

Mutational Screening of *RS1* gene in X-linked Retinoschisis in an South Indian Family

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Abstract

Purpose: To screen *XLRS1* gene for genetic mutations and describe the ocular phenotypes in an X-Linked retinoschisis family.

Methods: Ophthalmic examination, including best-corrected visual acuity, fundus photography, Ocular Coherence Tomography/Scanning Laser Ophthalmoscopy (OCT/SLO) and full-field Electroretinography (ERG) was performed in the family. Pedigree was obtained and peripheral blood was collected from the patient, unaffected husband and two children. All six exons of the *XLRS1* gene were amplified and screened for mutations by DNA sequencing.

Results: Thinning of macula was detected from both eyes. ERG revealed reduced rod response to dim white light. There was no history of consanguinity in family. Molecular analysis of *XLRS1* gene didn't reveal any mutation. There were no disease causing mutation in the exons or in other flanking introns of *XLRS1* and neither any single nucleotide polymorphisms (SNP's) were found in the disease family in exons or exon/intron boundaries of the *RS1* gene.

Conclusion: Our study show a case of isolated juvenile retinoschisis presented as bilateral macular atrophy in a female. Mutation screening did not detect any mutation in the *XLRS1* gene. Although juvenile retinoschisis is commonly

seen in males, females can also present in middle ages with isolated macular atrophy. The distribution of mutations in *RS1* gene was different to that reported in the literature.

Keywords: *XLRS1* gene, Juvenile Retinoschisis, DNA Sequencing, mutation, macular atrophy

Introduction

X-linked juvenile retinoschisis is the most common cause of macular dystrophy in males with an incidence ranging between 1: 5000 and 1: 25000 [1] [2-4]. Although juvenile retinoschisis is commonly seen in males, females can also present in middle ages with isolated macular atrophy. A full-field electro-retinogram can be diagnostic in such cases.

Juvenile retinoschisis is a hereditary retinal degenerative condition. The gene is on the distal short arm of X chromosome Xp22 [5]. Foveal schisis is present in almost all cases of X-linked retinoschisis [6].

The diagnosis is by clinical assessment and affected individuals show a significant loss in central and in some cases peripheral vision [7]. The patients have characteristic foveal schisis consisting of folds radiating outwards from the fovea in a stellate pattern containing microcystic schisis cavities [8]. The most important complications associated are vitreous hemorrhage, retinal detachment and neovascular glaucoma [2, 7, 9].

The *XLRS1* gene that causes X-linked Retinoschisis (XLRS) was identified in 1997 by positional cloning [5]. *XLRS1* consists of six exons and encodes a secretable extracellular adhesion protein called retinoschisin comprising of 224 amino acids. It is primarily present in photoreceptors and bipolar cells and is known to stabilize the organization of the retina by interacting with the surface of both photoreceptors and bipolar cells.

Here, we report a case of an isolated juvenile retinoschisis presented as bilateral macular atrophy in a female. Molecular analysis of the DNA of the patient did not have any mutation in the *XLRS1* gene.

Methodology

Clinical Case study report

A 50-year-old female presented with complaints of progressive diminution of vision in both eyes since 12 years. She was neither born to consanguineous parents nor married consanguineously. There had no relevant positive family history of any inherited retinal disorders. Her past medical and drug history were insignificant. General examination of the skin was normal. Best-Corrected Visual Acuity was 6/60 and 3/60 in the right and left eyes, respectively. Colour vision assessed by Hardy-Rand-Rittler charts showed severe unclassified trichanomaly. Fundus evaluation was normal except for dull macula in both eyes. The Optical Coherence Tomography/Scanning Laser Ophthalmoscope (OCT/SLO) showed thinned out

macula for both eyes with no evidence of retinoschisis. Full-field Electroretinogram (ERG) showed 60% reduction in rod response to dim white light stimulus, electronegative ERG to combined maximal stimulus and reduced cone bipolar cell response.

Genetic and Molecular Analysis Report

Pedigree was obtained from the family till third generation (Fig.1). In order to proceed with molecular analysis 8 ml of peripheral blood was collected in K2 EDTA Vacutainer (BD Biosciences) from affected female, her husband and their children with a prior-consent. Genomic DNA was extracted from the leucocytes according to the manufacturer's instructions Machery & Nagel Maxi kit Germany). Extracted DNA samples were stored at -20 °C freezer. For mutation screening all exons and the flanking intronic regions of *XLRS1* were amplified by the polymerase chain reaction (PCR) using standard primers (Table-1).

