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Molecular analysis of the XLRS1 gene in 4 females affected with X-linked juvenile retinoschisis

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ABSTRACT • RÉSUMÉ

Background: X-linked juvenile retinoschisis (XLRS) is the most common cause of juvenile macular degeneration in males. Because of its X-linked mode of transmission, the disease is rare in females. In this article, we describe a mutation screen conducted on a family in which 4 female patients affected with XLRS presented with an unusually severe phenotype.

Methods: DNA was extracted from peripheral blood, and the XLRS1 gene was amplified on DNA samples of all the available family members. The mutation screen was conducted by performing direct DNA sequencing using an MJ Research PTC-225 Peltier Thermal Cycler.

Results: A novel mutation, 588-593ins.C, was identified in exon 6 of the gene. The affected father was found to be heterozygous for the mutation, whereas all the female patients were homozygous for this mutation. The homozygosity of the mutation in the affected females led to severe phenotypes. The defective allele was expressed in infancy in 1 patient, whereas the disease manifested itself at variable ages in the other patients, reflecting a variation in the phenotype.

Interpretation: This report describes a novel mutation in a family in which consanguinity has led to XLRS in 4 females. A variation in the phenotype of the disease is consistent with the published literature and suggests the involvement of genetic modifiers or environmental factors in influencing the clinical severity of the disease.

Contexte : Le rétinoshisis juvénile du chromosome X (RJCX) est la cause la plus commune de dégénérescence maculaire juvénile chez les garçons. À cause de son mode de transmission, la maladie est rare chez les filles. Dans cet article, nous décrivons cependant un tableau de mutation dressé dans une famille dont 4 filles affectées par le RJCX ont présenté un phénotype anormalement grave.

Méthodes : L'on a extrait l'ADN du sang périphérique et amplifié le gène RJCX1 de tous les échantillons d'ADN des membres de la famille disponibles. Le dépistage de mutation a été dressé par le biais d'un séquençage direct de l'ADN selon la méthode du MJ Research PTC-225 Peltier Thermal Cycler.

Résultats : Une nouvelle mutation, 588-593ins.C, a été identifiée dans l'exon 6 du gène. Le père affecté était hétérozygote pour cette mutation, alors que toutes les patientes étaient homozygotes. L'homozygotisme de la mutation chez les patientes affectées a entraîné des phénotypes graves. L'allèle défectueux s'est exprimé dans l'enfance chez une patiente, alors que la maladie s'est manifestée à des âges variés chez les autres patientes, reflet de la variation du phénotype.

Interprétation : Ce compte-rendu décrit une nouvelle mutation dans une famille où la consanguinité a mené au RJCX chez quatre filles. La variation du phénotype de la maladie concorde avec la littérature publiée et suggère l'implication des modificateurs ou des facteurs environnementaux dans la gravité clinique de la maladie.

X-linked juvenile retinoschisis (XLRS) is the most common cause of juvenile macular degeneration in males.1 The disease usually manifests between the ages of 5 and 10 years, and patients present with vision loss and reading difficulties at school.1 In the majority of patients, diagnosis is clinical, and affected individuals show a significant loss in central, and in some cases peripheral, vision.1 On examination, they have characteristic foveal schisis consisting of folds radiating outwards from the foveola in a stellate pattern containing microcystic schisis cavities.2 The
disease is incurable and can be complicated by retinal detachment and vitreous hemorrhage leading to blindness.3 Positional cloning has identified the XLRS1 gene on chromosome Xp22 as the cause of this disease.4 The gene encodes a 224-amino acid protein known as retinoschisin and is primarily expressed in photoreceptor cells of the outer retina and bipolar cells of the inner retina.5,6 In this article, we describe a mutation screen of the XLRS1 gene conducted on a unique family (Fig. 1) in which 4 female patients were affected with XLRS due to consanguinity and had an unusually severe phenotype.

