Correlation of Genetic and Clinical Findings in Spanish Patients with X-linked Juvenile Retinoschisis

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Purpose. X-linked juvenile retinoschisis (XLRS) is one of the most common causes of juvenile macular degeneration in males, characterized by microcystic changes, splitting within the inner retinal layer (schisis), and the presence of vitreous veils. This study was conducted to describe and further correlate specific genetic variation in Spanish patients with XLRS with clinical characteristics and additional ophthalmic complications.

Methods. The study was performed in 34 Spanish families with XLRS, comprising 51 affected males. Thorough clinical ophthalmic and electrophysiological examinations were performed. The coding regions of the *RS1* gene were amplified by polymerase chain reaction and directly sequenced. Haplotype analyses were also performed.

Results. Twenty different mutations were identified. Ten of the 20 were novel and 3 were de novo mutational events. The most common mutation (p.Gln154Arg; 6/20) presented a common haplotype. *RS1* variants did not correlate with ophthalmic findings and were not associated with additional ophthalmic complications.

Conclusions. The prevalent p.Gln154Arg mutation is first reported in this work and presents a common origin in Spanish patients with XLRS. In addition, de novo mutations mainly occur in CG dinucleotides. Despite the large mutational spectrum and variable phenotypes, no genotype–phenotype correlations were found. Identifying the causative mutation is helpful in confirming diagnosis and counseling, but cannot provide a prognosis. (*Invest Ophthalmol Vis Sci.* 2009;50:4342–4350) DOI:10.1167/iovs.09-3418

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X-linked juvenile retinoschisis (XLRS; OMIM 312700; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is one of the most common causes of juvenile macular degeneration in males.[1] This X-linked trait affects only males; female carriers rarely have vision morbidity.[2,3]

XLRS is a symmetrical bilateral macular disorder with onset in the first decade of life, in some cases, as early as 3 months of age. Fundus examination shows microcystic changes of the macular region of the retina and areas of splitting within the nerve fiber layer, or schisis,[4,5] giving the impression of a spoked-wheel pattern, and the presence of vitreous veils, which are neural sheets that float in the vitreous cavity. Severe cases involve full-thickness retinal detachment.[4,6] More advanced stages of the disease are complicated by vitreous hemorrhage, retinal detachment, and neovascular glaucoma,[7] which may induce severe loss of vision. The electroretinograms (ERGs) of most affected males demonstrate normal or near-normal a-waves characteristic of photoreceptor function but often substantially reduced b-waves, originating from inner retinal cell activity.[8]

The *RS1* gene maps to chromosome Xp22.2-22.1, contains six exons, and encodes a 224-amino-acid protein called Retinoschisin,[9] with an N-terminal secretory leader peptide sequence[10,11] and a discoidin domain in exons four to six. Discoidin domains are highly conserved across species,[12] are found in a large family of secreted or membrane-bound proteins and have been implicated in cell adhesion and cell–cell interactions.[13]

It is known that Retinoschisin associates with retinal cell surface membranes,[14,15] acting as a cellular scaffold for the retinal architecture. Recently, Molday et al.[14] indicated that Retinoschisin forms a complex with Na/K-ATPase and SARM1, therefore being implicated in a novel signaling pathway important in the photoreceptor–bipolar synaptic structure and function and retinal cell organization.

Extensive investigations have led to the identification of numerous disease-causing mutations in the *RS1* gene, including missense, nonsense, deletions, insertions, and splice site mutations (www.hgmd.org/ Human Gene Mutation Database provided in the public domain by the Institute of Medical Genetics, Cardiff, Wales, UK). The correlation between the phenotype and genotype of XLRS remained unclear, according to reports.[16,17]

The purpose of this systematic screening was two-fold: to describe specific genetic variation in Spanish patients with XLRS and to further correlate the most frequent *RS1* variants with clinical characteristics and specific ophthalmic complications: retinal detachment, vitreous hemorrhage, and strabismus.

Methods

Ascertainment of Patients

This molecular study was reviewed and approved by the Ethics Committee of Fundacion Jimenez Diaz, and it was performed according to

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the tenets of the Declaration of Helsinki and further reviews (Edinburgh, 2000; www.wma.net). Thirty-four Spanish families presenting with XLRS due to mutations in the *RS1* gene were studied. The patient group consisted of 51 affected males.

The age at onset was defined as either the patient's age at which visual loss was first noted or the age documented in an ophthalmic record of the first diagnosis. In all cases, thorough clinical ophthalmic and electrophysiological examinations were performed, including a comprehensive ophthalmic and family history, funduscopic examination after pupillary dilation, static perimetry and best corrected visual acuity examination. For some patients, optical coherence tomography (OCT) images were obtained, which provided high-resolution, cross-sectional images of the macular region. Electrophysiological assessment included full-field electroretinogram (ERG), according to standards of the International Society for Clinical Electrophysiology of Vision (ISCEV).[18,19]

Ascertainment of Control Subjects

Healthy control individuals were recruited from anonymous male blood donors referred by the Blood Service of the Hospital. Subjects previously provided a signed informed consent, as well as their nationality, age, and sex. Thus, the ethnic background of recently immigrated individuals was ruled out. The control group was free of any ocular disease.

Molecular Analysis

DNA Extraction. Peripheral blood samples with EDTA anticoagulant were collected from each member of the family. Genomic DNA was extracted using an automated DNA extractor (BioRobot EZ1; Qiagen, Hilden, Germany). Before use, DNA samples were preserved frozen.

