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# A novel pathogenic *CRB1* variant presenting as Leber Congenital Amaurosis 8 and evaluation of gene editing feasibility

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**Conflict of interest** S.H.T receives financial support from Abeona Therapeutics, Inc and Emendo and is on the scientific and clinical advisory board for Nanoscope Therapeutics. Columbia University has filed patent applications related to *CRB1* for which B.L.D.C, S.H.T, and P.M.J.Q are listed as inventors. M.S., M.K. and I.H.M. have no conflicting interests.

**Ethical approval** The study was conducted under the Columbia University Institutional Review Board-approved protocol IRB AAAF1849. All procedures were performed in compliance with the tenets of the Declaration of Helsinki. Informed consent was waived due to the retrospective nature of the study and the minimal risk conferred to patients as per the Columbia University Institutional Review Board-approved protocol AAAR8743.

**Statement of human rights** The study was conducted under the Columbia University Institutional Review Board-approved protocol IRB AAAF1849. All procedures were performed in compliance with the tenets of the Declaration of Helsinki.

**Statement on the welfare of animals** No animals were used in this study.

**Informed consent** Informed consent was waived due to the retrospective nature of the study and the minimal risk conferred to patients as per the Columbia University Institutional Review Board-approved protocol AAAR8743.

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## Abstract

**Introduction—**Leber Congenital Amaurosis (LCA) is an inherited retinal disease that presents in infancy with severely decreased vision, nystagmus, and extinguished electroretinography findings. LCA8 is linked to variants in the *Crumbs homolog 1* (*CRB1*) gene.

**Case Description—**We report a novel *CRB1* variant in a 14-year-old male presenting with nystagmus, worsening vision, and inability to fixate on toys in his infancy. Color fundus photography revealed nummular pigments in the macula and periphery. Imaging studies revealed thickened retina on standard domain optical coherence tomography and widespread atrophy of the retinal pigment epithelium on autofluorescence. Full-field electroretinography revealed extinguished scotopic and significantly reduced photopic responses. Genetic testing demonstrated a novel homozygous variant, c.3057 T > A; p.(Tyr1019Ter), in the *CRB1* gene. This variant is not currently amenable to base editing, however, in silico analysis revealed several potential prime editing strategies for correction.

**Conclusion—**This case presentation is consistent with LCA8, suggesting pathogenicity of this novel variant and expanding our knowledge of disease-causing *CRB1* variants.

### Keywords

Leber Congenital Amaurosis; Novel *CRB1* variant; Gene therapy; Prime editing; Full-field electroretinography; Genotype–phenotype correlation

## Introduction

Leber congenital amaurosis (LCA) refers to a family of inherited retinal diseases (IRDs) affecting between 1 in 33,000 to 1 in 81,000 individuals worldwide [1, 2]. LCA presents in the first months of life with nystagmus, severely diminished or extinguished electroretinogram (ERG) readings, diminished pupillary responses, and characteristic eye rubbing or oculodigital reflex [1, 3, 4]. Autosomal recessive LCA is associated with variants in more than twenty genes including *GUCY2D*, *CEP290*, *RPE65,* and *Crumbs homolog 1* (CRB1) genes, with the latter accounting for 10% of cases [1, 5]. LCA8 is specifically associated with *CRB1* variants [5]. The *CRB1* gene is the human homolog of the *Drosophila melanogaster* Crumbs protein 1 [3]. In the human retina, three main CRB1 isoforms are expressed, CRB1-A (canonical), CRB1-B, and CRB1-C. CRB1 proteins regulate epithelial cell polarity, retinal development, and rod and cone photoreceptor morphogenesis [3, 6, 7].

*CRB1* variants have been shown to cause a wide variety of phenotypes, including autosomal recessive retinitis pigmentosa type 12 (RP12), maculopathy, Coats-like vasculopathy, and LCA8 [1, 5, 8]. Furthermore, variation has been described within each phenotype,

suggesting *CRB1*-related dystrophies exist on a spectrum with significant overlap between RP and LCA [4, 9]. This report correlates a case of LCA demonstrating nummular pigment deposits and para-arteriolar RPE sparing with a novel CRB1 variant.

