RESEARCH ARTICLE

Novel Mutations in XLRS1 Causing Retinoschisis, Including First Evidence of Putative Leader Sequence Change

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Juvenile retinoschisis is an X-linked recessive disease caused by mutations in the XLRS1 gene. We screened 31 new unrelated patients and families for XLRS1 mutations in addition to previously reported mutations for 60 of our families (Retinoschisis Consortium, Hum Mol Genet 1998;7:1185–1192). Twenty-three different mutations including 12 novel ones were identified in 28 patients. Mutations identified in this study include 19 missense mutations, two nonsense mutations, one intragenic deletion, four microdeletions, one insertion, and one intronic sequence substitution that is likely to result in a splice site defect. Two novel mutations, c.38T→C (L13P) and c.667T→C (C223R), respectively, present the first genetic evidence for the functional significance of the putative leader peptide sequence and for the functional significance at the carboxyl terminal of the XLRS1 protein beyond the discoidin domain. Mutations in 25 of the families were localized to exons 4–6, emphasizing the critical functional significance of the discoidin domain of the XLRS1 protein. Hum Mutat 14:423–427, 1999.

KEY WORDS: **X-linked juvenile retinoschisis; RS1; novel mutations; leader sequence**

INTRODUCTION

X-linked juvenile retinoschisis (RS; MIM# 312700) [McKusick, 1994] is a recessive hereditary retinal degeneration that is one of the more common causes of juvenile macular degeneration in males [RS Consortium, 1998; Sieving, 1998]. Carrier females are rarely affected [George et al., 1995], although we observed three affected females with a homozygous mutation state from apparent consanguinity [Mendoza et al., 1999]. RS disease in males frequently presents early in life as cystic macular degeneration. The cystic cavities may coalesce and cause further central vision loss later in life. Histopathology indicates that splitting of the retina occurs through the superficial neural layers [Condon et al., 1986] and affects peripheral vision. The electroretinogram (ERG) often shows a characteristic b-wave reduction but with normal a-wave, suggesting a defect in Müller or bipolar cell function [Arden et al., 1988].

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The XLRS1 gene at Xp22.2 [Pawar et al., 1996; Van de Vosse et al., 1996; George et al., 1996; Huopaniemi et al., 1997] has six exons that encode a 224 amino acid protein [Sauer et al., 1997]. Mutations in the XLRS1 gene have been identified in RS patients [Sauer et al., 1997; RS Consortium, 1998; Hotta et al., 1998; Rodriguez et al., 1998; Huopaniemi et al., 1998; Mashima et al., 1999; Duval et al., 1999; Mendoza et al., 1999]. The gene transcript is identified specifically in the retina [Sauer et al., 1997] and in the photoreceptor cells [Reid et al., 1999]. The predicted protein sequence contains a highly conserved discoidin domain in the carboxyl terminal region encoded by exons 4–6 [Sauer et al., 1997; RS consortium, 1998], and a putative amino terminal signal peptide is encoded by exons 1–2. Although the functional role of the XLRS1 protein has not yet been elucidated, the evolutionarily conserved discoidin domain is implicated in cell–cell interactions in several other proteins [Springer et al., 1984; He and Tessier-Lavigne, 1997; Vogel et al., 1997; Soker et al., 1998; Baumgartner et al., 1998]. More than 93 different mutations in XLRS1 have been reported so far in RS families. Most are missense mutations located in the discoidin domain, indicating its functional significance. Here we describe the analysis of the XLRS1 gene in 31 families in whom we identified 23 different mutations, including 12 novel mutations not previously described.