Polymerase chain reaction (PCR) was performed using genomic DNA in an Applied Biosystems 9700 thermal cycler. The reaction volume of 50 µl contained the following: 5 x Effi Taq reaction buffer (Bangalore Genei, India) 25 pmoles of each primer (MWG Biotech, Germany), 0.2 Mm each dNTP (Bangalore Genei, India) and 3 U Effi taq polymerase (Bangalore Genei, India). To the master mix 50 ng of DNA template was added before starting the PCR programme. PCR program for the *XLRS1* gene was as follows:

Pre – denaturation at 95 °c for 20 mins, followed by 35 cycles of denaturation at 94 ° C for 1 min, annealing at the appropriate temperature for 1 min, and 1 min elongation at 72 ° C. The final extension was performed at 72 ° c for 10 mins.

Table 1: Primer sequences, product size and Annealing temperatures of *XLRS1* gene. F and R denote, Forward/Sense primers and Reverse/Anti-sense primers, respectively. bp: base pair, Tm: melting temperature (Annealing temperature).

Exons	Primers (5' – 3')	Product size (bp)	Tm ° C
1	F: CTC AGC CAA AGA CCT AAG AAC R: GTA TGC AAT GAA TGT CAA TGG	216	58
2	F: GTG ATG CTG TTG GAT TTC TC R: CAA AGT GAT AGT CCT CTA TG	176	56
3	F: CGA TGC ATA AGG ACT GAG TGT GAT C R: GCA TTA ACA TAG GCT TAC TAA TAG	377	50
4	F: CGT GAG TAG TGA ACC GTT GAA GAC R: ACG CTG GTA GAG AGG CCT AT	381	50
5	F: GCA AGT TAA GTA TAA CGG AAG CTG G R: GGA AAG CGC AGA TGA TCC ACT GTG	508	50
6	F: GCA AAC TGC TTT AAC TAC TTC C R: CCA GCA CTG CAG TTA CAA TTG C	427	50

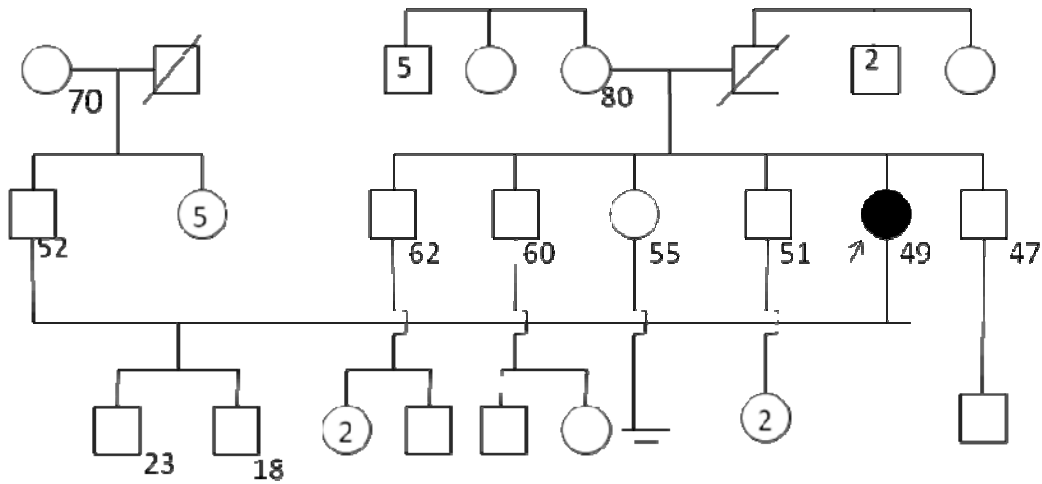


Figure 1: Pedigree of the family affected with X – linked Juvenile retinoschisis. Open circle, disease free female; black circle, female with disease; Open square, disease free male.

The amplicons were visualized on 2 % agarose gel incorporated with ethidium bromide (Fig.2). The gels were documented using Gel documentation system (Vilbert Lourmet).

