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Novel *CRB1* pathogenic variant in Chuuk families with Leber congenital amaurosis

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Abstract

The purpose of this article is to determine the cause of Leber congenital amaurosis (LCA) in Chuuk state, Federated States of Micronesia (FSM). In this prospective observational case series, five patients with early-onset vision loss were examined in Chuuk state, FSM, during an ocular genetics visit to study the elevated incidence of microphthalmia. Because of their low vision these patients were incorrectly assumed to have microphthalmia. A complete ophthalmological exam established a clinical diagnosis of LCA. Candidate gene exons were sequenced with a targeted

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

retinal dystrophy panel. Five subjects in three related families were diagnosed with LCA. All five were from Tonoas Island, within the Chuuk Lagoon, with ages ranging from 6 months to 16 years. DNA sequencing of affected individuals revealed a homozygous *CRB1* NM_201253.3:c.3134del pathogenic variant, which was heterozygous in their parents. *CRB1* genotypes were confirmed by a PCR restriction assay. We report identification of a founder pathogenic variant in *CRB1* responsible for autosomal recessive LCA in this isolated community. This discovery will lead to appropriate recurrence risk counseling.

Keywords

Chuuk; CRB1; Leber congenital amaurosis; Micronesia

1 | INTRODUCTION

Leber congenital amaurosis (LCA) is a genetically and phenotypically heterogeneous disorder causing severe early-onset childhood vision loss (MIM 613835). It accounts for 5% of all retinal dystrophies (Chung & Traboulsi, 2009; den Hollander et al., 2008; Kaplan et al., 1990; Kumaran et al., 2017). Causative pathogenic variants in 25 genes have been reported. LCA8 is associated with pathogenic variants in *CRB1* (crumbs homolog-1). *CRB1* encodes a structural protein in the retinal external limiting membrane, photoreceptor inner segments, and Muller glial villi that is deeply conserved in evolution and essential for retinal morphogenesis and apical-basal polarity (Henderson et al., 2011; Kowalczyk & Moses, 2002; Pellikka et al., 2002; Richard et al., 2006). We describe the discovery of a novel frameshift pathogenic variant in *CRB1*, as the cause of LCA in Chuuk, Micronesia, a geographically isolated population.

2 | METHODS

2.1 | Editorial policies and ethical considerations

This study including a recruitment invitation flyer and informed consent documents were approved by the Western Institutional Review Board (WIRB, IRB#2016-1215) before the recruitment. This study conforms to the Declaration of Helsinki. Consent was obtained from the subjects in the native language by a local pediatrician (AY).

2.2 | Study procedures

This is a prospective observational case series. This study was part of a larger project to investigate the etiology of anophthalmia and microphthalmia (A/M) in Chuuk, Federated States of Micronesia. In the course of recruitment, a group of subjects was ascertained on the basis of low vision, nystagmus and enophthalmos, under the incorrect assumption by local providers that they may have A/M. In April 2017, a multidisciplinary team spent 12 days on the island of Weno in Chuuk, one of four states comprising the Federated States of Micronesia. Clinical exams were performed at Shinobu M. Poll Memorial Center by a medical geneticist (AS), an ocular geneticist (AVL), a molecular geneticist (TG), genetic counselors (JC and SK), a low vision education consultant (DM), and a local pediatrician (AY), with assistance from local phlebotomists, nurses, educators, and Chuukese language

interpreters. Subjects were identified by Chuuk State Department Health Service staff through outreach, low vision, and educational service programs in anticipation of the team's visit.

All willing subjects with clinical features of A/M or low vision were included in the study, and evaluations were scheduled based on geographic accessibility to the clinic. Weno is one of >200 islands in Chuuk state, most of which are uninhabited. Subjects from other islands arrived by boat. We enrolled 29 participants from nine islands, five of whom are the focus of this report. These five subjects were found to have a clinically separate disorder from A/M upon exam, but were considered eligible to participate because they were given a tentative diagnosis of microphthalmia by referring physicians. Once the new suspected diagnosis was explained to families, along with our intent to find the genetic cause, we proceeded with their approval and additional counseling relevant to LCA disease.

