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A High Prevalence of Biallelic RPE65 Mutations in Costa Rican Children with Leber Congenital Amaurosis and Early Onset Retinal Dystrophy

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A High Prevalence of Biallelic *RPE65* Mutations in Costa Rican Children with Leber Congenital Amaurosis and Early Onset Retinal Dystrophy

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Abstract

Background: Leber Congenital Amaurosis (LCA) and Early Onset Retinal Dystrophy (EORD), are primary causes of inherited childhood blindness. Both are autosomal recessive diseases, with more than 25 genes explaining approximately ~70% of cases. However, the genetic cause for many cases remains unclear. Sequencing studies from genetically isolated populations with increased prevalence of a disorder has proven useful for rare variant studies, making Costa Rica an ideal place to study LCA/EORD genetics.

Materials and Methods: Twenty-eight affected children (25 LCA, three EORD) and their immediate family members, totaling 52 individuals (30 affected) from 22 families, were sequenced. Whole exome sequencing was performed on all affected individuals. Available parents were analyzed either by WES or Sanger sequencing to determine transmission.

Results: All affected individuals demonstrated compound heterozygous or homozygous mutations in known Inherited Retinal Disease (IRD) genes. Twelve variants were identified in at least one individual in three genes, *RDH12*, *RPE65*, and *USH2A*. Four recurrent *RPE65* mutations were observed in 97% of individuals and 95% of families. All patients with LCA and two of the three individuals with EORD had biallelic mutations in *RPE65*; one child with EORD had a homozygous *RDH12* mutation.

Conclusions: These data suggest that the majority of LCA/EORD in Costa Rica is due to four founder effects in *RPE65* which have been maintained in this genetically isolated population. This finding is of great clinical significance due to the availability of gene therapy recently approved in US and European Union for patients with biallelic *RPE65* deficits.

Key Words: *RPE65*, Costa Rica, Leber congenital amaurosis, inherited retinal disease

Introduction:

Leber congenital amaurosis (LCA) and Early Onset Retinal Dystrophy (EORD) have been reported as important causes of childhood blindness both worldwide and in Latin America (1, 2, 3). LCA and EORD are genetically and phenotypically heterogeneous diseases and are characterized by severe visual impairment in the first years of life with a markedly diminished or extinguished rod and cone electroretinogram (ERG) (4). Given the heterogeneity, clinical diagnoses of IRDs are challenging and molecular genetic testing is useful in establishing appropriate diagnoses for counseling and care. With the advent of gene therapy in patients with specific IRDs, identifying genetic etiology is increasingly important (4, 5, 6, 7).

LCA and EORD are inherited in an autosomal recessive manner. Causative mutations for LCA and EORD are reported in at least 25 genes including *RPE65*, *CEP290*, *RDH12*, and *CRB1* (8, 9, 10), revealing considerable genetic heterogeneity. However, approximately 20-30% of cases are not explained by the currently known causative genes indicating a need for further studies (10).

Given the significant heterogeneity and the fact that the list of IRD genes is incomplete, whole exome sequencing provides a comprehensive screen to detect known and novel mutations.

One proven method for increasing the power of rare variant analysis studies is to study genetically isolated populations with increased disease prevalence. The genetic admixture of the population of Costa Rica has been studied and has proved useful for genetic-mapping of some inherited diseases (11, 12, 13). Founder effects have been suggested in genetic mutations for glaucoma and other diseases (14, 15, 16). Populations descended from a relatively small migratory group allow researchers to identify and investigate disease resulting from infrequent genetic variants. The Costa Rican population is relatively homozygous (17) with a stable gene

pool and a high degree of distant consanguinity, increasing the likelihood of identifying autosomal recessive founder mutations responsible for IRDs.

The National Children’s Hospital (NCH) offers centralized tertiary care for all Costa Rican children less than 13 years old. The ophthalmology division hosts the Pediatric Retina Clinic and Costa Rican children are referred from throughout the country. We present results of molecular genetic analysis of a cohort of children with clinical LCA and EORD seen as patients at the National Children’s Hospital of Costa Rica.