Direct Sequencing. The six exons of the *RS1* gene, including intron–exon junctions were amplified by PCR with published primers.[9] These fragments were electrophoresed in a 3% agarose gel and purified with a DNA extraction kit (QIA-quick Gel Extraction Kit; Qiagen, Hilden, Germany). Sequencing reactions were performed with a four-dye terminator, cycle-sequencing, ready-reaction kit (BigDye DNA Sequencing Kit; Applied Biosystems, Inc., Foster City, CA). Sequence products were purified through fine columns (Sephadex G-501; Princetown Separations, Adelphia, NJ) and resolved on a sequencer (ABI Prism 3130; Applied Biosystems).

In addition, the presence of novel mutations located on exons 4, 5, and 6 of the *RS1* gene were analyzed in 100 male control chromosomes by direct sequencing.

Haplotype Analysis

Haplotypes were generated by using three microsatellite markers flanking the *RS1* gene (TEL-*DXS9911-RS1-DXS999-DXS989*-CEN). In several patients, four additional microsatellite markers were amplified (TEL-*DXS8022-DXS1053-DXS8019-DXS9911-RS1-DXS999-DXS1226-DXS989*-CEN) to determine whether they shared a common haplotype. After amplification by PCR, fluorescent-labeled products were mixed and electrophoresed (ABI Prism 3130; Applied Biosystems, Inc.). For haplotype reconstruction, an informatic program was used (Cyrillic ver. 2.1; Cyrillic Software, Wallingford, UK).

Statistical Analysis

For genotype–phenotype correlation studies, a χ^2 test was performed to examine the correlation between specific *RS1* variants, p.Gln154Arg, p.Glu72Lys, p.His194fsX263 and p.Pro203Leu, and specific ophthalmic complications: retinal detachment, vitreous hemorrhage, and strabismus. In addition, visual acuity was further compared among patients who did or did not have any of these complications. Statistical analyses were performed on computer (SPSS 10.0 Software; SPSS, Chicago, IL), and $P < 0.05$ was considered a statistically significant difference.

Results

Molecular Findings

Thirty-four Spanish families with XLRS, comprising 51 male patients, were evaluated, and 20 different hemizygous nucleotide substitutions were detected. Most disease alleles carried missense mutations (17/20; 85.0%). However, frameshift variants (2/20; 10.0%) and one nonsense mutation (1/20; 5.0%) were also identified in XLRS chromosomes. All mutations were clustered in exons 4, 5, and 6 encoding the discoidin domain. Most of the mutations detected have been reported as XLRS-associated variants, namely: p.Glu72Lys, p.Tyr89Cys, p.Arg141Cys, p.His194fsX263, p.Arg197Cys, p.Pro203Leu, p.Arg209His, p.Arg213Gln, p.Glu215Gln, and p.Leu216Pro. In addition, 10 novel disease-associated variants were identified: p.Gln80Ter, p.Leu137Pro, p.Thr138fsX, p.Gln154Arg, p.Pro192Leu, p.Ile194Asn, p.Arg197Ser, p.Arg200Ser, p.His207Asp, and p.Glu215Val (Table 1). These alleles were absent in 100 Spanish ethnically matched control chromosomes, and cosegregated within the families, suggesting that they are pathologic.

Common Haplotypes

The most frequent *RS1* variant in the Spanish XLRS population was the missense p.Gln154Arg (c.461A>G) mutation, being present in 17.64% (6/34) families. Haplotype analyses with seven microsatellite markers (TEL-*DXS8022-DXS1053-DXS8019-DXS9911-RS1-DXS999-DXS1226-DXS989*-CEN), spanning over 12.65 cM, were amplified in affected members from these six families. This molecular study showed that the common piece of DNA comprises 6.32 cM between the *DXS1053* and *DXS999* markers, which suggests a founder effect (Fig. 1A).

The missense p.Tyr89Cys mutation in exon 4 of the *RS1* gene was identified in two Spanish families. Allelic segregation analysis was performed in the index case of XLRS-44 and in all family members from XLRS-306. A common haplotype was demonstrated between the markers *DXS8022* and *DXS989* (12.65 cM), suggesting a common ancestry (Fig. 1B).

Recurrent Mutations

The second most frequent mutation was p.Glu72Lys (c.214G>A),[20] which was identified in five families. Nevertheless, these cohorts seemed not to be related, since they did not exhibit the same haplotype (Fig. 2A).

The third most frequent mutation was the p.His194fsX263 variant, which was identified in three unrelated families and most likely was of independent origin (Fig. 2B).

The novel missense p.Glu215Val (c.644A>T) mutation, in exon 6 of the *RS1* gene was identified in families XLRS-101 and XLRS-320. Allelic segregation analysis was performed in all family members, and the disease-associated haplotype segregated within the families. In addition, haplotype construction demonstrated an independent origin (Fig. 2C).

