

## CRB1-Related Leber Congenital Amaurosis: Reporting Novel Pathogenic Variants and a Brief Review on Mutations Spectrum

Mohammad Saberi<sup>1</sup>, Zahra Golchehre<sup>1</sup>, Arezou Karamzade<sup>1</sup>, Mona Entezam<sup>2</sup>, Yeganeh Eshaghkhani<sup>1</sup>, Elaheh Alavinejad<sup>1</sup>, Hassan Khojasteh Jafari<sup>3</sup> and Mohammad Keramatipour<sup>1\*</sup>

<sup>1</sup>Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; <sup>2</sup>Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; <sup>3</sup>Farabi Eye Hospital, Tehran, Iran

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### ABSTRACT

**Background:** Leber congenital amaurosis (LCA) is a rare inherited retinal disease causing severe visual impairment in infancy. It has been reported that 9-15% of LCA cases have mutations in *CRB1* gene. The complex of *CRB1* protein with other associated proteins affects the determination of cell polarity, orientation, and morphogenesis of photoreceptors. Here, we report three novel pathogenic variants in *CRB1* gene and then briefly review the types, prevalence, and correlation of reported mutations in *CRB1* gene. **Methods:** Whole exome sequencing and targeted gene panel were employed. Then validation in the patient and segregation analysis in affected and unaffected members was performed. **Results:** Our detected novel pathogenic variants (p.Glu703\*, c.2128+1G>A and p.Ser758SerfsX33) in *CRB1* gene were validated by Sanger sequencing. Segregation analysis confirmed the inheritance pattern of the pathogenic variants. **Conclusion:** Our findings show that emerging the next-generation sequencing-based techniques is very efficient in identifying causative variants in disorders with locus heterogeneity. **DOI:** 10.29252/ibj.23.5.362

**Keywords:** *CRB1*, Leber congenital amaurosis, Retinal dystrophies, Whole exome sequencing

**Corresponding Author:** Mohammad Keramatipour

Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; Tel. & Fax: (+98-21) 88953005; Email: mohammadkeramatipour@gmail.com

### INTRODUCTION

Leber congenital amaurosis (LCA) was first described by Theodore Leber in 1869. It is the most severe form of all inherited retinal diseases<sup>[1,2]</sup>. LCA is a group of inherited retinal dystrophies characterized mainly by severe visual impairment, nystagmus, and severely subnormal or non-detectable electroretinogram (ERG)<sup>[2-6]</sup>. Other common clinical features of the disorder are Franceschetti's oculo-digital sign (a pathognomonic sign of LCA), photophobia, hyperopia, and keratoconus<sup>[5,6]</sup>. Incidence of LCA is 2-3 per 100,000 newborns although it is more prevalent in isolated populations and in populations with high consanguinity rates<sup>[1,5]</sup>.

LCA is a genetically heterogeneous disease that, in most cases, is inherited in an autosomal recessive manner<sup>[2,4,5,7]</sup>. However, there are few autosomal dominant mutations, notably in *CRX* gene. Nearly, 30 genes have been identified to cause LCA, which *CEP290*, *GUCY2D*, and *CRB1* are the most frequently mutated genes<sup>[5,8-10]</sup>. Identified genes explain approximately 70% of molecular basis of the disease; however, the remaining 30% are unresolved cases<sup>[5,6]</sup>.

*Crumb* (*Drosophila*) homolog 1 (*CRB1*) gene encodes a transmembrane protein expressed in brain and retina. Protein CRB1 plays a role in determining and maintaining the apical polarity and adherent junction in embryonic epithelia<sup>[11]</sup>. For the first time, the causative effect of *CRB1* gene on LCA disease has been shown by Lotery et al.<sup>[12]</sup>. Their cohort study has

revealed that *CRB1* accounts for 9% of LCA cases. Other studies have reported its attribution to LCA patients ranged from 9% to 15%<sup>[6,11,13]</sup>. Based on a literature review, the spectrum of reported mutations in functional domains of *CRB1* gene can cause LCA (Fig. 1).

