Phenotypic Expression of Juvenile X-linked Retinoschisis in Swedish Families With Different Mutations in the XLRS1 Gene

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Objective: To describe the clinical phenotype of juvenile X-linked retinoschisis in patients with different mutations in the XLRS1 gene.

Methods: Thirty patients with 7 different XLRS1 mutations were examined. The genotype was determined by molecular genetics, which identified 6 known and 1 novel mutation (exon 5, 489 G→T). Ophthalmologic examination included full-field electroretinogram (ERG) recordings.

Results: The fundus appearance showed marked variations between, as well as within, families with different XLRS1 mutations. The ERG demonstrated typical reduction of B-wave amplitude, with relative A-wave preservation, causing a reduced B-A ratio in all affected males. The implicit time of the 30-Hz flicker ERG was prolonged in all patients examined. In a large family with a deletion of exon 1 and the promoter region, 12 affected males showed a phenotype ranging from moderate to severe vision impairment and a broad range of ERG abnormality, suggesting that additional factors may contribute to the disease severity.

Conclusions: Juvenile retinoschisis shows a wide variability in the phenotype between, as well as within, families with different genotypes. The ERG findings show reduced B-A ratios of dark-adapted recordings and prolonged implicit times of 30-Hz flicker response, which provide a useful clinical marker to confirm the clinical diagnosis.

Clinical Relevance: This study describes the wide variability in the phenotype in patients with juvenile retinoschisis and different mutations in the XLRS1 gene. The study emphasizes the importance of complementing the ophthalmologic examination with full-field ERG and molecular genetics in boys with visual failure of unknown etiology to determine the diagnosis early in the course of the disease.

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J U V E N I L E X-LINKED retinoschisis, also called congenital retinoschisis (congenital vascular veils or vitreous veils), is an X-linked inherited retinal disorder characterized by a splitting of the retina (schisis means splitting), leading to visual failure early in life. In juvenile retinoschisis, the splitting occurs in the nerve fiber layer. This differs from the schisis seen in acquired (senile) retinoschisis, in which the splitting is located in the middle layers of the sensory retina.1-3

Juvenile retinoschisis is considered to be a disorder of retinal development.4 The pathogenesis is still unknown, but previous histopathological5 and electrophysiological6 findings have suggested that the primary defect is located in the Muller cells, the principal glial cells of the retina.

Haas6 presented the first clinical description of the disease in 1898. Since then, the phenotype in juvenile retinoschisis and the variability in the phenotype have been described by several authors.2,3,7-11 Macular changes seem to be present in almost all cases. In most young patients, a wheel-like cystic formation is seen in the macula, whereas middle-aged and older patients more often have a nonspecific atrophic appearance in the macula. Peripheral schisis is seen in about 50% of the patients, predominantly in the inferotemporal part of the retina.2,7 The most important complications are retinal detachment (in about 10%) and vitreous hemorrhage (in about 5%).7,11 Although the disease is considered to be congenital, symptoms including visual failure, squint, or nystagmus usually appear at age 4 to 8 years. Visual acuity is frequently reduced to 20/100 (0.2) to 20/50 (0.4) but can range from light perception to 20/25 (0.9).7,10 Visual deterioration often progresses during the first 1 or 2 decades of life. After puberty, the disease is mainly stationary or slowly progressive un-
PATIENTS AND METHODS

Thirty patients from 9 families were examined (aged 5-70 years). All patients were informed about the objectives of the examination and volunteered to participate in the study. The genotype was determined by molecular genetics, which identified 6 known and 1 novel mutation in the XLRS1 gene (Table 1 and Figure 1). In the family with a deletion of exon 1 and the promoter region (family 50), a more extensive examination was performed that included 12 affected patients. This 5-generation family will therefore be described in more detail.

OPHTHALMOLOGIC EXAMINATION

Ophthalmologic examination included assessment of best-corrected visual acuity, kinetic perimetry with a Goldmann perimeter using standardized light targets \( V_{4e} \) and \( I_{4e} \), full-field ERG, slitlamp examination, fundus inspection, and fundus photography. Eight patients were examined at least twice, with a follow-up duration between 1.5 and 10 years. The same recording procedures were used in all examinations.