We collected family and personal histories, and constructed multigenerational pedigrees with assistance from local interpreters. We performed a full medical genetic physical examination on affected individuals to assess potential dysmorphology. The ophthalmic evaluation included Sheridan Gardiner single optotype visual acuity testing without lateral masking (Keeler Ophthalmic Instruments), pupillary assessment using the light of the indirect ophthalmoscope, hand-held slit lamp examination, eye alignment and movement assessment, cycloplegic refraction, and dilated fundus examination. As tolerated we obtained intraocular pressures by tonometry (Tono-Pen®, Reichert) and corneal pachymetry (PachPen®, Accutome). However, we were unable to obtain retinal photographs of adequate quality for publication.

Peripheral blood from probands and their parents was stabilized on-site with DNAgard reagent (Biomatrica, Inc.) and shipped to UC Davis, where genomic DNA was extracted using standard procedures (FlexiGene kit). Direct testing for pathogenic variants was conducted using the Retinal Dystrophy Panel version 11 RD chip (Molecular Vision Laboratory), which includes exons and intron/exon boundaries for 281 known or suspected retinal disease genes (Data S1), The analysis involved PCR amplification with multiplex primer sets printed on the RD SmartPanel chips (Chiang et al., 2015), followed by next-generation sequencing. Each primer set was duplicated to avoid random PCR failure or allele dropout, with an increased density of primer sets over known low-coverage regions. The RDv11 platform contains 5040 primer sets duplicated on two chips. We evaluated any region with <100× coverage after multiplex analysis, and confirmed all pathogenic variants and novel variants, by PCR Sanger sequencing. In our reports, exon 1 contains the start codon ATG (codon 1, with A as nucleotide 1).

To assess *CRB1* genotypes in subjects, obligate carriers, and other Chuuk DNA samples in our A/M study, we amplified a 428 bp segment of *CRB1* exon 9 by PCR using a touchdown protocol (55 to 58°C annealing × 45 cycles), with primers 5′-CACCTTCTCTCATTAGGTATTG and 5′-CTGTCTCCCACATAAATATCTG. The resulting products were digested with *Bmg*BI (^NNNCACGTG), which cleaves the LCA8 allele (giving 308 and 120 bp fragments) but spares the wild-type allele, and resolved by electrophoresis.

3 | RESULTS

A total of five subjects from three sibships were diagnosed with LCA (Figure 1). They live on Tonoas Island, range in age from 6 months to 16 years, and are related. All affected patients had severe limitations of vision since infancy. Clinical findings are summarized in Table 1. All subjects had nystagmus and were noted to have varying degrees of enophthalmos. Vision ranged from hand motion to light perception. All had normal anterior segment exams except one patient with corectopia in one eye, a superficial corneal scar in the fellow eye, and mild microcornea bilaterally. All other subjects had normal corneal diameters. Four had hyperopia. All subjects had retinal gliosis or pigmentary abnormalities but their optic nerves were normal. No other syndromic features were found in all subjects by a complete medical genetic physical examination. No other diagnosis was found in patients with low vision other than microphthalmia or LCA.

DNA sequencing revealed homozygous *CRB1* NM_201253.3: c.3134del pathogenic variants in all affected subjects tested, from three sibships (Figure 2), and heterozygous pathogenic variants in five of six parents available for testing (obligate carriers), who were clinically normal. To assess the potential for a larger founder effect and demonstrate the absence of this pathogenic variant in Chuuk population databases, we screened 47 additional Chuuk DNA samples in our A/M study for the *CRB1* pathogenic variant by PCR and *Bmg*BI digestion. Only one additional heterozygote was identified, the brother of an obligate carrier. The novel *CRB1* NM_201253.3:c.3134del allele thus appears limited to descendants of a common ancestor on Tonoas Island.