Materials and Methods:

Subjects

Children with a clinical diagnosis of LCA or EORD and no associated systemic disorders were recruited into the study (Table 1). Diagnosis was confirmed following clinical history, a complete ophthalmologic examination, fundus photography and full-field electroretinography (ERG). LCA was diagnosed in 25 children who were born with severe visual loss, inability to fixate in the first months of life, and found to have a diminished or flat dark-adapted ERG. EORD was diagnosed in three children with poor visual function and fixation, nyctalopia, and progressive vision loss, who presented in the first few years of life. Many patients had nystagmus and/or hypermetropia, but these clinical signs were not compulsory for the diagnoses (18). Patients with identified mutations were evaluated and reclassified, if appropriate, at the time of most recent examination. Representative examples of color fundus imaging and ERG results are presented in Supplemental Figure 1.

Ethics

Protocol approval was obtained from the Comité Ético Científico Institucional of the Caja Costarricense Seguro Social (CECI-CCSS) and was exempted by the Investigational Review Board of the Medical University of South Carolina. Following an educational session, full informed consent per the Comité Ético Científico Institucional of the Caja Costarricense Seguro Social (CECI-CCSS) was obtained and genetic counseling was provided by the Division of Genetics at NCH.

Genetic molecular screening

Blood was drawn from patients and chosen accompanying family members. Genomic DNA was extracted (Maxwell kit) and shipped to the Medical University of South Carolina. Whole exome sequencing was performed on 46 samples. Briefly, the overall procedure was as follows: DNA (200 ng) was sheared to an average size of 350 bp using a Corvaris S220 sonicator (Woburn, MA). Sheared DNA was exome enriched followed by next generation sequencing (NGS). Enriched libraries were prepared as described in the SureSelect XT Human All Exon V6 Target Enrichment System for Illumina Multiplexed Sequencing manual Agilent (Santa Clara, CA). Next Generation sequencing was performed following the manufacturer's protocol (Illumina, San Diego, CA). Paired-end (2X125 cycles) sequencing was performed on the Illumina HiSeq2500 utilizing version 4 reagents and software. 15Gb of data were obtained to ensure an overall average of 100X coverage. Upon completion of the sequencing run, basecalls were converted to Fastq files using the Illumina Bcl2 software. FASTQ files for each sample from multiple lanes were merged. Secondary analysis of sequencing files was performed on a Hadoop cluster and custom bioinformatics pipelines provided by OnRamp Bioinformatics (19). This

variant analysis pipeline aligned sequencing reads to hg19 by BWA-MEM (20). Picard (<https://broadinstitute.github.io/picard/>) was utilized to sort, index, and mark duplicate reads. GATK 3.3 (21) was used to realign bams for indel detection, and variants were called by HaplotypeCaller. Tertiary analysis was completed in VarSeq v2 (Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com) with custom analyses performed in Python (<https://www.python.org>). Variant filtration was done following GATK best practices for hard filtration. Annotations for gene consequence were calculated against RefSeq 105 Interim release (<https://www.ncbi.nlm.nih.gov/refseq/>), dbSNP 149 (<https://www.ncbi.nlm.nih.gov/projects/SNP/>) and gnomAD Exomes 2.0.2 (22) (<http://gnomad.broadinstitute.org/>) to identify common variants, Clinvar 2018-05-04 (<https://www.ncbi.nlm.nih.gov/clinvar/>) for clinical interpretation, and dbNSFP 3.0 (23, 24) and CADD scores 1.3 (25) for functional prediction. CADD scores utilize machine learning to model the likely impact of a variant on protein function by integrating dozens of observation based and computational factors. Candidate variants were required to be rare (gnomad AF < .001), likely to impact protein coding (missense, splice acceptor, splice donor, premature start codon gains, stop gained, initiator codon variant, frameshift variant, inframe insertion/deletion), predicted damaging by at least 2/6 of our dbNSFP markers and a CADD score > 20 (top 1% of deleterious variants). Four dbNSFP splice site algorithms were also used (MaxEnt, PWM, NNSplice and GeneSplicer) in order to estimate potential disruption of gene splicing. Variants from a list of known retinal disease-causing genes (26) (<https://sph.uth.edu/retnet/>) were prioritized in analysis. Because LCA/EORD are autosomal recessive disorders, variants which did not occur as either compound heterozygous or homozygous in an affected individual were excluded.