In 4 patients it was possible to examine only 1 eye. Full-field ERGs were recorded with an analysis system (Nicolet Biomedical Instruments, Madison, Wis). After dilation of the pupil with topical 1% cyclopentolate hydrochloride and 10% phenylephrine hydrochloride, a Burian-Allen bipolar contact lens ERG electrode was applied together with a ground electrode on the forehead. The patient was dark adapted for 40 minutes before the testing. Responses were obtained with a wide-band filter (−3 dB at 1 Hz and 500 Hz), stimulating with single full-field flash (30 microseconds) with blue light (Wratten filters 47, 47A, and 47B; Nicolet Biomedical Instruments), and with white light (0.81 candela [cd]–s/m²). Cone responses were obtained with 30-Hz flickering white light (0.81 cd-s/m²) averaged from 20 sweeps. If responses measuring less than 10 µV were recorded with single white flashes, recordings were also obtained with computer averaging (30 flashes), a bipolar artifact rejecter, and a line frequency notch filter (50 Hz).

MOLECULAR GENETICS

Lymphocyte DNA was extracted from blood samples of individuals in these families. The 6 exons of the XLRS1 gene were amplified by polymerase chain reaction (PCR) with the use of primers described by Sauer et al.\(^1\)\(^6\) The PCR products were sequenced by means of these same primers with a cycle-sequencing reaction kit (Amersham, Arlington Heights, III). Mutations were identified in comparison with 100 normal chromosomes from unrelated individuals.

In family 50, exons 2 to 6 amplified appropriately, and no mutations were found in the coding sequence or the splice sites; however, we were unable to amplify by PCR either exon 1 or the 1117–base pair sequence upstream of the coding sequence of exon 1. Exon 1 was amplified by means of the primers described by Sauer et al.\(^1\)\(^6\) Two pairs of primers were designed to amplify the upstream region: (1) GCC CAT ACT GTT CAT TCT ATC (at position +3 to −16) and AGC AGG GGA TTA AGT TGA G (at position −568 to −586) and (2) CCT CCC ATC AAC TTT ATT C (at position −489 to −509) and AAT GAT GGC GAA GAA AGG (at position −1099 to −1117). Position of these primers was counted by taking the position of the starting site of the transcription initiation as 0. Neither of these primer pairs resulted in the formation of amplified product. The upstream region was expected to contain the promoter. Although this was a negative result, positive controls from other families were run at the same time, and only this family showed absence of PCR product with these primer sets.

FUNDUS APPEARANCE

We found a marked interfamilial and intrafamilial variability of the fundus appearance. All patients had macular changes, although these were extremely discreet in some cases. In some of the younger patients, cystic, stellate foveal changes were seen, whereas, in the older patients, macular changes varied from blunt foveal reflex or subtle pigment motting to atrophic macular degeneration (Figure 2). However, in other patients, the macular alterations seemed to be partially independent of the patients’ ages (Figure 2, A and B, and Figure 3, A and B). Peripheral retinoschises were seen in only 9 of the patients at the time of examination. With the exception of the youngest and the oldest patients, the most striking feature ophthalmoscopically was the golden-white fun-

dex reflex, predominantly localized to the midperipheral retina, especially temporal to the macula (Figure 2, A).

Twelve patients were examined from the family with the deletion of exon 1 and the promoter region (family 50). Within this family there was a marked variability in the clini-
cl phenotype, spanning from patients with minor macular changes and a moderate reduction of visual function (20/32 [0.7]) to patients with severe retinal degeneration, optic atrophy, peripheral bone spicule pigmentation, and a visual acuity reduced to hand motions (Figure 3).

**FULL-FIELD ERGS**

Full-field ERGs were obtained in all 30 patients. There were marked variations in the ERG recordings between, as well as within, families with different mutations in the XLRS1 gene. However, 29 patients demonstrated remaining rod responses to dim blue light. The typical response to white light single flash, with a reduction of the B-wave amplitude and a relative preservation of the A-wave, causing a reduced B-A ratio, was seen in all families (Figure 4). In several of the older patients, there was a reduction of the A-wave amplitude as well. The B-A ratio was less than 2.0 in 29 patients. One patient had a B-A ratio greater than 2.0 (2.29). The cone B-wave amplitudes were subnormal in most cases, but also in these responses there were large variations between the patients. All patients examined showed a prolongation in implicit times for cone B wave on 30-Hz flicker white-light stimulation.

The typical response to a single flash of white light, with a reduction of the B-wave amplitude and a relative preservation of the A-wave, was also seen in the large family with the deletion of exon 1 and the promoter region of the XLRS1 gene (family 50). Within this family, there was a marked variability in the ERG recordings and the reduction in amplitude seemed to be partially independent of the patients’ ages (Table 2). However, in this family, 2 patients were reexamined after 8 to 10 years. The full-field ERGs in these 2 patients demonstrated a marked reduction in the retinal function during this time (Table 3).

