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ARTICLE

Three widespread founder mutations contribute to high incidence of X-linked juvenile retinoschisis in Finland

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X-linked juvenile retinoschisis (RS) is a recessively inherited disorder causing progressive vitreoretinal degeneration in males. The gene defective in retinoschisis, *XLRS1*, has recently been identified and characterised. This gene consists of six exons encoding a protein with a putative role in cell–cell adhesion and phospholipid binding. Juvenile retinoschisis has been actively studied in Finland over the past 30 years, with over 300 diagnosed RS patients. Based on genealogical studies, approximately 70% of the Finnish RS patients originate from Western Finland and 20% from Northern Finland. In this study, one third of the known Finnish RS patients were screened for mutations of the *XLRS1* gene. Haplotype analysis, using nine microsatellite markers spanning 1 cM in Xp22.2, suggested the segregation of eight different mutations in these families. To identify mutations, the six exons were amplified by PCR and analysed by single strand conformation analysis, followed by direct sequencing of the PCR products. We identified seven distinct missense mutations, all in exons 4 and 6. The mutations in exon 4, 214G > A and 221G > T, are accountable for RS in Western Finland. A third mutation in exon 4, 325G > C, gives rise to RS in Northern Finland. These three founder mutations are the predominant cause of RS in Finland and their existence explains the high incidence of the disease. The identification of mutations common in genetically isolated populations, such as Finland, allows the diagnosis of patients with an atypical RS phenotype and enables nationwide carrier testing and improved genetic counselling.

Keywords: X-chromosome; retinoschisis; mutation screening; haplotypes

Introduction

X-linked juvenile retinoschisis (RS; MIM 312700) belongs to the group of vitreoretinal dystrophies. The disease causes progressive impairment of vision with variable degrees of severity. The onset of the disease appears to be from the abnormal development of the cortical vitreous, probably already at the foetal stage.[1] Patients are typically diagnosed at school age, although retinal changes may be present at birth.[1,2] The visual acuity is commonly poor (0.1–0.6) in children, and deterioration of vision occurs in the fourth and fifth decades of life because of macular atrophy. The most serious sight-threatening complications are vitreous

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Table 1 STSs used in this study

Marker	*Primer sequences (5'–>3')*	*Annealing temperature*	*Product size (bp)*
CA1	TCCTGATTTAAGGATCCCCC TTATTGGGGGAAGAGTTCCA	57°C	163–153
CA3	TCAAAAATCAGCACCAAAAGAA TCCATGTGTTTTCATAGCTTGG	57°C	273–259
TAAA	CAGCTATTTGGGAGGCTGAG TTGAGGTGCAAGTGTCAAGG	58°C	204–188
CA6	CTTAGCTGGCCATTTAGGGA TCCCCTTTCAAAAACAGGAA	55°C	187–179
TAAA2	AATTGCTTGAATCCAGGAGG GGCTCTTTCCACTCCAGTCA	58°C	222–206

haemorrhage and retinal detachment.[3,4] There is no known therapy to cure the degenerative process.

Females heterozygous for the *RS* gene are symptomless, and clinical detection of carriers is not definitive with present methods. A woman can get the disease only if she has an affected father and a heterozygous mother, usually as a result of a consanguineous marriage. A few affected females have been described in the literature,[5–7] including one with Turner syndrome.[8]

Several linkage studies have localised the *RS* gene to a 1 cM interval DXS418–DXS999/DXS7161 on Xp22.[9–13] This region has further been covered with YAC clones[12,14–16] and a PAC contig.[16] The entire RS candidate region has now also been sequenced by the Sanger Centre,[17] in collaboration with the Retinoschisis Consortium.