**CASES**

The cases have been described previously.7 Briefly, a 3-year-old girl (IV-4 in Fig. 1) presented with bilateral nystagmus with light perception only in the right eye. On examination, both the eyes were found to have complete retinal detachment. XLRS had been diagnosed in her father (III-4 in Fig. 1) when he was a child, and his best-corrected visual acuities were 6/18 in the right eye and counting fingers at 2 m in the left eye. He had a left divergent squint and relative afferent papillary defect in the amblyopic left eye. There was extensive macular scarring and signs of peripheral retinoschisis in both eyes.

All the patient’s sisters (patients IV-2, IV-3, and IV-5 in Fig. 1) had presented earlier with similar visual problems. Patient IV-2 presented at the age of 10 years and had best-corrected visual acuities of 6/60 in the right eye and 3/60 in the left. There were punctuate lens opacities in the right eye, but the left lens was clear. She had a typical macular schisis consisting of folds radiating outwards in a stellate pattern extending to the inferior arcades in both eyes (Fig. 2A). Patient IV-3 was 5 years old at the time of presentation and had best-corrected visual acuities of 6/36 in the right eye and 6/60 in the left. She had bilateral inferior scars with pigmentary changes accompanying peripheral and macular retinoschisis (Fig. 2B). Her optic disks were healthy, and there was no evidence of retinal detachment. Patient IV-5 presented at the age of 1 year, and she was observed to have left esotropia when she was 3 months old. She had bilateral retinal detachment and retinoschisis with macular involvement. There were secondary changes in the areas of detachment with markedly atrophic retinae. Subjects III-5 and IV-1 were clinically normal with unaided 6/6 vision in both eyes, clear media, and no retinal abnormalities.

**Mutation screen of the XLRS1 gene**

This study was approved by the Ethical Review Committee, Aga Khan University, Karachi. A mutation screen of the XLRS1 gene was performed on all the available family members. DNA was isolated from peripheral blood using a phenol-chloroform protocol as described in an earlier report.8 All exon–intron boundaries of the XLRS1 gene were amplified using the primer sequences already published.4 Ten microlitres of the amplified product was purified and subjected to DNA sequencing. Sequencing reactions were performed in an MJ Research PTC-225 Peltier Thermal Cycler using ABI PRISM BigDye Terminator Cycle sequencing kits with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems, Foster City, Calif.), following the protocols supplied by the manufacturer. A novel frameshift mutation, 588-593ins.C, was identified in exon 6 of the gene (Fig. 3), which disrupted the stop codon site and resulted in the abnormal elongation of the transcript (Fig. 4).
This mutation was present in a heterozygous state in both the mother (III-5 in Fig. 1) and the affected father (III-4 in Fig. 1), whereas all the female patients were found to be homozygous for the mutation. Patient IV-1 was found to be negative for the mutation.

**INTERPRETATION**

The presence of 2 defective alleles is required for X-linked recessive diseases to express themselves in females as compared with male patients, who express the phenotype if the only allele present on the single X-chromosome becomes nonfunctional. The present study has revealed a novel frameshift mutation in a family in which consanguinity has led to homozygosity of this novel mutation in 4 females, resulting in an X-linked recessive disorder. Both the parents (subjects III-5 and III-4) were found to be heterozygous for the mutation; however, the disease was only manifest in the father (subject III-4) because of the X-linked mode of transmission of this disorder. Moreover, the presence of 2 defective alleles in the affected females led to a severe phenotype as compared with the presence of 1 defective allele in their father, suggesting a phenotypic gradient due to the presence of the mutation.

**Fig. 3**—Electropherograms of exon 6 of the XLRS1 gene. Arrow indicates a frame-shift mutation in the patient; an insertion of additional “C” in exon 6 can be seen here.

**Fig. 4**—A segment of the amino acid sequence of XLRS1. The frameshift mutation leads to disruption of the stop codon resulting in the elongation of amino acid.

**Fig. 5**—Alignment of XLRS1 between species. XLRS1, especially the discoidin domain of the gene, is highly conserved in different species, suggesting an important functional and essential role of this domain throughout evolution. The highlighted region is an area within the discoidin domain where the frameshift mutation has been discovered in the family discussed.

**REFERENCES**


**Key words:** X-linked juvenile retinoschisis, XLRS1, juvenile macular degeneration, mutation, X-linked recessive