Application of next-generation sequencing (NGS) has been very advantageous for detecting causative genetic variants in monogenic diseases, especially disorders with locus heterogeneity<sup>[14,15]</sup>. Accordingly, this technology has been employed to detect the molecular causes of retinal dystrophies in many studies and showed impressive detection rates<sup>[16-19]</sup>.

In this study, we report three novel pathogenic variants in three Iranian families with LCA, which has been detected by whole exome sequencing (WES) and targeted gene panel.

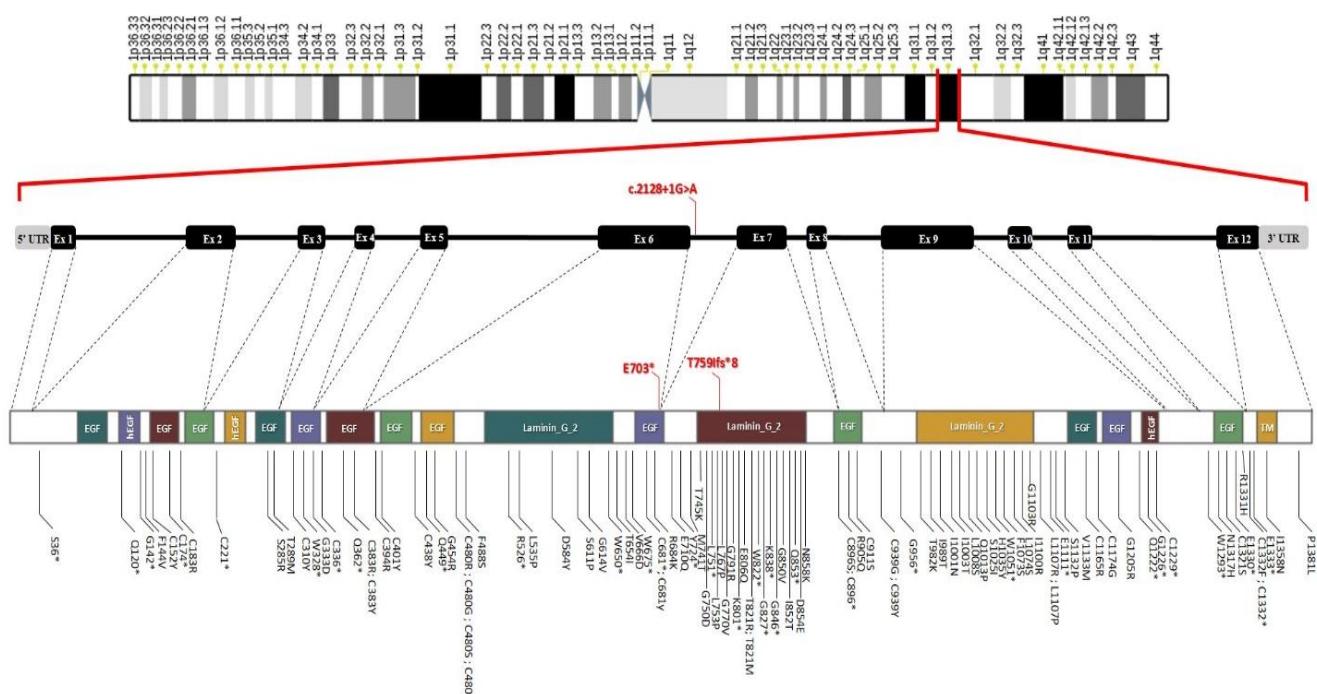
## MATERIALS AND METHODS

### Subjects

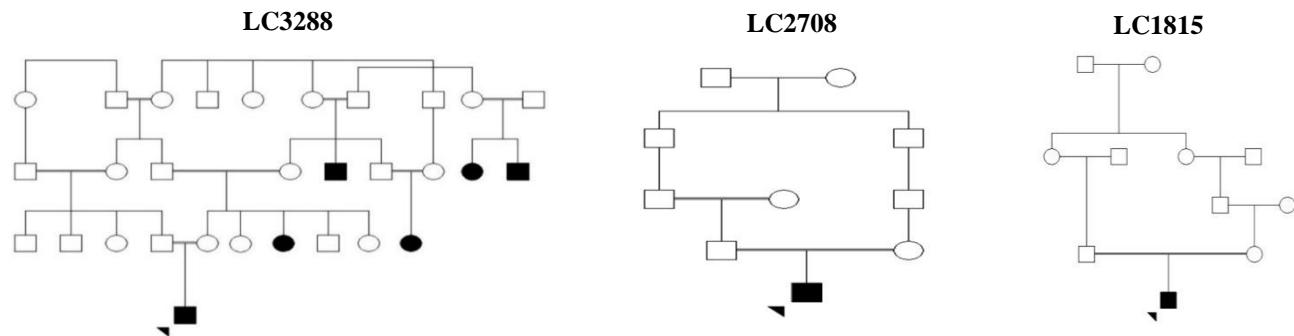
Here, we report three pedigrees of our cohort for 117 patients with inherited retinal diseases, which will be published in near future. Primary clinical diagnosis was established by an expert ophthalmologists through medical history, clinical vision evaluations such as funduscopic, family history, and paraclinical

investigations such as ERG. Available relatives (affected or unaffected) were invited to submit a blood sample for segregation analysis. The pedigrees of the investigated cases are illustrated in Figure 2. An informed consent was obtained from all the participants or their parents/guardians. This study was approved (IR.TUMS.MEDICINE.REC.1396.4198) by the Research Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

Genomic DNA was extracted from whole blood using Exgene™ Blood SV DNA purification kit (GeneAll®, Korea) according to the manufacturer's instructions. Considering great genetic heterogeneity of the suggested diagnosis, cone-rod dystrophy (CRD), NGS-based methods were used for the probands. WES was done for proband of LC3288 pedigree, and targeted panel including 19 genes were performed for LC1815 and