4 | DISCUSSION

LCA is a genetically and phenotypically heterogeneous disorder causing severe early-onset childhood blindness. It was first described by Theodore Leber in 1869 (Hanein et al., 2004) and accounts for 5% of all severe retinal dystrophies (Chung & Traboulsi, 2009; den Hollander et al., 2008; Kaplan et al., 1990; Kumaran et al., 2017). Children usually present with poor vision and nystagmus. The fundus can appear almost normal or exhibit a wide range of pigmentary abnormalities, but the electroretinogram is severely abnormal in all cases. Pathogenic variants in 28 genes have been reported to cause LCA or early-onset retinal dystrophy (Kondkar & Abu-Amero, 2019). The disorder is usually autosomal recessive, but autosomal dominant LCA pedigrees are known (Arcot Sadagopan et al., 2015).

LCA8 is caused by loss-of-function pathogenic variants in *CRB1* (crumbs homolog-1). The *CRB1* gene spans 210 kb on chromosome 1q31 and has >12 exons, which can be alternatively spliced to generate 10 transcript variants (Quinn et al., 2017). The encoded proteins range in size from 870 to 1406 amino acids, and include membrane-tethered and secreted isoforms. *CRB1* is expressed by rod and cone photoreceptors and Muller glia within the retina and is localized to their subapical regions. It plays critical roles in retinal morphogenesis, establishment of apical-basal polarity, formation and integrity of the external limiting membrane, and apoptosis (Gosens et al., 2008; Jacobson et al., 2003). *Crumbs* proteins and the Crb cell polarity complex have been deeply conserved

during metazoan evolution (Kowalczyk & Moses, 2002). In mice, but not in humans, the phenotypic severity of *Crb1* null pathogenic variants is limited by expression of paralog *Crb2* in the retina (Luhmann et al., 2015; Mehalow et al., 2003; Pellissier et al., 2014).

Human *CRB1* pathogenic variants were first reported in 21 of 233 patients with LCA in a 2001 study (Lotery et al., 2001). The severity of *CRB1* ocular phenotypes is notably variable, even within pedigrees (Bujakowska et al., 2012; Henderson et al., 2011). However, the most common anatomical features include hyperopia, nummular (round) pigmentary clumps and increased retinal thickness upon optical coherence tomography (Henderson et al., 2011). Our subjects share this pattern of retinal pigmentation findings and hyperopia. A new feature in our cases is superficial retinal gliosis.

Microphthalmia is defined as a small eye with an anatomical malformation that may affect the anterior segment, such as microcornea and/or the posterior segment. Because microphthalmia is relatively common in Chuuk state (Yomai & Pavlin, 2010), in the course of recruitment, a group of low-vision subjects was incorrectly assumed by local providers to have microphthalmia based on an enophthalmos. After the eye exam by an ophthalmologist, microphthalmia was not suspected because all subjects had normal anterior segment exams except one patient with corectopia in one eye, a superficial corneal scar in the fellow eye, and mild microcornea bilaterally. All other subjects had normal corneal diameters.

Our patient with LCA and microcornea has a first cousin with microphthalmia. We were unable to measure axial length to determine if this LCA patient had microphthalmia or isolated microcornea. It is possible that the patient has both disorders. *CRB1* pathogenic variants have been reported in patients with nanophthalmos and retinal dystrophy (Zenteno et al., 2011), but are not responsible for A/M disease in Chuuk. Our study to determine the genetic cause of microphthalmia in this region is ongoing.

All five patients with LCA were found to harbor a novel homozygous pathogenic variant, NM_201253.3:c.3134del, p.Leu1045ArgfsTer18, in exon 9 of *CRB1*. The 1-bp deletion causes a frameshift in exon 9, truncating the protein in the extracellular segment, within interspersed EGF (epidermal growth factor) and laminin G domains. However, given its location, the premature stop is likely trigger nonsense-mediated decay (NMD) of all *CRB1* mRNAs, creating a null allele. This particular pathogenic variant has not been previously reported and is not listed by the NHLBI Exome sequencing project among 6500 individuals of European (66%) or African (34%) ancestry (evs.gs.washington.edu). However, a downstream frameshift NM_201253.3:c.3347del, p.Phe1116SerfsTer25 was reported to cause LCA (Hanein et al., 2004). Exon 9 is a relative hotspot for LCA8 pathogenic variants (Bujakowska et al., 2012).

