

# R102W mutation in the *RS1* gene responsible for retinoschisis and recurrent glaucoma

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## Abstract

• **AIM:** To identify the mutations in *RS1* gene associated with typical phenotype of X-linked juvenile retinoschisis (XLRS) and a rare condition of concomitant glaucoma.

• **METHODS:** Complete ophthalmic examinations were performed in the proband. The coding regions of the *RS1* gene that encode retinoschisin were amplified by polymerase chain reaction and directly sequenced.

• **RESULTS:** The proband showed a typical phenotype of XLRS with large peripheral retinal schisis in both eyes, involving the macula and combined with foveal cystic change, reducing visual acuity. A typical phenotype of recurrent glaucoma with high intraocular pressure (IOP) and reduced visual field was also demonstrated with the patient. Mutation analysis of *RS1* gene revealed R102W (c.304C>T) mutations in the affected male, and his mother was proved to be a carrier with the causative mutation and another synonymous polymorphism (c.576C>CT).

• **CONCLUSION:** We identified the genetic variations of a Chinese family with typical phenotype of XLRS and glaucoma. The severe XLRS phenotypes associated with R102W mutations reveal that the mutation determines a notable alteration in the function of the retinoschisin protein. Identification of the disease-causing mutation is beneficial for future clinical references.

• **KEYWORDS:** X-linked retinoschisis; glaucoma; *RS1* gene; mutation

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## INTRODUCTION

X-linked juvenile retinoschisis (XLRS; OMIM 312700) is one of the most common genetic causes of macular degeneration in males and is an X-linked recessive inherited disorder with an estimated the worldwide prevalence of 1 in 15 000 to 1 in 25 000. It is characterized by a splitting of the inner layers of the neurosensory retina and microcystic-like changes of the macular region, leading to reduced visual acuity early in life. Lesions in the peripheral retina have been observed in nearly half of the cases [1]. The clinical course generally causes a moderate decrease of visual acuity, but complications of severe cases include full-thickness retinal detachment, vitreous hemorrhage and rarely neovascular glaucoma, which may induce severe loss of vision [2,3]. The full-field electroretinogram typically shows a normal or sub-normal a-wave with a reduced amplitude b-wave originating from inner retinal cell activity [4]. Heterozygous female carriers rarely have visual morbidity, while females who are homozygous for an *RS1* mutation show the similar phenotypes to affected males [5,6].

The disease-causing retinoschisin 1 (*RS1*) gene localizes at chromosome Xp22.1 identified by positional cloning in 1997 [7]. It is the only gene known to be responsible for XLRS to date. The *RS1* gene contains six exons that encode a 224 amino acid extracellular adhesion protein called retinoschisin, which is secreted from photoreceptor and bipolar cells [8]. The protein contains a highly conserved discoidin domain which is encoded by exon 4 to exon 6. Though the function of retinoschisin is unknown, previous studies have revealed it may be involved in cellular adhesion and cell-cell interactions in retina and plays a role in retinal development [9].

So far more than 189 disease-causing mutations in *RS1* gene have been reported ([http://grenada.lumc.nl/LOVD2/eye/home.php?select\\_db=RS1](http://grenada.lumc.nl/LOVD2/eye/home.php?select_db=RS1)), including missense/nonsense

mutations, deletions, insertions, and splice site mutations. The majority of these are missense mutations in exons 4 to 6 and most of them are reported in Western populations. Furthermore, few studies have elucidated the genotype-phenotype correlation of XLRS. In this study, we report molecular and clinical findings in a Chinese XLRS family with concomitant glaucoma.

