

CERKL Mutations and Associated Phenotypes in Seven Spanish Families with Autosomal Recessive Retinitis Pigmentosa

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PURPOSE. Retinitis pigmentosa (RP) is a genetically heterogeneous group of inherited retinopathies. Up to now, 39 genes and loci have been implicated in nonsyndromic RP, yet the genetic bases of >50% of the cases, particularly of the recessive forms, remain unknown. A novel gene (*CERKL*) has been described as associated with RP26. It encodes a ceramide kinase that is assumed to be involved in sphingolipid-mediated apoptosis in the retina. This is a report of the phenotypes and genotypes of persons carrying disease-causing mutations in *CERKL*.

METHODS. Two hundred ten unrelated Spanish families with nonsyndromic autosomal recessive RP were analyzed for sequence variations. Seven of these families presented a mutation in *CERKL*. Nine affected persons of these families were clinically investigated, including visual field, electrophysiology, and fundus examination.

RESULTS. The mutation p.Arg257ter was identified in the homozygous state in all seven affected families. The patients with this variation in *CERKL* presented a common phenotype with characteristic macular and peripheral lesions.

CONCLUSIONS. This study presents the first genotype-phenotype correlation for persons carrying p.Arg257ter mutation and provides clues for a characteristic phenotype of these mutations among persons with autosomal recessive cases. (*Invest Ophthalmol Vis Sci.* 2008;49:2709–2713) DOI:10.1167/iov.07-0865

Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies caused by the loss of photoreceptors and clinically characterized by retinal pigment deposits at the mid periphery that are visible on fundus examination. Prevalence of nonsyndromic RP is approximately 1 in 4000. The most common form of RP is rod-cone dystrophy, in which

the first symptom is night blindness, followed by the constriction of the visual field and progressive loss of visual acuity, leading to blindness after several decades.¹ The condition may segregate as an autosomal dominant, autosomal recessive, or an X-linked recessive trait, and it may also occur on a sporadic basis in up to 50% of cases.² All genes identified to date are believed to account for roughly 50% of all retinal dystrophy cases.³ A new gene encoding for a ceramide kinase-like protein (*CERKL*) was identified and described to be associated with RP26.^{4,5}

CERKL is assumed to be involved in sphingolipid-mediated apoptosis in the retina. This gene is composed of a diacylglycerol kinase catalytic domain and two nuclear localization signal domains, which are thought to be responsible for nucleolar retention of the protein. In fact, *CERKL* could be demonstrated to be localized in the nucleolus, nucleus, and cytosol. *CERKL* is moderately expressed in different tissues, including retina, kidney, lung, and pancreas and weakly in brain, placenta, and liver.⁵

Although *CERKL* has been reported to be a homologue of *CERK*, two studies have demonstrated that *CERKL* has no CERK activity. In fact, it has been shown that *CERKL* does not phosphorylate either ceramide or diacylglycerol.^{6,7}

Proteins encoded by four recently identified RP genes corresponding to loci RP 9, 11, 13, and 18 are transported into the nucleus and involved in pre-mRNA splicing, acting as splicing factors.^{8–11} Proteins encoded by RP1 and RP14 are known to be localized in the nucleolus.^{12,13} Therefore, one could hypothesize that *CERKL* also plays a similar important role for retinal functions by its action in the nucleus and nucleolus.⁶

Tuson et al.⁵ identified a homozygous nonsense mutation, p.Arg257ter (c.769C>T), in exon 5 of the *CERKL* gene, which introduced a stop codon, causing a prematurely truncated protein (position 257 of 532 amino acids) within the presumed catalytic domain. The homozygous mutation in the gene was shown to cause retinal degeneration; however, the molecular mechanisms by which this mutated protein causes retinal degeneration remain unknown.

In the present study, we investigated the retinal phenotypes of persons with nonsyndromic autosomal recessive RP (arRP) due to the p.Arg257ter mutation.

PATIENTS AND METHODS

Patients

A total of 210 unrelated Spanish families affected with arRP were clinically examined at the Fundacion Jimenez Diaz Hospital (Madrid, Spain). Informed consent was obtained from all persons involved in the molecular study or from their legal guardians in accordance with the tenets of the Declaration of Helsinki (Edinburgh, 2000).

