X-Linked Retinoschisis: Phenotypic Variability in a Chinese Family

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X-linked juvenile retinoschisis (XLRS), a leading cause of juvenile macular degeneration, is characterized by a spoke-wheel pattern in the macular region of the retina and splitting of the neurosensory retina. Our study is to describe the clinical characteristics of a four generations of this family (a total of 18 members) with X-linked retinoschisis (XLRS) and detected a novel mutations of c.3G>А (p.M1?) in the initiation codon of the RS1 gene. By direct sequencing. Identification of this mutation in this family provides evidence about potential genetic or environmental factors on its phenotypic variance, as patients presented with different phenotypes regardless of having the same mutation. Importantly, OCT has proven vital for XLRS diagnosis in children.

X-linked juvenile retinoschisis (XLRS, MIM 312700), first described in 1898 by German ophthalmologist Josef Haas, is a relatively common retinal degenerative disease that affects male patients early in life. XLRS affects approximately 1 in 5,000–25,000 men1,2. XLRS is caused by mutations in the RS1 gene in Xp22.2 and was identified by positional cloning in 19972. Human RS1 contains six separate exons and encodes for the 224 amino acid cell-surface protein retinoschisin (RS1)3,4. RS1 is highly expressed by retinal photoreceptors and bipolar cells and interacts with the surfaces of these cell types to stabilize the organization of the retina4–6.

Patients with severe cases and older individuals display atrophic macular in the retinal pigment epithelium7. White flecks and dots, and fundus albipunctatus are associated with the severe foveal and peripheral retinoschisis7. Complications include vitreous hemorrhage, choroidal sclerosis, retinal detachment, and neovascular glaucoma, and often leads to a poor prognosis8. Electroretinogram (ERG) can be used for the clinical diagnosis of XLRS. Because the main functional defect may occur after photo transduction, the full-field ERG in patients with XLRS exhibits a relative preservation of an a-wave amplitude which indicate the function of photo transduction. But they have a substantial reduction of the dark-adapted b-wave amplitude originating in inner retinal cell activity9. Because XLRS patients with RS1 mutations may have normal ERG, the diagnosis of XLRS cannot be based on ERG alone10. Retinal fundus examination, optical coherence tomography (OCT), microperimeter, and fundus fluorescein angiography (FFA), as well as RS1 mutational analysis, are increasingly used in its diagnosis10.

In this study, we report finding in a Chinese family with XLRS. We identified a novel mutation, c.3G>А (p.M1?), in the RS1 gene. This mutation co-segregates with disease in the family and likely results in loss of RS1 protein production. This mutation expands the mutational spectrum of XLRS and the phenotypes of the affected members of this family provide evidence of potential genetic or environmental factors on XLRS prognosis.

Methods
Clinical examination. The clinical protocol was approved by the Second Xiangya Hospital Institutional Review Board, and the tenets of the Declaration of Helsinki were followed. Informed, written consent was obtained before blood sample acquisition. Eighteen members of the family were enrolled in the study. All the individuals underwent standard ophthalmic examinations including evaluation of best-corrected visual acuity (BCVA), non-contacted tonometry, slit-lamp biomicroscopic examination, computerized perimetry (Oculus Twinfield). After pupil dialation with tropicamide (1%) and phenylephrine hydrochloride (2.5%), patients were examined by indirect ophthalmoscopy, OCT (Zeiss Cirrus HD OCT – 2000, Carl Zeiss meditec, Inc. Dublin, CA; Spectralis, Heidelberg, Germany), fundus photography (TOPCON TRC 50 DX). Some patients received intravenous fluorescein angiography (FA. Spectralis, Heidelberg, Germany) and Goldman applanation tonometry. ERG (Espion V5, Diagnosys LLC, MA, USA) was performed according to standard testing protocols recommended by

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Diagnosis of XLRS was based upon history, clinical examination, and electroretinogram findings when available. Molecular genetic studies were performed using PCR amplification and DNA sequencing. Genomic DNA was isolated from peripheral leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturers’ instructions.