**Table 1. Patients With Identified Mutations in the XLRS1 Gene Examined in This Study**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Family Identification</th>
<th>No. of Patients Examined</th>
<th>Predicted Protein Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1 deletion</td>
<td>50</td>
<td>12</td>
<td>...*</td>
</tr>
<tr>
<td>Exon 3, 120 C→A</td>
<td>122, 221, 244</td>
<td>8</td>
<td>C 40 X</td>
</tr>
<tr>
<td>Exon 4, 214 G→A</td>
<td>64</td>
<td>5</td>
<td>E 72 K</td>
</tr>
<tr>
<td>Exon 4, 221 G→T</td>
<td>240</td>
<td>1</td>
<td>G 74 V</td>
</tr>
<tr>
<td>Exon 4, 233 G→T</td>
<td>48</td>
<td>1</td>
<td>E 75 X</td>
</tr>
<tr>
<td>Exon 5, 489 G→T</td>
<td>220</td>
<td>1</td>
<td>W 163 C</td>
</tr>
<tr>
<td>Exon 6, 574 C→T</td>
<td>222</td>
<td>2</td>
<td>P 192 S</td>
</tr>
</tbody>
</table>

*Part of the upstream region and the start codon are missing, and this may result in the formation of no protein product.
†New mutation.

**Figure 1. Pedigrees of 5 Swedish families with juvenile X-linked retinoschisis and identified mutations in the XLRS1 gene. A, Family 50 (exon 1 deletion). B, Families 122, 221, and 244 (exon 3). C, Family 64 (exon 4). ERG indicates electroretinogram.**

**Figure 2. Fundus photographs from 3 patients with mutations in the XLRS1 gene. A, A 45-year-old man (exon 3, III:1 in family 244 [Figure 1, B]). Visual acuity was 20/100 (0.2). A typical microcystic appearance is seen in the macular region. B, A 56-year-old man (exon 4, III:2 in family 64 [Figure 1, C]). Visual acuity was counting fingers. Advanced retinal changes are present, with areas with pigmentation and atrophy, as well as narrow arteries. C, A 69-year-old man (exon 4, II:3 in family 64 [Figure 1, C]). Visual acuity was 20/125 (0.15). There is atrophic macular degeneration.**
Figure 3. Fundus appearance and full-field electroretinogram (ERG) recordings from 3 patients with the exon 1 deletion in the XLRS1 gene (family 50, Figure 1, A). A, Patient VI:1, an 18-year-old man. Visual acuity was 20/100 (0.2). The typical wheel-like microcystic formation is seen in the macular region. B, Patient IV:1, a 60-year-old man. Visual acuity was hand motions. Atrophic macular degeneration with pigmentedations, a pale optic disc, and narrowing of the arteries are seen. C, Patient III:4, a 69-year-old man. Visual acuity was 20/63 (0.3). There is an almost normal fundus appearance, with minor macular changes.
Only a few previous reports have been published describing the clinical features in families with defined mutations in the XLRS1 gene. In this study, patients with 7 different mutations in the XLRS1 gene were examined regarding the clinical phenotype.

Previous studies regarding juvenile X-linked retinoschisis have described the typical clinical features including macular changes, retinoschisis, retinal pigmentations, and vitreous veils, and also the wide variability in the fundus appearance. The variation in the ophthalmoscopic findings makes diagnosis based on fundus examination difficult. This study demonstrates the marked variation in the phenotype regarding the fundus appearance and the full-field ERG between, as well as within, families with different mutations in the XLRS1 gene. These variations seemed to be partially independent of the patients' ages. In the family with the deletion of exon 1 and the promoter region (family 50), the variation in the clinical phenotype was very pronounced. This family is of particular interest because the deletion is considered to include the promoter of the XLRS1 gene. A deletion including the promoter of the gene would result in the absence of the XLRS1 gene and, thereby, no production of the XLRS1 protein. The variation within this family suggests that additional factors, perhaps other genetic influences, may contribute to the disease severity.

As mentioned above, there was marked variability in the fundus appearance between, as well as within, families with different genotypes. However, a prominent feature observed in almost all patients examined, with different genotypes, was the golden-white fundus reflex. This finding has been described before in juvenile retinoschisis.
sis and is considered to be secondary to an increase in extracellular potassium concentration in the retina. The Müller cells are, among other things, responsible for the regulation of extracellular potassium levels in the retina, and, as mentioned above, previous studies have indicated that juvenile retinoschisis is likely associated with a defect in these cells.

The Müller cells have previously been considered to be important for the generation of the B wave of the ERG, but more recent studies regarding this show that the bipolar cells may produce the B wave directly, either selectively or in association with the Müller cells. However, there is other ERG evidence of Müller cell involvement in juvenile retinoschisis. The Müller cells are involved in generating the scotopic threshold response of the ERG. The reduction of scotopic threshold response amplitudes in juvenile retinoschisis implicates Müller cells in the pathophysiology in at least some cases of juvenile retinoschisis.