Sanger sequencing was performed on the DNA of 12 parents to confirm transmission of variants observed in affected children. Oligonucleotides (Supplemental Table 1) were designed for PCR amplification of exons 3, 4, 5 and 12. Oligos 1 and 2 were used to amplify exons 3, 4 and 5 as one amplicon. Oligos 3 and 4 were used to amplify exons 12 and 13 as one amplicon. PCR amplification was performed with the above oligo pairs at 0.32 μ M of each oligo in a 50 μ L reaction using HiFi HotStart ReadyMix (KAPA Biosystems). The conditions for the PCRs were as follows: three minutes at 95°C followed by 30 cycles of 98°C for 20 seconds, 66.7°C 30 seconds and 72°C for 90 seconds with a final cycle of 72°C for five minutes. Pooled amplicon and sequencing oligo were sent out for sequencing (Eurofins Genomics, Louisville, KY). Exon 3 was sequenced using oligos 1 and 5; Exon 4 was sequenced using oligos 6 and 7; Exon 5 was sequenced using oligos 2 and 6; Exon 12 was sequenced using oligos 3 and 4. Representative chromatograms have been included for novel RPE65 variants in Supplemental Figure 2.

Results:

Population:

Samples were analyzed from 52 individuals from 22 different families with a history of Leber congenital amaurosis (LCA), early onset retinal dystrophy (EORD), or retinitis pigmentosa (RP). A total of 30 individuals were affected, 28 of whom were children. The affected children had a mean age at time of examination of 8.8 years (range 1-16) and 16 were female (57.1%).

Clinical Presentation:

Clinical results are summarized in Table 1. The mean age of first symptoms was 5.4 months (range one month to 24 months). The most frequent behavior noted by parents was light gazing

and attraction to bright lights (82%). During the course of the disease, nyctalopia was present in 24 (86%), and 16 demonstrated nystagmus (57%). At most recent examination, retinal findings included mottling of the retinal pigment epithelium in 25 patients (89%), bony spicules in two patients, and yellow granules in one patient. The macula appeared normal in 22 (79%), 12 (43%) had vascular attenuation and optic nerve pallor was found in five (18%). No cataracts were present. The ERG was extinguished in 93% and diminished in two children (RPCR-I-1 and -2, see supplemental Fig. 1). Given clinical findings, 25 of the children were diagnosed with LCA and three with EORD (RPCR-I-1 and -2, and RPCR-XX-1

Molecular Genetic Analysis

Mutations in known IRD-associated genes were detected in each of the 30 affected individuals with a total of 12 variants identified (Table 2). Detected variants were classified according to ACMG guidelines (27) as either pathogenic, likely pathogenic, or of unknown significance. A variety of details concerning the specific variants, including HGVS nomenclatures, classification and basic statistics on each variant are summarized in Table 3. For each affected individual, we reported a positive finding if a compound heterozygous or homozygous variant of pathogenic or likely pathogenic classification were found (Table 4). All 30 affected individuals had homozygous or putative compound heterozygous variants classified as either pathogenic or likely pathogenic in a known IRD gene. Variants were submitted as Clinvar submission group CGL-MUSC-001 and submissions are ongoing to the relevant LOVD databases.

RPE65, a gene associated with a clinical diagnosis of LCA, was mutated in over 95% of cases in our cohort. Of the 6 variants observed in *RPE65*, (two pathogenic and four likely pathogenic variants), four recurrent variants occurred at least once in all *RPE65* positive cases.

Compound heterozygous or homozygous mutations in two other genes associated with a clinical diagnosis of IRD were also detected in at least one individual: one likely pathogenic variant in *RDH12*, and four variants of unknown significance in *USH2A*.

Clinical Characteristics by Mutation

Recurrent *RPE65* Mutations

Four *RPE65* mutations were found to be homozygous or heterozygous in more than one individual and across families.

p.(Ile98Hisfs*26) was classified as a pathogenic frameshift deletion. It was observed in 14 affected individuals from 10 families. Parents and/or affected siblings were available for three families and in all cases showed consistent inheritance patterns and cosegregation with affection status. Three children in two families with homozygous *RPE65* mutation p.(Ile98Hisfs*26) presented at one month of age, two with nystagmus. All demonstrated nyctalopia and light gazing with poor vision in the 20/200-20/400 range.