## Results

### Clinical presentation

A 14-year-old male was referred to the Department of Ophthalmology at Columbia University Irving Medical Center for further evaluation. The patient was diagnosed with Retinitis pigmentosa/Leber congenital amaurosis in infancy after an inability to fixate on toys, severe vision loss and sensitivity to light. He describes his vision as severely blurred but unchanged since age of onset. Reading and face recognition are severely impaired without magnification glasses or accommodation tools such as zoom functionality. Navigation is challenging but the patient has never relied on walking canes or service dogs. The family history was significant for remote consanguinity and similar eye problems in an undiagnosed maternal cousin in Ecuador.

At presentation, visual acuity was best corrected to Snellen 20/400 in both eyes. Horizontal nystagmus was noted bilaterally, and pupillary dilation response was poor. Axial length measured 19.75 and 19.86 mm in the right and left eyes, respectively. Fundus color photography revealed widespread mottling of the retinal pigment epithelium (RPE) and nummular intraretinal pigment migration in the macula and peripheral retina (Fig. 1). Para-arteriolar RPE sparing was observed on funduscopic examination (Fig. 2A, red arrows). Spectral-domain optical coherence tomography (SD-OCT) revealed severe outer retinal layer atrophy with near complete loss of the photoreceptor layer, symmetrical on both eyes compared to control (Fig. 2B and C). Despite the loss of lamination, macular thickness measurements using Heidelberg software showed a globally thickened retina. Full-field ERG testing revealed extinguished scotopic rod-specific (Dark adapted 0.01) and maximal responses (Dark adapted 3.0) (Fig. 3). Isolated cone responses were nearly extinguished and Photopic 30-Hz flicker ERG had amplitudes and implicit times of 6.395 microvolts and 46 ms and 6.396 microvolts and 42 ms in the right and left eyes, respectively (Fig. 3). Genetic testing via whole exome sequencing revealed a homozygous *CRB*1 variant, c.3057 T > A;p.(Tyr1019Ter). Based on the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) variant criteria and guidance from the ClinGen Sequence Variant Interpretation (SVI) working group we classify the c.3057 T > A;p.(Tyr1019Ter) mutation as likely pathogenic (PVS1 plus PM2 supporting) [10].

The patient returned for a 3-year follow-up complaining of severe photophobia at which time he was best corrected to 20/400 on the right and 20/800 on the left eye. Color fundus photography revealed further progression of RPE mottling and nummular pigment migration (Fig. 1B). Short-wave autofluorescence (SW-AF) imaging was obtained which showed a wide central area of hypoautofluorescence, consistent with RPE atrophy, with surrounding hyperautofluorescence in the periphery (Fig. 2A). OCT imaging showed further degeneration of the Ellipsoid zone (Fig. 2D).

Similar findings were observed on color fundus photography and SW-AF and SD-OCT at a seven-year follow-up (Figs. 1C; 2E), but visual acuity had worsened to count fingers at 3 feet on both eyes although the patient reported no changes in vision. Follow-up photopic ERG showed stable cone responses, evidenced by comparable 30-Hz flicker responses of 7.213 microvolts and 22 ms in the right eye, 5.424 microvolts and 21 ms in left eye (Fig. 3).

### Gene editing analysis

In silico analysis of the c.3057 T > A; p.(Tyr1019Ter) loci for prime editing showed two nearby protospacers leading to edits at positions + 19 (Fig. 4A) and + 15 (Fig. 4B) from corresponding nick sites that could be utilized for the correction of this variant.

## Discussion

Variants in the *CRB1* gene are reported to cause a range of clinical phenotypes with significant overlap between RP12 and LCA8, making definitive diagnosis ambiguous [8, 11]. Preserved para-arteriolar retinal pigment epithelium (PPRPE), characterized by sparing of RPE surrounding retinal arterioles, is a characteristic feature of RP12 but can also be present in LCA8 [12]. Similarly, intraretinal pigment migration is a shared feature between RP12 and LCA8 [13]. The proband presented with both nummular pigment deposits and para-arteriolar sparing. Nonetheless, distinctive clinical features in the patient make the diagnosis of LCA much more likely.

