Original Article

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Mutations in the *RS1* gene in a Chinese family with X-linked juvenile retinoschisis

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Summary **The purpose of our study was to identify the mutations in the retinoschisis 1 (*RS1*) gene, which was associated with X-linked retinoschisis (XLRS) in a four-generation Chinese family, and to provide the theoretical basis for gene diagnosis and gene therapy. Genomic DNA was extracted from peripheral leukocytes. All six exons and flanking intronic regions were amplified by polymerase chain reaction (PCR), followed by direct sequencing. Through our genetic analysis, one frameshift 573delG mutation was identified in the patients of this four-generation pedigree; however, this mutation was absent in normal or non-carrier subjects. In conclusion, this 573delG mutation is reported in the Chinese population for the first time. This mutation widens the mutational spectrum of *RS1* in Asians. Identification of mutations in the *RS1* gene and expanded information on clinical manifestations will facilitate early diagnosis, appropriate early therapy, and genetic counseling regarding the prognosis of XLRS.**

Keywords: X-linked juvenile retinoschisis, polymerase chain reaction, frameshift mutation, foveal schisis

1. Introduction

X-linked retinoschisis (XLRS) is one of the most common causes of juvenile macular degeneration in males, with an incidence of 1 in 15,000 to 1 in 30,000 (*1*). The major clinical characteristics of affected males are visual deterioration, foveal-schisis in almost every patient due to the splitting of the retinal cell layers, and a decrease in the b-wave amplitude of the electroretinogram (ERG). Unfortunately, there is no medication to halt the progression of maculopathy, which may induce complete blindness.

XLRS, a recessively inherited disorder, is caused by mutations in the retinoschisis 1 (*RS1*) gene localized at chromosome Xp22.2. The *RS1* gene consists of six exons, and encodes a 224 amino-acid protein named retinoschisin. This protein, has an N-terminal secretory leader peptide and a conservative discoidin domain encoded in exons four to six, and is expressed and secreted from photoreceptors and bipolar cells, where this protein is anchored to the surface of the secreting cells themselves. This protein is presumed to be responsible for normal adhesion and signaling within the retinal cell layers (*2,3*).

On the basis of previous genetic examinations, a wide spectrum of different mutations in *RS1* have been recorded in the Leiden Open Variation Database, majority of these reported mutations are missense mutations, which primarily exist within exons 4-6 of *RS1* (*4*). Exons 1-3 tend to have mainly translation truncating nonsense mutations (*5*). However, the precise correlation between the phenotype and genotype of XLRS still remains unclear.

In our study, we encountered a large XLRS Chinese family where one family member had already lost his sight completely. In the current study, we aimed to identify the molecular variations of XLRS in this Chinese family. We detected the mutations in the *RS1* gene by direct sequencing.

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2. Materials and Methods

2.1. *Patients and controls*

The study protocol, which was carried out in accordance with the tenets of the Declaration of Helsinki, was approved by the ethical committee of Henan provincial people's Hospital. Twenty-four members of the family with XLRS and 40 eligible volunteers as normal controls were recruited for this research. Family history was collected from the proband. Comprehensive ophthalmic examinations included best-corrected visual acuity (BCVA), ERG, optical coherence tomography (OCT), fluorescein angiogram, slit lamp biomicrocopy, and fundus examinations, in order to confirm the diagnosis. Informed consent was obtained from all participants prior to their evaluations in the course of the study.

2.2. *Blood collection and DNA extraction*

For each subject, venous blood samples (5 mL) were collected into tubes containing EDTA and genomic DNA was extracted using the DNA blood isolation kit. For all probands, exons 1-6 of *RS1* PCR was carried out to amplify the exonic regions of *RS1*, using the primers listed in Table 1. The PCR reaction was performed in a DNA thermocycler (Eppendorf, Hamburg, Germany). The reaction was performed in a total volume of 25 μL containing 2.5 μL 10× buffer (25 mM $MgCl_2$), 0.2 μM each of dNTP, 0.5 μM of each primer, 1 units of Taq DNA polymerase, and 100 ng of genomic DNA. The cycling procedure for the *RS1* gene were as follows: 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30 sec, annealing for 30 sec at the appropriate temperature for each primer pair, and extension at 72°C for 30 sec. The final extension step was lengthened to 5 min.

2.3. *Direct sequencing*

All amplified fragments were electrophoresed in a 1.5% agarose gel and purified with a DNA extraction kit. Exons and the flanking intronic regions of *RS1* were directly sequenced on an automated sequencer (ABI prism 3130 Genetic Analyzer, Applied Biosystems, CA, USA). The results were compared with the reference sequence of the XLRS sequence variation database.