The ERG in juvenile retinoschisis has been shown to manifest a reduction in B-wave amplitude and a relative preservation of the A wave on single white-flash stimulation, leading to a reduction in the B-A ratio. In this study, the B-A ratio was in the lower range, or less than the B-A ratio in normal subjects. One patient had a B-A ratio higher than 2.0. This patient had very subtle macular changes as well and would have been very hard to diagnose without the help of molecular genetics studies, confirming mutations in the XLRS1 gene. Two of the patients were followed up for periods of 8 and 10 years. The full-field ERGs in these 2 patients demonstrated a marked decrease in retinal function during this period. A prolongation in the implicit time of the cone B wave for stimulation by 30-Hz flicker white-light has only been reported in a few cases of juvenile retinoschisis. In this study, a prolongation in the implicit time for 30-Hz flicker light was found in all patients.

Since the ophthalmoscopic appearance in juvenile X-linked retinoschisis is variable, it is important to complement the ophthalmologic examination with a full-field ERG in young boys with visual impairment. The combination of full-field ERG and molecular genetics makes clinical diagnosis possible early in the course of the disease.

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REFERENCES


Table 2. Visual Acuity and Full-Field Electroretinogram Recordings From 12 Patients From Family 50 (Deletion of Exon 1, Figure 1, A) Compared With Values From 59 Normal Control Subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Visual Acuity (Snellen)</th>
<th>White Light B Wave, µV</th>
<th>White Light B-A Ratio</th>
<th>30-Hz Flicker White Light, µV</th>
<th>30-Hz Flicker White Light Implicit Time, ms</th>
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<tbody>
<tr>
<td>VI:1</td>
<td>6</td>
<td>Counting fingers</td>
<td>113</td>
<td>1.32</td>
<td>12</td>
<td>34.8</td>
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<tr>
<td>V:3</td>
<td>7</td>
<td>20/50 (0.4)</td>
<td>40</td>
<td>0.67</td>
<td>20</td>
<td>36.4</td>
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<tr>
<td>V:3</td>
<td>15</td>
<td>20/50 (0.4)</td>
<td>108</td>
<td>1.34</td>
<td>24</td>
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<tr>
<td>V:2</td>
<td>16</td>
<td>20/40 (0.3)</td>
<td>103</td>
<td>1.22</td>
<td>31</td>
<td>34.6</td>
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<tr>
<td>VI:1</td>
<td>18</td>
<td>20/100 (0.2)</td>
<td>61</td>
<td>0.98</td>
<td>18</td>
<td>35.2</td>
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<tr>
<td>V:8</td>
<td>24</td>
<td>20/100 (0.2)</td>
<td>143</td>
<td>1.51</td>
<td>23</td>
<td>35.4</td>
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<tr>
<td>V:7</td>
<td>26</td>
<td>20/125 (0.15)</td>
<td>95</td>
<td>1.62</td>
<td>25</td>
<td>36.8</td>
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<tr>
<td>V:4</td>
<td>29</td>
<td>20/40 (0.5)</td>
<td>128</td>
<td>1.85</td>
<td>18</td>
<td>33.8</td>
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<td>V:2</td>
<td>42</td>
<td>20/63 (0.3)</td>
<td>134</td>
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<td>IV:10</td>
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<td>Hand motion</td>
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<td>41.8</td>
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<tr>
<td>III:4</td>
<td>69</td>
<td>20/63 (0.3)</td>
<td>92</td>
<td>1.65</td>
<td>18</td>
<td>37.6</td>
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<tr>
<td>Mean*</td>
<td>. . .</td>
<td>. . .</td>
<td>228 ± 125</td>
<td>2.95 ± 1.79</td>
<td>49.8 ± 34.2</td>
<td>29.2 ± 3.6</td>
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* Mean values (± 2 SDs) from 59 normal control subjects.

Table 3. Follow-up Electroretinogram From 2 Patients From Family 50 (Exon 1 Deletion)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Blue Light, µV</th>
<th>White Light A Wave, µV</th>
<th>White Light B Wave, µV</th>
<th>White Light B-A Ratio</th>
<th>30-Hz Flicker White Light, µV</th>
<th>30-Hz Flicker White Light Implicit Time, ms</th>
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<tr>
<td>VI:1</td>
<td>10</td>
<td>66</td>
<td>125</td>
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<td>1.0</td>
<td>41</td>
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<tr>
<td>V:4</td>
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<td>63</td>
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<tr>
<td>V:4</td>
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<td>137</td>
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<td>254</td>
<td>1.58</td>
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<tr>
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