The remaining three recurrent *RPE65* mutations were missense variants classified as likely pathogenic. The variants showed consistent inheritance patterns and cosegregation with affection status. All three missense variants were predicted damaging by multiple functional prediction algorithms and had CADD scores greater than 20, suggesting significant impact on protein function.

p.(Arg81Ile) was observed in 11 affected individuals from seven families. Two sisters (RPCR-XXII-1 and 2), homozygous for *RPE65* mutation p.(Arg81Ile), had symptom onset at one month of age, light-gazing behavior, nyctalopia, nystagmus, mottled retinal pigment epithelium, poor vision (20/400 and 20/600) and demonstrated a flat ERG. Four children in three families were found to have the p.(Arg81Ile) mutation in combination with the p.(Ile98Hisfs*26) mutation. These children presented between 4 and 6 months with nyctalopia and light gazing, and were found to have mottled retinal pigment epithelium and a flat ERG.

p.(Gly140Glu) was present in four affected individuals from four families, including one female (RPCR-XIII-1) homozygous for p.(Gly140Glu) *RPE65* mutation. This individual presented with light gazing at 5 months of age, low vision, and demonstrated RPE mottling and a flat ERG.

p.[(Arg446Ser); (?)] was present in 12 affected individuals from nine families, with five children in three families homozygous for the p.[(Arg446Ser); (?)] *RPE65* mutation. These children presented at age 3-6 months and vision ranged from 20/100 to no fixation. Of these, two demonstrated nystagmus, and three nyctalopia.

Functional Prediction of p.[(Arg446Ser); (?)] required special attention as it is a change in the final base before a splice site. Four algorithms were used to estimate disruption of splicing. MaxEnt and PWM predicted disruption while NNSplice and GeneSplicer did not.

Nonrecurrent *RPE65* variants

Two *RPE65* variants were only observed in single families. **p.(Ser121Phefs*10)**, a frameshift insertion, was classified as pathogenic. This variant was only observed in one individual (RPCR-

XVI-1) , who also exhibited p.[(Arg446Ser); (?)]. Finally, **p.(Val240Phe)**, classified as a likely pathogenic missense variant, was observed in two brothers (RPCR-I-1 and -2), both also containing p.(Ile98Hisfs*26). These boys demonstrated nyctalopia at age two and diminished ERGs. Diagnosed with EORD, they have maintained acuity at the 20/20-20/50 range into their teenage years.

Other identified IRD mutations

One patient (RPCR-XX-1) maintained visual acuity of 20/60 and was found to be homozygous for a likely pathogenic missense variant in *RDH12*, **p.(Ser175Leu)**. This alteration is located in the predicted binding site for the substrate (28) (<http://www.uniprot.org/uniprot/Q96NR8>) and another missense variant at this position, p.(Ser175Pro), has been reported as a pathogenic mutation (29, 30). Furthermore, this variant was predicted damaging by all functional prediction algorithms evaluated and had a CADD score of 30, strongly suggesting an impact on protein function.

Four different *USH2A* variants were also observed in our cohort. Pathogenic variants in *USH2A* are known to cause Usher syndrome, Type IIA (OMIM # 276901) and retinitis pigmentosa 39 (OMIM # 613809). Three individuals were heterozygous carriers of *USH2A* and two individuals had compound heterozygous mutations in *USH2A*, in addition to having biallelic mutations in the *RPE65* gene. While several of the *USH2A* variants showed sufficient evidence for a likely pathogenic classification, all of these variants were classified as variants of unknown significance due to conflicting lines of evidence. Specifically, two individuals in the same family (XIV-1 and XIV-2) had the same *RPE65* mutations that were thought to be causative of the childrens' eye disease. Only one of the children (XIV-2) was also a compound heterozygote for a

USH2A mutation, suggesting that the *USH2A* mutations were not disease causing in this family. The other individual with compound heterozygous mutations in *USH2A* (XIX-1) had a known pathogenic mutation and a likely pathogenic mutation in *RPE65*, again suggesting that the *USH2A* mutations were not the primary cause of the individual's eye disease.

DISCUSSION

We report genetic evaluation and clinical findings of children with IRD in Costa Rica, including LCA and EORD and RP. Of over 25 genes currently known to be associated with LCA/EORD (4, 31), we found a very high prevalence of biallelic *RPE65* mutations. (32, 33). This cohort within the National Health system of Costa Rica suggests a higher prevalence of *RPE65* mutations than has been estimated in other populations around the world (31, 34, 35, 36, 37, 38, 39, 40), though some studies have suggested population specific increases in *RPE65* mutation prevalence (31, 41). This study identified four recurrent *RPE65* mutations, at least one of which was seen in 29 out of 30 cases (97%), and 20 out of 21 families (95%). *RPE65* mutations in the general population are thought to explain ~6% of LCA cases (42).