3. Results

3.1. *Clinical findings*

The pedigree of interest was a four generation family with twenty-four family members, including four affected males and twenty unaffected individuals based on clinical evaluations (Figure 1). An X-linked pattern of inheritance of disease was shown in the family pedigree. However, a high degree of clinical variability is observed among the patients. The mean age at disease onset was 7.5 years, and the initial clinical presentation was poor visual acuity. The clinical characteristic of the proband was mainly bilateral foveal schisis and no peripheral schisis. The grandfather of the proband (I_1, Figure 1), therefore, was identified with a more severe clinical manifestation. The patient had typical foveal and peripheral schisis in both eyes, combined with neovascular glaucoma in the right eye, which required surgical intervention. The age of onset, visual acuity, and peripheral and macular involvements for each affected individual are described in Table 2.

Figure 1 depicts the pedigree of the Chinese family with X-linked juvenile retinoschisis. Squares indicate males, and circles indicate females. Dark-shaded boxes represent affected subjects with X-linked juvenile retinoschisis, while non-shaded boxes and circles mark unaffected family members. Circles with a dot denote

Table 1. Sequence of primers used to amplify the coding regions of the *RS1* gene

Exon	Size of exon (bp)	Size of amplified fragment (bp)	Primer (5'-3')	Annealing temperature (°C)
1	52	216	F-ctcagccaaagacctaag R-gtatgcaatgaatgtcaatgg	58
2	26	175	F-gtgatgctgttggatttctc R-caaagtgatagtcctctatg	56
3	106	177	F-ctgccctgcctctctggttg R-ggtgcttgttgagtattgag	60
4	142	219	F-ggtgcttgttgagtattgag R-aaaatccccgggccctgc	56
5	196	310	F-gagagccgacacctgcgg R-gggtgcgagctgaagttgg	65
6	153	412	F-cccgatgtgatggtgacagg R-ctttgttctgactttctctggc	62

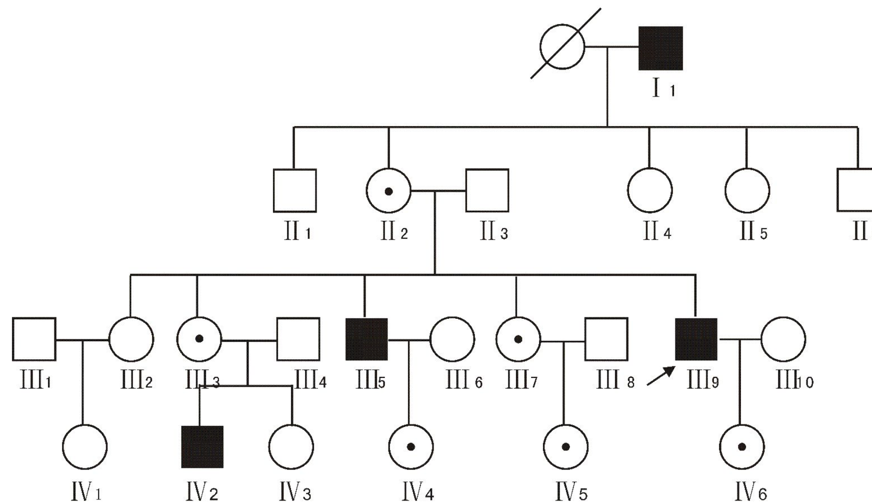

Figure 1. Pedigree of the Chinese family with X-linked juvenile retinoschisis.

Table 2. Clinical features of four affected males in the Chinese pedigree with XLRS

Patient ID	Age at onset (years)	Symptom	Visual acuity		Foveal schisis	Peripheral schisis	Ocular complications
			Right	Left			
I$_1$	12	Poor VA	Enucleation	LP	Yes	Yes	Neovescular glaucoma
III$_5$	6	Poor VA	0.15	FC	Yes	No	Not found
III$_9$	7	Poor VA	0.1	0.3	Yes	No	Not found
IV$_2$	5	Poor VA	0.2	0.2	No	Yes	Not found

LP, light perception; VA, visual acuity; FC, finger counting.

obligate carriers. A slashed circle indicates a deceased family member. The arrow points to the proband (III$_9$, Figure 1).

3.2. *Genetic analysis*

All exons and the flanking sequence of the *RS1* gene were screened for mutation detection in the family. A frameshift mutation at exon 6 due to a G deletion at the 573 base position was identified by direct sequencing of the PCR products in the proband (Figure 2B). All other exons were found to be normal. The proband's mother (II$_2$, Figure 1), who was clinically normal, showed a heterozygous frameshift mutation (Figure 2C). In addition, this mutation was also detected in the other three affected males (I$_1$, II$_5$, and IV$_2$; Figure 1), and the other five female carriers were found to have a heterozygous condition (II$_2$, III$_3$, III$_7$, IV$_4$, IV$_5$, and IV$_6$; Figure 1). All the remaining family members and the matched controls showed a normal sequence (Figure 2A).

