X-Linked Retinoschisis With Point Mutations in the XLRS1 Gene

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Background: X-linked retinoschisis (XLRS) is a relatively rare vitreoretinal dystrophy that causes visual loss in young men. Recently, a gene responsible for this disease, designated XLRS1, was identified, and several deleterious gene mutations were reported.

Objective: To analyze Japanese patients clinically diagnosed as having XLRS formutational changes in the XLRS1 gene.

Methods: Ten patients with XLRS underwent full ophthalmologic examination, including slitlamp biomicroscopy and dilated funduscopy. Genomic DNA was isolated from leukocytes, and all exons of the XLRS1 gene were amplified by polymerase chain reaction and analyzed using a direct sequencing method.

Results: Point mutations in the XLRS1 gene were identified in all 10 patients. The mutations were identical in each of 2 pairs of brothers. Six of the point mutations represented missense mutations, 1 was a nonsense mutation, and 1 was a frameshift mutation. Five of the mutations are newly reported herein.

Conclusions: The discovery of new point mutations in this study increases the available information regarding the spectrum of genetic abnormalities and clinical manifestations of XLRS. However, the limited data failed to reveal a correlation between mutation and disease phenotype.

Clinical Relevance: Identification of mutations in the XLRS1 gene and expanded information on clinical manifestations will facilitate early diagnosis, appropriate early therapy, and genetic counseling regarding the prognosis of XLRS.


X-Linked retinoschisis (XLRS), first described by Haas1 in 1898, is a relatively rare vitreoretinal dystrophy that causes visual loss in young men. Worldwide prevalence of the disease is reportedly 1:15 000 to 1:30 000,2 and it is characterized by perifoveal radial retinoschisis in a cartwheel-like pattern, peripheral retinoschisis with inner-leaf breaks, and “golden glistening” of the peripheral retina.3 The exact pathogenesis of XLRS is unknown, although histopathologic and electrophysiologic studies have shown that splitting of the retina occurs superficial to the nerve fiber layer and inner limiting membrane. Furthermore, an inherited defect has been identified that affects the innermost portion of the cytoplasm of the Müller cell,4-7 a cell necessary for neuronal growth and connectivity,8,9 resulting in failure to establish proper neuronal interactions.10

Electroretinography (ERG) in patients with XLRS shows a reduced b wave in most cases,7,11 whereas fluorescein angiography occasionally shows avascular areas or retinal vascular leakage, even in ophthalmoscopically normal-appearing fundi.12

Recently, a gene responsible for XLRS, designated XLRS1, was identified by Sauer and colleagues.13 The XLRS1 gene is composed of 6 coding exons that span approximately 15 kilobases of genomic DNA. Subsequent mutational studies13-16 in patients with XLRS have revealed 5 one-exon deletions, 12 splice site mutations,11 frameshift insertions or deletions, 7 nonsense mutations, 51 missense mutations, and 1 rare mutation caused by an early Okazaki fragment sequence replacement.

In the present study, 10 Japanese patients diagnosed as having XLRS based on clinical findings were examined for possible mutations in the XLRS1 gene.

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Clinical characteristics and ERG results of patients with XLRS are summarized in Table 1. Eight eyes of 5 patients had foveal retinoschisis, 8 eyes of 5 patients had peripheral retinoschisis, 3 eyes of 3 pa-
PATIENTS AND METHODS

HISTORY AND CLINICAL EXAMINATION

Ten patients (from 8 Japanese families) diagnosed clinically as having XLRS were enrolled in the study. Each patient and family were interviewed extensively, followed by full ophthalmic examination, including slitlamp biomicroscopy and dilated funduscopy. Bright-flash ERG was performed on each patient. This investigational study was approved by the Osaka University Hospital Human Research Review Board, and informed consent was obtained from all participants.

DNA ANALYSIS

Twenty milliliters of venous blood was collected from each patient. Genomic DNA was extracted from lymphocytes, and 50 ng of DNA was amplified by polymerase chain reaction with 500 mmol/L of each forward and reverse primer in an amplification mixture containing Tris-hydrochloride, 10 mmol/L, (pH 8.3), potassium chloride, 50 mmol/L, magnesium chloride, 1.5 mmol/L, deoxyribonucleoside triphosphate, 0.2 mmol/L, and Taq polymerase (AmpliTaq Gold; Perkin-Elmer, Norwalk, Conn), 0.5 U. The primer pairs, based on published sequences,13 amplified each exon of the XLRS1 gene, including flanking introns. All exons from each patient were sequenced using a direct sequencing method. Amplified DNA was purified using a polymerase chain reaction purification kit (Qiagen GmbH, Hilden, Germany) and sequenced with an automated fluorescent DNA sequencer (ABI Prism 377; Perkin-Elmer) using a dye terminator cycle sequencing kit (Perkin-Elmer). To exclude polymorphic variants, all coding sequences were also analyzed in 100 normal X chromosomes.