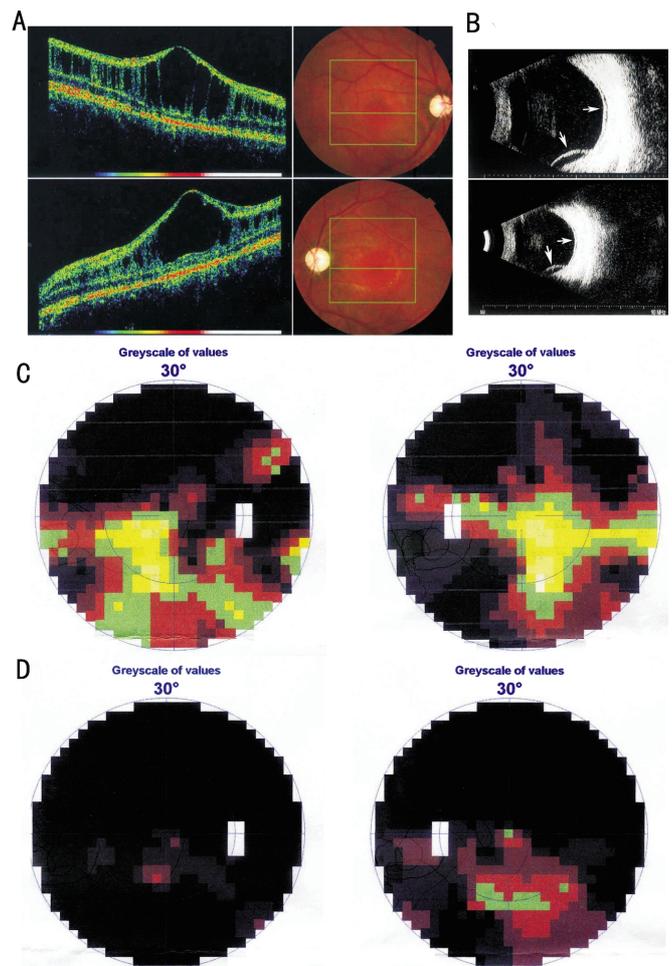
**MATERIALS AND METHODS**

**Proband Patient and Clinical Examinations** An 11-year boy with a complaint of blurred vision visited this facility 10 years ago and was diagnosed with juvenile retinoschisis. The patient was followed up longitudinally with a comprehensive ophthalmic examinations, including fundoscopy, ultrasonography, optical coherence tomography (OCT), fluorescein angiography (FA) and Goldman or automated perimetry. The parents were also examined with regular eye examinations.

**Molecular Screening** Informed consent and study protocol were obtained from the participants and local Ethics Committee. Genomic DNA was extracted from peripheral leukocytes using a genomic DNA extraction kit (RelaxGene Blood DNA System, TIANGEN Biotech, Beijing, China) according to the manufacturer's protocol. The six exons of the *RS1* gene were amplified by polymerase chain reaction (PCR) with previously reported primers [1], including intron-exon junctions. The PCR products were electrophoresed in a 1.5% agarose gel and purified with a PCR Purification Kit (AxyPrep PCR Cleanup Kit, Hangzhou, China). The PCR products were directly sequenced on an automated sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems). Sequencing results were compared with an *RS1* reference sequence (GeneBank No: NM\_000330).