De Novo Mutational Events

In XLRS-108, a novel nonsense mutation was identified in an affected child: a C-to-T change at nucleotide 238 in exon 4 of the *RS1* gene, where glutamine was replaced by a stop codon at codon 80 (p.Gln80Ter [c.238C>T]) (Table 1). This change was not detected in his mother, indicating a de novo event. In addition, p.Glu72Lys was detected as a de novo mutation in XLRS-43. In this family, this variant occurred for the first time in a carrier mother who had two affected male twins (Fig. 2A). The p.Pro203Leu variant was identified in two families. Haplotype analysis demonstrated independent origin for this *RS1* mutation. Similar to family XLRS-43, in family XLRS-199, this mutation was described for the first time in a carrier mother of

TABLE 1. Summarized *RS1* Mutations in Spanish X-Linked Juvenile Retinoschisis Pedigrees

Pedigrees	*n*	Exon	Nucleotide Change	Amino Acid Change	Predicted Effect	Frequency (%)
43*, 93, 104, 185, 189	5	4	c.2I4G>A	p.Glu72Lys	Missense	14.70
108*	1	4	**c.238C>T**	**p.Gln80Ter**	Nonsense	2.94
44, 306	2	4	c.266A>G	p.Tyr89Cys	Missense	5.90
102	1	5	**c.410T>C**	**p.Leu137Pro**	Missense	2.94
250	1	5	**c.412_421del**	**p.Thr138fsX145**	Frameshift	2.94
95	1	5	c.421C>T	p.Arg141Cys	Missense	2.94
30, 70, 97, 143, 161, 219	6	5	**c.461A>G**	**p.Gln154Arg**	Missense	17.64
217	1	6	**c.575C>T**	**p.Pro192Leu**	Missense	2.94
53, 55, 124	3	6	c.578_579insC	p.His194fsX263	Frameshift	8.82
321	1	6	**c.581T>A**	**p.Ile194Asn**	Missense	2.94
162bis	1	6	**c.589C>A**	**p.Arg197Ser**	Missense	2.94
145	1	6	c.589C>T	p.Arg197Cys	Missense	2.94
322	1	6	**c.598C>A**	**p.Arg200Ser**	Missense	2.94
199*, 268	2	6	c.608C>T	p.Pro203Leu	Missense	5.90
276	1	6	**c.619C>G**	**p.His207Asp**	Missense	2.94
112	1	6	c.626G>A	p.Arg209His	Missense	2.94
45	1	6	c.638G>A	p.Arg213Gln	Missense	2.94
29	1	6	c.643G>C	p.Glu215Gln	Missense	2.94
101, 320	2	6	**c.644A>T**	**p.Glu215Val**	Missense	5.90
107	1	6	c.647T>C	p.Leu216Pro	Missense	2.94
Total	34					

Novel mutations are in bold. Mutations occurring at C or G from CG dinucleotides are underscored; *n*, number of families.
* Pedigrees with a de novo mutational event.

two affected twins (Fig. 2D). Of interest, these de novo mutations were located on CG dinucleotides (Table 1).

Genotype–Phenotype Correlation

The summary of clinical and genetic findings is presented in Table 2. In general, clinical data demonstrated intra- and interfamilial variability (i.e., age at onset ranged from congenital to the 2nd decade of life). Also, patients' visual acuity varied from counting fingers to 60/100, or a wide variation was observed between both eyes.

Three main general symptoms were observed: (1) Retinal schisis: 92.7% of the patients presented macular schisis, whereas only 24.4% also showed peripheral schisis; (2) vitreous abnormalities: 46.3% of the affected individuals presented vitreoretinal proliferation, vitreous degeneration, or persistent hyperplastic primary vitreous; (3) abnormal ERG: in every patient in whom ERG was performed, it showed alterations, mainly in the b-wave amplitude, ranging from reduced to extinguished. Besides, additional ophthalmic findings were observed in less proportion: cataracts (31.8%), amblyopia (31.8%), scotomas—mainly associated with schisis areas—(31.8%), photophobia (27.3%), or nyctalopia (27.3%). Finally, in elder subjects (>40 years old), retinal pigment epithelium (RPE) atrophy, pigment spots, optic pallor, and vascular abnormalities were observed.

In family XLRS-112, we identified two brothers bearing the p.Arg209His (c.626G>A) mutation. Of interest, one of them presented with typical retinoschisis symptoms, whereas his brother was asymptomatic at the age of 39.

Despite typical clinical features of XLRS—macular schisis, microcysts, and vitreous veils—additional ophthalmic findings of retinal detachment, vitreous hemorrhage, and strabismus were evaluated in patients presenting the most frequent *RS1* variants: p.Gln154Arg, p.Glu72Lys, p.His194fsX263, and p.Pro203Leu. To determine whether these retinal complications were associated with specific mutations in the patients, we further compared both parameters by χ^2 test ($P = 0.05$). This analysis revealed that *RS1* variants did not correlate with these ophthalmologic complications and were not associated with retinal detachment, vitreous hemorrhage, or strabismus.

DISCUSSION

In this study, we examined *RS1* gene mutations from 51 affected males belonging to 34 Spanish families and evaluated genotype–phenotype correlations in these patients. Twenty different hemizygous nucleotide substitutions in the coding region of the gene were identified, of which 14 were found only once. The majority of these (17/20) were missense mutations, although nonsense mutations (1/20), insertions (1/20), and deletions (1/20) have all been found. Of these 20 variants, 10 were not previously reported.

All mutations were not distributed randomly over the gene, as all them were found in exons 4, 5, and 6 encoding the discoidin domain, which extends from amino acids 63-219[21] and has been considered critical for *RS1* function. Our mutation analysis also revealed a high prevalence of mutations involving cysteine residues, pointing to sites important in the tertiary folding and protein function (Table 1).

Common Ancestries versus Hot Spots

The most frequent substitution was the novel p.Gln154Arg variant, identified in six Spanish families, affecting five males and one carrier female. Once haplotypes were constructed, all affected members demonstrated a common haplotype, therefore suggesting identity by descent (Fig. 1A). Of interest, only another nucleotide change has been described at codon 154, p.Gln154Ter, in one family from the UK.[21] As these variants do not lie on a CpG dinucleotide, this site may be less frequently hit by mutations. Consequently, we suspected that once it was mutated, p.Gln154Arg was inherited from a common ancestor. This finding may indicate the possibility of a founder effect in the Spanish population. Five (71.4%) of the seven patients carrying the p.Gln154Arg mutation presented symptoms early in life, and three (42.8%) of them also showed such ophthalmic complications as retinal detachment (2/7), vitreous hemorrhage (1/7), and strabismus (1/7; Table 2).

