Electroretinographic Findings in Three Family Members with X-linked Juvenile Retinoschisis Associated with a Novel Pro192Thr Mutation of the XLRS1 Gene

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Purpose: To present ocular findings in three family members with X-linked juvenile retinoschisis (XLRS) associated with a novel Pro192Thr mutation.

Cases: We examined 21- (Case 1), 17- (Case 2), and 10-year-old (Case 3) male patients who showed wheel-like cystic lesions in the macula and a silver-gray reflex in the peripheral retina. Case 2 was a cousin of Case 1. Case 3 was a brother of Case 2.

Methods: Scotopic electroretinogram (ERG) (dim and bright flash), oscillatory potentials, photopic ERG, and 30-Hz flicker responses were recorded in each patient. The XLRS1 gene was analyzed in patient blood samples by a direct sequencing method.

Results: A novel missense mutation (Pro192Thr) was identified in the XLRS1 gene in each patient. Variable b/a ratios upon scotopic bright flash stimulation were evident (Case 1: right 1.16, left 1.20; Case 2: right 0.98, left 1.01; Case 3: right 0.81, left 0.83). Only Case 3 showed the typical “negative” waveform. The amplitude of rod b-waves was significantly decreased in all patients.

Conclusions: Three cases with a novel Pro192Thr mutation showed the phenotypic variation in ERG, especially in b/a ratio, which has been considered an important diagnostic parameter.

Key Words: Electroretinogram, retinoschisis, XLRS1 gene.

Introduction

X-linked retinoschisis (XLRS) is characterized by splitting of the retinal nerve fiber layer in the macular area, causing a “spoke-wheel” pattern of macular cysts.1,2 This condition may be clinically apparent at birth.3-6 The inheritance of XLRS follows a recessive X-chromosomal pattern.7 Under dark-adapted conditions, a negative electroretinogram (ERG), i.e., reduced b-wave amplitude in spite of a normal a-wave, has been frequently described in XLRS patients.8-13 The pathogenesis is not yet clear; however, electrophysiologic results have suggested an underlying defect in Müller cells11 and in the proximal retina, postsynaptic to the photoreceptors,14 and histopathologic studies also suggest this hypothesis.1,2,15 XLRS is thought to be a progressive disease.4

The retinoschisis gene (XLRS1) has six exons that encode the 224-amino-acid protein. The predicted XLRS1 protein contains a highly conserved motif implicated in cell-cell interaction, and this may be active in cell adhesion processes in retinal development.16 Many types of mutations of XLRS1 have been investigated.17-22
In this report, we present the ERG findings from a single family who showed the novel missense mutation, Pro192Thr.

**Report of Cases**

**Case 1**

Case 1 was a 21-year-old man. Poor corrected visual acuity in the right eye was found when he was 7 years old. He was first examined in our clinic when he was 11 years old. His corrected visual acuity was 0.6 in the right eye and 1.0 in the left. The cornea and lens were normal in both eyes. Both maculas showed radial retinal folds, i.e., wheel-like cystic lesions. Goldmann kinetic perimetry showed no abnormal findings. Automatic static perimetry showed decreased sensitivity in the central retina bilaterally. His inferotemporal peripheral retina showed a silver-gray reflex in the right eye. There was no peripheral retinoschisis, retinal tear or retinal detachment in either eye.

**Case 2**

Case 2 was a 17-year-old man, who was the cousin of Case 1. Reduced corrected visual acuity in both eyes was found when he was 7 years old, and he was first examined in our clinic when he was 9 years old. His corrected visual acuity was 0.3 in the right eye and 0.2 in the left. However, his near visual acuity was 1.2 in both eyes. Wheel-like cystic lesions like those of Case 1 were found in both eyes. Silver-gray reflex was found in all the surrounding areas of the peripheral retina except the temporal quadrant. Peripheral retinoschisis, retinal tear, and retinal detachment were not evident. Goldmann kinetic perimetry was not examined.