The gene responsible for retinoschisis, *XLRS1*, was recently isolated by positional cloning strategies.[18] In this original study, nine mutations of the *XLRS1* gene were identified in nine individual RS families. In addition, the Retinoschisis Consortium has recently performed a collaborative study and identified 82 disease-causing mutations.[19] The *XLRS1* gene encodes a protein of 224 residues whose expression is limited to the retina. The XLRS1 protein contains a 23 amino acid residue leader sequence as well as a conserved discoidin motif at the C-terminus. Since other peptide sequences with a discoidin motif in the carboxyterminal part of the protein are known to mediate cell-to-cell interactions, it is assumed that the XLRS1 protein is involved in cell-to-cell adhesion.[18,20] The precise function of the XLRS1 protein, or the pathophysiology of the disease, is still largely unknown.

X-linked retinoschisis is very common in Finland with a prevalence of > 1:17 000.[21] Moreover, the majority of patients come from the Western province of Satakunta, while the province of Oulu in the Northern part of the country is another area of considerable density.[22] This report describes the mutations found in the *XLRS1* gene of Finnish RS patients. The aim of the study was to identify all Finnish founder haplotypes and mutations as well as to estimate the age of the founder mutations. These results will benefit all Finnish RS families. Also, the mutation findings in a thoroughly studied isolated population can be useful when estimating the world-wide frequency of RS.

Materials and Methods

Patients and Control DNAs

Members of 55 Finnish retinoschisis families (117 affected males, one female) were included in the haplotype and mutation analyses. Thirty-nine of these families had already taken part in our previous linkage studies.[13] Blood samples were obtained from 16 new families. High molecular weight genomic DNA was prepared from blood leukocytes of the RS patients by standard methods. One ophthalmologist (HF) saw all the patients and confirmed the diagnosis. The birthplaces of the ancestors were determined by genealogical studies. Clinical studies of the RS families, as well as the classification of the patients as severe, moderate and mild cases, have been published earlier.[22,23] DNAs from 75 healthy blood donors were used as normal controls.[24]

Genetic Analysis with New Microsatellite Markers

The Sanger Centre had sequenced the PAC clones which cover the RS candidate region. We screened the entire sequence[17] and found 13 new polymorphic markers. Primer sequences and PCR conditions for the markers which had more than three alleles, ie CA1, CA3, TAAA, CA6, and TAAA2 are listed in Table 1. Primers were designed using the Primer 3 program.[25] The data on allele sizes and frequencies were derived from 100 normal X chromosomes (Table 2). The alleles were numbered consecutively according to decreasing size. Primer sequences and PCR amplification conditions for

Table 2 Allele sizes and frequencies for the new microsatellite markers. Allele frequencies were obtained from 100 normal X chromosomes

Markers									
CA1		*CA3*		*TAAA*		*CA6*		*TAAA2*	
Size (bp)	*Freq.*	*Size*	*Freq.*	*Size*	*Freq.*	*Size*	*Freq.*	*Size*	*Freq.*
1. 163	0.04	1. 273	0.02	1. 204	0.01	1. 187	0.59	1. 222	0.01
2. 161	0.04	2. 271	0.06	2. 200	0.28	2. 185	0.02	2. 218	0.04
3. 159	0.72	3. 269	0.13	3. 196	0.62	3. 183	0.24	3. 214	0.30
4. 157	0.01	4. 267	0.48	4. 192	0.08	4. 181	0.01	4. 210	0.63
5. 155	0.02	5. 265	0.06	5. 188	0.01	5. 179	0.14	5. 206	0.02
6. 153	0.17	6. 263	0.16						
		7. 261	0.05						
		8. 259	0.04						