Besides, the p.Tyr89Cys variant was identified in two unrelated families (Fig. 1B). Affected members from both cohorts shared a larger piece of DNA (12.65 cM) than did the p.Gln154Arg families (6.32 cM). Therefore, a more recent origin for p.Tyr89Cys was suspected.

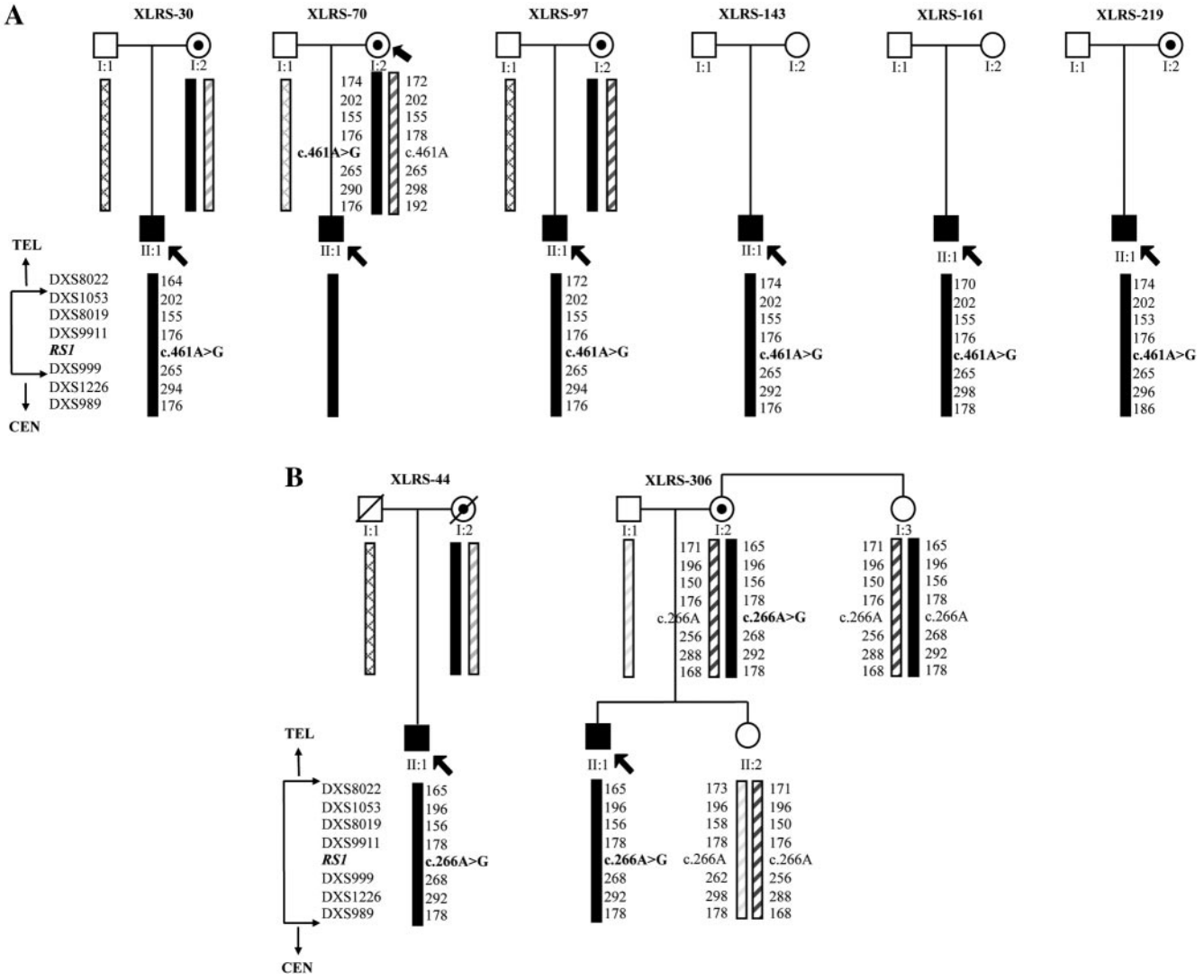

Figure 1. XLRS pedigrees presenting common haplotypes for p.Gln154Arg and p.Tyr89Cys variants. (**A**) Haplotype analyses from p.Gln154Arg affected males and one carrier woman (simplified pedigrees). The displayed region shows seven microsatellite markers spanning over 12.65 cM. p.Gln154Arg-mutation-bearing patients shared a common piece of 6.32-cM DNA (between the markers *DXS1053* and *DXS999*), whereas the distal markers (*DXS8022*, *DXS1226,* and *DXS989*) differed among them. In family XLRS-219, the *DXS8019* marker (153 bp) differed by two base pairs from that in the remaining families (155 bp) which could be explained by microsatellite instability. (**B**) The missense p.Tyr89Cys mutation, in exon 4 of the *RS1* gene, was identified in two Spanish families (simplified pedigree from XLRS-44). Haplotype construction demonstrated a common haplotype between *DXS8022* and *DXS989*, suggesting a common ancestry. In family XLRS-306, we observed two sisters, one carrier (I:2) and one noncarrier (I:3), who shared the same haplotype. A de novo mutational event is possible in the carrier. However, it cannot be ruled out that markers were not informative for the women's parents.