**Case 3**

Case 3 was a 10-year-old boy who was a brother of Case 2. His corrected visual acuity was 1.0 in both eyes when he was 8 years old. He became aware of decreased corrected visual acuity at the age of 9 when he was first examined in our clinic. His corrected visual acuity was 0.4 in the right eye and 0.7 in the left. Wheel-like cystic lesions like those of Case 1 were found in both eyes. Color vision was normal (panel-D15). As in Case 2, silver-gray reflex was found in the peripheral retina of the inferior quadrant in both eyes, although peripheral retinoschisis, retinal tear and retinal detachment were not observed. Goldmann kinetic perimetry showed no abnormal findings.

The pedigree of these 3 cases is shown in Figure 1. A recessive X-chromosomal inheritance pattern was considered.

Macular degeneration without cystic lesions was found in both eyes of the 85-year-old grandfather (gray square in Figure 1). He told that his corrected visual acuity had been decreased since his childhood. Informed consent for further examination could not be obtained from him, so we could not confirm whether or not he had the mutation of XLRS1.

**Materials and Methods**

**ERG Recording and Analysis**

The ERG procedure complied with the International Society for Clinical Electrophysiology of Vision (ISCEV) standard protocol. The methods were similar to those described in a previous study. Both eyes were dilated with a mydriatic and subjects were dark-adapted for at least 45 minutes before testing. The responses were obtained from Burian-Allen bipolar electrodes (Hansen Ophthalmic Instruments, Iowa City, IA, USA). The stimulus was a 10-μs xenon flash (ERG Photic Stimulator, SLS-4100, Nihon Kohden, Tokyo) delivered by means of a Ganzfeld dome (Sanso, Tokyo). Stimulus intensity was controlled by means of neutral density filters (Fuji Film, Tokyo). Scotopic rod b-wave responses and scotopic bright flash ERGs were recorded with a 0.5 to 100 Hz filter setting. Oscillatory potentials (OPs) were recorded with a 50–500-Hz filter setting. The photopic ERGs and 30-Hz flicker responses were recorded.
under 30 cd/m² background illumination after at least 15 minutes of light adaptation. Amplitudes and/or implicit times from the responses were calculated and compared with the values from 15 age-matched normal subjects aged 7–31 years (mean age = 21.3 years) (Table 1).

We analyzed rod and cone a-waves by fitting them to a model proposed by Hood and Birch. Rod-only responses were obtained by computer subtraction of photopic ERGs from scotopic ERGs (flashes under 30 cd/m²). Rod td and cone td were fixed at 25–27 ms; log S, t₅d, and Rₘp₃ for rods and cones were determined by the Dye Terminator Cycle Sequencing method (PE Applied Biosystems, Foster City, CA, USA) and an ABI Prism 310 Genetic Analyzer. For the molecular genetic study, procedures followed the tenets of the Declaration of Helsinki and were approved by the Ethics Committee for Medical Research of Hirosaki University School of Medicine.

