Clinical and molecular characterisation of females affected by X-linked retinoschisis

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ABSTRACT

Background: X-linked Retinoschisis (XLRS) is a leading cause of juvenile macular degeneration associated with mutations in the RS1 gene. XLRS has a variable expressivity in males and shows no clinical phenotype in carrier females.

Design: Clinical and molecular characterisation of male and female individuals affected with XLRS in a consanguineous family.

Participants: Consanguineous Eastern European-Australian family

Methods: Four clinically affected and nine unaffected family members were genetically and clinically characterised. DNA analysis was conducted by the Australian Inherited Retinal Disease Register and DNA Bank (AIRDR).

Main Outcome Measures: Clinical and molecular characterisation of the causative mutation in a consanguineous family with X-linked retinoschisis

Results: By direct sequencing of the RS1 gene, one pathogenic variant, NM_000330.3: c.304C>T, p. R102W, was identified in all clinically diagnosed individuals analysed. The two females were homozygous for the variant whilst the males were hemizygous.

Conclusion: Clinical and genetic characterisation of affected homozygous females in X-linked retinoschisis affords the rare opportunity to explore the molecular mechanisms of XLRS and the manifestation of these mutations as disease in humans.

Key Words: X-linked retinoschisis, clinical genetics, molecular genetics
INTRODUCTION

X-linked Retinoschisis (XLRS) is caused by mutations in the Retinoschisin (RS1) gene and is a leading cause for inherited macular degeneration in juvenile males.\(^1,2\) It typically manifests with foveal schisis and splitting of outer plexiform layers by ten years of age.\(^3,4\) Disease phenotype can show significant intra-familial variability. Though the cause of this is poorly understood, 5 genetic modifiers are likely to play a role. While these remain elusive in humans, a major genetic modifier of retinoschisin1 (Mor1) has been identified using a mouse model for XLRS.\(^6\)

The RS1 gene, located on chromosome Xp22.2 has been found to be expressed exclusively in retinal tissues. RS1 encodes an extracellular protein, important in photoreceptor membrane adhesion.\(^7\) Interestingly, disease causing variants in RS1 have been found to predominate in the highly conserved discoidin domain.\(^8,9\) This domain is encoded by exons four to six of RS1 and homologous to other cell adhesion and cell signalling proteins.\(^7,10,11\) Missense mutations in RS1 generally cause protein misfolding which leads to intracellular retention and degradation of retinoschisin.\(^12\) Only one case of XLRS in females from a consanguineous family has been previously reported in the literature.\(^13,14\) We present a further consanguineous immigrant Australian family with two males, as well as two females, clinically diagnosed with XLRS and show variable disease phenotypes.

METHODS

A consanguineous Australian family, (Figure 1) containing individuals clinically diagnosed with XLRS who had not before participated in similar research, were analysed for disease causing mutations in the RS1 gene. Informed consent was obtained from all research participants in accordance with the Human Research
Ethics Committee’s approval guidelines of both the Sir Charles Gairdner Hospital (Western Australia) and the Royal Victorian Eye and Ear Hospital (Victoria).

**Molecular Genetic Studies**

Saliva samples were collected from four individuals clinically diagnosed with XLRS and nine reportedly unaffected family members (Oragene® DNA Self-Collection kit, DNA Genotek). Genomic DNA was extracted by the Western Australian DNA Bank (www.wadb.org.au), and analysed by the Australian Inherited Retinal Disease Register and DNA Bank (AIRDR) for disease causing mutations in the RS1 gene by amplification and bidirectional sequencing of coding and flanking intronic regions using published primers. Sequences were analysed against human reference sequence, NM_000330.3 (Assign ATF v1.5. Conexio Genomics, Fremantle, Australia).

**Clinical Characterisation and Electrophysiology**

Nine previously unexamined family members underwent full ophthalmological examination, including dilated fundus examination. Previous clinical findings for the known affected family members were retrospectively collected on chart review. Reported results of electrophysiological tests for XLRS-1 and XLRS-3 (Figure 1) were performed to International Society for Clinical Electrophysiology of Vision (ISCEV) standards.

**RESULTS**

This study focussed on the clinical and molecular characterisation of a consanguineous family containing both males and females clinically diagnosed with XLRS. (Figure 1). This pedigree contains one non-consanguineous nuclear family with an affected male (XLRS-12) having inherited XLRS from his obligate carrier mother (XLRS-9) and maternal grandmother (XLRS-8). The second nuclear family consists of two affected females (XLRS-3, XLRS-4) presumably due to their parents consanguineous relationship (first cousins); their father (XLRS-1) has retinoschisis.

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whilst their mother (XLRS-2) is thought to be a carrier, with a normal ophthalmic examination. XLRS-2 also had a previous union resulting in two clinically unaffected daughters (XLRS-5, XLRS-6). Additional, reportedly affected, family members reside in eastern Europe were not included in this study.