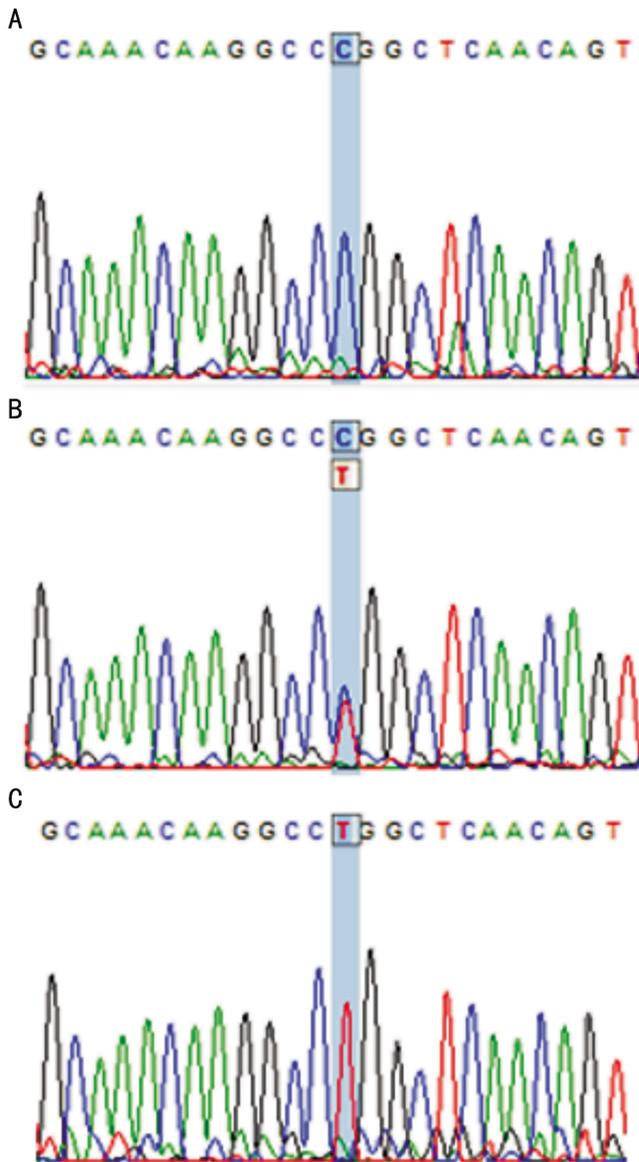
**RESULTS**

**Clinical Features and Longitudinally Follow-up** The patient (aged 11) was found significant retinoschisis in the left eye and mild interlayer schisis in the right eye at the first visit based on the examinations of fundoscopy and OCT (Figure 1A). A 1-3mm detachment line was revealed in the left eye, detected by B-ultrasonography (Figure 1B). High intraocular pressure (IOP) was found in both eyes and fundoscopic examination revealed typical sign of optic nerve atrophy at the age of 12. Ocular hypotensive agents were administrated to lower the IOP thereafter. The right eye developed severe macular schisis similar as that in the left eye when the patient was 14-year-old. The IOP increased to 40mmHg bilaterally and perimetry revealed significant visual field defect (Figure 1C, 1D). The patient undertook iridectomy and trabeculectomy sequentially to reduce IOP in both eyes. Because the anterior chamber was shallow and IOP remained high in the right eye, ultrasonic phacoemulsification and intraocular lens implantation were undertaken and successfully reduced the IOP.



**Figure 1 Fundus photographs, optical coherence tomography, ultrasonography and visual field images of the proband** A: Optical coherence tomography images in the left row showed the retina schisis in the nerve fiber layer in the both left and right eyes. Fundus photographs in the right row showed microcystic-like changes of the macular region in the both eyes; B: The arrows in the ultrasonography images showed the retinal schisis; C: The examination of visual field (2010-01-05), the left panel showed the left eye and the right panel showed the right eye; D: The examination of visual field (2010-09-09).

**Genotyping** The proband patient and his mother were enrolled in the study and analyzed for *RS1* mutations (Figure 2). All six exons of the *RS1* including intron-exon junctions were screened. A missense mutation of C to T at position 304 in the exon 4 (c.304C > T) was identified by direct sequencing of the PCR products in the proband, which revealed a substitution that replaced the arginine (CGG) with the tryptophan (TGG) at amino acid position 102 (p. R102W). No additional mutations were detected in other coding regions. The mother was unaffected and carried a heterozygous c.304C > T mutation. The mutation detected has been reported as an XLRS-associated variant. A synonymous polymorphism in exon 6, c.576C > CT, was also demonstrated in the female carrier. We also sequenced an unrelated female without any eye disease to display the wild-type genotype.