In addition, 100 randomly selected DNA samples (200 chromosomes) from a healthy Spanish control population were analyzed to

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TABLE 1. SNP Markers Analyzed

Gene	SNP	Location
<i>CERKL</i> 182109650-182229996	rs720453	chr2:181860076
	rs155107	chr2:182060025
	rs1047307	chr2:182109996int
	rs1449255	chr2:182136280int

The SNP nomenclature and position are according to NCBI dbSNP database. INT, intragenic.

assess the frequency of respective sequence changes in the normal population.

Methods

Peripheral blood samples were collected in EDTA tubes. DNA was extracted from peripheral blood leukocytes with an automated DNA extractor (model BioRobotEZ1; Qiagen, Hilden, Germany).

Genotyping Microarray

Mutational screening was performed by genotyping microarray based on arrayed primer extension (APEX) technology (Asper Ophthalmics, Tartu, Estonia). Sixteen genes associated with arRP (*CERKL*, *CNGA1*, *CNGB1*, *MERKT*, *PDE6A*, *PDE6B*, *PNR*, *RDH12*, *RGR*, *PLBP1*, *SAG*, *TULP1*, *CRB1*, *RPE65*, *USH2A*, and *USH3A*.) were analyzed for variants on this arRP chip, with which a total of 518 mutations could be simultaneously tested.

Direct Sequencing

The exon 5 of the *CERKL* gene, including the intron-exon junctions, was amplified by PCR primers previously reported,⁵ to confirm the results obtained from the microarray. Sequencing reactions were performed using the four dye terminator cycle sequencing ready reaction kit (dRhodamine DNA Sequencing kit; Applied Biosystems, Inc. [ABI], Foster City, CA). Sequence products were purified through fine columns (Sephadex G-501; Princetown Separations, Adelphia, NJ) and resolved (Prism model 3100; ABI).

Restriction Analysis

A restriction assay was performed for the described mutation, by screening a panel of 200 Spanish control chromosomes. The exon 5 of *CERKL* was amplified by PCR using primers containing a mismatch nucleotide that created a restriction site for the endonuclease *AvaII*.⁵ The 157-bp PCR fragments were digested and resolved onto a 3% agarose gel.

Haplotype Analysis

Haplotypes were constructed using the four most informative SNPs for *CERKL*⁵ (Table 1). The SNPs were genotyped (SnaPshot kit; ABI) according to the protocol provided by the manufacturer.

Clinical Evaluation

Phenotype analysis consisted of clinical ophthalmic examination, static perimetry, panel D15 testing, and Ganzfeld electroretinography. Ganzfeld electroretinography was recorded according to ISCEV (International Society for Clinical Electrophysiology of Vision) standards^{14,15} (UTAS 2000 system; LKC Technologies, Gaithersburg, MD) and jet electrodes. White flashes were used at a standard flash intensity of 1 and 2 cd/m².

RESULTS

Mutation Identification

Using, the arRP mutation testing chip (Asper), we identified a homozygous nonsense mutation (c.769C>T) identified at

codon 257 in exon 5 in 7 of 210 Spanish families. This sequence change creates a stop codon that presumably produces a truncated protein (p.Arg257Ter). The change was absent in the Spanish control population.

Direct sequencing of DNA from the affected individual, was performed to validate the result obtained from the microarray. Segregation analysis was undertaken in the family by using a restriction enzyme to verify further that this was the disease causing mutation, and it was during this analysis that two additional affected individuals were identified.

Haplotype Analysis

After direct DNA sequencing, haplotype analysis was performed in all the patients, and it revealed that all of them presented the same haplotype, which supports the hypothesis of a common ancestry.

Clinical Evaluation

The studied persons shared a relatively uniform phenotype, characterized by a symptom-free interval in the first two decades of life (median age at onset, 23 years) followed by a fast decline in visual function. In eight cases, night blindness was the first noticeable symptom. In most patients, the initial diagnosis was made in the second or third decade of life, due to severe visual impairment including central visual field loss and reduction of visual acuity. Six of nine persons were initially diagnosed as having RP and one as having cone-rod dystrophy, macular dystrophy, and gyrate atrophy, respectively. Fundus findings showed characteristic changes with macular atrophy and well demarcated partly confluent patches of chorioretinal atrophy with pigment clumping in the far periphery. Patients did not show extra ocular symptoms. The clinical data are summarized in Table 2.