LC2708 pedigrees. SureSelect V6™ Target Enrichment Kit (Agilent, USA) with the 61-Mb target region was used in WES and NimbleGen, Roch™ comprised 19 genes (69,628 base pairs) for targeted panel. The enriched libraries were sequenced on the Illumina Hiseq 4000™ platform. Sequencing reads were generated in the FASTQ format after nucleotide calling. Read pairs were aligned to the human reference genome (hg19) using the Burrows-Wheeler Aligner, and duplicate reads were marked



**Fig. 1** Spectrum of mutations in functional domains of *CRB1*. Our novel pathogenic variants are illustrated with red color. The schematic representation of *CRB1* domains was obtained from Phosphosite database (<https://www.phosphosite.org/proteinAction?id=11964200&showAllSites=true>). EFG, EGF-like domain (Pfam: PF00008); hEGF, human growth factor-like EGF (Pfam: PF12661); Laminin\_G\_2, laminin G domain (Pfam: PF02210); TM, transmembrane.



**Fig. 2.** Pedigrees of the investigated families. Probands were indicated by arrowheads.

with PICARD tools. The GATK UnifiedGenotyper module was applied for Indel realignment, base recalibration, variant calling, and variant filtering. Then variants were annotated using SnpEff tool. Variant prioritization based on population frequency, effect or nature of the variants, and zygosity were performed.

Polymerase chain reaction (PCR) followed by Sanger sequencing was conducted to confirm the variants of interest in the probands. Besides, the investigations of the detected variants in their available members of the pedigrees were carried out by PCR-Sanger sequencing. The PCR products were purified by Expi<sup>TM</sup> Combo GPMini purification kit (GeneAll Biotechnology, Seoul, South Korea) and sequenced by ABI 3500 automated sequencer (Pishgam Biotech Company, Tehran, Iran).