LCA8 is considered more severe than RP12 [4]. LCA8 presents at birth with blindness, nystagmus, occulodigital reflex, and microphthalmos [1, 3]. Similarly, these features of LCA8 are evidenced in the proband by the clinical findings of horizontal nystagmus, decreased axial length bilaterally, and reports of difficulty tracking toys in infancy [3]. ERG is another distinguishing tool. Functional testing in LCA8 typically demonstrates severely diminished rod and cone function in infancy, while RP12 patients exhibit a slower, attenuated progression of their rod-cone dystrophy [3]. ERG in the latter case demonstrates early, but not congenital decrease, in ERG signal amplitude and implicit time delay [13]. Rod function is typically affected first, followed by cone dysfunction and central vision deterioration.

Management of LCA8 has been challenging, as no therapeutic option is available for patients with *CRB1*-mediated IRDs. Studies aiming to evaluate the optimal therapeutic window have demonstrated a window appropriate for early interventional therapies of *CRB1*-linked IRDs [14, 15]. In fact, successful proof-of-concept data have been made using CRB family member CRB2 in *Crb1Crb2* conditional knockout mice and *CRB1* patient induced pluripotent stem cell (iPSC)-derived retinal organoids [16–19]. However, the CRB1 isoform diversity, characterized by different isoforms predominately expressed in different retinal cell types, makes the choice of gene augmentation unclear, especially in variants affecting multiple CRB1 isoforms. In this context, double-strand break (DSB)-independent gene editing approaches become viable alternative strategies, especially for variants like c.3057 T > A;p.(Tyr1019Ter) that affect both CRB1 isoform A and B [7, 20].

Transversions, as in the proband, are not amenable to base editing [7]. However, prime editing using a NGG protospacer-adjacent motif (PAM) was feasible with the edit at the + 19 position. Further evaluation revealed the variant is also amenable to a prime editing strategy using a NGA PAM with a closer edit at the + 15 position, which offers higher editing efficiency relative to the + 19 position (NGG PAM strategy). Nonetheless, this NGA prime editing design reveals a series of four T nucleotides (T4) within the 3' extension of the prime-editing guide RNA (pegRNA). T4 is interpreted by DNA polymerase III as a minimal termination sequence, while T6 indicates full termination [21]. T4 stretches may truncate the pegRNA sequence, reducing editing efficiency. This limitation can be overcome through the delivery of mRNA prime editing machinery in place of plasmid DNA [22–24]. Alternatively, delivery of type 7 polymerase-mediated mRNA, with more restricted termination sequences than the type 3 polymerases, seems to overcome the complication of repeat T sequences, thus being preferential for prime editing in vivo [24]. Further, incorporating silent mutations close to the edit would disrupt the T4 stretch enabling pegRNA delivery as plasmid DNA and may also enhance prime-editing efficiency (Fig. 4) [25].

The present case expands on the phenotypic presentations of *CRB1* variants by correlating the proband's novel *CRB1* variant, c.3057 T > A;p. (Tyr1019Ter), with his clinical presentation consistent with LCA8. No current treatment for LCA8 has been developed, however, we propose prime editing as a promising correction method of this variant. Future studies are necessary to evaluate the feasibility of prime editing in vitro and in vivo.

## Materials and Methods

### Clinical evaluation

Patient evaluation included measurement of Snellen visual acuity (in feet). Comprehensive retinal examination was completed following pupillary dilation with 1.0% mydriacyl and 2.5% phenylephrine. Imaging studies included SD-OCT, SW-AF, and color fundus photography (Optos 200Tx unit). Full-field ERG was performed using Dawson-Trick-Litzkow (DTL) recording electrodes and Ganzfeld stimulation according to the International Society for Clinical Electrophysiology of Vision standards [26].

### Prime editing analysis

Prime editing designs were evaluated as previously described [8]. In brief, the variant was analyzed by two different individuals in SnapGene (Version 4.3.11) using both the canonical NGG PAM and the NGA PAM prime editors. Designs were made only for a PE2 strategy with the 3'-extensions of the pegRNAs having a fixed primer binding site of thirteen nucleotides in length and reverse transcription template of 29 (NGG design) and 25 nucleotides (NGA design) in length.