Night Blindness and Photophobia. Eight of the nine persons carrying *CERKL* mutations reported night blindness with ages at onset ranging from 16 to 34 years. Photophobia was reported by seven of the nine individuals.

Visual Acuity. Visual acuities on examination varied considerably and ranged from light perception to 20/50, where reasonable differences between both eyes were observed due to different stages of macular involvement. Relatively well-preserved visual acuity up to early adult age, followed by a fast decline, appeared to be a common feature in these persons.

Refractive Error. Of the nine patients, refractions were determined in six and revealed myopia ≥ 3 D in three and emmetropia or mild refractive error < 3 D in three persons.

Visual Fields. Visual fields were constricted symmetrically, although the extent seemed to be better correlated with age than with visual acuity. Although no longitudinal data were available showing visual field test results in the patients, case histories showed that a relatively fast disease progression over time is likely. Visual fields were uninformative because of absolute scotoma in four patients; central scotoma related to maculopathy was found in three individuals.

ERG Recordings. At the time of ERG recordings according to ISCEV standards for this study (available in six persons; median age, 43 years), no retinal potentials were recorded that were discernible from noise in any person.

Retinal and Macular Findings. Retinal changes seemed to be relatively uniform among the studied persons. These changes were characterized by macular atrophy of various degrees, sometimes in combination with pigment clumping and hyperpigmentation, as well as by retinal changes in the periphery. In five persons examined, characteristic roundish, well-demarcated lesions of chorioretinal atrophy were present.

TABLE 2. *CERKL* Homozygous p.Arg257ter Mutation and Associated Phenotypic Characteristics

Family and Origin	First Symptoms (Age) and Course	Age (y)	Visual Acuity	Refractive Error	Visual Field	ERG	Anterior Segment and Fundus
211 (Almeria)	NB (20 y), field constriction (22 y), progressive loss of VA (30 y), glare sensitivity, photopsia	45	LP-HM	No data	Absolute scotoma	Scv: not detectable Pho: not detectable	AS normal, normal optic disc, narrowed vessels, moderate bone spicule HP in the mid periphery, in the far periphery intensive pigment clumping and round, well-demarcated areas of chorioretinal atrophy; macular RPE atrophy with annular hyperpigmentation (RE) and pigment clumping (LE). RE: chorioretinal scar after traumatic retinal detachment
828 (Toledo)	NB (23 y), field constriction (32 y), progressive loss of VA (37 y), glare sensitivity, color deficiencies, photopsia; ID cone dystrophy	38	HM-20/50	-9.50/-11.0	Concentric constriction and central scotoma with remaining peripheral islands	Scv: not detectable Pho: not detectable	SC (bilateral), pale optic disc, narrowed vessels, well-demarcated chorioretinal atrophy in the macula, single bone spicule HPs in the mid periphery; round, well-demarcated areas of chorioretinal atrophy and PE clumping in the far periphery
218 (Burgos)	NB and glare sensitivity (30 y), field constriction (33 y)	55	No data	No data	No data	No data	No data
320 (Burgos)	Progressive loss of VA (33 y), color deficiencies, glare sensitivity (29 y); NB (33 y); ID, RP	37	30/100-10/100	-1.75/0	Concentric constriction and central scotoma with small remaining central island (RE); remaining peripheral islands (LE)	Scv: not detectable Pho: not detectable	AS normal, slightly pale optic disc, narrowed vessels, sparse bone spicule HPs in the mid periphery, well-demarcated chorioretinal macular atrophy
325 (Sevilla)	NB (21 y), field constriction (26 y), progressive loss of VA (21 y), glare sensitivity, color deficiencies	32	CF-CF	Myopia >3 D	Concentric constriction and central scotoma with remaining peripheral islands	No data	AS normal, normal optic disc, narrowed vessels, sparse bone spicule HPs in the mid periphery; round, well-demarcated, partly confluent areas of chorioretinal atrophy and PE clumping in the far periphery; well-demarcated macular RPE atrophy with hyperpigmentation
325 (Sevilla)	NB (30 y), field constriction (40 y), progressive loss of VA (40 y); ID: RP	53	LP-LP	+2.0/+0.5	Absolute scotoma	No data	AS normal, exotropia; glaucomatous excavation of the optic disc, narrowed vessels, well-demarcated chorioretinal atrophy in the macula, sparse bone spicules in the mid periphery; round, well-demarcated areas of chorioretinal atrophy and PE clumping in the periphery
335 (Burgos)	NB (16 y), field constriction (20 y), progressive loss of VA (22 y), color deficiencies, glare sensitivity (22 y); ID: RP	42	LP-LP	-5.0/-5.0	Absolute scotoma	Scv: not detectable Pho: not detectable	AS: SC, exotropia, pale optic disc, narrowed vessels, dense bone spicule and spotty hyperpigmentations covering the entire retina, even within the vascular arcades, macular with dense hyperpigmentation and underlying chorioretinal atrophy
595 (Ciudad Real)	Progressive loss of VA (13 y), field constriction (15 y), color deficiencies, glare sensitivity (20 y); ID: macular dystrophy	43	LP-LP	No data	Absolute scotoma	Scv: not detectable Pho: not detectable	AS: SC, exotropia (RE), traumatic iris defect and cataract (LE); Fd: pale optic disc, narrowed vessels, dense bone spicule and spotty hyperpigmentations covering the entire retina even within the vascular arcades, macular with dense hyperpigmentation and underlying chorioretinal atrophy
595 (Ciudad Real)	NB (34 y), field constriction (36 y), progressive loss of VA (37 y), glare sensitivity (37 y); ID: RP	49	40/200-CF	+0.5/+0.75	Concentric constriction with remaining central island (RE); absolute scotoma (LE)	Scv: not detectable Pho: not detectable	AS normal; slight pallor of the optic disc, narrowed vessels, well-demarcated polymorph chorioretinal atrophy in the macula, sparse bone spicules in the mid periphery, single round, well-demarcated areas of chorioretinal atrophy in the periphery

