Novel RS1 mutations associated with X-linked juvenile retinoschisis

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Abstract. To identify mutations in the retinoschisin (RS1) gene in families with X-linked retinoschisis (XLRS). Twenty families with XLRS were enrolled in this study. All six coding exons and adjacent intronic regions of RS1 were amplified by polymerase chain reaction (PCR). The nucleotide sequences of the amplicons were determined by Sanger sequencing. Ten hemizygous mutations in RS1 were detected in patients from 14 of the 20 families. Four of the ten mutations were novel, including c:176G>A (p:Cys59Tyr) in exon 3, c:531T>G (p:Tyr177X), c:607C>G (p:Pro203Ala) and c:668G>A (p:Cys223Tyr) in exon 6. These four novel mutations were not present in 176 normal individuals. The remaining six were recurrent mutations, including c:214G>A (p:Glu72Lys), c:304C>T (p:Arg102Trp), c:436G>A (p:Glu146Lys), c:544C>T (p:Arg182Cys), c:599G>A (p:Arg200His) and c:644A>T (p:Glu215Val). Our study expanded the mutation spectrum of RS1 and enriches our understanding of the molecular basis of XLRS.

Introduction

X-linked retinoschisis (XLRS, MIM 312700) is a hereditary retinal disease characterized by a splitting of the neurosensory retina, with a prevalence of 1:5,000 to 1:25,000 males worldwide (1). Typical fundus changes include radiating cystic maculopathy in most cases and peripheral retinoschisis in half of the cases (2). However, the disease has a high degree of phenotypic variability (3-6), in which genetic testing is of value in confirming the diagnosis (4).

XLRS accounts for most congenital retinoschisis (2,7) and is due to mutations in the retinoschisin gene (RS1, OMIM 312700) localized on Xp22.13 (8,9). The encoded protein, retinoschisin, is secreted from photoreceptors and bipolar cells as a functional homo-octameric complex that is thought to play a role in cellular adhesion and cell-to-cell interaction (10).

Gene transference to mouse models of X-linked juvenile retinoschisis, which suggest gene replacement may be a possible future therapy for patients (11-13). Genetic diagnosis is the basis for gene transference in the future. Therefore, we have to fully understand the molecular basis of XLRS. To date, more than 160 different RS1 mutations have been identified in patients with XLRS (http://www.dmd.nl/rs), including small intragenic deletions, nonsense and missense mutations, frame shift insertions and deletions, and splice site mutations. However, there are still some RS1 mutations that remain unknown.

In this study, we analyzed the coding exons and the adjacent regions of RS1 in patients from 20 unrelated Chinese families with XLRS. Ten hemizygous mutations, including 4 novel mutations, were detected in 14 families.

Subjects and methods

Probands with XLRS from 20 unrelated families were enrolled in this study. Written informed consent was obtained from the participating individuals or their guardians prior to the collection of clinical data and genomic samples. This study was approved by the Internal Review Board of the Zhongshan Ophthalmic Center.

Mutation detection. Genomic DNA was prepared from venous leukocytes. Six pairs of primers (Table I) were used to amplify the six coding exons and the adjacent intronic sequence of RS1 (NCBI human genome build 37.2, NC_0008659.1 for genomic DNA, NM_000330.3 for mRNA, and NP_000321.1 for protein). Touchdown polymerase chain reaction (PCR) was performed with decreasing 0.5˚C per cycle from 64˚C for the first 15 cycles then down to 57˚C (the annealing temperature) for the remaining 21 cycles. GC buffer was used. DNA sequences of the amplicons were identified with ABI BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA) on an ABI 3130 Genetic Analyzer (Applied Biosystems). Sequencing results
and consensus sequences from the NCBI human genome database were compared by using the SeqMan II program of the Lasergene package (DNA Star, Inc., Madison, WI) and then aligned to identify variations. Each variation was confirmed by bidirectional sequencing. Mutation description followed the recommendation of the Human Genomic Variation Society (HGVS). Variations detected in patients were further evaluated in controls by sequencing 176 normal individuals.

The Sorting Intolerant From Tolerant (SIFT) program and the Polymorphism Phenotyping (PolyPhen-2) were used to
predict whether an amino acid substitution was likely to affect the protein function (14,15).

Results

Mutation analysis.

Ten hemizygous mutations in *RS1* were detected in patients from 14 of the 20 families with retinoschisis (Table II and Fig. 1), including c:176G>A (p:Cys59Tyr) in exon 3, c:214G>A (p:Glu72Lys) and c:304C>T (p:Arg102Trp) in exon 4, c:436G>A (p:Glu146Lys) in exon 5, c:531T>G (p:Tyr177X), c:544C>T (p:Arg182Cys), c:599G>A (p:Arg200His), c:607C>G (p:Pro203Ala), c:644A>T (p:Glu215Val) and c:668G>A (p:Cys223Tyr) in exon 6. Of the 10, the c:176G>A, c:531T>G, c:607C>G and c:668G>A were novel. These novel mutations occurred in highly conserved regions (Fig. 2) and were predicted to be pathogenic (Table II). They were absent in 176 normal individuals.

All 10 probands with hemizygous *RS1* mutations (the clinical data of 4 probands were not available) had clinical symptoms and signs of retinoschisis (Table III). The four probands with novel mutations showed macular and peripheral retinoschisis.