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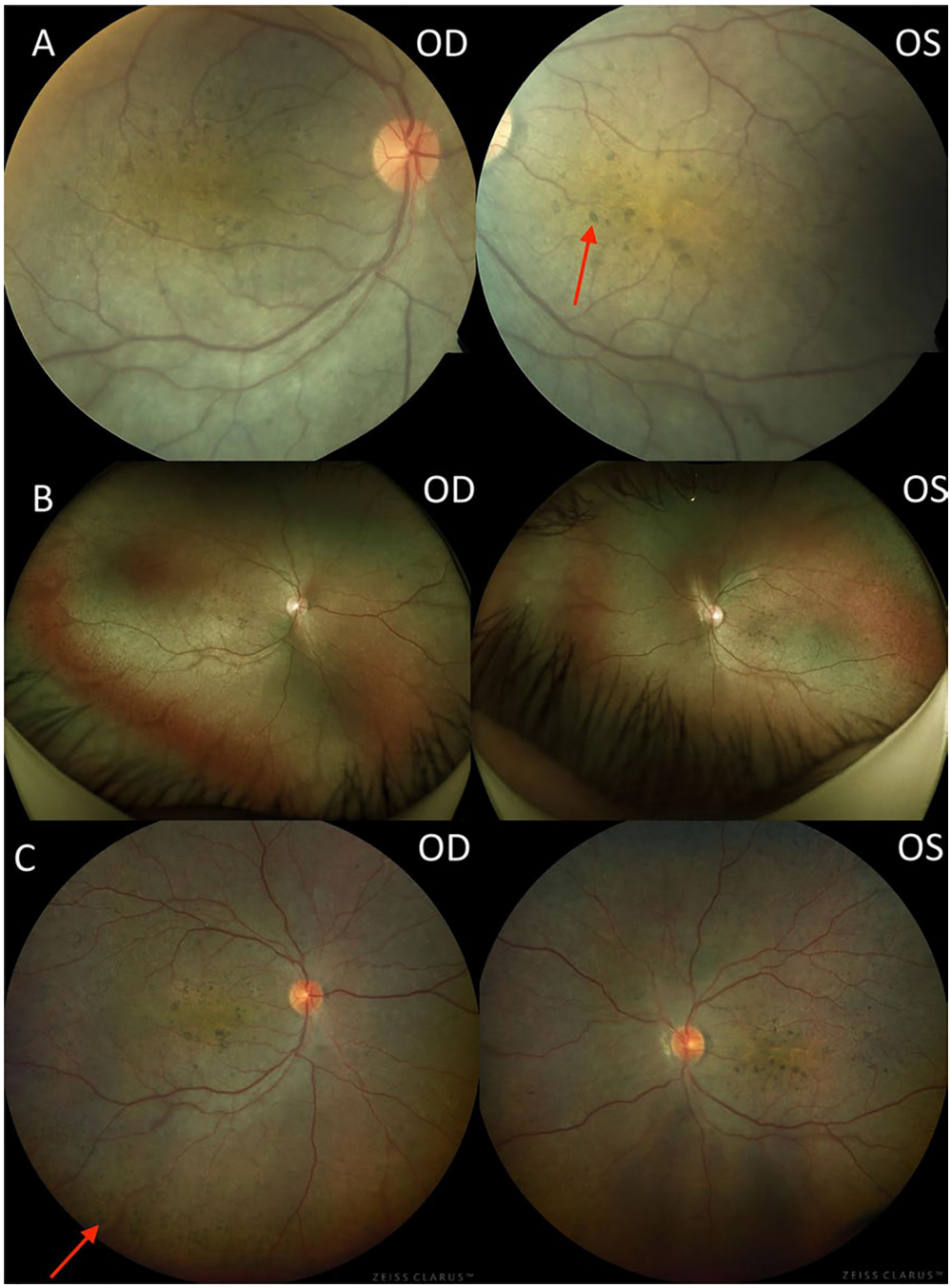


**Fig. 1.**

Fundus photography of a patient with homozygous c.3057 T > A;p.(Tyr1019Ter) variants in the *CRB1* gene. Color fundus photography of the right and left eyes at presentation **A** revealed rare nummular pigments at the macula (red arrows). Follow-up widefield fundus imaging at three **B** and seven **C** years after presentation show further pigmentary deposits at the macula and periphery (red arrows)