### Variant interpretation

Allelic frequency of the variants was investigated in population databases viz dbSNP (<http://www.ncbi.nlm.nih.gov/snp>), 1000 genome project (1000GP) (<http://browser.1000genomes.org>), Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>), NHLBI GO Exome Sequencing Project (ESP) (<http://evs.gs.washington.edu/EVS>), and our local database that includes more than 1000 exome of Iranian population. A variety of computational (*in silico*) predictive tools, including MutationTaster (<http://www.mutationtaster.org>)<sup>[20]</sup>, Combined Annotation-Dependent Depletion (CADD; <http://cadd.gs.washington.edu>)<sup>[21]</sup>, and DANN Score<sup>[22]</sup> were used to evaluate the effect of the detected nucleotide exchange on the basis of evolutionary conservation, protein structure, and protein function. Moreover, the variant was investigated in RetNet (<https://sph.uth.edu/retnet/>), HGMD (Human Gene Mutation Database), ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>), GeneReviews<sup>®</sup> (<https://www.ncbi.nlm.nih.gov/books/NBK1116/>), and OMIM<sup>®</sup> databases and also in literature for any previously reported record.

## RESULTS

### Clinical findings

Based on clinical and para-clinical examinations, LCA was suggested as primary clinical diagnosis of patients. Moreover, there was no evidence for the involvement of other organs; no signs of intellectual disability, skeletal abnormality, hearing impairment, and renal malfunction were observed.

### Genetic findings

Performed WES generated approximately 161 million reads, with 137 million non-redundant reads mapped to the target regions. More than 99% of the target regions had  $>10\times$  coverage, and more than 96% of the target regions had  $>30\times$  coverage. In average, performed targeted panel covered 98.8% of target regions with an average depth of  $250\times$ . Moreover, 93.5% of the target regions were covered by  $30\times$ . Step by step analysis and filtering of the NGS data left three homozygous variants in *CRB1* gene (Table 1). The variants were absent from population databases (ExAC, 1000G, dbSNP, and our local database) and have not previously been reported in the literature. In addition, as shown in Table 1, multiple lines of *in silico* computational analysis support the deleterious effect of the variants on the gene or gene product (MutationTaster, CADD, and DANN scores). Homozygosity of the probands was also confirmed by PCR-Sanger sequencing (Fig. 3).

## DISCUSSION

LCA, as the most severe and early onset form of inherited retinal diseases, is responsible for approximately 20% of blind children studying in schools<sup>[1,2,4]</sup>. Genetic testing is a crucial step for patients suspected to have LCA because it has the ability to confirm diagnosis and makes distinction between LCA and other retinal diseases with similar

**Table 3.** Description of the causative variants in probands

Pedigree	Gene/RefSeq	Chromosome position	Variant features			Zigosity	Location	Population databases		Predictive tools			
			CDS change	Amino acid change	dbSNP			1000GP	GnomAD	Pishgam	CADD	DANN	Mutation Taster
LC2708	CRB1/ NM_201253.2 Chr1: 197,396,730	LC1815 Chr1: 197,391,087	c.2107G>T	p.Glu703*	Hom	Exon 6	-	-	-	-	24.9	0.8736	DC
			c.2128+1G>A	-		Hom	Intron 6	-	-	-	26.9	0.9892	
			c.2276_2279dupCTTA	p.Ser758Ser fsX33								24.8	-