According to the American College of Medical Genetics and Genomics (ACMG) guidelines for the interpretation of sequence variants, the NM_201253.3:c.3134del variant was determined to be pathogenic by fulfilling these criteria (PVS1, PM2, PM4, and PP1) (Richards et al., 2015).

Chuuk state is a cluster of volcanic islands in western Pacific Ocean, and the word Chuuk means “high mountains.” The ancestors of current Micronesians settled over 4000 years ago.

The islands were sighted by the Spanish explorer Á lvaro de Saavedra in 1528. European explorers, first the Portuguese and then the Spanish, incorporated the archipelago into the Spanish East Indies and in 1887 the first town was founded. All LCA subjects in our study are from Tonoas Island, which has an area of only 8.8 km^2 and a population of 3910 at the time of the last census in 2000 (Chuuk State Census Report 2000, 2002). Chuuk is the most populous state in the Federated State of Micronesia with 50,000 inhabitants on 120 square kilometers. Isolated and remote from its closest neighbor countries (Philippines and Indonesia), Micronesia has a naturally restricted gene pool, and a history of population bottlenecks (Hussels & Morton, 1972; Sheffield, 2000; Sundin et al., 2000). Consanguineous marriages are common.

We report the identification of the founder pathogenic variant in the *CRB1* responsible for autosomal recessive LCA in an isolated community. This discovery will lead to appropriate testing of family members and recurrence risk counseling, with future hope of gene-based therapeutic intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research, supporting data are not available beyond that which appears in the publication.

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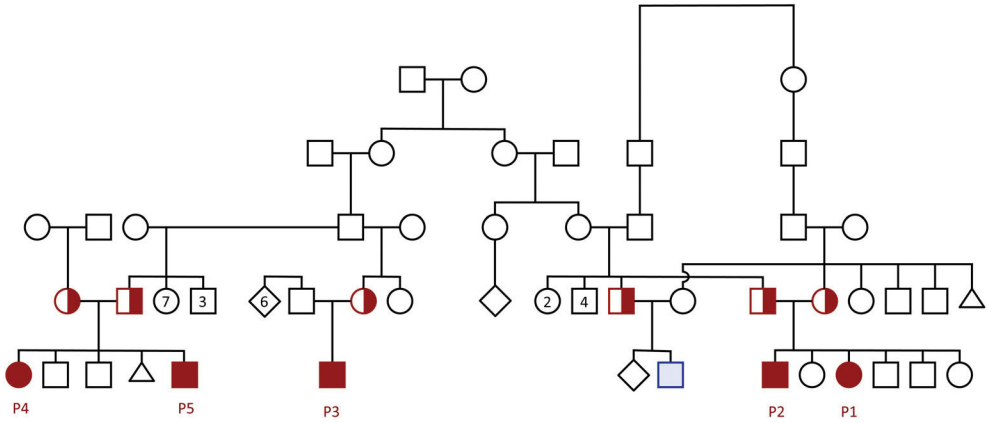

FIGURE 1.

Pedigree diagram showing three interconnected families with LCA subjects (P1 to P5). Genotyped homozygotes (solid) and carriers (half-solid) are shaded dark red; one subject with microphthalmia but wild-type *CRB1* genotype is shaded light blue