In columns four through eight (from the left), the most recent ophthalmic evaluation is described; in the fourth column, visual acuity of the right (RE)/left eye (LE); in the fifth column, the refractive error (spherical equivalent) is listed; in the sixth column, visual field data of the right and left eyes; in the seventh column the ERG data are provided; and in the eighth column anterior segment and fundus characteristics are described. NB, night blindness; VA, visual acuity; LP, light perception; HM, hand movements; np, not possible; Scv, scotopic; Pho, photopic; amp, amplitude; IT, implicit time; AS, anterior segment; SC, subcapsular cataract; HP, hyperpigmentations.

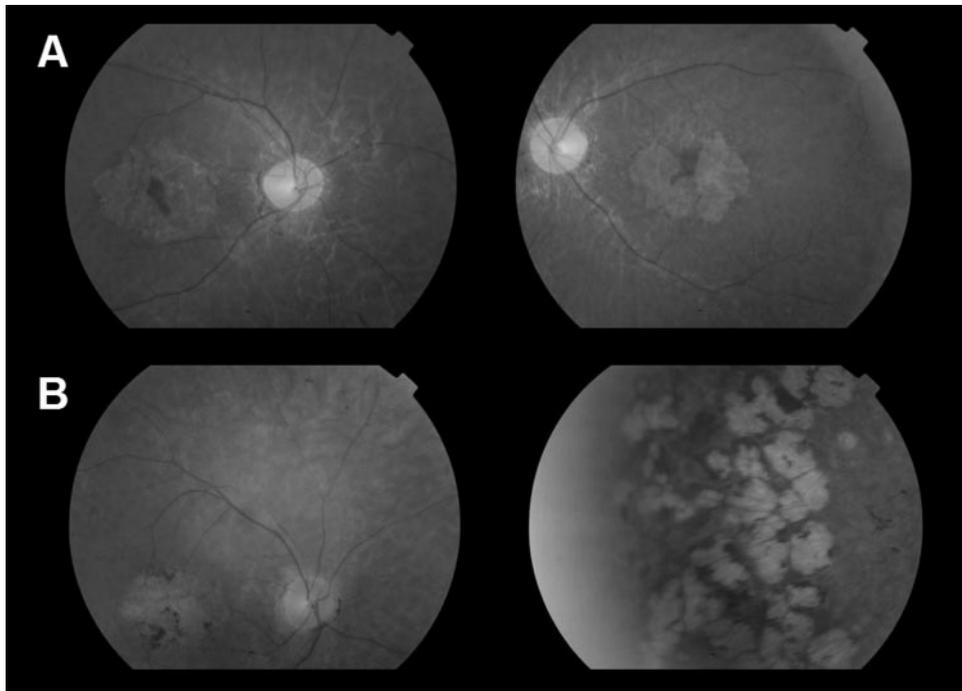


FIGURE 1. Funduscopy of patients carrying the *CERKL* homozygous p.Arg257ter mutation. (A) In our set of patients, macular atrophy of various intensities, pigment clumping, and retinal changes in the periphery were appreciated. (B) Fundus photograph in one patient shows the typical appearance of gyrate atrophy.

In some persons, they were numerous and partly confluent, resembling the typical appearance of gyrate atrophy. All persons showed pigment clumping in these lesions, some of them had heavy pigment accumulation. Various amounts of bone spicule-like pigments were found in all persons in the mid periphery (Fig. 1).

Of note, the optic disc appeared relatively vital and well preserved, and the arterioles more than the venules showed mild to moderate attenuation.

Cataracts and Anterior Segment. Posterior subcapsular cataract was found in three of nine patients carrying *CERKL* mutations. Otherwise, anterior segments appeared normal (except for posttraumatic changes in one eye).

DISCUSSION

The purpose of this work was to describe a point mutation [p.Arg257ter (c.769C>T)] in the *CERKL* gene in Spanish arRP families and its associated phenotype. This change has been reported,⁵ but it has been identified only among Spanish patients.

Although RP is known to be a clinically heterogeneous disorder, the persons studied seem to share a relatively uniform phenotype.

The p.Arg257ter mutation was present in 7 of 210 families, which represents a frequency of ~3.3%. In addition, this is the only mutation described in *CERKL* so far. We believe this frequency is relevant because *CERKL* is the second most mutated gene in Spanish patients with arRP.