**Molecular Genetic Findings**

Sequencing of the male proband (XLRS-1, Figure 1) identified a known disease-causing variant, c.304C>T (rs61752067; HGMD#CM971319) occurring in exon four of the RS1 gene. This change is predicted to cause a p.Arg102Trp substitution in the encoded protein. No other variants were detected. Further analyses confirmed the absence of wild-type signal at position c.304 in all affected family members. The two males (XLRS-1 and XLRS-12) are hemizygous, whilst the two affected females (XLRS-3 and XLRS-4) are homozygous for this mutation. The mother and father (XLRS-2 and XLRS-1) of the affected sisters (XLRS-3 and XLRS-4) were found to be heterozygous and hemizygous for this familial variant respectively. Targeted sequencing of the two daughters of XLRS-2 from a previous marriage, identified one as a carrier (XLRS-6) and the other wild-type (XLRS-5) for the familial variant. (Figure 2)

**Clinical Findings**

Clinical findings for the four affected individuals in the pedigree studied are described in Table 1. Of note, the females (XLRS-3 and -4) both presented from birth with high hypermetropia, accommodative esotropia and nystagmus. The proband XLRS-1 was thought to have had strabismic amblyopia from childhood, with a diagnosis of retinoschisis being made only after his daughter (XLRS-3) was diagnosed. Due to subsequent complications from the schisis and retinal detachments, XLRS-1 now meets the criteria for legally blind status. XLRS-12 also experienced a delayed diagnosis, having had reduced vision from age 6 years attributed to astigmatism. Following a blunt force head injury, he sustained a retinal
detachment, requiring multiple surgeries. No significant clinical findings were identified in the remaining nine family members.

**DISCUSSION**

In this study we revealed the genetic diagnosis of affected males and females within a consanguineous XLRS family, and comparatively analysed their disease phenotypes. The detected RS1 variant c.304C>T has been previously reported in two Western European families, a cluster of families from the United Kingdom 5,8,16 and various other populations including China. 3,8,17 The encoded non-synonymous change p.Arg102Trp is located within the phylogenetically conserved discoidin domain of the retinoschisin protein. This and other substitutions in this region result in severe protein misfolding, which reportedly interferes with secretion of the encoded retinoschisin protein from the cell.12,18,19,20

Compared to the affected males, the affected females in this family were diagnosed at an earlier age, both being diagnosed with nystagmus by the age of 1 year. A similar observation was made for the previously reported consanguineous family containing affected females.13,14 The reason for this remains unclear, but may be associated with a disparate inheritance of RS1 genetic modifiers (between affected males and females), still yet to be elucidated in humans. Alternatively it is possible that there may be some other mechanism inherent to the females possessing two faulty alleles, which render them susceptible to a more severe disease phenotype. This seems less likely, particularly in light of the well established phenotypic variability of this and other discoidin domain substitutions.5,21,22

The success of RS1 gene-therapy trials in animal models23,-26 holds promise for the development of an effective gene-based therapeutic strategy in humans in the foreseeable future. Genetic characterisation, as well as the establishment of a clinical baseline, is an important preceding step for participation in such trials and for taking advantage of family planning options, including prenatal or pre-implantation genetic
diagnosis. Affected homozygous females are exceptionally rare in x-linked recessive disorders, so this study represents an important special case of such a genetic and clinical characterisation.

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19. Wu W W, Molday R S. Defective discoidin domain structure, subunit assembly, and endoplasmic reticulum processing of retinoschisin are primary mechanisms responsible for X-linked retinoschisis. J Biol Chem 2003;278:28139-28146


**Figure 1:** Pedigree of consanguineous XLRS family
**Figure 2:** Electropherograms

- **XLRS-1**: c.304C>T, Affected male
- **XLRS-12**: c.304C>T, Affected male
- **XLRS-3**: c.304C>T, Affected female
- **XLRS-4**: c.304C>T, Affected female
- **XLRS-6**: c.304C>T, Carrier female
- **XLRS-9**: c.304C>T, Carrier female
- **XLRS-10**: c.304C>T, Carrier female
- **XLRS-11**: c.304C>T, Carrier female
- **XLRS-13**: c.304C, Non-carrier female
- **XLRS-5**: c.304C, Non-carrier female
Table 1: Clinical data of male and female family members hemizygous and homozygous for familial RS1 mutation c.304 C>T.

<table>
<thead>
<tr>
<th>DEMOGRAPHICS</th>
<th>XLR1-3</th>
<th>XLR1-4</th>
<th>XLR1-1</th>
<th>XLR1-12</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>11</td>
<td>9</td>
<td>51</td>
<td>19</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age Onset</td>
<td>Birth</td>
<td>Birth</td>
<td>0-5y (treated for strabismus and amblyopia as a child)</td>
<td>6y</td>
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</table>

<table>
<thead>
<tr>
<th>SYMPTOMS</th>
<th>XLR1-3</th>
<th>XLR1-4</th>
<th>XLR1-1</th>
<th>XLR1-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyctalopia</td>
<td>Y (birth)</td>
<td>Y (birth)</td>
<td>Y (≤childhood)</td>
<td>Yes (≤childhood)</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>Y (birth-sensory)</td>
<td>Y (birth-sensory)</td>
<td>N</td>
<td>N</td>
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<tr>
<td>Hyperopia</td>
<td>Y (high, birth)</td>
<td>Y (high, birth)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Amblyopia</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Not recorded</td>
</tr>
<tr>
<td>Cataract</td>
<td>N</td>
<td>N</td>
<td>Y (post-vitrectomy)</td>
<td>N</td>
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<tr>
<td>Floaters</td>
<td>N</td>
<td>N</td>
<td>Y (childhood, intermittent)</td>
<td>N</td>
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<tr>
<td>Photophobia</td>
<td>N</td>
<td>N</td>
<td>Y (≤ teens)</td>
<td>N</td>
</tr>
<tr>
<td>Light Flashes</td>
<td>Y (intermittent)</td>
<td>N</td>
<td>Y (intermittent)</td>
<td>N</td>
</tr>
<tr>
<td>Schisis</td>
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<td>N</td>
<td>Y</td>
<td>Not examined prior to detachment</td>
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<tr>
<td>Retinal Detachment</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y (11 yo. Ball hit head)</td>
</tr>
<tr>
<td>Electroneg b-wave (ERG)</td>
<td>Y</td>
<td>not done</td>
<td>Y</td>
<td>not done</td>
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