Table 3 Haplotypes associated with Finnish RS patients at nine marker loci

Distances between markers	*Marker*	*Western I*	*Western II*	*Northern*	*Fam 41*	*Fam 36*	*Fam 39*	*Fam 5*	*Fam 2*
		214G>A	*221G>T*	*325G>C*	*no mut*	*625C>G*	*554C>A*	*608C>T*	*312C>G*
	DXS418	8 7 8	7 8 8	12	7	8	8	9	7
30 kb									
	CA1	3 3 3	3 3 3	3	3	3	3	3	5
270 kb									
	DXS9911	6 6 6	7 7 7	5	5	5	5	6	5
2 kb									
	CA3	5 5 5	3 3 4	1	3	3	1	5	4
18 kb									
	TAAA	2 2 2	3 3 3	3	3	3	3	2	1
180 kb									
	CA6	3 3 3	1 1 1	1	1	1	5	3	3
100 kb									
XLRS1									
50 kb									
	TAAA2	2 2 1	4 4 4	3	2	3	3	4	3
50 kb									
	DXS999	3 3 3	1 1 1	1	3	5	7	9	7
100 kb									
	DXS7161	5 5 5	5 5 5	7	5	6	6	2	2
No. of families with this haplotype		26 2 2	7 2 1	10	1	1	1	1	1

the eight additional less informative markers are available on request.

For haplotype analysis, all the families were genotyped with the markers DXS418, CA1, DXS9911, CA3, TAAA, CA6, TAAA2, DXS999 and DXS7161 (Table 3). DNA samples were amplified by PCR using standard protocols and the above mentioned markers. Amplified products were separated on 6% polyacrylamide gels in 1 × TBE as described earlier.[13] After electrophoresis, the gels were visualised by silver staining.[26]

Mutation Analysis

For each patient, exons 1–6 and flanking sequences were amplified by PCR from genomic DNA. PCR primers and amplification conditions were those published earlier.[18] Mutation search was performed by single strand conformation analysis (SSCA).[27] PCR products were separated on MDE gels (Mutation Detection Enhancement, FMC (FMC BioProducts, Rockland, ME, USA); 0.7 × and 1 × MDE) in 0.6 × TBE buffer. Gels were run at room temperature with 10 W constant power for 14–20 hours and stained by silver staining.[26] PCR products exhibiting aberrant SSCA patterns (Qiagen, Valencia, CA, USA) were purified by QIAquick purification columns (Qiagen) and sequenced directly in both directions by fluorescent automated sequencing (ABI373A). PCR products spanning the mutations found were also amplified from 100 control chromosomes, followed by SSCA.

Some of the mutations found could be detected by restriction enzyme analysis. These mutations had either created or caused a loss of a particular restriction site (Table 4). Genomic DNA was amplified as described earlier.

In general, digestions of amplified exon products were performed in 1 × reaction buffer using 10–20 U of the restriction enzyme. The resulting fragments were electrophoresed through 2.5% agarose gels (Sea Kem, FMC) and DNA visualised with ethidium bromide (Figure 1a).

Results

Haplotype Analysis and Genealogical Investigations

Based on the present and previous genealogical studies[22] and the new haplotype data, the 55 kindreds studied could be divided into four groups: A) Western I families (n = 30) form a group of families whose ancestors were born in the Western part of Finland, near Pori. Almost all ancestors of these families can be traced to one large pedigree,[22] (H Forsius, personal communication, 1998). B) Most ancestors of the Western II families (n = 10) originate in the neighbouring parishes near the town of Pori. Some families are at present living in Southern Finland. C) The Northern families (n = 10) are all related to each other, although the families are scattered over a large area.[28] D) Individual families (n = 5) from the Southern, Eastern and Northern parts of Finland are not related to any known RS family.

Table 3 summarises the genotypes of the RS patients in this study. If the most distal, highly mutable marker locus DXS418 is left out of the analysis,[13] and mutations are allowed at the loci TAAA2 and CA3, a total of eight different haplotypes can be observed. We were not able to estimate the degree of linkage disequilibrium by allelic association due to the lack of recombinations in the ancestral haplotypes in each group.

Identification of Mutations

Since the haplotype data suggested that there might be eight different mutations, we searched for mutations in the representatives of these eight groups by SSCA. Direct sequencing of PCR fragments showing mobility shifts revealed seven missense mutations (Table 4), all in exons 4 and 6.

