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

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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<sub>40</sub> and L<sub>40</sub>, 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.

Full-field ERGs were obtained in all patients. 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% pil with topical 1% 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 candelas [cd]·s/m<sup>2</sup>). Cone responses were obtained with 30-Hz flickering white light (0.81 cd·s/m<sup>2</sup>) 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 rejector, 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 primaries described by Sauer et al. The PCR products were sequenced by means of these same primaries with a cycle-sequencing reaction kit (Amersham, Arlington Heights, Ill). 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 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. 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 –1117 to –489) 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.
cal 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 pigmention, 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, 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).

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**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, 223 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.

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**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 2/100 (0.2). A wheellike microcystic appearance is seen in the macular region. B, A 66-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 pigmention 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 wheellike 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 pigmentations, 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\textsuperscript{18-21} 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\textsuperscript{2,7-9,11} 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.\textsuperscript{17} 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.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Full-field electroretinogram recordings from 5 patients with different mutations in the XLRS1 gene.}
\end{figure}
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