**Table 1.** Electroretinogram Results of 3 Cases with X-linked Juvenile Retinoschisis and Normal Controls

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Range</td>
<td>Right Eye</td>
<td>Left Eye</td>
</tr>
<tr>
<td>Scotopic bright flash a-wave amplitude (µV)</td>
<td>315.9 (51.3)</td>
<td>193.6–396.4</td>
<td>261.8</td>
<td>274.6</td>
</tr>
<tr>
<td>Implicit time (ms)</td>
<td>13.1 (0.8)</td>
<td>11.6–14.8</td>
<td>13.5</td>
<td>12.7</td>
</tr>
<tr>
<td>Scotopic bright flash b-wave amplitude (µV)</td>
<td>485.8 (84.8)</td>
<td>276.1–606.6</td>
<td>303.1</td>
<td>328.6</td>
</tr>
<tr>
<td>Implicit time (ms)</td>
<td>55.4 (3.3)</td>
<td>49.0–59.5</td>
<td>47.7*</td>
<td>47.5*</td>
</tr>
<tr>
<td>b/a wave ratio</td>
<td>1.45 (0.15)</td>
<td>1.29–1.85</td>
<td>1.16*</td>
<td>1.20*</td>
</tr>
<tr>
<td>Photopic bright flash a-wave amplitude (µV)</td>
<td>61.3 (13.9)</td>
<td>38.7–82.2</td>
<td>60.7</td>
<td>46.7</td>
</tr>
<tr>
<td>Implicit time (ms)</td>
<td>14.6 (0.5)</td>
<td>13.4–15.5</td>
<td>14.9</td>
<td>13.7</td>
</tr>
<tr>
<td>Photopic bright flash b-wave amplitude (µV)</td>
<td>133.1 (33.5)</td>
<td>71.9–184.4</td>
<td>69.2*</td>
<td>98.8</td>
</tr>
<tr>
<td>Implicit time (ms)</td>
<td>33.8 (1.2)</td>
<td>31.4–35.9</td>
<td>33.3</td>
<td>32.1</td>
</tr>
<tr>
<td>30-Hz flicker amplitude (µV)</td>
<td>83.2 (23.3)</td>
<td>47.5–133.0</td>
<td>5.0*</td>
<td>6.0*</td>
</tr>
<tr>
<td>Implicit time (ms)</td>
<td>14.5 (1.7)</td>
<td>11.6–17.4</td>
<td>24.4*</td>
<td>23.6*</td>
</tr>
<tr>
<td>Oscillatory potentials (O1+O2+O3) amplitude (µV)</td>
<td>186.8 (54.6)</td>
<td>108.0–275.8</td>
<td>92.3*</td>
<td>143.9</td>
</tr>
<tr>
<td>Rod log</td>
<td>Rₘp₃</td>
<td>2.39 (0.09)</td>
<td>2.18–2.51</td>
<td>2.31</td>
</tr>
<tr>
<td>Rod log S</td>
<td></td>
<td>1.02 (0.07)</td>
<td>0.90–1.11</td>
<td>1.04</td>
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<tr>
<td>Cone log</td>
<td>Rₘp₃</td>
<td>1.85 (0.09)</td>
<td>1.70–2.03</td>
<td>1.79</td>
</tr>
<tr>
<td>Cone log S</td>
<td></td>
<td>0.91 (0.09)</td>
<td>0.81–1.20</td>
<td>1.01</td>
</tr>
</tbody>
</table>

*Value outside normal range.

**DNA Analysis**

About 10 mL of venous blood was drawn from each patient into a small volume of heparin solution. High-molecular-weight genomic DNA was extracted from leukocytes of each patient by a QIAamp® column (Qiagen GmbH, Hilden, Germany). All 6 exons of the XLRSI gene were amplified by polymerase chain reaction (PCR) with the use of the oligonucleotide primers and conditions described previously. The PCR products were electrophoresed and visualized in 1.5% agarose gels. Nucleotide sequences of the PCR products were directly determined by the Dye Terminator Cycle Sequencing method (PE Applied Biosystems, Foster City, CA, USA) and an ABI Prism 310 Genetic Analyzer. For the molecular genetic study, proceedings followed the tenets of the Declaration of Helsinki and were approved by the Ethics Committee for Medical Research of Hirosaki University School of Medicine.

**Results**

**ERG**

In scotopic bright flash ERG, a-wave amplitudes were normal in all cases, and b-wave amplitudes were reduced in both eyes of Case 2. B/a ratios upon...
scotopic bright flash stimulation were decreased in all cases. The values were variable (Case 1: right 1.16, left 1.20; Case 2: right 0.98, left 1.01; Case 3: right 0.81, left 0.83) (Figure 2 and Table 1).