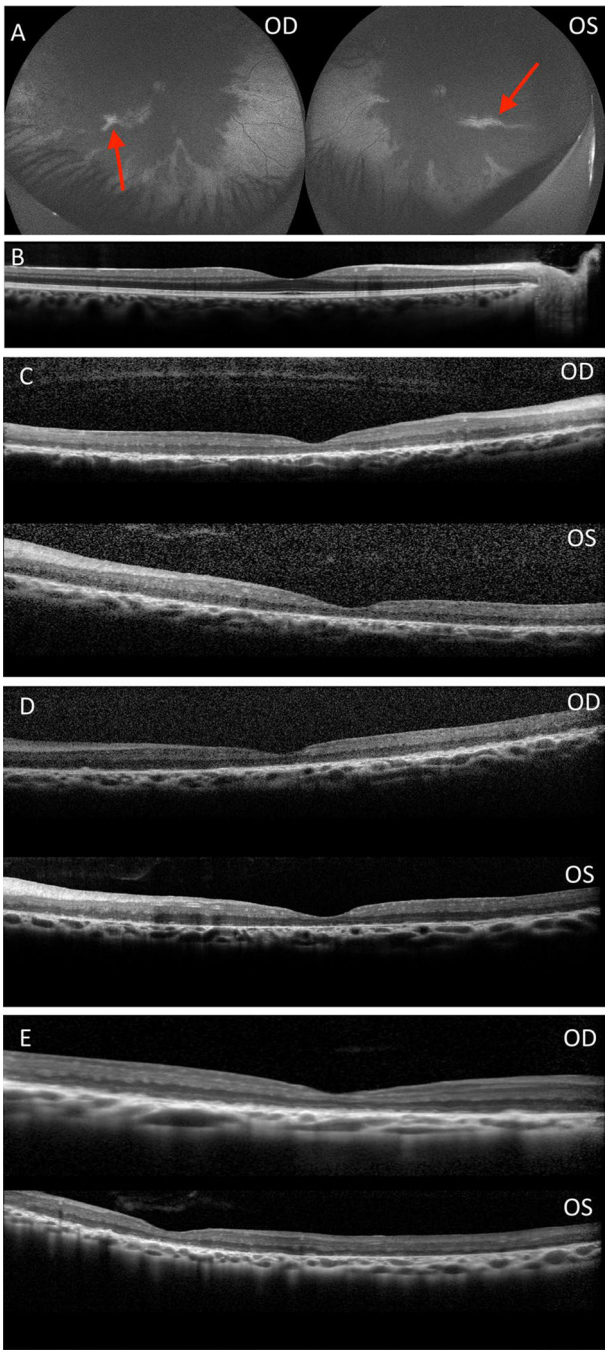


**Fig. 2.**

Short-wave autofluorescence (SW-AF) and standard domain optical coherence tomography (SD-OCT) imaging studies in a patient with homozygous c.3057 T > A;p. (Tyr1019Ter) variants in the *CRB1* gene. Short-wave autofluorescence (SW-AF) shows a wide central area of retinal pigment epithelium (RPE) atrophy bilaterally **A**. Red arrowheads indicate para-arteriolar sparing at the inferior arcades on both eyes. SD-OCT at presentation, compared to control **B**, revealed abnormal lamination with widespread outer nuclear layer atrophy and

extensive loss of the ellipsoid zone bilaterally **C**. Follow-up OCT at three **D** and seven (E) years after presentation show further atrophy of the remaining ellipsoid zone over time