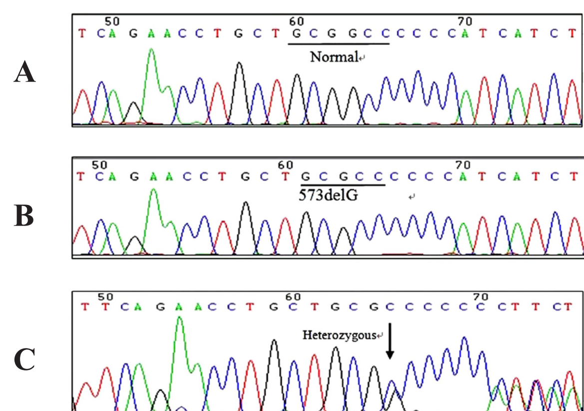

Figure 2. Photograph of DNA sequence of the mutation. (**A**) Normal control; (**B**) DNA sequencing shows a frameshift mutation at exon 6 due to a G deletion at the 573 base position in the proband and the other 6 affected males; (**C**) Heterozygous mutation of the site mentioned above in female carriers.

4. Discussion

XLRS is an X-linked macular disorder caused by mutations in the *RS1* gene. Identification of these mutations is becoming increasingly important in a variety of clinical settings. To the best of our knowledge, over 150 mutations have been recognized (*6*). To gain insight into the genetic mechanisms and pathogenesis of XLRS, we investigated a large Chinese family with XLRS.

Through our thorough clinical examinations and genetic analysis, all four affected males were identified with a single base (G) deletion at the 573

base position (573delG) in exon 6. Moreover, the other three female carriers were found to have a heterozygous condition, while other members of the family and all the healthy controls did not display this mutation. Considering that female carriers are unaffected and the matched controls are not detected to have this mutation, it seems that the absence of a functional *RS1* gene was responsible for retinoschisis in affected males in this family and that the disease was transmitted as an X-linked recessive trait (*7*).

Consequently, this 573delG mutation might shift the open reading frame, resulting in the C-terminal containing a novel set of forty-two amino acids, eleven amino acids longer than the normal protein. It is hypothesized that the activity of this mutant protein may be reduced or completely lost, which would disrupt the signaling pathway and thus lead to destabilization of retinal cell organization and structure (*8*). To date, this is the first report of the 573delG mutation in the Chinese population, which widens the mutational spectrum of *RS1* and is expected to help with diagnosis of this rare genetic disease XLRS in Asians. This identified mutation is present in the hotspot region at amino acid position 192, belonging to the highly conserved discoidin motif of retinoschisin protein, which supports the notion that this domain has functional significance in cell-to-cell adhesion (*9*). Our results are in agreement with previous findings reported in an Australia population by Hewitt *et al*. (*10*).

Although the same frameshift mutation was identified in all four affected males in this family, clinical manifestation of patients within the family exhibited remarkable phenotypic variability, ranging from foveal stellate cystic change to severe bilateral peripheral retinoschisis and highly elevated bullous retinoschisis with vitreous hemorrhage, as well as great variation in age at onset and progression. However, a few studies implied the possibility of a genotype-phenotype relationship. Phenotypes of XLRS were more severe in cases associated with upstream mutations (exons 1-3) in the *RS1* gene. Li *et al*. also reported that the severity of the phenotypes are more likely to relate to the particular mutation types, such as frameshift, splice site, or some missense mutations (*11*). Comparison of our findings with these previously reported patients of other ethnicities, together with the clinical findings within the reported Chinese family in the current study, presumably reflects that there is no simple phenotype-genotype correlation and that disease severity is not mutation dependent only in XLRS. It is therefore presumed that additional factors, perhaps other genetic influences or unique environmental factors might act as contributors to disease severity (*12-14*). More investigations on the possibility of a genotype-phenotype correlation and factors causing phenotypic variation are still needed.

In summary, we identified a frameshift deletion 573delG mutation in the Chinese population for the first time. These findings will provide precise diagnosis when a clinical presentation is uncertain early in the course of the disease, as well as confirmation for genetic counseling and prenatal diagnosis for carriers without any symptoms. Elucidation of the mutation spectrum and phenotypic variability in XLRS is still in progress to better understand the pathogenesis of the disease and to facilitate *RS1* gene replacement therapy.

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