**Figure 2 DNA sequencing of the proband, his mother, and a normal control** A: Wild type in a normal control; B: Heterozygous mutation in the proband's mother; C: Hemizygous mutation in the proband.

## DISCUSSION

To date, more than 189 unique disease-causing mutations have been identified in different populations. Most of them are missense mutations located in exons 4-6 which is the highly conserved region known as the discoidin domain (Figure 3A). Retinoschisin is currently believed to function as a retinal cell adhesion protein, since knockout mice deficient of *Rsl1h* the murine ortholog of *RS1*, manifested splitting of the inner nuclear layer and overall disorganization of the retinal cell layers with irregular displacement of cells<sup>[10]</sup>.

In the present study, we examined the *RS1* gene in a Chinese family with XLRs. Mutation analysis of *RS1* revealed a missense mutation and a non-disease-related polymorphism (SNP) as reported previously<sup>[1]</sup>. The R102W mutation was



**Figure 3 Sequence alignment and structural homology model of retinoschisin, and localization of disease-linked mutations**

A: An amino acid sequence alignment of *RS1* including position 102; B: Homology model of the discoidin domain of retinoschisin, the blue ribbon showed the p.R102W mutation.

detected in the affected male and a heterozygous mutation in his mother, who is a female carrier. This mutation was first reported in Western families in 1998, and then identified in Asian families<sup>[1,11,12]</sup>. Previous studies have shown that missense mutations, including R102W, in the domain can prevent proper protein folding and secretion<sup>[2,13]</sup>. Deposition of misfolded mutant proteins leads to endoplasmic reticulum (ER) stress triggering unfolded protein response and eventually cell apoptosis. The discoidin domain of retinoschisin was modeled using SWISS-MODEL (Figure 3B). The predicted location of 102 also revealed the mutation affected the structure of the Retinoschisin. To the best of our knowledge, a total of 189 unique mutations in the *RS1* gene had been reported. Taken together, the R102W mutation identified in this study indicates a mutation hot spot in the *RS1* gene, which should be a priority to be screened for gene diagnosis.

The proband patient showed a rare condition of concomitant glaucoma, except for typical phenotype of retinal schisis. Through a long-term follow-up history, we were able to investigate early-onset retinoschisis and recurrent glaucoma based on the comprehensive ophthalmic examinations. Three years after diagnosis of retinoschisis, IOP was found in both eyes. After a treatment of pharmaceuticals and surgeries, the IOP was successfully reduced.

Generally, treatment of XLRs is limited to the prescription of low-vision aids. In recent research, a marked reduction of retinoschisis at the posterior pole was reported following local application of 2% dorzolamide<sup>[14-16]</sup>. Approximately half of the eyes in this trial showed a response to this treatment

with improvement of visual acuity, but in some cases, a response is only seen after several months of application. The response to treatment is not associated with the genotype. However, the efficacy of long-term treatment still has to be established. As XLRS is a recessive disease caused by the impairment in retinoschisin function, gene therapy has been considered as a potential and valid treatment for this disease. In recent studies, two independent groups have revealed the potential of gene therapy to restore retinal structure and function: slow retinal degeneration in *Rs1h* knockout mice, an animal model for XLRS<sup>[17-19]</sup>. The trials need to be extended to large animals to optimize the process in the next step.

In brief, we successfully performed genetic diagnosis of X-linked juvenile retinoschisis in a simplex pedigree with a very rare phenotype of recurrent glaucoma, which may be helpful for future clinical references. Genetic diagnosis is important for two main reasons: genetic counseling and personalized treatments such as gene therapy<sup>[17-18]</sup>. Based on the precise genetic diagnosis, the family received genetic counseling. Moreover, the finding laid a foundation for the potential gene therapy in the future.

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**Conflicts of Interest:** Huang XF, None; Tu CS, None; Xing DJ, None; Gan DK, None; Xu GZ, None; Jin ZB, None.

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