The amplitude of each OP (O1, O2, and O3) was measured from a baseline drawn as a first-order approximation between the troughs of successive wavelets. The sum of the amplitudes of the OPs (O1 + O2 + O3) was decreased in the right eye of Case 1.

The amplitudes of rod b-waves were decreased bilaterally in Cases 2 and 3, and were nonrecordable in Case 1.

In photopic bright flash ERG, the amplitudes of cone a-waves in all cases were within the normal range. The implicit time in the left eye of Case 3 was delayed. The amplitudes of cone b-waves were decreased in the right eye of Case 1 and in both eyes of Case 3. The implicit times of cone b-waves were delayed in both eyes of Case 3.

In 30-Hz flicker ERG, amplitudes were severely decreased in both eyes of Case 1. The implicit times were delayed only in Case 1.

Rod and cone functions were analyzed with fitting models (Figure 3). Log of $R_{mp}$ and log of S of both rod and cone were within the normal ranges in all 3 cases. Table 1 shows a summary of ERG findings. The ERGs in Case 1 were most affected.

**DNA Analysis**

DNA analysis was performed after informed consent was obtained from the patients following a thorough explanation of this study.

Nucleotide sequencing analysis showed a novel hemizygous transversional change from a cytosine res-
due to adenine at nucleotide 574 of the \textit{XLRS1} gene in all 3 cases examined (Figure 4). This mutation predicts an amino acid substitution of threonine for proline at codon 192 in exon 6 (Pro192Thr) of the gene.

The 3 cases in this report showed wheel-like cystic lesions in the macula, silver gray reflex in the peripheral retina, and the \textit{XLRS1} mutation. These findings indicate XLRS.

\textbf{Discussion}

\textbf{Figure 3.} (A) Results of the rod a-wave fitted to a model proposed by Hood and Birch.\textsuperscript{25} Solid curves are the raw data, and dashed curves are the models after fitting to the leading edge of the rod a-waves. $S$ and $Rm_{p3}$ values are given in the panel. (B) Results of the cone a-wave fitted to a model proposed by Hood and Birch.\textsuperscript{34} Solid curves are the raw data, and dashed curves are the models after fitting to the leading edge of the cone a-waves. $S$ and $Rm_{p3}$ values are given in the panel.
Electrophysiologic and histopathologic studies\textsuperscript{1,11,14,15} have suggested an underlying defect in Müller cells and in the proximal retina, postsynaptic to the photoreceptors in XLRS. Recently, Grayson et al\textsuperscript{28} reported that \textit{XLSI} mRNA was detected only in the photoreceptor layer, but the protein product of the gene, retinoschisin, was detected both in the photoreceptors and within the inner layer of the retina. Although the function of the retinoschisin, which contains a discoidin domain, is unknown, the protein might have effects not only on the inner layer of the retina but also on the photoreceptors. Some patients showed the ERG change in the outer retina\textsuperscript{10,11,18} It might be secondary in XLRS, as also proposed by
Miyake et al. Thus the state of photoreceptors is important for the interpretation of responses from the inner retina. The amplitude and latency of the trough of the a-wave are often used as parameters that represent photoreceptor function clinically. However, it should be noted that the peak a-wave amplitude does not represent the maximal receptor response. In addition, the response of the rod a-wave of the human ERG to moderately intense flashes is partially postreceptoral in origin as was originally shown for the cone. Photoreceptor activity can be assessed by fitting the leading edge of the rod and cone a-waves to a model. In our 3 cases, the left eye of Case 3 showed prolonged implicit times of photopic a-wave, although the functions of rods and cones were within the normal range.

Our results showed that the rod b-waves that might derive from bipolar cells were reduced in all cases (nonrecordable in Case 1). Dysfunctions of bipolar cells might exist in all 3 cases in different degrees. The OPs that might derive from amacrine cells were decreased only in the right eye of Case 1. In the right eye of Case 1, the damage might exist not only in bipolar cells but also in amacrine cells.