Table 1. Clinical Characteristics of Patients With X-Linked Retinoschisis

<table>
<thead>
<tr>
<th>Patient/ Age/Sex</th>
<th>Visual Acuity OD</th>
<th>Visual Acuity OS</th>
<th>Fundus Findings and Electroretinography</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/2 (10 mo)/M</td>
<td>Not tested</td>
<td>Not tested</td>
<td>Bullous retinoschisis OU, macular involvement OS, reduced b wave OU</td>
</tr>
<tr>
<td>B/32 (29)/M</td>
<td>20/100</td>
<td>20/100</td>
<td>Peripheral and foveal retinoschisis OU, reduced b wave OU</td>
</tr>
<tr>
<td>C/12 (2)/M</td>
<td>20/250</td>
<td>20/200</td>
<td>Peripheral retinoschisis with large inner retinal breaks OU, reduced b wave OU</td>
</tr>
<tr>
<td>D/49 (40)/M</td>
<td>20/200</td>
<td>20/200</td>
<td>Perifoveal round cysts OU, reduced b wave OU</td>
</tr>
<tr>
<td>E1†/58 (49)/M</td>
<td>20/30</td>
<td>20/200</td>
<td>Chorioretinal macular degeneration OD, bull’s-eye macular lesion OS, reduced b wave OU</td>
</tr>
<tr>
<td>E2†/55 (46)/M</td>
<td>20/200 Light perception</td>
<td></td>
<td>Turbid, flat fovea OD, diffuse pigmentary chorioretinal degeneration OS, reduced b wave OD, extinguished electroretinography OS</td>
</tr>
<tr>
<td>F1†/13 (8)/M</td>
<td>20/200 Light perception</td>
<td>20/200</td>
<td>Foveal retinoschisis with vitreous vells OU, reduced b wave OU</td>
</tr>
<tr>
<td>F2†/11 (6)/M</td>
<td>20/50</td>
<td>20/50</td>
<td>Foveal retinoschisis with vitreous vells OU, reduced b wave OU</td>
</tr>
<tr>
<td>G/25 (15)/M</td>
<td>20/50</td>
<td>20/150</td>
<td>Foveal and peripheral retinoschisis OD, bull’s-eye macular lesion, diffuse chorioretinal degeneration OS, reduced b wave OD, extinguished electroretinography OS</td>
</tr>
<tr>
<td>H/18 (11 mo)/M</td>
<td>20/400</td>
<td>20/50</td>
<td>Peripheral retinoschisis with a large inner retinal break, macular degeneration OD, peripheral golden reflex, foveal retinoschisis OS, reduced b wave OU</td>
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*Age in years (unless otherwise indicated) at time of study, with age at first diagnosis in parentheses.
†Brothers from the same family, previously reported by Wakabayashi et al.19
‡Brothers from the same family.
ciliary testing, such as ERG and fluorescein angiography, may be helpful, although its findings in XLRS also vary considerably. In particular, the ERG may reveal a reduced b wave with single bright-flash stimulus or a negative rectangular configuration with repetitive rectangular photopic stimuli.20

In the present study, a negative response was revealed by flash ERG in all patients. Miyake and colleagues21 reported that, despite a reduced b wave, the a wave was normal in early stages of XLRS, whereas later stages were associated with a reduction in the a wave as well. They suggested that photoreceptors were not the primary target in the pathogenesis of this disease. However, in our study, patient G showed reduction of the a and b waves in the left eye, despite being only 25 years of age. His left eye was noted to have diffuse chorioretinal atrophy from childhood. It is possible that early progression of disease in this patient is related to the particular frameshift mutation that he had in his XLRS1 gene. However, the patient who had a nonsense mutation (patient B) did not have a particularly severe phenotype compared with the others. In our study, significant phenotype-genotype correlation was not identified.

Following identification of the XLRS1 gene,13 several mutations of the gene have been reported.13-16 In the present study, we were able to identify 8 different point mutations, 5 of which have never, to our knowledge, been previously reported. Mutations in the XLRS1 gene in Japanese patients have been previously reported by Hotta and colleagues,15 who identified 6 missense mutations and 1 nonsense mutation; only 1 of these (the W92C mutation) was identified in our study. Hotta and colleagues suggested that the E72K mutation may be common among Japanese patients; however, none of our 10 patients had this mutation. The Retinoschisis Consortium14 reported a "hotspot" at a stretch of 6 C residues (positions 574-579). Our study revealed 1 missense mutation (C574T) in this location. Approximately 100 mutations in the

<table>
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<th>Table 2. Mutations Detected in the XLRS1 Gene</th>
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<td>Patient</td>
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<tr>
<td>A</td>
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<td>B</td>
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<td>C</td>
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<tr>
<td>D</td>
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<td>E1, E2</td>
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<td>F1, F2</td>
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<tr>
<td>G</td>
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<td>H</td>
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*Mutations are named according to standard nomenclature.
†Same mutation as Hotta et al15 reported.
‡Same mutation as the Retinoschisis Consortium14 reported.
§Same mutation as Sauer et al13 and the Retinoschisis Consortium14 reported.

Figure 1. Left, Classic bull’s-eye pattern of retinoschisis in the left eye of patient E1. Right, Foveal retinoschisis and vitreous veils in the left eye of patient F1.

Figure 2. Pedigrees of families A, B, E, F, and H with X-linked retinoschisis (XLRS). Solid symbols indicate individuals with XLRS as diagnosed by clinical examination; open symbols, unaffected individuals.
XLRS1 gene have been reported, which are mainly located in exons 4, 5, and 6, although they occur in other exons as well. A definitive phenotype-genotype correlation has not been identified to date. This suggests that the entire XLRS1 protein may play an important role in this disease. Interestingly, 6 of the mutations we found involved codon 101-203, which is a highly preserved domain with the discoidin motif, a protein considered to function in phospholipid binding and cell-cell interactions on membrane surfaces.13,22

Direct sequencing succeeded in identifying mutations in the XLRS1 gene in all 10 patients. It may be expected that the accumulation of genetic information using methods such as direct sequencing will reveal further insight into the pathogenesis and clinical spectrum of XLRS. Furthermore, it is hoped that identifying various mutations in the XLRS1 gene will lead to improved early diagnosis and appropriate early therapy where possible, depending on the phenotype. Finally, disease classification, combined with long-term studies in prognosis, will aid the clinician in providing genetic counseling for affected patients and families.

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