Considering that our patients share the same sequence variant, the possibility of a common ancestry was suspected. As those SNPs are closely linked to this gene, a possible recombination event would not be very likely. Because all the studied patients share the same haplotype, we assume identity by descent in those families.

CERKL is moderately expressed in adult human retina, kidney, lung, and pancreas. Low levels of expression have also been detected in brain, placenta, and liver.⁵ Of interest, our patients did not present extraocular symptoms. The age of onset in the second decade, the course, and the retinal findings

seemed to be characteristic features of the p.Arg257ter mutation. All persons had a symptom-free interval until the second decade of life (median age at first symptoms, 23 years), followed by a rapid decline of visual function. At the time studied (median age, 43 years), no person had recordable retinal potentials. In that, according to history, the subjects had near-normal visual function up to a median age of 23 years, the data show a rapid decline of visual function after the first symptoms occurred.

Nearly all persons reported having night vision difficulties followed by concentric restriction of the field, glare sensitivity, and color discrimination difficulties. Therefore, it may be speculated that in these persons carrying *CERKL* mutations, the rod and cone system is affected equally.

As described previously, *CERKL* is predominantly expressed in the retina ganglion cell layer, although light levels of this protein have also been detected in the inner nuclear and photoreceptor cell layers.⁵ The absence of extraocular symptoms in adult patients with arRP, suggest that this gene may interact with a specific retinal protein of the visual pathway not defined yet.

Further longitudinal studies or studies of less-affected persons may result in better knowledge of the underlying pathomechanism. Retinal findings showed well-demarcated round lesions of chorioretinal atrophy in the far periphery, partly confluent, with various amounts of pigment clumping or aggregation. These findings resemble those in gyrate atrophy; however, in the studied persons carrying *CERKL* mutations, a macular atrophy was present as an additional characteristic feature. Only sparse bone spicules were found in the mid periphery in most of the subjects.

This study presents the first genotype-phenotype correlation of persons carrying *CERKL* mutations and provides clues for identifying a characteristic phenotype of these mutations among persons with arRP.

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