Western I mutation was found in all the patients with a Western I haplotype. This mutation is caused by a G to A transition at position 214 of the cDNA. This change eliminates a TaqI restriction site and alters the amino acid at position 72 from Glu to Lys (Table 4). The loss of a TaqI site in exon 4 PCR products was used to identify the 214G > A alleles in all the RS patients with the same haplotype (Table 3; Figure 1a). Our female RS patient had the Western I mutation in both of her X chromosomes.

Western II mutation in exon 4 is caused by a G to T transversion at position 221 of the cDNA, resulting in a Gly to Val change at amino acid position 74. The mutation in all the patients with the Western II haplotype could be identified by restriction enzyme assay. The mutation creates a TspRI recognition site that can be detected by TspRI digestion since the normal sequence lacks the recognition site for this enzyme. Because the restriction enzyme based method is not yet reliable enough to be used routinely in mutation detection we use the SSCA method (Figure 1b). All the Northern patients carried the 325G > C mutation which alters the amino acid at position 109 from Gly to Arg, and can only be detected by SSCA or sequencing (Figure 1c).

Unrelated families 2, 5, 36 and 39 had the mutations 312C > G (ex 4), 608C > T (ex 6), 625C > G (ex 6) and 554C > A (ex 6), respectively. Additional mutation data are shown in Table 4. We did not find any mutations in

Table 4 RS patients and XLRS1 mutations identified in this study

Mutation at nucleotide level	*Affected exon*	*Mutation at amino acid level*	*Method used for rapid detection*	*Patient group*	*% of known Finnish patients*
214G>A	4	Glu72Lys	Loss of TaqI	Western I	70%[a]
221G>T	4	Gly74Val	Creation of TspRI	Western II	6%
312C>G	4	Asn104Lys	Creation of HinfI	Family 2	2%
325G>C	4	Gly109Arg	SSCA	Northern	19%[a]
554C>A	6	Thr185Lys	SSCA	Family 39	1%
608C>T	6	Pro203Leu	Loss of MaeII	Family 5	2%
625C>G	6	Arg209Gly	Loss of AciI	Family 36	0.3%

[a]denotes that the number is derived including patients which belong to the same pedigree. Mutation analysis was not performed on all of these patients; 36% of the Western RS patients and 44% of the Northern RS patients were included in the analysis.