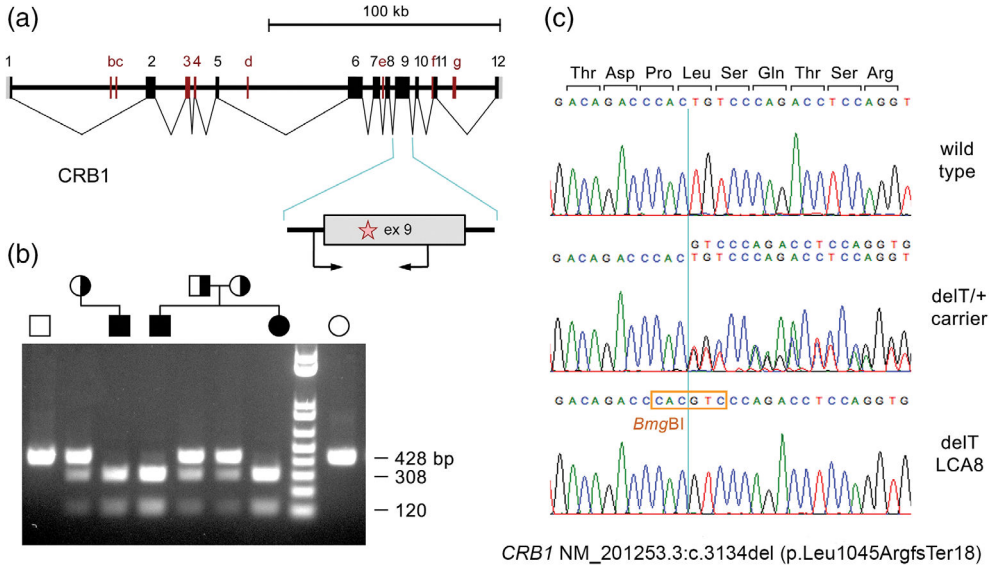

FIGURE 2.

(a) Map of human *CRB1* gene on chromosome 1q31, showing spliced exons 1–12 of prototypic transcript (den Hollander et al., 2004) NM201253.3, which encodes a 1406-amino acid isoform (NP957705.1). Alternative exons b-g and differentially spliced exons are indicated in dark red. The exon 9 amplicon, PCR primers (arrows) and NM_201253.3:c.3134del pathogenic variant (star) are shown below. (b) Gel photograph of diagnostic PCR products amplified from Chuuk LCA family members, digested with *Bmg*BI. (c) Sanger chromatograms of PCR products showing the *CRB1* mutated region in wild-type, heterozygous and LCA individuals

TABLE 1

Summary of clinical features

F/P	Age (years)	Sex	VA	HCD (mm)	A/S	RVA	Retinal pigmentation	CR (diopters)	Other
F1/P1	4	F	LP OU	11.00 OU	WNL	+2	WNL	OD +2.00 + 1.50 × 60 OS hyperopic[a]	Diffuse superficial retinal gliosis OU
F1/P2	16	M	HM OU	11.75 OU	WNL	+3	Marked 360° mid periphery, nummular pigmentation	OD +1.50 + 6.00 × 120 OS +3.50 + 3.00 × 60	Diffuse superficial retinal gliosis OU
F2/P3	15	M	LP OU	10.50 OD 10.25 OS	corectopia OD central superficial corneal scar OS	?	Scattered nummular pigment clumps mid periphery, encroaching macula	OD −0.50 OS[a]	RPE stippling in macula OD Macular coloboma[b] OS
F3/P4	7	F	LP OU	12.00 OD 11.50 OS	WNL	+2	Scattered nummular pigment clumps including macula	OD +4.50 OS +5.50	Diffuse superficial retinal gliosis OU Vascular stalk or vitreous veil OS
F3/P5	0.5	M	CNSM OU	12.00 OU	WNL	+1	WNL	OU +6.50	Diffuse grayish retinal appearance OU

Note: Optic nerves for all subjects were normal.

Abbreviations: ?, questionable; A/S, anterior segment; CNSM, central, maintained, not steady; CR, cycloplegic refraction; F, family; HCD, horizontal corneal diameter; HM, hand motion; LP, light perception; OD, right eye; ON, optic nerve; OS, left eye; OU, both eyes; P, patient; RPE, retinal pigment epithelium; RVA, retinal vascular attenuation; VA, visual acuity; WNL, within normal limits.

[a]Unable to obtain accurate retinoscopy.

[b]In this context, macular coloboma indicates a geographic, generally circular, chorioretinal disruption in the central macula.