As previously mentioned, several of the variants in *RPE65* reported here have been observed in the gnomAD database. Interestingly, gnomAD (22) indicated a clear increased prevalence of all four of the recurrent *RPE65* variants specifically within the population designated as “Latino” within this database . These variants occurred in 31 alleles from the ~15k “Latino” individuals in gnomAD, while only occurring twice in the ~105k “non-Latino” individuals. In all gnomAD cases, the *RPE65* variants were heterozygous. This data shows a clear increase in the prevalence of these mutations in the “Latino” samples which may indicate that these findings are relevant outside of Costa Rica. However, the “Latino” classification is very broad and poorly defined.

Without specific demographic information on the gnomAD individuals carrying these variants, which is not publicly available, it is impossible to arrive at a clear conclusion at this time.

However, this increased prevalence in the “Latino” population is not sufficient to explain the results found in our Costa Rican cohort. A rough application of the Hardy-Weinberg Principle with the summed allelic frequency of these variants in the “Latino” individuals in gnomAD would predict 5 cases of RPE65 homozygotes in the total population of ~5 million Costa Ricans, while we have observed 29 cases in our cohort alone.

OMIM defines LCA2 as LCA caused by RPE65 inactivation. LCA2 patients are characteristically night blind, as was found in our population. Consistently, light-gazing and light-seeking behavior was notable and frequent in our young children. LCA2 has been described with a range of clinical findings including variation in nystagmus and nyctalopia, as we report in our population (43, 44). The range of phenotypic severity may correlate with consequences of specific mutations within the *RPE65* gene (45, 46). Heterogeneity in clinical presentation within our group of patients is consistent with an evolving characterization of diseases with a genetic component (47). Indeed, clinical heterogeneity amongst those found to have identical mutations and even amongst the same families confirms the diversity of genetic pathophysiology and the complexity of gene expression (6, 8, 44). Our findings suggest that the *RPE65* mutation p.(Val240Phe) is associated with a milder phenotype and the p.(Arg81Ile) and p.(Ile98Hisfs*26) mutations are found in more severe disease. Novel mutations are continually being added to the knowledge base (8, 35). Further study is necessary to confirm our findings and to better define the pathophysiology of specific mutations in the *RPE65* gene.

We were able to detect likely causative mutations in a large percentage of children in our cohort, probably due to the fact that they represented a well selected phenotype and were confirmed as

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LCA/EORD with visual loss in the first 24 months of life. It should be noted that patients in our study were selected, in part, for their ability to travel to the hospital, and thus may be biased towards families in closer proximity to San Jose. Expanded genetic testing is needed to corroborate and expand our results.

The centralized national medical care system of Costa Rica facilitates data collection and treatment logistics. Our large cohort of Costa Rican families with biallelic *RPE65* mutations, and others yet to be identified, provide an important opportunity to gain insight into treatment efficacy. Finally, given the development of gene therapy recently approved in both the United States and the European Union for patients with biallelic *RPE65* deficits, our study gives hope to these families that gene therapy may be available to their population in Costa Rica in the future (5, 43, 48, 49).