Table 1. Pedigree of the family in this study. BCVA: best corrected visual acuity; M: male; F: female; NAD: No Abnormality Detected; S: sphere; C: cylinder. For example OD 20/50: +2.0 S +0.5 C at 155 means that best corrected visual acuity of IV-3 was 20/50 with +2.0 sphere and +0.5 cylinder at 155 degree in right eye. II-3 had trauma when he was young, and there is lens dislocation and retinal detachment now.

The International Society for Clinical Electrophysiology of Vision (ISCEV). Diagnosis of XLRS was based upon history, clinical examination, and electroretinogram findings when available.

Polymerase chain reaction and molecular genetic studies. Genomic DNA was isolated from peripheral leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturers’ instructions.
protocol. Mutational screening of each coding exon was performed using the primer sequences that were previously published11. After purification, amplicons were sequenced using an ABI 3730 Genetic Analyzer (ABI, Foster City, CA, USA) at the Beijing Institute of Genomics (Shenzhen, China). Mutations were identified by comparing coding sequences with those of 100 normal X chromosomes and unaffected family members. Sequences were assembled and analyzed with Lasergene SeqMan software (DNASTAR, Madison, WI, USA). The results were compared with an RS1 reference sequence (GeneBank accession number: NM_000330). Mutations were annotated following the nomenclature recommended by the Human Genome Variation Society (HGVS).

Results

Clinical Findings. Three juvenile and two adult patients were affected in this family (Fig. 1d, Table 1), and none had a history of premature delivery. Panel D in Fig. 1 shows the proband (IV-3) of a 12-year-old male suspected to have retinoschisis when decreased vision occurred four years earlier. Best-corrected visual acuity was 20/50 in the right eye (OD) and 20/40 in the left eye (OS). Patient had normal intraocular pressure and slit-lamp biomicroscopy finding in both eyes. Under funduscopic examination, bullous retinoschisis was present in temporal and foveal regions in both eyes, which was confirmed by OCT (Fig. 2i,j). Full-field ERGs demonstrated borderline reduction in the rod-specific ERG, normal maximal ERG a-waves but mild electronegative waveforms. Photopic 30 Hz flicker ERGs were slightly delayed without significant amplitude reduction; transient photopic ERG showed a low b/a ratio 0.56 OD and 0.64 OS.

Figure 2. Images of the proband (IV-3). Panels (A–D) show grayish pigmentation in temporal quadrant both eyes. Schisis is only observed under fundusoscopic examination; (E–H) FA present no infusion in peripheral retina, with little inferotemporal linkage in right eye and no macula linkage in both eyes; (I,J) The thickness of macular retina is 540 μm in the left eye and 580 μm in right eye determined by OCT. GCL: ganglion cell layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer.
sclerotic, cortical changes of the lens, and bullous retinoschisis was present in right eye inferotemporal and foveal regions (Fig. 5b,f). The left eye presented no retinoschisis, but had pigmentary abnormalities in the peripheral retina (Fig. 5a).

All affected males had severe retinoschisis, except the patient’s uncle (III-2). III-2 complained of uncorrected vision acuity and OCT showed mild cystic in macula, which was easily could be missed by the fundus examination (Fig. 3e–h).

**Molecular Genetic Findings.** Direct Sanger sequencing identified a novel hemizygous mutation, c.3G > A (p.M1), in the RS1 gene in the proband (IV3). Our results indicate that this mutation is likely to be
the disease-causing mutation. First, further Sanger sequencing of all available family members suggests that this mutation co-segregates with the disease in the family, given that all the affected males are hemizygous, and all the unaffected individuals are either heterozygous (female carriers) or wild type for this mutation (Fig. 1a–c). Second, mutations affecting the same initiation codon p.M1 have been extensively reported in XLRS patients. Third, this methionine is well conserved across different vertebrate species, suggesting functional importance. Lastly, this mutation is likely to cause no protein production since other ATG sequences in the RS1 gene are not likely to be used as the alternative initiation codon (see discussion). Overall these results suggest that this mutation is the underlying cause of XLRS in this family.