Hot Spots and De Novo Mutational Events

The second most frequent alteration was p.Glu72Lys, which was identified in five unrelated Spanish families. As previously described, p.Glu72Lys is the most common RS founder mutation in Finland[20] and has also been reported numerous times in many different populations.[21] Nevertheless, in our population this variant resembled a recurrent mutational event rather than a founder effect, as haplotypes demonstrated independent origins. Moreover, this mutation arose de novo in a carrier woman who had two affected twins (Fig. 2A). The patients presented various ages at onset, ranging from a few months to >20 years. Of them, 28.5% showed vascular abnormalities and 57.1% presented additional complications that required treatment: surgical correction of retinal detachment and strabismus and photocoagulation for vitreous hemorrhage (Table 2).

The third most frequent variant was the c.578_579insC (p.His194fsX263) frameshift mutation, identified in three Spanish families presenting different haplotypes (Fig. 2B). This insertion has been described in three unrelated families from Austria, the United Kingdom, and the United States. Moreover, in this stretch of cytosine nucleotides (positions 574-579) another five different mutations have been additionally identified in 18 unrelated families.[21] These recurrent mutational events across populations further suggest that this tract is a definite hot spot in *RS1*.

Finally, the missense p.Glu215Val substitution was observed in two Spanish families. Although this mutation was first described in these cohorts and a possible common ancestor was possible, haplotype analysis demonstrated independent origins (Fig. 2C).

De Novo Mutational Events and CpG Dinucleotides

Three de novo mutations were identified in our set of families, representing a frequency of 8.8% (3/34; Table 1). These three