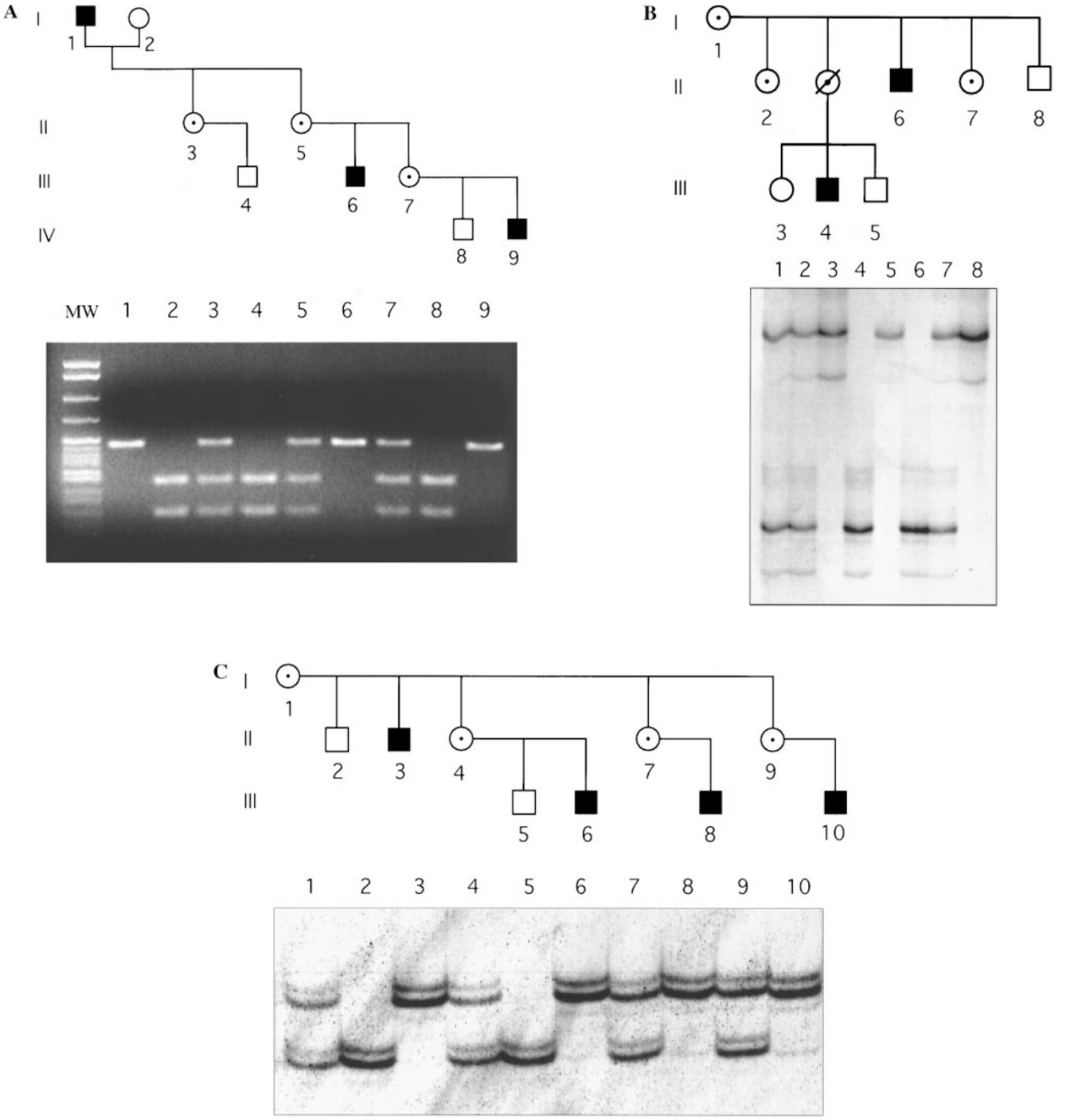

Figure 1 *Detection methods for the Finnish founder mutations, showing the segregation of each mutation in a representative family.* **a***) TaqI restriction enzyme analysis of the Western I mutation. In affected family members (1, 6, 9) the 220 bp PCR product is uncut due to the loss of the TaqI recognition site. In healthy individuals (2, 4, 8) the 220 bp product is digested into 135 bp and 85 bp fragments. Heterozygous carriers (3, 5, 7) show all the three fragments. Molecular weight marker is pBR322/MspI.* **b***) SSCA analysis of the Western II mutation. The numbers above the lanes correspond with those in the pedigree.* **c***) SSCA analysis of the Northern mutation. The numbers above the lanes correspond with those in the pedigree.*

one RS patient (unrelated family 41) after sequencing all exons, their flanking regions, and the 3' UTR.

The segregation of each mutation in a family was confirmed either by SSCA or restriction enzyme based assay. After the seven mutations were identified, mutation analysis was performed for all the 118 patients according to their haplotype. All exons were sequenced only in a few representatives of each mutation group. We did not find any second alterations. None of the XLRS1 mutations identified in this study, 214G > A, 221G > T, 312C > G, 325G > C, 554C > A, 608C > T, 625C > G, were found in 100 control X-chromosomes and are therefore likely to be pathogenic. Mutation 325G > C has been described earlier by Sauer *et al*[18] and mutations 214G > A, 221G > T, and 608C > T, by the Retinoschisis Consortium.[19]

