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• Brief Report •

X-linked juvenile retinoschisis: phenotypic and genetic characterization

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Abstract

• Juvenile X-linked retinoschisis (XLRS, MIM#312700) belongs to a group of the vitreoretinal dystrophies. We aimed to describe the phenotype-genotype correlation of three XLRS cases in juveniles with different novel mutations from the Lithuanian population. The patients demonstrated macular retinoschisis and typical cyst-like cavities on spectral-domain optical coherence tomography (SD-OCT) images. The mean central foveal thickness was 569.7 μm. Two patients presented with peripheral retinoschisis. Flash electroretinogram demonstrated a reduced b/a ratio (<1.0) in all patients. *RS1* (NM_000330.3) gene coding exons Sanger sequencing was performed. *RS1* c.599G>T (p.R200L) mutation was detected in one case, showing to be pathogenic in silico analysis. c. (92_97) insC (p.W33fs) mutation was identified for another patient, indicating the variant is possibly damaging in silico analysis. The third case was identified with a pathogenic mutation c.422C>G (p.R141H), HGMD CM981753. These are the first cases of XLRS in the Lithuanian population confirmed by molecular genotyping. Presented patients had a different genotype but similar phenotypic traits.

• KEYWORDS: X-linked retinoschisis; *RS1* mutation; optical coherence tomography; electroretinogram
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INTRODUCTION

Juvenile X-linked retinoschisis (XLRS, MIM#312700) is a relatively common vitreoretinal dystrophy affecting males between the ages of 5-10y with reading difficulties[1]. The worldwide prevalence is ranging from 1:5000 to 1:20 000[2]. Juvenile retinoschisis is characterized by bilateral maculopathy-radial streaks arising from foveal schisis, with associated splitting of inner retinal layers in the peripheral retina in 50% of patients[2]. Eyes with peripheral schisis show a characteristic negative electroretinogram (ERG) arising from a marked reduction in b-wave amplitude[1]; however, the ERG response is more variable than previously expected[3]. Best-corrected visual acuity (BCVA) typically ranges from 20/50 to 20/120 and may remain stable until the 5th or 6th decade of life. The prognosis of the disease is poor due to progressive maculopathy-the cavities tend to resolve and the visual acuity (VA) drops[4]. Disease progression and severity is highly variable even within families. During the course of the disease, secondary complications including vitreous or intra-schisis hemorrhage, neovascularization, subretinal exudation and rarely retinal detachment (RD) and traumatic rupture of foveal schisis can occur. Female carriers are asymptomatic but detailed ophthalmological examination can reveal minor retinal abnormalities[1].

XLRS is an X-linked recessive disease, with almost full penetrance, but it exhibits a high degree of intra- and interfamilial variability[3]. Molecular genetic studies identified the only disease causative *RS1* gene on chromosome Xp22 in 1997[1]. This gene encodes a 224 amino acid protein known as retinoschisin (RS1), expressed exclusively by photoreceptors and bipolar cells in the retina and pineal gland[1]. Over 200 disease-causing mutations in the *RS1* gene are known with most mutations occurring as non-synonymous changes in the major protein unit, *i.e.* discoidin domain[1].

Retinoschisin is expressed in photoreceptors as a disulphide-linked homo-octameric complex, but the protein is found in both inner and outer retina. RS1 binds to the surface of photoreceptors and bipolar cells-it acts like a cell adhesion protein and plays a crucial role in maintaining the structural integrity of the inner retina[1,3].

Clinical diagnosis of XLRS can be challenging due to young age of the patients, variable phenotype and limited correlation in genotype-phenotype studies[2].

We aim to present the phenotypic and genetic correlations of three juvenile XLRS cases with different novel and described *RS1* mutations from the Lithuanian population.

METHODS

Three adolescent male patients presented at Vilnius University Hospital Santaros Klinikos with a suspicion of a juvenile retinoschisis. Genealogical data showed no ophthalmic disease and the parents have no clinical symptoms.