Discussion

In this study, ten different hemizygous mutations in *RS1* were identified in 14 families with XLRS. These mutations are predicted to be pathogenic. All patients with mutations demonstrated typical signs of XLRS. The ten mutations affected different domains of retinoschisin, including the RS1 domain (1 mutation), discoidin domain (8 mutations) and C-terminal segment (1 mutation). These mutations were not randomly distributed over the gene (Fig. 3) because 80% of mutations were clustered in the discoidin domain (16). The two novel mutations, Tyr177X and Pro203Ala in the discoidin domain, may cause a shorter retinoschisin form or protein misfolding (13). The cysteine mutations in the RS1 domain (Cys59Tyr) and C-terminal segment (Cys223Tyr) may cause failure of the discoidin domain to assemble into a normal multisubunit complex (17,18). Most of RS1 mutation loci were hot mutation spots, while the Cys59, Glu72, Arg102, Glu146, Arg182, Arg200, Pro203, Glu215 and Cys223 could be substituted by 1-2 other kinds of amino acids and be reported more frequently (19-30). However, the mutations in the present study also differed from those reported previously. The RS1 mutations accounts for 70% of the Chinese retinoschisis (14/20) cases in our study. The

![Figure 3. Distribution of the mutations detected a linear diagram of RS1 showing the organization of retinoschisin into domains and segments.](image-url)
Table III. Clinical information on individuals with RS1 variations.

| Patient ID | Mutations | Age (years) | BCVA |
|------------|------------|-------------|------|
|            | Nucleotide | Protein     | Exam | Onset | Family history | OD | OS | Macular change | Peripheral change | Retinal hole | Strabismus | OCT | ERG(b/a) |
| QT042      | 176G>A     | Cys59Tyr    | N/A  | N/A   | No           | N/A | N/A | N/A          | N/A             | N/A          | N/A        | N/A | N/A     |
| QT335      | 176G>A     | Cys59Tyr    | 11   | 6     | No           | 0.4 | 0.2 | mRS          | pRS             | No           | No         | RS  | N/A     |
| QT221      | 214G>A     | Glu72Lys    | 19   | EC    | Yes          | 0.1 | 0.2 | mRS          | PD              | No           | No         | N/A | N/A     |
| QT232      | 214G>A     | Glu72Lys    | 18   | 8     | No           | 0.4 | 0.2 | mRS          | Degeneration     | No           | No         | N/A | N/A     |
| QT653      | 214G>A     | Glu72Lys    | 5    | 3     | No           | 0.3 | 0.7 | mRS          | pRS             | Yes          | No         | N/A | Reduced |
| MD015      | 304C>T     | Arg102Trp   | N/A  | 7     | No           | 0.2 | 0.3 | PD, FRB      | No              | No           | No         | N/A | N/A     |
| RP006      | 436G>A     | Glu146Lys   | 5    | 4     | No           | FC  | 0.03| PD, FRB      | No              | No           | Yes        | N/A | Reduced |
| MD030      | 531T>G     | Tyr177X     | 6    | 5     | No           | 0.3 | FC  | mRS          | pRS             | No           | Yes        | N/A | Reduced |
| QT212      | 544C>T     | Arg182Cys   | N/A  | N/A   | N/A          | N/A | N/A | N/A          | N/A             | N/A          | N/A        | N/A | N/A     |
| QT417      | 544C>T     | Arg182Cys   | 12   | EC    | No           | 0.3 | 0.03| No           | pRS             | Yes          | No         | N/A | N/A     |
| QT848      | 599G>A     | Arg200His   | 21   | EC    | No           | 0.6 | 0.4 | mRS          | No              | No           | No         | N/A | Reduced |
| QT911      | 607C>G     | Pro203Ala   | 22   | EC    | No           | 0.2 | 0.4 | mRS          | pRS             | No           | Yes        | N/A | N/A     |
| QT219      | 644A>T     | Glu215Val   | N/A  | N/A   | N/A          | N/A | N/A | N/A          | N/A             | N/A          | N/A        | N/A | N/A     |
| QT758      | 668G>A     | Cys223Tyr   | 9    | 6     | No           | 0.4 | 0.3 | mRS          | pRS             | Yes          | No         | RS  | N/A     |

BCVA, best-corrected visual acuity; mRS, macular retinoschisis; pRS, peripheral retinoschisis; RS retinoschisis; EC, early childhood; N/A, not available; PD, pigmental disorder; FRB, foveal reflex was blunted; FC, figure counting; ERG(b/a), the ratio of b wave amplitude to a wave amplitude.
Cys59Tyr, Tyr177X, Pro203Ala, Glu215Val and Cys223Tyr mutations only are present in the Chinese population (31), and the Cys59Tyr mutation was more common (10% frequency in our retinoschisis cases). The Glu72Lys mutation is the most common among Chinese (15%) as well as other populations (19,32), while another very common mutation, Pro192Ser (33), which was reported from people of different ethnic backgrounds was not found. We do not know whether the spectrum and frequency of RS1 gene in the Chinese is different from others. Our study contributes to the current state of knowledge.

In summary, we identified ten mutations in 14 of 20 families with XLRS. Our results expand the mutation spectrum of RS1 that might enrich our understanding of the molecular basis of XLRS in the Chinese population.

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