Geographical Distribution and Age Estimations of the Finnish XLRS1 Mutations

The geographical distribution of the birthplaces of 54 carrier females, including all mutation types, is shown in Figure 2. We detected a large clustering of Western I mutations in a well defined south-western region of Finland. A relatively high frequency of RS was also noted in the region of Oulu where the Northern mutations have accumulated. A third smaller clustering of mutations was detected in the parishes neighbouring the town of Pori, very close to Western I families. Two of the unrelated mutations were identified in families living in the Northern parts of Finland, overlapping with the region of the Northern mutation. The other two unrelated mutations were found in the families living farthest to the East. All in all, most Finnish RS patients are clearly clustered in Northern and South Western Finland (Figure 2), and have one of the three major mutations.

Our earlier haplotype analyses, performed using RFLP markers spanning a region of 11 cM, had suggested the existence of two distinct founder haplotypes and therefore two founder mutations.[24] In those studies 75% of the Northern families and 76% of the Western families shared a common haplotype between the markers DXS207–DXS41 (11 cM). Combining our previous and present haplotype data with the new mutation data, the Northern families share a common haplotype between the markers DXS7161–DXS207 (7 cM)[15,24] and the Western I families between the markers DXS7161–DXS1195 (1 cM; provided that we postulate mutations in markers DXS418 and TAAA2), suggesting that the Western I mutation is older than the Northern mutation.[13,24]

Discussion

This paper describes the Finnish mutations found in the *XLRS1* gene. One third of all known Finnish RS patients, 55 families (118 patients) were analysed in this study. Haplotype analysis with eight markers covering the 770 kb interval CA1–DXS7161 revealed eight

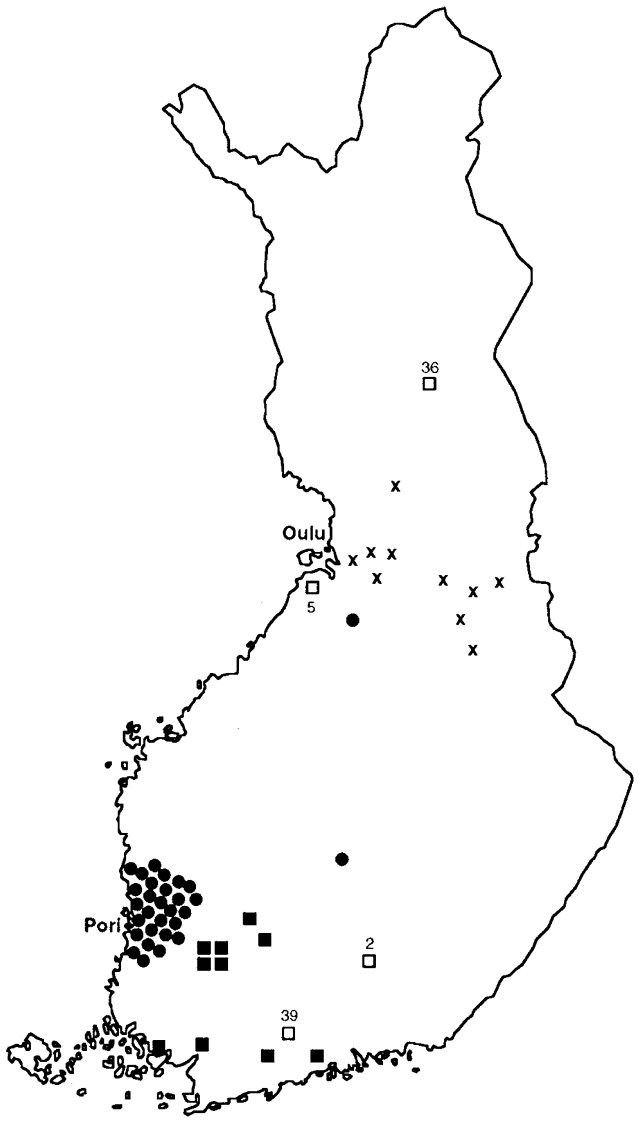

Figure 2 *A map of Finland showing the birthplaces of 54 carrier females. The different mutation types are indicated by:* • = Western I mutation; ■ = Western II mutation; ***X*** = Northern mutation; □ = unrelated families; 2 = Fam 2; 312C > G; 5 = Fam 5; 608C > T; 36 = Fam 36; 625C > G; 39 = Fam 39; 554C > A