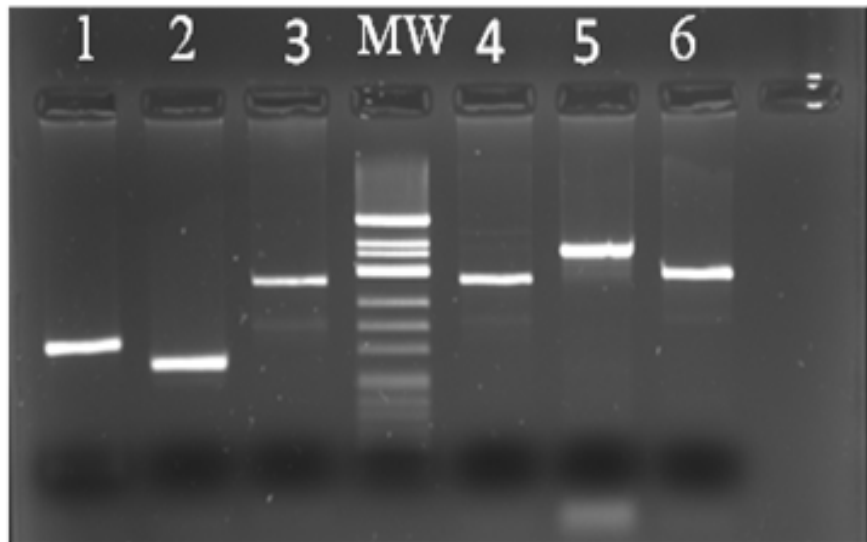


Figure 2: 2% Agarose gel showing PCR Products of all six exons of RS1 Gene. Lane-1: Exon 1 – 216 bp; Lane-2: Exon 2-176 bp; Lane-3: Exon 3-377 bp; Lane-4: Exon 4-381 bp; Lane-5: Exon 5-508 bp; Lane-6: Exon 6 – 427 bp

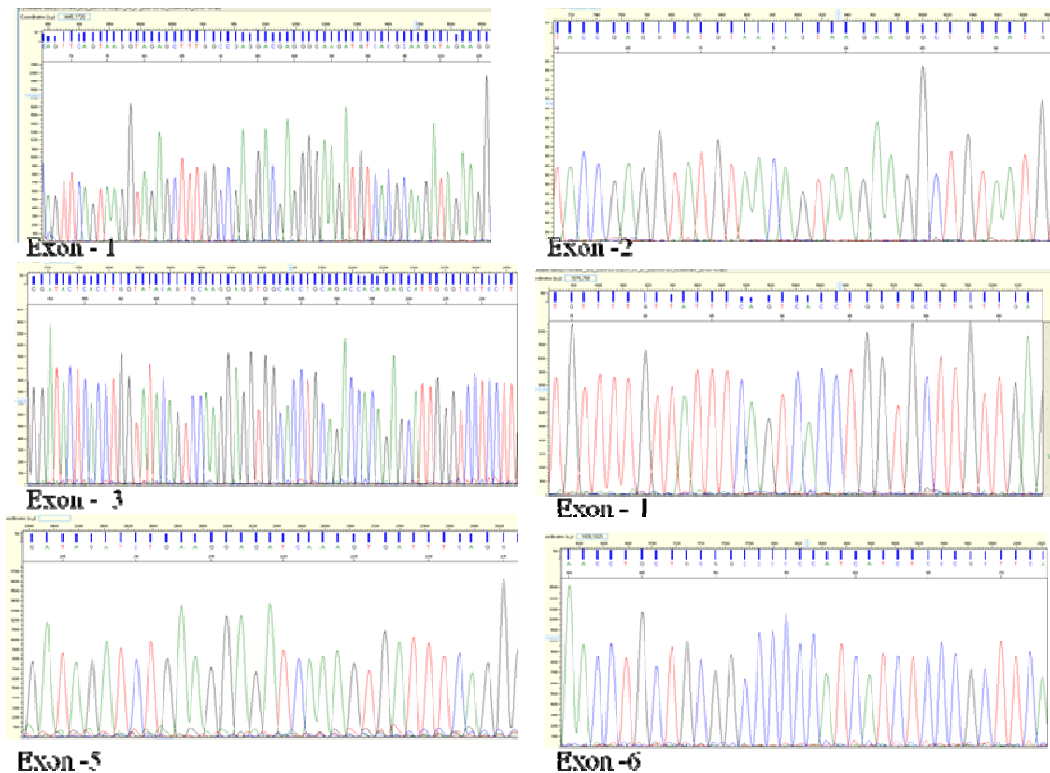


Figure 3: Normal Electropherogram of all exons of *RS1* Gene.