MATERIALS AND METHODS

The clinical diagnosis of RS disease was made by ophthalmic examination that included fundoscopy, fluorescein angiography, and ERG recordings [Pawar et al., 1996; Sieving 1998]. Informed consent was obtained from participants in conjunction with blood samples obtained for genetic analysis. DNA was purified from lymphocytes using Puregene® DNA isolation kit (Gentra Systems, Minneapolis, MN). No female carriers in these RS families reported visual symptoms or visual impairment. All six exons of the XLRS1 gene were PCR-amplified using primers previously described. PCR products were purified by agarose gel electrophoresis and sequenced using a Thermosequanase radiolabeled terminator cycle sequencing reaction kit (Amersham, Arlington Hts., IL), and the same primers as in PCR amplification [Sauer et al., 1997]. Both the coding regions and intron–exon boundaries were read. The nature of the sequence changes in affected individuals were analyzed using the data on 100 normal chromosomes from unrelated individuals that we and the members of the RS Consortium tested as controls [RS Consortium, 1998]. Further, we have not observed the mutations described here as benign polymorphisms in about 161 more patients and unaffected family members that we have sequenced fully or partially (results not shown). Computational analysis of the mutations were carried out using programs in Protean (DNASTAR, Madison, WI) GCG (Genetics Computer Group, Madison, WI), and ExPASy Molecular Biology Server (www.expasy.ch/).

RESULTS AND DISCUSSION

DNA sequencing of the RS gene in 31 patients and their available family members not previously investigated [RS Consortium, 1998] identified 23 different mutations in 28 of these patients (Table 1, Fig. 1). Twelve of these mutations are novel. For three RS patients who show a characteristic clinical phenotype, no mutations were found in the six exons and intron–exon junctions, and these families are being analyzed further in the untranslated and regulatory regions of the gene.

Of the 23 different XLRS1 mutations (Table 1, Fig. 1), 14 are missense, two are nonsense, one is an intragenic deletion involving exon 1 and extending into the putative promoter region, four are microdeletions, one is an insertion and one is a single base substitution in an exon–intron junction sequence that is likely to result in a splice site defect. All but two mutations were in exons 4–6. Exons 1 and 3 each had one mutation. Mutations in exon 2 are rarely reported, and none were seen in these families. All the mutations associated with the disease were found only in the affected males and the related carrier females that were available for the study.

The 12 novel mutations included five missense changes, four deletions, one intragenic deletion, one insertion, and one splice site mutation. These novel mutations increase the total number of different RS mutations reported worldwide to at least 105 for now. The remaining 11 mutations in these families were among those previously reported [RS Consortium, 1998; Sauer et al., 1997; Hotta et al., 1998; Mashima et al., 1999], and all except for one nonsense change c.120C→A (C40X) were localized to the discoidin domain.

Several of the novel mutations are particularly interesting for understanding the possible functional significance. Missense mutation c.38T→C changes an amino acid residue (L13P) in the amino terminal part of the protein predicted to be the signal peptide encoded by exons 1–2 (amino acids 1–23).

TABLE 1. XLRS1 Gene Mutations in Patients With X-Linked Juvenile Retinoschisis

Exon/ IVS	Nucleotide change[1]	Effect on protein/ translation	Tested families/ sporadics	Geographic origin of patients	Novel/ reported[2]
1	c.1-52delExon1+promoter	No expression?	1	Sweden	Novel
1	c.38T→C	L13P	1	USA	Novel
3	c.120C→A	C40X	1	Sweden	Reported
4	c.194delA	Frame shift	1	USA	Novel
4	c.208G→A	G70S	2	USA	Reported
4	c.208G→C	G70A	1	USA	Novel
4	c.214G→C	E72K	1	USA	Reported
4	c.219delA	Frameshift	1	USA	Novel
4	c.221G→T	G74V	1	USA	Reported
4	c.223G→T	E75X	1	USA	Reported
4	c.253-255delAAC	N85del	1	USA	Novel
4	c.286T→C	W96R	2	USA	Reported
4	c.305G→A	R102Q	4	2 USA, Belgium S. Africa	Reported
5	c.371-374delAGAT	Frameshift	1	USA	Novel
5	c.418G→A	G140R	1	USA	Reported
5	c.426T→G	C142W	1	Saudi Arabia	Novel
5	c.489G→T	W163C	1	Sweden	Novel
IVS5	c.522+5G→A	Splice variant?	1	USA	Novel
6	c.549-550ins2C	Frameshift	1	USA	Novel
6	c.574C→T	P192S	1	Sweden	Reported
6	c.598C→T	R200C	1	USA	Reported
6	c.599G→A	R200H	1	Belgium	Reported
6	c.667T→C	C223R	1	USA	Novel