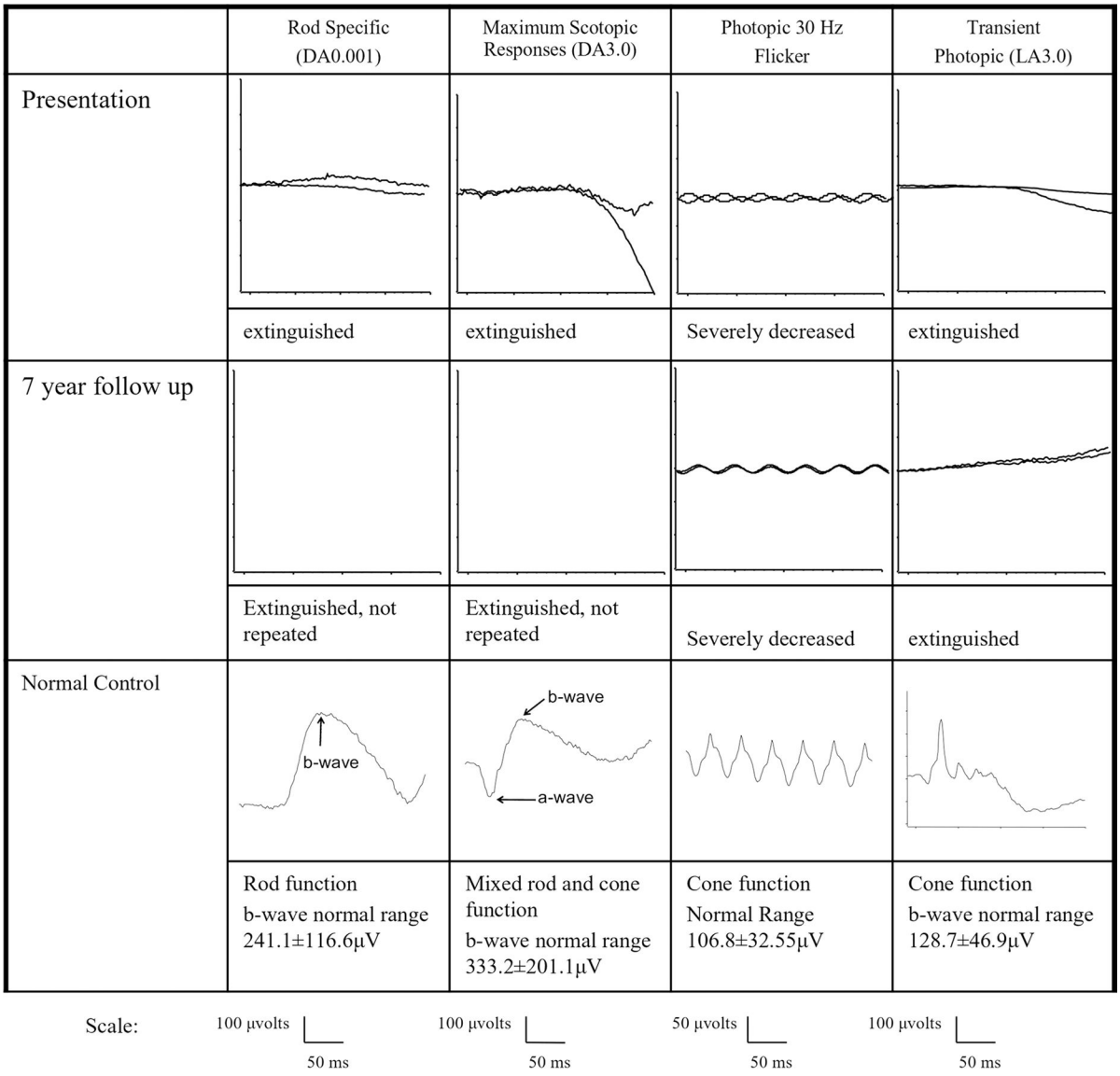


**Fig. 3.**

Full-field electroretinogram (ffERG) recordings of a patient with homozygous c. 3057 T > A; p. Tyr1019Ter variants in the *CRB1* gene. Baseline ffERG revealed a pattern of rod-cone dysfunction. Scotopic rod-specific and maximal responses were extinguished bilaterally (left and right eye responses were superimposed). Photopic 30-Hz flicker ERG had amplitudes and implicit times of 6.395 microvolts and 46 ms and 6.396 microvolts and 42 ms in the right and left eyes, respectively. Follow-up photopic ERG shows comparable 30-Hz flicker ERG amplitudes and implicit times of 7.213 microvolts and 22 ms and 5.424 microvolts and 21 ms in the right and left eyes, respectively