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For Peer Review Only

Subject code	age, years	sex	onset mo	Va OD at last exam	Va OS at last exam	diagnosis	nyctalopia	light gazing	nystagmus	retinal findings	vessels	optic nerve	refraction	ERG
RPCR-I-1	12	m	24	20/50	20/40	EORD	Yes	No	No	yellow granules	normal	normal	astigmatism	diminished potentials
RPCR-I-2	16	m	24	20/20-1	20/25	EORD	Yes	No	Yes	mottled RPE	normal	normal	emmetropia	diminished potentials, cone fx preserved
RPCR-II-1	9	f	1	20/150	20/400	LCA	Yes	Yes	No	mottled RPE	attenuated	normal	myopic astigmatism	flat ERG
RPCR-III-1	14	m	6	20/1200	20/400	LCA	Yes	Yes	Yes	mottled RPE	attenuated	normal	emmetropia	flat ERG
RPCR-IV-1	12	f	1	20/60	20/70	LCA	Yes	Yes	Yes	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG
RPCR-V-1	15	f	1	20/200	20/1200	LCA	Yes	Yes	Yes	mottled RPE	attenuated	palido	myopic astigmatism	flat ERG
RPCR-VI-1	5	m	18	20/400	20/1200	LCA	Yes	No	No	mottled RPE	normal	normal	myopia	flat ERG
RPCR-VIII-1	9	f	1	20/400	20/200	LCA	Yes	Yes	Yes	mottled RPE	attenuated	normal	hyperopia	flat ERG
RPCR-VIII-2	4	f	1	20/400	20/200	LCA	Yes	Yes	Yes	mottled RPE	normal	normal	emetropie	flat ERG
RPCR-IX-1	4	m	4	fixation	fixation	LCA	Yes	Yes	No	mottled RPE	normal	normal	myopic astigmatism	flat ERG
RPCR-IX-2	7	f	1	20/70	20/80	LCA	Yes	Yes	Yes	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG
RPCR-X-1	10	f	6	20/400	20/400	LCA	Yes	Yes	Yes	mottled RPE	normal	normal	myopic astigmatism	flat ERG
RPCR-XI-1	4	f	1	20/200	20/200	LCA	Yes	Yes	No	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG
RPCR-XIII-1	5	m	6	20/100	20/200	LCA	No	Yes	No	mottled RPE	attenuated	normal	hyperopic astigmatism	flat ERG
RPCR-XIV-1	6	f	3	fixation	fixation	LCA	Yes	Yes	No	mottled RPE	normal	normal	hyperopia	flat ERG
RPCR-XIV-2	10	m	6	CF	CF	LCA	Yes	Yes	Yes	mottled RPE	attenuated	pallor	emmetropia	flat ERG
RPCR-XV-1	5	m	3	20/100	20/100	LCA	Yes	Yes	No	mottled RPE	attenuated	pallor	hyperopic astigmatism	flat ERG
RPCR-XVI-1	11	m	1	20/150	20/3600	LCA	Yes	Yes	Yes	mottled RPE	attenuated	normal	hyperopic astigmatism	flat ERG
RPCR-XVII-1	4	f	1	fixation	fixation	LCA	Yes	Yes	No	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG
RPCR-XVII-2	9	m	1	20/4200	20/150	LCA	Yes	Yes	Yes	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG
RPCR-XVIII-1	8	f	3	20/200	20/200	LCA	Yes	No	Yes	mottled RPE	attenuated	normal	hyperopic astigmatism	flat ERG
RPCR-XIX-1	13	m	1	20/100	20/150	LCA	No	Yes	Yes	bony spicules	attenuated	pallor	myopic astigmatism	flat ERG
RPCR-XX-1	15	f	18	20/60	20/70	EORD	Yes	No	No	bony spicules	attenuated	normal	myopic astigmatism	flat ERG
RPCR-XXI-1	1	f	6	no fixation	no fixation	LCA	No	Yes	No	mottled RPE	normal	normal	myopic astigmatism	flat ERG
RPCR-XXI-2	6	m	5	20/600	20/900	LCA	No	Yes	Yes	mottled RPE	normal	normal	myopic astigmatism	flat ERG
RPCR-XXII-1	11	f	1	20/400	20/400	LCA	Yes	Yes	Yes	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG
RPCR-XXII-2	15	f	1	20/700	20/600	LCA	Yes	Yes	Yes	mottled RPE	attenuated	pallor	hyperopic astigmatism	flat ERG
RPCR-XXIII-1	7	f	5	20/150	20/150	LCA	Yes	Yes	No	mottled RPE	normal	normal	hyperopic astigmatism	flat ERG

Table 1: Clinical Findings of Participating Children with Lebers Congenital Amaurosis (LCA) and EORD (Early Onset Retinal Dystrophy) presenting at the National Children's Hospital in Costa Rica.

Abbreviations: Va= Visual Acuity, m= male, f=female, mo= months, ERG= Electoretinogram, RPE= Retinal Pigment Epithelium, Fx= Function, LP= Light Perception, CF= Count Fingers

Gene Symbol	Number of Variants	Number of Affected Individuals	Number of Families	Transcript Details
<i>RDH12</i>	1	1	1	NP_689656.2, NM_152443.2
<i>RPE65</i>	6	31	20	NP_000320.1, NM_000329.2
<i>USH2A</i>	4	2	2	NP_996816.2, NM_206933.2

Table 2: Genes with reported variants

Descriptive statistics concerning genes with reported variants. The number of unique variants, number of affected individuals with at least one reported variant, and the number of families with at least one reported variant within at least one person are provided. Also, the NCBI reference protein and sequence accession numbers by which coding variant nomenclatures are defined in Table 3 are also provided.