After a full medical and ophthalmic history, an ophthalmological examination including external examination, slit-lamp and dilated fundus examination was performed. BCVA (evaluation with ETDRS chart), color vision with Ishihara plates were assessed. Two patients underwent static Humphrey 30-2 visual field test (Carl Zeiss Meditec, Humphrey Field Analyzer), one patient do to its small age-a kinetic Goldmann visual field test. Colour fundus photography was performed with a TRC-50DX retinal fundus camera (Topcon, Tokyo, Japan).

Cross-sectional retinal images were recorded horizontally through the fovea using spectral domain optical coherence tomography (SD-OCT) enhanced depth imaging (EDI) (Heidelberg Engineering, Heidelberg, Germany). Flash-ERG (RETI-port gamma plus, Roland Consult) were recorded using gold foil electrodes. Protocols followed the recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV).

DNAs from the patients and possibly their carrier mothers have been extracted using phenol-chloroform method (protocol available upon request) from blood samples. *RS1* (NM_000330.3) gene coding exons Sanger sequencing using specific primer sequences (available upon request) was performed. Both strands of polymerase chain reactions (PCR) products were sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, USA). Capillary electrophoresis was performed on 3130xL Genetic Analyser (Applied Biosystems, USA) and primary sequence analysis performed using Sequence Analysis v5.2 software (Applied Biosystems, USA).

This research was adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Vilnius University Faculty for Medicine. Informed consents for ophthalmic and genetic investigations were obtained from the participating families.

RESULTS AND DISCUSSION

Cases Presentation Three adolescent male patients, aged 9 (1st patient), 12 (2nd patient) and 17 (3rd patient) years old, presented with complains of near vision difficulties and had never had a full far vision. They mostly complained of reading difficulties, also tiredness of the eyes and text shimmering while reading. The BCVA for distance ranged from 0.32 to 0.6, all patients had near BCVA of 0.6. The refraction of all patients was hyperopic with astigmatism. The eye movements for all patients were full, no squint, anterior segment was unremarkable. In fundus photography (Figure 1) the macular reflex was lacking bilaterally for all patients, in fundus examination binocular optic papilla was oval, with clear boundaries, pink in color, cup/disc ratio (C/D) was physiological. SD-OCT scans (Figure 2) of all patients revealed macular schisis with large intraretinal separation mainly in the inner nuclear layer (INL), less in the outer plexiform, outer nuclear layer and ganglion cell layer, extending beyond the foveal area and a certain amount and size of cavities formation. The mean central foveal thickness (CFT) was 569.7 μm. Peripheral retinal changes were variable: from peripheral schisis inferiorly and nasally, with light traction, additional lamellar hole in the inner layer and obliterated vessels in the left eye (LE) for the first patient, demarcation lines nasally in the right eye (RE) and paramacular temporal in the LE for the second patient and no changes in the peripheral retina for the third patient.

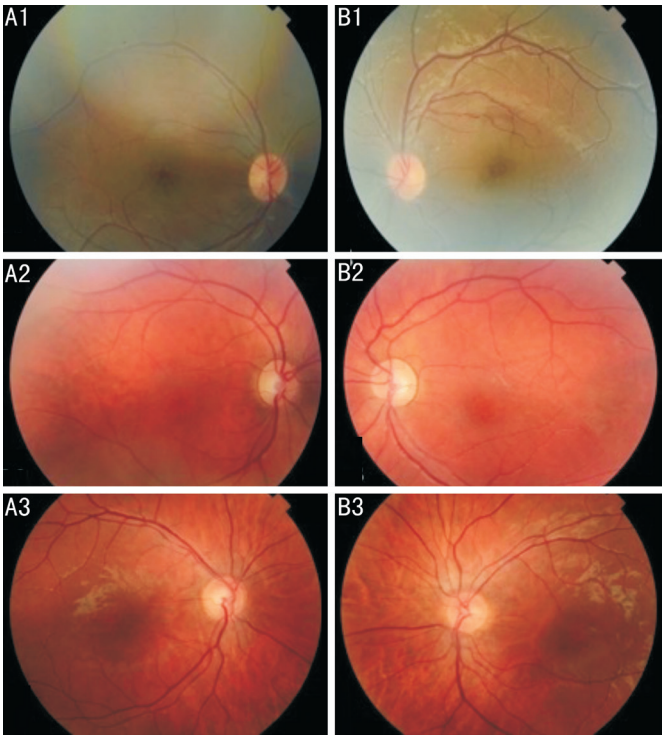

Figure 1 Fundus photography and macular appearance of all patients At the macula of the first patient dystrophic changes, pigment mottling, marked cystic changes can be seen (A1, B1), for the second patient clear spoke-wheel pattern foveal schisis, cystic edema, star-shaped pigment mottling (A2, B2) could be seen, third patient showed foveal schisis and cystic changes (A3, B3).