Polymerase chain reaction (PCR) amplified DNA of *XLR51* gene were sequenced using both the sense and anti-sense primers by direct nucleotide sequencing. The Big Dye Terminator cycle sequencing was run on an automated sequencer (ABI Prism 310 Genetic Analyzer, Applied Biosystems).

The results were compared with the reference sequence from X-linked retinoschisis sequence database (ENSEMBL Transcript ID ENST 00000379984). Analysis of the detailed sequence after obtaining from the sequencer was done by manual screening and comparison with the existing sequence of *XLR51* gene.

Results

Full-field Electroretinogram (ERG) showed 60% reduction in rod response to dim white light stimulus, electronegative ERG to combined maximal stimulus and reduced cone bipolar cell response. The outcome of the clinical investigations lead to conclude that an isolated juvenile retinoschisis be established.

We compared the sequence results of both sense and anti-sense primers with the reference sequence (ENSEMBL Transcript ID ENST 00000379984) and found no disease causing mutation in the exons or in other flanking introns of *XLR51*. There were no SNPs found in the disease family in or near the *XLR51* gene locus.

Discussion

Juvenile retinoschisis is a progressive bilateral X-linked retinal degeneration [10]. The progression of the disease is rapid in childhood and then it slows down to become stable by the age of 20. Further loss of vision may happen in the fourth or fifth decade due to macular atrophy. Although, almost exclusively seen in males, there are reports of autosomal dominant [11] and recessive [12] modes of inheritance in females. Isolated foveal retinoschisis [13] has also been reported in females.

Mutations of the *XLRS1* gene are known to cause many cases of inherited and sporadic XLRs. Over 150 *XLRS1* gene mutations have been identified in different XLRs patients to date [1]. Around 75% of the known mutations affect the discoidin domain of the protein [1, 14]. Exons 4-6 of *XLRS1* gene encodes for the discoidin domain and is highly conserved throughout evolution providing a strong correlation of the vitality of the domain and its evolutionarily conserved property.

In our studied family no mutations could be identified despite sequencing both the complete gene coding and immediate flanking intronic regions. In our case study one possible explanation for not detecting any disease causing mutation in *XLRS1* gene could be attributed to misdiagnosis. Lack of stringent and disease specific clinical findings would explain a plausible misdiagnosis of the disease condition. Our finding of an affected female did not categorize in the classical classification of retinoschisis where the disease is transmitted in X-linked, autosomal dominant and autosomal recessive pattern in females. Here, we have addressed a case where the affected female is most likely a X-linked carrier. Unfortunately, we could not collect the sample from proband parents for sequencing the gene of interest but sequencing *XLRS1* gene in her kids revealed a normal electro-chromatogram. Lack of carriers in the pedigree contradict the argument against the disease being transmitted in this pedigree in the X-linked form, most likely the disease phenotype in the proband could be an outcome of sporadic mutation in other genes instead of *RS1*. A large size study would most likely be needed to identify the mutations in the *XLRS1* gene and to correlate with the phenotype and genotype.

Conclusion

The study can be concluded with the impression that a subject visited our ophthalmic clinic and presented a case of isolated juvenile retinoschisis presented as bilateral macular atrophy in a female. Retinoschisis disease causing mutation in *XLRS1* gene could not be detected on extensive mutational screening efforts. Although juvenile retinoschisis is commonly seen in males, females can also present in middle ages with isolated macular atrophy.

Recommendations

Our mutation screening protocol didn't reveal any mutations in the *XLRS1* gene. This is a report of an isolated case and hence, might restrict the mutation detection process on population basis. Hence, a study with higher sample size would be ideal for detecting not only existing disease causing mutation as well identifying novel

mutations of *XLRS1* gene. However, the distribution of mutations in *RS1* gene was different in our study to that reported in the literature.

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