Discussion
XLRS is the leading cause of juvenile macular degeneration in males. Different phenotypes were present in this family, such as one individual with asymmetry clinical symptoms and another with pigmentation. However, all had the same point mutation c.3G > A (p.M1), at the initiation codon of the XLRS1 gene, resulting in a change from an ATG codon to an ATA codon (from Met to Ile). In humans, ATG (methionine), as an initiation codon, signals the initiation of translation. Therefore, its abolishment may result in a dramatically truncated protein or loss of protein production. There are 11 other ATG sequences, including this one, throughout the XLRS1 coding region. Nine of them, which are not in frame, are at the following positions: 47 to 49 base pairs (bp), 80 to 81 bp, 111 to 113 bp, 140 to 142 bp, 186 to 188 bp, 266 to 268 bp, 434 to 436 bp, 473 to 475 bp, and 530 to 532 bp. Two sequences in frame (442 to 444 bp and 640 to 642 bp) result in Met148 and Met214. However, both Met148 and Met214 are not imbedded in a proper Kozak sequence to facilitate the initiation of translation. Therefore, due to the lack of this proper ribosomal binding sequence, this mutation is likely to result in loss of protein production.

Figure 4. Images of IV-1. (A–D) There is float (green arrow) and no linkage in FA. (E–F) OCT results revealed the macula thickness is 651 um in right eye and 548 in left eye.
Three other initiation codon mutations (1 A > G 1 A > T and 2 T > C) in the RS1 gene have been previously reported14–16.

Over the last 16 years, significant progress has been made in understanding XLRS at the clinical, genetic, molecular, and cellular levels. To date 196 different mutations in the RS1 gene are known to cause XLRS (Leiden

