping and sequencing. In: Davies KE, Tilghman SM (eds) Genome analysis. Vol 1: Genetic and physical mapping. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 39–81

McKusick VA (1994) Mendelian inheritance in man: a catalog of human genes and genetic disorders, 11th ed. Johns Hopkins University Press, Baltimore, London

Nagaraja R, Kere J, MacMillan S, Masisi W, Johnson D, Molini B, Wein K, et al (1994) Characterization of four human YAC libraries for clone size, chimerism, and X chromosome sequence representation. Nucleic Acids Res 22:3406–3411

Nelson DL, Ballabio A, Cremers F, Monaco AP, Schlessinger D (1995) Report of the Sixth International Workshop on Human X Chromosome Mapping 1995. Cytogenet Cell Genet 71:307–342

Nevanlinna HR (1972) The Finnish population structure: a genetic and genealogical study. Hereditas 73:195–236

Norio R (1981) Diseases of Finland and Scandinavia. In: Rotchild H (ed) Biocultural aspects of disease. Academic Press, New York, pp 359–415

Oudet C, Weber C, Kaplan J, Segues B, Croquette M-F, Ollagnon Roman E, Hanauer A (1992) Characterisation of a highly polymorphic microsatellite at the DXS257 locus: confirmation of very close linkage to the retinoschisis disease gene. J Med Genet 30:300–303

Pawar H, Bingham EL, Lunetta KL, Segal M, Richards JE, Boehnke M, Sieving PA (1995) Refined genetic mapping of juvenile X-linked retinoschisis. Hum Hered 45:206–210

Peevo R (1977) Noormarkku. In: Öhling M, Lahtinen M, Osala T, Ahrner L (eds) Kotiseutuni. ISBN 951-95005-6-1. Suomen Maakuntajulkaisu Oy, Vaasa, Finland, pp 231–241

Pennaccio LA, Lehesjoki A-E, Stone NE, Willour VL, Virtaneva K, Miao J, D'Amato E, et al (1996) Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). Science 271:1731–1734

Pertola E (1977) Kokemäki. In: Öhling M, Lahtinen M, Osala T, Ahrner L (ed) Kotiseutuni. ISBN 951-95005-6-1. Suomen Maakuntajulkaisu Oy, Vaasa, Finland, pp 170–178

Puech B, Kostrubiec B, Hache J-C, Francois P (1991) Épidémiologie et prevalence des principales dystrophies rétiniennes héréditaires dans le Nord de la France. J Fr Ophtalmol 14: 153–164

Sieving PA, Bingham EL, Roth MS, Young MR, Boehnke M, Kuo C-Y, Ginsburg D (1990) Linkage relationship of X-linked juvenile retinoschisis with Xp22.1-Xp22.3 probes. Am J Hum Genet 47:616–621

Sulisalo T, Klockars J, Mäkitie O, Francomano CA, de la Chapelle A, Kaitila I, Sistonen P (1994) High-resolution

linkage-disequilibrium mapping of the cartilage-hair hypoplasia gene. Am J Hum Genet 55:937–945

Tahvanainen E, Forsius H, Damsten M, Karila E, Kolehmainen J, Weissenbach J, Sistonen P, et al (1995) Linkage disequilibrium mapping of the cornea plana congenita gene CNA2. Genomics 30:409–414

Terwilliger JD (1995) A powerful likelihood method for the analysis of linkage disequilibrium between trait loci and one or more polymorphic marker loci. Am J Hum Genet 56: 777–787

Trump D, George NDL, Walpole SM, Bill RM, Moore AT, Yates JRW (1996) Further genetic and physical mapping of X-linked retinoschisis. Eur J Hum Genet 4:62

van de Vosse E, Bergen AAB, Meershoek EJ, Oosterwijk JC, Gregory S, Bakker B, Weissenbach J, et al (1996) An Xp22.1-Xp22.2 YAC contig encompassing the disease loci for RS, KFSD, CLS, HYP and RP15: refined localization of RS. Eur J Hum Genet 4:101–104

van de Vosse E, Booms PFM, Vossen RHAM, Wapenaar MC, van Ommen GJB, den Dunnen JT (1993) A CA-repeat polymorphism near DXS418 (P122). Hum Mol Genet 2:2202

Weber BHF, Janocha S, Vogt G, Sander S, Ewing CC, Roesch M, Gibson A (1995) X-linked juvenile retinoschisis (RS) maps between DXS987 and DXS443. Cytogenet Cell Genet 69:35–37

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, et al (1992) A second-generation linkage map of the human genome. Nature 359:794–801

Wieacker P, Wienker TF, Dallapiccola B, Bender K, Davies KE, Ropers HH (1983) Linkage relationships between retinoschisis, Xg, and a cloned DNA sequence from the distal short arm of the X chromosome. Hum Genet 64: 143–145