Ishihara color test was unremarkable for all patients.

First and second patients underwent the standard automated perimetry of 30 degrees, but it couldn‘t be evaluated correctly due to a high amount of fixation losses and errors.

Third patient underwent Goldman visual field investigation, which results were normal.

All patients underwent an ISCEV standard full-field ERG. In all the patients flash ERG showed the typical selective decrease

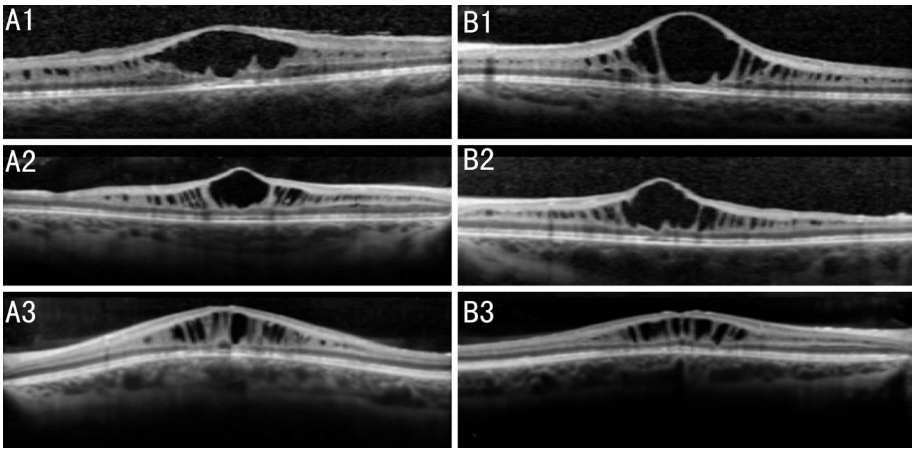

Figure 2 SD-OCT scans revealed macular schisis with large intraretinal separation for all patients The CFT of the first patient RE was 600 μm, LE was 661 μm (A1, B1); second patient CFT of the RE was 570 μm, LE was 777 μm (A2, B2); third patient CFT of the RE was 373 μm, LE was 434 μm (A3, B3).

or absence of the b-wave as compared to the a-wave (negative ERG). The multifocal ERG was performed for the second and third patients and showed a great reduction of central responses in both eyes.

Second and third patients were treated with Nepafenaci 0.1% eye drops (Nevanac eye drops, 1 mg/mL, S.A. Alcon-Couvreur N.V., Rijksweg 14, B-2870 Puurs, Belgium) three times daily for a 2y of follow-up period every three months and discontinuing the drug in case of maximal positive effect or ocular side effects. CFT dropped and the retinal cavities diminished while using Nepafenac eye drops and regained their thickness while discontinuing the drug.

NM_000330.3:c.599G>T (p.R200L) mutation was detected in one patient, *in silico* analysis showing to be pathogenic [disease causing (mutationtaster.org); PROVEAN score - 6.883 – deleterious; probably damaging]. Human Genome Mutation Database (HGMD) involves three other different mutations at the same position (CM095237[5], CM981767[6], CM981768[6]) supporting the pathogenicity of the identified variant. NM_000330.3:c.(92_97) insC (p.W33fs) mutation creating a frame shift was identified for another patient, again *in silico* analysis indicating the variant is possibly damaging and disease causing (mutationtaster.org). The third patient was identified with a described pathogenic mutation NM_000330.3:c.422G>A (p.R141H), HGMD CM981753[6]. The mothers of all three patients have been confirmed to be the heterozygous carriers of the identified mutations by Sanger sequencing.