clearly distinct haplotypes. After sequencing the coding region of the *XLRS1* gene, we identified seven missense mutations, all in exons 4 and 6. Based on the geneological data and the mutation results, 70% of the Finnish RS patients carry the Glu72Lys (Western I) mutation. The high frequency of this missense mutation has also been observed in another study, where it was identified in 34 apparently unrelated patients, comprising 14% of all the RS mutations reported.[19] The Northern mutation (Gly109Arg) accounted for approximately 19% of the Finnish RS mutations, being the second most common mutation in Finland. Surprisingly, a third founder mutation (Gly74Val) was identified in a group of families from Western Finland. Gly74Val accounts for 6% of all Finnish XLRS1 mutations. Thus, the three amino acid substitutions Glu72Lys (Western I), Gly109Arg (Northern), Gly74Val (Western II) are responsible for approximately 95% of all RS cases in Finland. In addition to these founder mutations, four mutations (Asn104Lys, Pro203Leu, Arg209Gly, Thr185Lys) were identified in individual families. All the nucleotide changes found in this study occurred at C or G nucleotides. However, only the mutation in family 5 (608C > T, CCG > CTG) shows the typical CG to TG transition affecting a CpG dinucleotide, which is known to be prone to C > T transitions.[29] Three of the mutations reported in this paper, 312C > G (Fam 2) in exon 4, 554C > A (Fam 39) in exon 6, and 625C > G (Fam 36) in exon 6, have not been found in any other European or North American RS patient,[18,19] and are thus novel XLRS1 mutations. All the Finnish mutations are clustered in the C-terminus of the *XLRS1*-gene product, supporting the notion that the N-terminal part of the protein is functionally less important.[18,19]

Intragenic deletions, splice site mutations, nonsense mutations and frameshift mutations can cause major changes in the protein structure, often resulting in severe dysfunction of the protein. By contrast, the functional consequences of missense mutations are usually harder to predict. The *XLRS1* gene has a well conserved discoidin domain.[18] Based on the alignment of the discoidin domain sequences, it has been observed that some of the RS amino acid substitutions introduce amino acids which are never used at this particular position in discoidin domains of other proteins.[19] From this point of view, the most interesting amino acid substitution is the proline to leucine change (608C > T), found in family 5. The wild type proline at position 203 is one of the most conserved amino acids in discoidin motifs. It is present in all the protein sequences containing discoidin domains that were aligned by the RS Consortium.[19] Replacement of a highly conserved proline residue by a leucine is likely to change the functional structure of the XLRS1 protein dramatically.

Although some mutations, like our most common Western I mutation Glu72Lys, change amino acids at less conservative positions, they can still cause considerable changes in proteins. In this mutation an acidic glutamic acid residue is changed to a basic lysine. Charged groups of basic and acidic amino acids have key roles in stabilising specific protein conformations.[30] Therefore, changes from acidic to basic amino acids at certain positions are likely to cause major changes of protein conformation. Mutations in families 2 and 39 (Asn104Lys and Thr185Lys) result in a change of an uncharged amino acid residue Asn/Thr to a positively charged Lys residue. Glycine to arginine (Gly109Arg) and arginine to glycine (Arg209Gly) substitutions were detected in Northern patients and in family 36, respectively. These changes are expected to have conformational effects since glycine, the smallest of the amino acids, can fit into the 3-dimensional structure in regions that are inaccessible to other amino acids.[30] It is difficult to predict the effect of the Gly74Val change (Western II mutation), because both the amino acids, Gly and Val, are small, nonpolar neutral residues. The mutant proteins as well as the wild type XLRS1 protein have to be characterised further before one can draw any firm conclusions about the functional consequences.