Chromosome: Position	Symbol	Clinvar Accession	LOVD	Novel	Sequence Ontology	HGVSp	HGVSc	Classification	Allele Count	Family Count
1:68896965	<i>RPE65</i>	SCV000803382	RPE65_000167	no	missense	p.[(Arg446Ser); (?)]	c.1338G>T	Likely Pathogenic	17	9
1:68905251	<i>RPE65</i>	SCV000803383	pending	no	missense	p.(Val240Phe)	c.718G>T	Likely Pathogenic	2	1
1:68910290	<i>RPE65</i>	SCV000803384	pending	yes	missense	p.(Gly140Glu)	c.419G>A	Likely Pathogenic	10	5
1:68910348	<i>RPE65</i>	SCV000803385	RPE65_000073	no	frameshift	p.(Ser121Phefs*10)	c.361dup	Pathogenic	1	1
1:68910501	<i>RPE65</i>	SCV000803386	RPE65_000028	no	frameshift	p.(Ile98Hisfs*26)	c.292_311del	Pathogenic	18	11
1:68912396	<i>RPE65</i>	SCV000803387	pending	yes	missense	p.(Arg81Ile)	c.242G>T	Likely Pathogenic	14	7
1:215821979	<i>USH2A</i>	SCV000803388	pending	yes	missense	p.(His4825Tyr)	c.14473C>T	Uncertain Significance	1	1
1:216138718	<i>USH2A</i>	SCV000803389	USH2A_000178	no	missense	p.(Arg2354His)	c.7061G>A	Uncertain Significance	1	1
1:216424277	<i>USH2A</i>	SCV000803390	USH2A_000197	no	stop gained	p.(Ser712*)	c.2135del	Uncertain Significance	1	1
1:216462632	<i>USH2A</i>	SCV000803391	pending	yes	missense	p.(Leu654Pro)	c.1961T>C	Uncertain Significance	4	3
14:68193773	<i>RDH12</i>	SCV000803392	RDH12_000005	no	missense	p.(Ser175Leu)	c.524C>T	Likely Pathogenic	2	1

Table 3: Reported Variant Details

All variants positions and consequences are provided against GRCh37/hg19. Variants are indicated as novel if they were in neither LOVD or Clinvar at the time of submission. The allele count statistics include both affected and unaffected individuals. The family count statistics provide the total number of families in which the individual variant was observed in at least one individual.

Patient Name	HGVSp	Gene	Allele origin	Zygotity
RPCR-I-1	p.(Val240Phe)	RPE65	Mother	Compound Heterozygous
	p.(Ile98Hisfs*26)	RPE65	Father	Compound Heterozygous
RPCR-I-2	p.(Val240Phe)	RPE65	Mother	Compound Heterozygous
	p.(Ile98Hisfs*26)	RPE65	Father	Compound Heterozygous
RPCR-II-1	p.[(Arg446Ser); (?)]	RPE65	Unknown	Compound Heterozygous*
	p.(Ile98Hisfs*26)	RPE65	Unknown	Compound Heterozygous*
RPCR-III-1	p.(Ile98Hisfs*26)	RPE65	Mother	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
RPCR-IV-1	p.[(Arg446Ser); (?)]	RPE65	Unknown	Compound Heterozygous*
	p.(Ile98Hisfs*26)	RPE65	Unknown	Compound Heterozygous*
RPCR-V-1	p.(Gly140Glu)	RPE65	Unknown	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
RPCR-VI-1	p.(Arg81Ile)	RPE65	Both Parents	Homozygous
	p.(Leu654Pro)	USH2A	Mother	Heterozygous
RPCR-VI-2	p.(Gly140Glu)	RPE65	Unknown	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
RPCR-VI-3	p.(Ile98Hisfs*26)	RPE65	Unknown	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
	p.(Leu654Pro)	USH2A	Unknown	Heterozygous
RPCR-VIII-1	p.(Ile98Hisfs*26)	RPE65	Mother	Homozygous
RPCR-VIII-2	p.(Ile98Hisfs*26)	RPE65	Mother	Homozygous
RPCR-IX-1	p.(Ile98Hisfs*26)	RPE65	Mother	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
RPCR-IX-2	p.(Ile98Hisfs*26)	RPE65	Mother	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
RPCR-X-1	p.(Ile98Hisfs*26)	RPE65	Unknown	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Unknown	Compound Heterozygous*
RPCR-XI-1	p.(Ile98Hisfs*26)	RPE65	Both Parents	Homozygous
RPCR-XIII-1	p.(Gly140Glu)	RPE65	Unknown	Compound Heterozygous*
	p.(Arg81Ile)	RPE65	Mother	Compound Heterozygous*
RPCR-XIV-1	p.[(Arg446Ser); (?)]	RPE65	Both Parents	Homozygous
RPCR-XIV-2	p.[(Arg446Ser); (?)]	RPE65	Both Parents	Homozygous
	p.(Arg2354His)	USH2A	Unknown**	Compound Heterozygous*
	p.(Ser712*)	USH2A	Unknown**	Compound Heterozygous*
RPCR-XV-1	p.[(Arg446Ser); (?)]	RPE65	Unknown	Homozygous
RPCR-XVI-1	p.[(Arg446Ser); (?)]	RPE65	Mother	Compound Heterozygous
	p.Ser121Phefs	RPE65	Father	Compound Heterozygous
RPCR-XVII-1	p.[(Arg446Ser); (?)]	RPE65	Mother	Compound Heterozygous
	p.(Ile98Hisfs*26)	RPE65	Father	Compound Heterozygous
RPCR-XVII-2	p.[(Arg446Ser); (?)]	RPE65	Mother	Compound Heterozygous