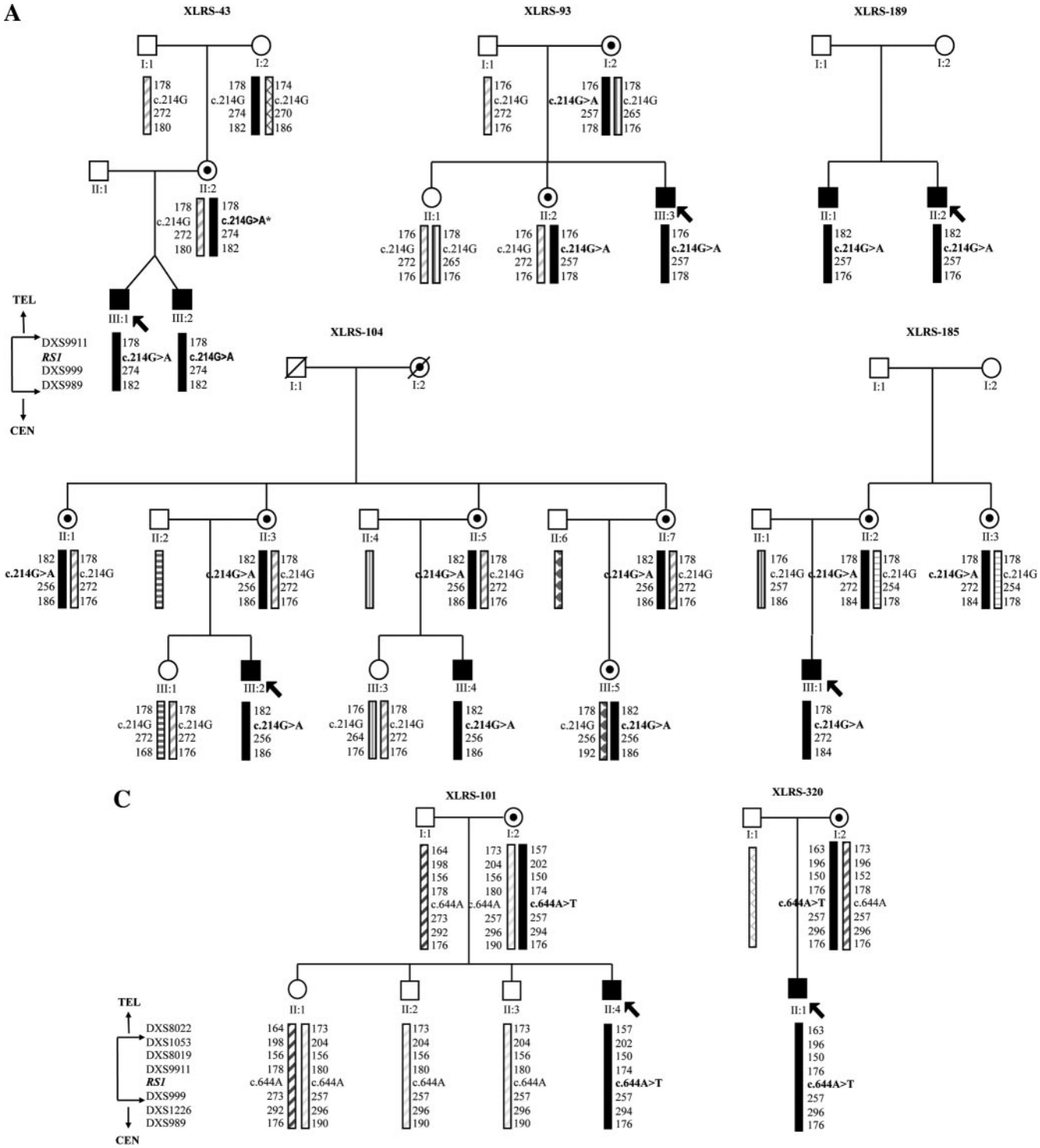

FIGURE 2. Pedigrees from Spanish families presenting the same *RS1* variants but different haplotypes. (**A**) Pedigrees from families having the p.Glu72Lys mutation (*bold*). Haplotypes indicated an independent origin for this variant. *Individual II:2 from XLRS-43, with a de novo mutational event. (**B**) Pedigrees from families presenting the p.His194fsX263 mutation (*bold*). Haplotypes indicate an independent origin for this variant. (**C**) The novel missense p.Glu215Val (c.644A>T) mutation (*bold*), in exon 6 of the *RS1* gene was identified in families XLRS-101 and XLRS-320. Allelic segregation analysis was performed in all family members, and the disease-associated haplotype segregated within the families. In addition, haplotype construction demonstrated an independent origin. (**D**) The p.Pro203Leu (c.608C>T) variant was identified in two families (*bold*). Haplotype analysis demonstrated independent origin for this *RS1* mutation. *Individual II:2 from XLRS-199, with a de novo mutational event. (**A**, **B**, **C**, **D**) *Arrows* indicate the index case.

variants—p.Gln80Ter (c.238C>T); p.Glu72Lys (c.214G>A); and p.Pro203Leu (c.608C>T)—lie on CG dinucleotides.

RS1 contains 26 CpG dinucleotides, accounting for ~7.7% of the coding sequence of the gene. We analyzed all point mutations identified in *RS1* so far (www.hgdm.cf.ac.uk) and we found that ~18.5% of them occur at these sites. This percentage could suggest one of the main causes for mutational events in the *RS1* gene, therefore providing direct evi-

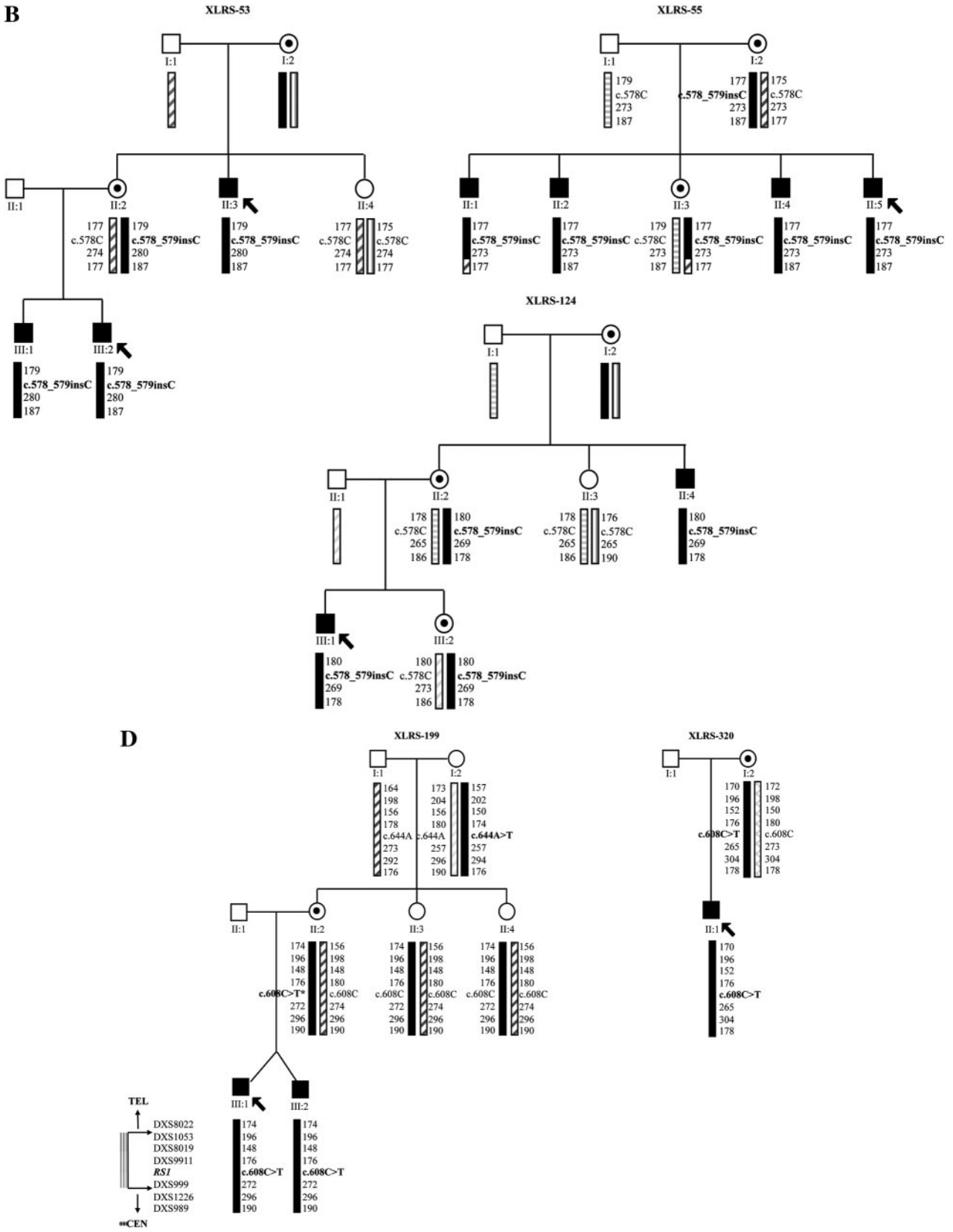

FIGURE 2. (*Continued*)

TABLE 2. Summarized Clinical and Genetic Data of 43 Male Patients Belonging to 34 Spanish XLRS Families