[1]Mutation descriptions follows the standard nomenclature [Antonarakis, 1998] and the XLRS1 cDNA (GenBank AF014459) was used as a reference according to Sauer et al. [1997] and the RS Consortium, 1998.
[2]These and the other mutations previously reported from Kellogg Eye Center and other laboratories could be found on the Retinoschsis DB at http//www.dmd.nl/rs/rshome.html

This mutation is the first reported genetic indication of a functional role played by the amino terminal part of the protein. The disease association with this change inside the signal peptide sequence indicates a critical role for this secretory motif in the biological expression of XLRS1 protein function. The substitution of a new proline residue in this sequence is expected to disrupt the folding of the signal peptide domain (see below). The only mutations previously reported in this region involved the initiation codon, and since these probably terminate translation [RS Consortium, 1998], they do not provide any information about the possible significance of this region in the XLRS1 protein structure and/or function.

The XLRS1 discoidin domain, which extends from amino acids 63–219 [RS Consortium, 1998], has been considered critical for XLRS1 function since many missense mutations are found within the discoidin domain and are associated with the disease. Two of the novel missense mutations c.426T→G (C142W) and c.489G→T (W163C) eliminate or create a cysteine residue, respectively. These two mutations inside the discoidin domain are expected to severely affect the tertiary structure of the protein, as predicted for previously re-

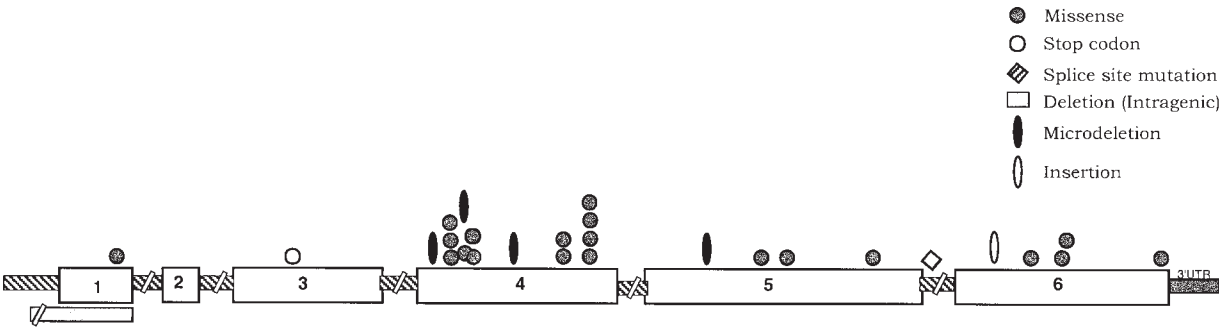

FIGURE 1. Mutations in XLRS1 gene. Schematic diagram of the XLRS1 gene with exons (1–6) and splice site junctions showing the distribution of the newly identified XLRS1 mutations in 28 patients and families (see details in Table 1 and the text).

ported mutations involving cysteine residues [RS Consortium, 1998].

No mutations have thus far been reported for the five residues (amino acids 220–224) at the carboxy terminal end that are just beyond the conserved discoidin domain. Here we report for the first time a novel mutation, c.667T→C, that eliminates a cysteine (C223R) residue on the carboxyl terminal side of the discoidin domain. This cysteine at 223 is not observed in any of the other 28 known discoidin domain-containing proteins [RS Consortium, 1998]. By its disease association, C223 seems to play a critical role in the functional or structural integrity of the XLRS1 protein. C223R is the most carboxyl terminal amino acid change that has been associated with RS pathology. It is not yet clear whether the five residues in the extreme carboxyl terminal region of the protein are an integral part of the discoidin domain or whether they contribute in some other fashion to XLRS1 protein interactions.