Patient Name	HGVSp	Gene	Allele origin	Zygosity
	p.(Ile98Hisfs*26)	<i>RPE65</i>	Father	Compound Heterozygous
RPCR-XVIII-1	p.[(Arg446Ser); (?)]	<i>RPE65</i>	Unknown	Compound Heterozygous*
	p.(Gly140Glu)	<i>RPE65</i>	Unknown	Compound Heterozygous*
RPCR-XIX-1	p.[(Arg446Ser); (?)]	<i>RPE65</i>	Mother	Compound Heterozygous*
	p.(Ile98Hisfs*26)	<i>RPE65</i>	Unknown	Compound Heterozygous*
	p.His4825Tyr	<i>USH2A</i>	Unknown	Compound Heterozygous*
	p.(Leu654Pro)	<i>USH2A</i>	Unknown	Compound Heterozygous*
RPCR-XX-1	p.Ser175Leu	<i>RDH12</i>	Unknown	Homozygous
RPCR-XXI-1	p.[(Arg446Ser); (?)]	<i>RPE65</i>	Both Parents	Homozygous
	p.(Leu654Pro)	<i>USH2A</i>	Mother	Heterozygous
RPCR-XXI-2	p.[(Arg446Ser); (?)]	<i>RPE65</i>	Both Parents	Homozygous
RPCR-XXII-1	p.(Arg81Ile)	<i>RPE65</i>	Both Parents	Homozygous
RPCR-XXII-2	p.(Arg81Ile)	<i>RPE65</i>	Both Parents	Homozygous
RPCR-XXIII-1	p.(Gly140Glu)	<i>RPE65</i>	Both Parents	Homozygous

Table 4: Patient Report Details

Details on the specific variants reported for all affected individuals, including transmission status when available.

* Assumed compound heterozygous variants in the absence of allele origin data.

** Only RPE65 status was examined by sanger sequencing in RPCR-XIV-2 parents; USH2A transmission status is unknown.

Supplemental Table 1: PCR and sequencing Oligos

Oligos	RPE65 Sequence Name	Oligo Sequence
1	RPE65 PCR Exon 3F	cacctgttgatgggcaagc
2	RPE65 PCR Exon 5R	gggtctctgtgcaagcgtagt
3	RPE65 Exon 12F	agtttcctcaaataaccagaagt
4	RPE65 Exon 13R	tcattcttccaaggcatctg
5	RPE65 Seq Exon 3R	atgtccctcgatccatgc
6	RPE65 Seq Exon 4F	ccagctggccacatatgc
7	RPE65 Seq Exon 4R	aacattcagttgggttcagtaacc

Supplemental Figure 1: Representative patient RPCR XVIII-1. Eight year old girl with onset of nystagmus at age 3 months with nyctalopia. A) Electroretinogram (ERG): Extinguished scotopic ERG, right eye. B) Color Fundus Photograph: Mottling of equatorial Retinal Pigment Epithelium (RPE)

Supplemental Figure 1. Representative Clinical Data

Two novel variants were found in RPE65. These variants were confirmed via sanger sequencing. Provided are representative chromatograms for (A) c.242G>T p.(Arg81Ile) and (B) c.419G>A p.(Gly140Glu).

Supplemental Figure 2. Sanger Chromatograms.