Figure 5. OCT and fundus image of II4. Fundus images of patient II-4 show pigment changes with RPE atrophy in right eye and favela schisis with peripheral schisis in left eye. (E,F) OCT results showed 82 µm macular retina in the left eye, however the right eye was 340 µm. The OCT results had low quality due to the presence of cataracts, thus a representative image is shown.
fluid accumulation in the extracellular environment forming a fluid-filled cystic cavity that is observed in OCT
mouse model. In another study, Shastry named modifier of Rs1 1 (Mor1) has been found to be responsible for the phenotypic variation in the XLRS they also had different phenotypes, such as the pigmentation only happened in their grandfather. One possible
more than 40% of patients with RS1 mutations did not have a negative ERG, thus the appearance of OCT has
who show normal ERG results. Rnner could in turn disrupt the organization of retinal layers resulting in a dysfunction of the photoreceptor-bipolar
signature characteristic for cellular proteins processed through the secretory pathway, a dominant amino
nuclear layer), most bipolar cells (INL, inner nuclear layer), as well as the two plexiform layers (OPL, outer plex-
retinoschisin (RS1) protein, expressed and localized in the rodent and human retina and pineal gland, was
Open Variation Database, LOVD version 2.0, Build 31; http://grenada.lumc.nl/LOVD2/eye/home.php?select_ db=RS1). The RS1 gene spanning 32.4 kb of genomic DNA is organized in six exons and five intervening regions. Retinoschisin (RS1) protein, expressed and localized in the rodent and human retina and pineal gland, was
specifically found at the extracellular surfaces of the inner segments of rod and cone photoreceptors (ONL, outer nuclear layer), most bipolar cells (INL, inner nuclear layer), as well as the two plexiform layers (OPL, outer plexiform layer; IPL, inner plexiform layer). RS1 is organized with four distinct regions: a 23-amino-acid N-terminal
state domain (DS domain), a 39 amino acid RS1 domain upstream of the discoidin domain, and a 5 amino acid C-terminal segment. It is thought that the DS domain of RS1 anchors to the surface of photoreceptors and bipolar cells. The whole protein is proposed to be the cell adhesion protein which maintains the cellular organization of the retina or may regulate the fluid balance between the intracellular and extracellular environment through its interactions with a Na/K ATPase-SARM1 complex. Thus the lack of functional RS1 may cause fluid accumulation in the extracellular environment forming a fluid-filled cystic cavity that is observed in OCT and histology, particularly within the photoreceptor and bipolar cell layers (INL and ONL). The cystic cavities could in turn disrupt the organization of retinal layers resulting in a dysfunction of the photoreceptor-bipolar
The penetrance of XLRS is almost complete but its expressivity is highly variable. First, different mutations are associated with different severity. For example, it has been reported that deletion in exon 1 causes more attenuated ERG, whereas missense mutations are associated with a more benign, slowly progressive disease course. Second, the same mutation in RS1 can be associated with different clinical presentations, which is the case for our patients. Comparing his uncle, the proband and his brother had much more severe presentation. What’s more, they also had different phenotypes, such as the pigmentation only happened in their grandfather. One possible explanation is that there are genetic modifiers. Indeed, a quantitative trait locus (mapped on chromosome (Chr) 7, named modifier of Rs1 1 (Mor1) has been found to be responsible for the phenotypic variation in the XLRS mouse model. In another study, Shastry et al. found that the common polymorphisms of the CFH, LOC387715/ ARMS2 and HTRA1 genes do not contribute to the phenotypic variability of the XLRS disorder.
Environmental factors may contribute to the phenotypic variability of XLRS. Although there are some reports about uneven progress of the both eyes, most of them are due to complications, such as vitreous hemorrhage and retinal detachment. Our patients had none of them, and it seems he got two different diseases in both eyes (II4). In another family with initiation codon mutation, inconformity of phenotype in both eyes has also been reported. Two of the family members had a history of “trauma” and one had ocular surgery and the other developed retinal detachment. Neovascularization and vitreous hemorrhage occurred in another patient with diabetes mellitus. Hence the patients’ experience also play some kind of effect on disease's progression. (Thus the patient's medical history also plays an important role in disease progression).
In the past, ERG was used to differentiate the XLRS from other diseases. However, with development in ophthalmic technologies, spectral domain OCT (SD-OCT) is an important diagnostic tool in diagnosis of this disease. The OCT, not only can depict the vivid change of foveal schisis, but can also be used for the diagnosis of young children who are generally uncooperative with ERG. Just a single scan of the foveal area is sufficient to detect cystoid changes and to distinguish XLRS from other differential diseases associated with visual loss in uncooperative children. Importantly, it can be used to detect foveal schisis alone in the eyes with clinical XLRS who show normal ERG results. Rnner et al. observed ERG results in the two-year follow-up study and believed more than 40% of patients with RS1 mutations did not have a negative ERG. Thus the appearance of OCT has changed the diagnostic approach for XLRS. Menke et al. also found SD-OCT might show absence of retinoschisis in older patients which increases the difficulty to differentiate it from other forms of macular dystrophy (i.e. II-4). Overall, molecular genetic tests is most precise diagnostic method.
In summary, we identified a new mutation of XLRS to expand the mutation spectrum and explored the important role of OCT on its diagnosis. Importantly, the inconformity of genotype and phenotype reports deepen our understanding of the molecular basis of XLRS. Finally, OCT proved it is vital in XLRS diagnosis especially in children. A combination of ERG, FAF, OCT, and molecular-genetic testing is advised to verify the diagnosis in clinically suspected XLRS.

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Corrigendum: X-Linked Retinoschisis: Phenotypic Variability in a Chinese Family

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The original version of this Article contained typographical errors in the spelling of the author Terry G. Coursey, which was incorrectly given as Terry Coursy. This has now been corrected in the PDF and HTML versions of the Article.

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