Mutation	Family		Age at Onset	Age (y)	Visual Acuity (RE/LE)	Vitreous Findings	Vascular Abnormalities	Macular Findings	Peripheral Schisis
	Number	Subject							
p.Glu72Lys	XLRS-43*	Proband	4 Months	10	20/100	Normal	Yes	Normal	Yes
p.Glu72Lys	XLRS-93	Proband	12 Years	36	50/100	Vitreous veils	No	Macular microcysts	Yes
p.Glu72Lys	XLRS-104	Proband	8 Years	19	10/100	Vitreous veils	No	Macular schisis, macular cycts	Yes
p.Glu72Lys	XLRS-104	Cousin	7 Years	11	40/100	Normal	No	Macular schisis	No
p.Glu72Lys	XLRS-185	Proband	6 Months	4	—	Vitreous veils	No	Normal	Yes
p.Glu72Lys	XLRS-189	Proband	21 Years	59	10/100	Vitreous veils	Retinal vasculitis	Macular schisis	No
p.Glu72Lys	XLRS-189	Brother	12 Years	42	—	Normal	—	Macular schisis	No
p.Gln80Ter	XLRS-108*	Proband	9 Years	9	—	—	—	—	—
p.Tyr89Cys	XLRS-44	Proband	10 Years	54	10/100	Vitreous veils	No	Macular RPE atrophy, optic pallor	No
p.Tyr89Cys	XLRS-306	Proband	16 Years	25	20/100	Vitreous veils	No	Macular schisis	No
p.Leu137Pro	XLRS-102	Proband	6 Years	24	20/100	Normal	No	Macular schisis, macular cysts	Yes
p.Thr138fsX145	XLRS-250	Proband	Congenital	48	10/100	Vitreoretinal degeneration	No	Macular schisis	No
p.Arg141Cys	XLRS-95	Proband	4 Years	42	10/100	Vitreoretinal degeneration	Dendritiform blood vessels	Macular RPE atrophy, chorioretinal atrophy, pigment spots	No
p.Gln154Arg	XLRS-30	Proband	18 Months	24	—	Vitreoretinal proliferation	No	Schisis of the macula, cavities in the inner retina	No
p.Gln154Arg	XLRS-30	Grandfather	15 Years	—	10/100	Normal	No	Macular schisis	No
p.Gln154Arg	XLRS-70	Son	1 Year	5	—	Vitreous veils	Peripheral exudates	Microcystic macular degeneration, separation of the inner retinal layers	No
p.Gln154Arg	XLRS-97	Proband	6 Months	6	—	Vitreous veils	No	Macular schisis	Yes
p.Gln154Arg	XLRS-143	Proband	2 Years	9	20/100	Normal	No	Macular schisis	Yes
p.Gln154Arg	XLRS-161	Proband	1 Year	5	—	Normal	No	Macular schisis	No
p.Gln154Arg	XLRS-219	Proband	11 Years	15	70/100	Vitreous veils	No	Normal	Yes
p.Pro192Leu	XLRS-217	Proband	2 Years	4	—	Normal	No	Macular schisis	No
p.His194fsX263	XLRS-53	Proband	3 Years	12	30/100	Macular folds	No	Macular schisis	No
p.His194fsX263	XLRS-53	Brother	15 Months	14	50/100; CF	Macular folds	Yes	Macular schisis	No
p.His194fsX263	XLRS-53	Uncle	5 Years	33	—	—	—	—	—
p.His194fsX263	XLRS-55	Proband	4 Years	45	5/100	Vitreoretinal degeneration	Thin vessels	Optic pallor, peripheral pigment spots	No
p.His194fsX263	XLRS-124	Proband	7 Years	17	60/100; 70/100	Normal	No	Macular schisis	No
p.Ile194Asn	XLRS-321	Proband	12 Years	13	40/100	Normal	No	Macular schisis	No
p.Arg197Ser	ARRP-162bis	Proband	7 Years	49	10/100	Normal	Thin vessels	Optic pallor, pigment spots, macular microcysts	No
p.Arg197Ser	ARRP-162bis	Brother	25 Years	45	40/100; CF	Vitreous veils	No	Macular schisis, macular cysts	No
p.Arg197Cys	XLRS-145	Proband	3 Years	27	40/100	Normal	No	Macular schisis	No
p.Arg200Ser	XLRS-322	Proband	10 Years	11	5/100; 40/100	Normal	No	Macular schisis	No
p.Arg200Ser	XLRS-322	Brother	14 Years	14	—	Normal	No	Macular schisis	No
p.Pro203Leu	XLRS-199*	Proband	7 Years	10	40/100	Normal	No	Radial pattern of macular schisis, macular cycts	No
p.Pro203Leu	XLRS-199*	Brother	7 Years	10	40/100	Normal	No	Radial pattern of macular schisis, macular cysts	No
p.Pro203Leu	XLRS-268	Proband	6 Months	2	—	Normal	No	Macular cysts	Yes
p.His207Asp	XLRS-276	Proband	3 Years	15	30/100	Vitreous veils	No	Radial pattern of macular schisis, macular cysts	No
p.Arg209His	XLRS-112	Proband	Congenital	36	2/100	Persistent hyperplastic primary vitreous	No	Macular schisis	No
p.Arg213Gln	XLRS-45	Proband	7 Years	20	10/100	Normal	No	Macular schisis	Yes
p.Glu215Gln	XLRS-29	Proband	5 Years	23	20/100; 10/100	Normal	No	Microcysts in maculas	No
p.Glu215Gln	XLRS-29	Second uncle	28 Years	55	10/100	Normal	No	Microcysts in maculas	No
p.Glu215Val	XLRS-101	Proband	2 Years	25	30/100; 40/100	Normal	No	Bilateral macular lesion	No
p.Glu215Val	XLRS-320	Proband	8 Years	9	50/100	Normal	No	Macular schisis	No
p.Leu216Pro	XLRS-107	Proband	13 Years	28	30/100; 20/100	Vitreous veils	No	Macular schisis	No