A novel in-frame deletion, c.253-255delAAC, eliminates the amino acid residue N85 of XLRS1 in one family. The amino acid sequence alignments made based on conserved structural motifs of 28 different discoidin domains indicate that the residue corresponding to N85 of the XLRS1 protein is preserved during evolution, although in most cases it is a serine [Baumgartner et al., 1998; RS Consortium, 1998]. No deletion at this position has been observed except in coagulation factor V discoidin domain 1 and for which there seems to be a difference of opinion [Baumgartner et al., 1998; RS Consortium, 1998]. Further, no missense changes have so far been reported that alter N85 in RS families. These observations suggest that, indeed, the residue at this position is critical for structure/function of the protein and is consistent with the deleterious effect of N85del in XLRS1 protein. The shortened peptide could affect the proper protein folding, leading to an inactive product.

The novel missense mutation c.208G→C (G70A) affects the same amino acid residue as the c.208G→A (G70S) mutation found in two other families in this study (Table 1). These mutations show an interesting distribution. We previously reported c.208G→A mutations in eight other apparently unrelated RS families, all of USA origin [RS Consortium, 1998]. However, the possibility of a distant commonality of their origin has not yet been ruled out. Similarly, the XLRS1 missense mutation c.286T→C (W96R) reported here in two USA families (Table 1) and previously in seven other RS families [RS Consortium, 1998] of USA origin. The only other report of c.286T→C is a single case from Germany [Sauer et al., 1997].

The mutation c.522+5G→A is predicted to cause a splice site defect. The estimated frequency for G at +5 position in the consensus splice site is 0.84, as compared to 1.0 for +1G, making it a significantly critical base in the precursor mRNA splicing process [Padget et al., 1986; Shapiro and Senapathy, 1987]. A splice site "consensus value" or the score [Shapiro and Senapathy, 1987; Krawczak et al., 1992], calculated using an on-line splice site prediction program by neural network (http://www-hgc.lbl.gov/projects/splice), for the mutated splice site was 0.79, compared to 1.0 for the normal XLRS1 splice site. A base change at +5G in the donor splice site has been implicated to reduce significantly the stability of the base pairing of the splice site with complementary region of U-Sn RNA [Cooper et al., 1995]. Other novel RS-causing lesions include two single nucleotide deletions (c.194delA and c.219delA), a 4 bp deletion (c.371-374delAGAT) and a 2 bp insertion (c.549-550ins2C). All of these either shift the translation frame and either cause premature termination or extend the translation product by several amino acids.

The missense mutation c.214G→A (E72K) is the most common RS mutation known to date and has been found in more than 40 families reported thus far [RS Consortium, 1998], but it is present in only a single family in this study. The c.214G→A is in a CpG mutation hot spot, and the prevalence of this RS mutation suggests that it may have multiple origins [RS Consortium, 1998]. In the present study, mutations in the other reported CpG hot spot include one c.574T→C (P192S) and four cases of c.305G→A (R102Q). c.305G→A was previously identified in 15 other families.

A computer analysis was used to explore possible effects of different mutations on the XLRS1 peptide secondary structure. Chou-Fasman and Garnier-Robson algorithms were used for predicting putative alpha and beta regions and regions of turns and coils. Amphipathic and flexible regions of the peptide were predicted using the algorithms of Eisenberg and Karplus-Schultz. Most of the missense mutations described above seem to induce only mild to moderate deviations from the predicted protein secondary structure, and these changes were often confined to the region around the mutation site. The novel mutation c.38T→C (L13P), however, is predicted to induce more severe changes in several of the structural parameters mentioned above. In contrast, the c.253-

255delAAC (N85del) mutation that shortens the peptide by one residue deviates little from the normal peptide in the predicted secondary structural features and we cannot predict why this results in disease. Characterization of additional mutations in RS may be beneficial in establishing RS genetic diagnostics, genotype–phenotype correlations and in furthering the understanding of the pathophysiology of this disease.

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