According to the literature, macular schisis in XLRS patients ranges from 68% to 100%[7] and peripheral retinoschisis-from 43% to 60%[8]; similar to macular schisis, peripheral schisis may also be influenced by the variable age of the cohort, since schisis cavities usually resolve with time. Second and third patients in our study had peripheral schisis, and all of them were affected by foveal schisis, which had the main negative impact to life quality.

Other peripheral retinal findings with reported rates by George *et al*[8] (56 patients) may include metallic sheen (38%), retinal pigment epithelium (RPE) pigmentary changes (29%), white spiculations (11%), vascular sheathing (9%) and vitreous veils (39%)[7]. Just in two out of our three patients vitreous veils and no other peripheral findings were observed.

The natural history of disease is macular schisis, which can resolve with time and be followed by macular atrophy. Our patients were still too young to experience macular atrophy. George *et al*[8] reports that 100% of eyes showed bilateral maculopathy on examination-schisis, blunted fovea, dispigmentation or RPE atrophy.

This chronic clinical course can be interrupted by a sudden deterioration of vision due to complications explained earlier[7]. Optical coherence tomography (OCT), as a non-invasive imaging, helps to elucidate the precise location of the schisis, in which INL was found to be the most common site for schisis[9]. Yu *et al*[10] results were similar to ours and he showed that INL, outer plexiform layer and outer nuclear layer schisis were found in XLRS. Other structural alterations of photoreceptors like thinning, ellipsoid zone defects with the RPE alterations, atrophy as well as normal macular structure on SD-OCT (Fahim *et al*[7], 8% of eyes) were also noted. The cavity size can vary according to the individual regardless of age, and the causes for that could include specific mutation, other eye disorders, or medication[4].

It is well established that an electronegative bright flash dark-adapted ERG is a characteristic, classic finding in XLRS, although it is not universal[11]. According to Bradshaw *et al*[12], the amplitude of the a-wave is reduced in up to 30% of XLRS patients. All of our patients had an electronegative bright flash dark-adapted ERG.

Studies show that schisis can involve different layers of retina, supporting the idea of a phenotypical variability. This variability can present: as reduction in amplitudes of either

a or b wave or both under scotopic or photopic conditions in different eyes. The decrease of b-wave amplitudes may imply disruption of the inner retinal morphology and this may cause the dysfunction of photoreceptor and Müller/bipolar cells. Abnormal oscillatory potentials seen can point to a possible role of amacrine cells in the pathogenesis of retinoschisis. Issues of ERG are the large test-retest variability of ERG recordings and the specific limitations associated with ERG assessment in children, even worse in cases of squint and nystagmus. So future prospective studies of bright flash dark-adapted a- and b-wave ERG amplitudes should concentrate in patients with peripheral schisis and detection of a beneficial marker to avoid the secondary complication like vitreous hemorrhage or RD[13].

Mutations in the *RS1* gene are responsible for the inherited and sporadic XLRS. All the molecular genetic analysis of the gene and protein expression in the retina have directly included photoreceptors and bipolar cells in the disease process[1]. Over 200 disease-causing mutations in the *RS1* gene are known with most mutations occurring as non-synonymous changes in the major protein unit, *i.e.* discoidin domain.

Previous investigations of genotype-phenotype correlations have found that less or almost no macular abnormalities are found for patients carrying missense mutations, although the inverse is not true[6]. Other genotype-phenotype investigations have also revealed worse VA and more frequent ERG abnormalities among patients with nonsense, splice-site and frame shift mutations compared to those with missense mutations[14].

The eye represents a proper target for gene therapy due to the immune privilege provided by the blood-ocular barrier, the ability to easily visualize, access and locally treat the cells and the minimal amount of vector needed due to the size of this organ. Several active gene therapy clinical trials are now ongoing for inherited retinal dystrophies, including juvenile XLRS[15].

The present study describes three XLRS adolescent male patients with XLRS different mutations with very similar central morphological and functional changes, but different peripheral changes. It indicates that careful clinical examination, including SD-OCT, retinal imaging, and *RS1* mutation screening, remain the basic procedures for a correct diagnosis. To conclude, although the phenotype-genotype correlation of XLRS is still under debate, further investigations of genetic and/or epigenetic factors that may affect peripheral changes, clinical causes of the disease and perhaps possible treatment are needed.

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