Only in both eyes of Case 1 the amplitudes of 30-Hz flicker response were diminished and the implicit times were delayed. Reduced 30-Hz flicker amplitude and delayed implicit times of 30-Hz flicker response have been typical findings in XLRS. Recently, the on-pathway dominant impairment was described in XLRS using a sinusoidal flicker under photopic condition. The authors suggested that the reduced amplitude and delayed implicit time of the 30-Hz flicker ERG were due, in part, to a relative reduction in an ERG ON response. The on-pathway dominant impairment was also described using a long flash under photopic conditions. In our 3 cases, it is very interesting that 30-Hz flicker responses were different even though photopic bright flash responses were similar. Kondo and Sieving reported on the characteristics of photopic sine wave flicker ERG in detail. They analyzed flicker response as the harmony of three vectors, the photoreceptor component, the ON-component and the OFF-component. The photoreceptor component becomes very small at flicker stimuli near 30-Hz, so 30-Hz flicker response is thought to be a result of the harmony of postreceptoral ON- and OFF-components. ON- and OFF-components depend on the function of photoreceptors. In our 3 cases, cone functions (cone log and cone log S) were within the normal range. The results of changes in the ON- and OFF-components at 32-Hz flicker ERG have been described. Changes only in the ON-component do not result in a large reduction of flicker amplitudes. However, a mild OFF-component change (OFF-component delay) will result in reduced amplitudes and delayed implicit times.

![Figure 4](image)

**Figure 4.** DNA analysis of the patients. One of representative nucleotide sequence data is shown. Identical hemizygous mutation was found in all affected patients examined. Arrows point to the nucleotide at position 574 of the XLRS1 gene, which is a C (cytosine) in the normal subject but is altered to A (adenine) in the patients. This base change results in an amino acid substitution of threonine for proline at codon 192 (Pro192Thr).
implicit times of the flicker ERG. Therefore, the variability of 30-Hz flicker ERG in our cases might be influenced by the changes of not only ON- but also OFF-bipolar cells.

Under dark-adapted conditions, a negative ERG has frequently been described in XLRS.6-13,18,20 Park et al20 reported that three male siblings with a missense mutation, Arg141His, showed severely reduced scotopic b-waves (b/a ratio 0.77, 0.58, and 0.66, respectively). Bradshaw et al18 reported that two cases with Arg141Cys showed reduced scotopic b-waves. B/a ratios were 0.57 in one case, about 0.7 in the other.

On the other hand, several cases of XLRS with mildly affected or normal ERG have been reported.17,18,37 Sieving et al37 reported a family with a missense mutation Arg213Trp. They described that a 54-year-old grandfather demonstrated typical “negative ERG” (b/a ratio 0.58), although a 13-year-old grandson showed a normal ERG (b/a ratio 1.62). Taketani et al17 reported a 3-year-old boy with a missense mutation, Arg102Gln. The patient had a macular hole and retinal detachment in his left eye, and the lesion was treated surgically. At 8 years of age his ERG was recorded using conventional scotopic bright implicit times of the flicker ERG. Therefore, the variability of 30-Hz flicker ERG in our cases might be influenced by the changes of not only ON- but also OFF-bipolar cells.

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It has been reported that the severity of the ERG abnormality in XLRS was variable, without clinical, genetic or age correlation.18,19,38 We also found the phenotypic variation in a family with XLRS associated with a novel Pro192Thr mutation. The interest in a genotype-phenotype correlation has risen after XLRS1 gene mutation was first reported.18 Bradshaw et al18 studied clinical findings in 19 patients with XLRS in 17 families and found significant variability across the patient population and no correlation between ERG changes, clinical features, and genetic mutation. As described above, we also found variable ERG findings, for example b/a ratio, even within a family. Further electrophysiological and genetic studies will be required to clarify the disease mechanisms in XLRS.

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References