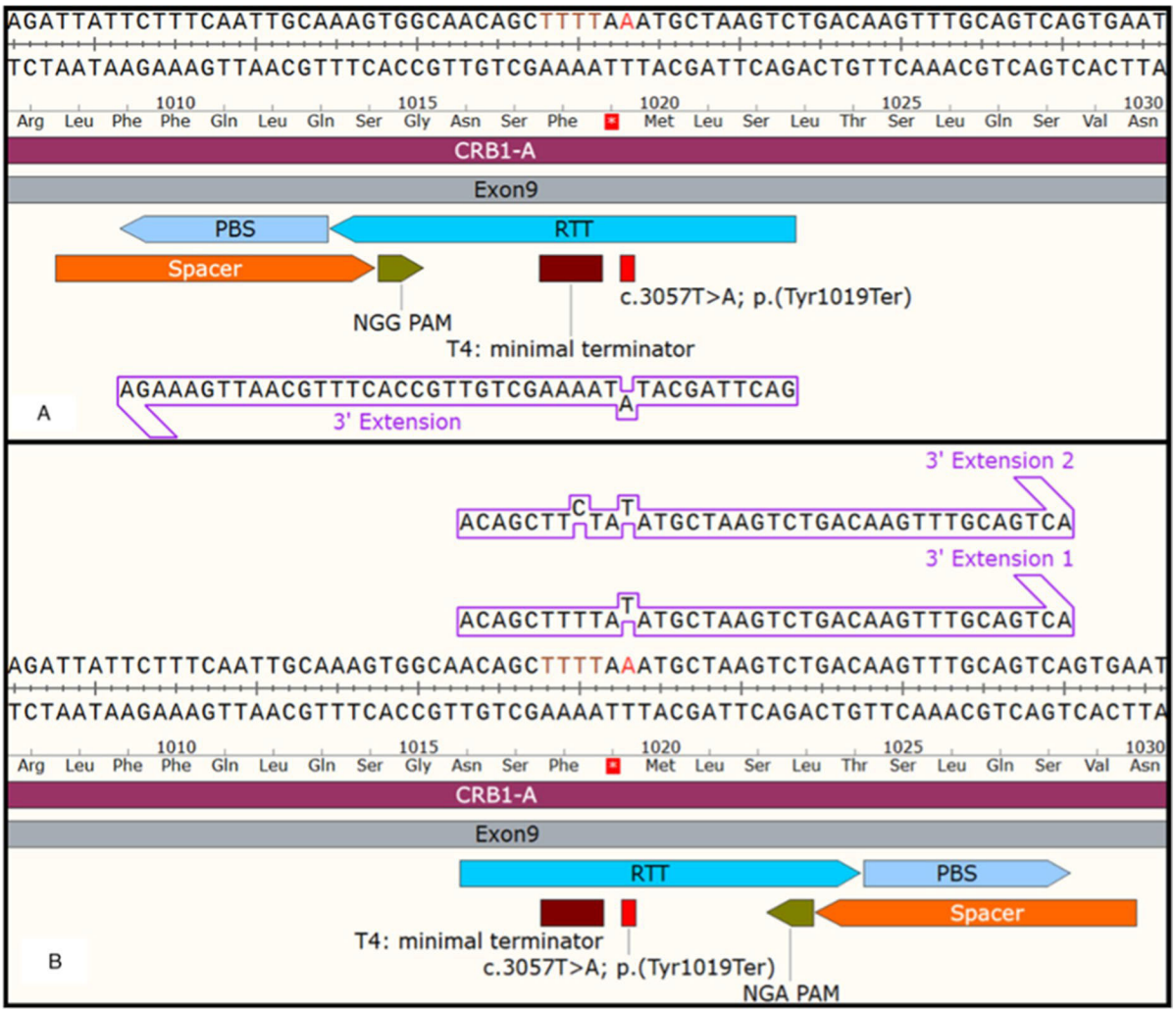


**Fig. 4.**

Analysis of prime editing approaches for the correction of the c.3057 T > A;p.(Tyr1019Ter) *CRB1* variant. Prime editing designs are shown utilizing the NGG prime editor with the edit at + 19 position **A** and the NGA prime editor with the edit at + 15 position **B**. 3' extension 1 with the T4 stretch and 3' extension 2 with the silent mutation that would disrupt the TTTT and enable delivery as plasmid DNA (**B)**