		Additional Ophthalmic Complications					
Electroretinogram	**Others**	**Retinal Detachment**	**Age of Surgical Correction**	**Vitreous Hemorrhage**	**Age at Surgical Correction**	**Strabismus**	**Age at Surgical Correction**
Reduced	Astigmatism, hypermetropia	Yes	4 Months	No		Yes	5 Months
—	Amblyopia	No	—	Yes	14 Years	Yes	Childhood
Reduced b-wave amplitude	—	No	—	Yes	14 Years	No	—
—	—	No	—	No	—	No	—
—	Amblyopia	Yes	1 Year	No	—	Yes	4 Months
—	Cystoid macular edema, dyschromatopsia, photophobia	No	—	No	—	No	—
—	—	No	—	No	—	No	—
—	—	—	—	—	—	—	—
No recordable b-wave	Cataracts, absolute scotomas	No	—	No	—	No	—
Reduced a-wave and b-wave ampitudes	—	No	—	No	—	No	—
No recordable b-wave	—	No	—	Yes	6 Years	No	—
—	Myopia	Yes	46 Years	No	—	No	—
Extinguished	Cataracts	No	—	No	—	No	—
Extinguished	—	Yes	18 Months	Yes	2 Years	No	—
—	Cataracts	Yes	15 Years	No	—	No	—
Reduced b-wave amplitude	Hypermetropia, strabismus	No	—	No	—	No	—
—	—	No	—	No	—	Yes	6 Months
Reduced b-wave amplitude	—	No	—	No	—	No	—
Reduced b-wave amplitude	—	No	—	No	—	No	—
Reduced b-wave amplitude	Amblyopia, astigmatism, myopia, scotomas	No	—	No	—	No	—
—	—	No	—	No	—	No	—
—	Hyperopia	No	—	No	—	Yes	3 Years
—	—	No	—	Yes	15 Months	No	—
—	—	—	—	—	—	—	—
Reduced	Amblyopia, myopia, mystagmus, scotomas	No		No	—	No	—
No recordable b-wave	Dyschromatopsia, photophobia	No		No	—	No	—
—	—	No		No	—	No	—
Extinguished	Cataract, dyschromatopsia, nyctalopia, photophobia	Yes	7 Years	No	—	No	—
No recordable b-wave	Amblyopia, nyctalopia, photophobia, scotomas	No	—	No	—	Yes	32 Years
Reduced b-wave amplitude	—	No	—	No	—	No	—
—	Congenital cataracts	No	—	No	—	No	—
—	Congenital cataracts	No	—	No	—	No	—
—	—	No	—	No	—	No	—
—	—	No	—	No	—	No	—
—	—	No	—	No	—	No	—
—	—	No	—	No	—	No	—
—	Congenital cataracts	No	—	No	—	No	—
Reduced b-wave amplitude	Nyctalopia	No	—	No	—	No	—
No recordable b-wave	Amblyopia, nyctalopia, photophobia, scotomas	No	—	Yes	5 Years	No	—
No recordable b-wave	Nyctalopia, hemeralopia, photophobia, scotomas	No	—	No	—	No	—
Extinguished	Amblyopia, nyctalopia, photophobia, strabismus	No	—	No	—	No	—
—	—	No	—	No	—	No	—
Reduced b-wave amplitude	—	No	—	No	—	No	—

CF, counting fingers.
* De novo mutational event.

dence of multiple origins of XLRS and different mutation frequencies across populations.

In conclusion, we observed that *RS1* mutations identified in two or more families and presenting different haplotypes, occurred at CG dinucleotides in 50% of the cases. Nevertheless, de novo mutations occurred in CG dinucleotides in 100% of the cases.

Phenotype–Genotype Correlation

In our Spanish patients with XLRS, we found that the *RS1* mutation spectrum is large. Their phenotype is relatively uniform for three main ophthalmic findings—retinal schisis, vitreous abnormalities, and altered ERG b-wave amplitude—although showed wide inter- and intrafamilial variation in terms of age of onset and progression. Moreover, this study revealed that *RS1* variants did not correlate with additional ophthalmic complications, being not significantly associated with retinal detachment, vitreous hemorrhage, or strabismus. Nevertheless, the knowledge of Spanish mutational spectrum led to the identification of a founder effect for a novel *RS1* variant (p.Gln154Arg), which mainly causes early onset of the disease.

Thus, identifying the causative mutation in patients with XLRS is helpful for confirming diagnosis and counseling of family members, although an asymptomatic male is reported in this work, but cannot predict the likely course of the disease for an individual patient.

Although crucial, extensive phenotyping of Spanish patients with XLRS did not allow the assessment of a certain prognosis for the wide spectrum of *RS1* variants, since no genotype–phenotype correlation was demonstrated. Nevertheless, patients could receive accurate counseling and be informed about possible ophthalmic complications that can be surgically treated (i.e., retinopexia for retinal detachment, surgical correction of strabismus). Therefore, patient education and close follow-up are the only clinical alternatives for early identification and treatment of vision-threatening complications.

To our knowledge, this study represents the first report of *RS1* mutations in Spanish patients with XLRS and provides further evidence of different mutation frequency across populations.

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