In one Finnish RS patient the molecular change underlying the disease is still unknown. We were unable to identify a mutation by direct sequencing of all six coding exons, their immediate flanking sequences and the 3' and 5' UTR regions. Because the patient is a sporadic case, this mutation might be a *de novo* rearrangement in the patient. Since we have not determined the mRNA levels, we cannot exclude mutations in enhancer regions or other changes that might influence the transcription. Other mutations might reduce the stability of the mRNA. The patient may also carry a mid-intron mutation which remains to be discovered. Although the clinical findings of the patient are typical for X-linked retinoschisis, it cannot be ruled out that the patient might have autosomal recessive retinoschisis.[31]

Because the haplotypes of the patients in each mutation type are almost completely identical, we conclude that each mutation arose only once. As stated

earlier, the Western I mutation is also very common in other European and North American RS patients. It may be unlikely, though, that there is a common founder for all of these patients. We have identified the RS haplotypes of one Swedish and one Danish patient with a 214G > A (Western I) mutation (unpublished, 1998). Although the Swedish patient shared the same haplotype with the Finnish patients, it is probable that this patient's family emigrated from Finland to Sweden. The Danish patient had a different haplotype and the mutation most probably arose independently, as in many other European countries (haplotype data not available). The retinoschisis mutations do not seem to have any selective effects. As stated earlier,[21] the disease is most likely underdiagnosed in most parts of the world. The high number of RS mutations identified in an isolated population (this study) gives further support to the idea that the frequency of RS must be higher than previously thought.

Our studies suggest that most of the Finnish XLRS1 mutations are not very old. Only a few Finnish disease-causing mutations, occurring throughout Finland, have expanded for over 100 generations.[32] It has been estimated that mutations that occur in certain small geographical regions in Finland have existed only for 15–30 generations.[33,34] Due to the fact that our RS families are closely related, and because of the scarcity of recombinations in the ancestral haplotypes of each mutation, we were not able to estimate the ages of the mutations by statistical analyses.[35,36] Our age estimations of the mutations are thus based on the genealogical studies only. The progenitors of Western I, Western II and Northern pedigrees were born in 1732, 1806 and 1715, respectively. Ancestors can be easily traced back to the beginning of the 18th century but often there are no church registers preserved from earlier times. The Northern parts of Finland were inhabited in the 1700s,[37,38] and we estimate that the age of the Northern mutation is > 300 years. With a typical human generation span of 25 years, this suggests that the mutation has segregated over 12 generations. The Western II mutation is much younger, about 200 years (approximately eight generations). The oldest founder mutation is clearly the Western I mutation. Based on the fact that the Western parts of Finland have been inhabited for at least 1000 years and approximately 70% of the Finnish patients carry the Western I mutation, this may have existed in the population at least twice as long as the Northern mutation. We estimate, but cannot yet prove, that the Western I mutation is > 600 years old, corresponding to > 24 generations. Based on the family data, the four individual mutations have existed for 1–5 generations.

One of the early aims of this study was to determine whether there is a correlation between the molecular defect and the disease phenotype. No such correlation could be observed; the whole spectrum of retinoschisis phenotypes from almost total blindness to just a mild reduction of visual acuity were found in the groups of families carrying either the Northern, the Western I or the Western II mutation. These observations strongly suggest that other genetic factors, such as modifying genes or epigenetic factors most likely influence the phenotypic severity of this disorder.

Although we cannot predict the severity of the disease by identifying a specific mutation, we can offer the Finnish RS families reliable carrier and pre- and postnatal diagnostics. The analysis is presently done by restriction enzyme based methods and SSCA, but the most common Finnish mutations will soon be conveniently diagnosed by DNA microchip technology.

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