DC, disease causing

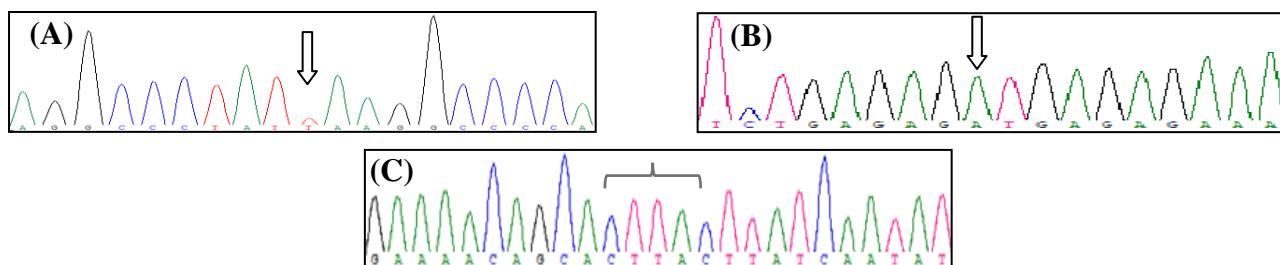
clinical presentations. Determining the causative variant is very helpful in genetic counselling and future reproductive planning for the family. It may also help in defining the prognosis of the disease, as well as making personalized decision for the patient, including mutation or gene-specific treatments in near future.

LCA is a genetically and clinically highly heterogeneous disease. Some syndromic features, such as hearing impairment, involvement of nervous system, renal abnormalities, and skeletal anomalies in Senior-Løken syndrome, Joubert syndrome, and conorenal syndrome may accompany with retinal manifestation of LCA. Therefore, these diseases need to be discriminated from LCA as an isolated ocular disease. However, occasionally, this differentiation cannot be achieved by clinical and paraclinical investigations, especially in the early childhood<sup>[23,24]</sup>. Moreover, to date, 321 genes and loci on RetNet database have been linked to inherited retinal dystrophies, which some of

them such as early onset Retinitis pigmentosa (RP) and CRD may have similar features with LCA. In addition, nearly 30 LCA causative genes have been reported<sup>[5,8-10]</sup>.

Considering the above mentioned issues, we need a robust, high-throughput, and cost-effective technique for the genetic analysis of LCA or this kind of spectrum disorders. The advent of NGS technology and dramatic decrease in its cost make it an extraordinary diagnostic tool for Mendelian disorders with high locus heterogeneity<sup>[14,15]</sup> including retinal diseases. In case of genetic diagnosis for retinal diseases, this technology has been successfully applied in panel-based manner or through WES<sup>[16-18]</sup>. NGS has higher detection rates than array-based genotyping and is extremely more cost-effective than Sanger sequencing for LCA disease<sup>[25]</sup>.

*CRB1* was identified through the candidate gene approach, since its implication has previously been



**Fig. 3** Genotypes of the probands. (A) c.2107G>T in LC3288; (B) c.2276\_2279dupCTTA in LC2708; (C) c.2128+1G>A in LC1815. Arrows show position of the variants.

identified in RP12<sup>[12,26]</sup>. Indeed, mutations in *CRB1* gene cause a spectrum of hereditary retinal dystrophies, including LCA type 8 (LCA8), early-onset rod-cone dystrophy, RP12, autosomal dominant pigmented paravenous chorioretinal atrophy, recessive RP with para arteriolar preservation, CRD, and isolated autosomal recessive foveal retinoschisis<sup>[8,9,27-30]</sup>.

LCA8 (OMIM: # 613835) frequency among LCA cases varies from 0% in Indian to 17% in Spanish patients<sup>[1,28]</sup>. Gene-specific indications for *CRB1*-associated retinal dystrophy may be some fundus features: deep nummular pigmentations, preservation of the para-arteriolar retinal pigment epithelium, Coats-like vasculopathy, and retinal telangiectasia with exudation<sup>[26,31,32]</sup>. Another paraclinical sign suggesting *CRB1* mutation is retinal thickening on OCT<sup>[30,33,34]</sup>.

The causative gene for LCA8, *CRB1*, is located in 1q31.1 and encodes a 1406-amino acid protein containing 19 epidermal growth factor (EGF)-like domains and three Laminin A globular-like domains in extracellular region, and a short cytoplasmic tail<sup>[35]</sup>. More than 300 pathogenic variants have been reported in *CRB1* gene, which the most common ones are missense (about 60%)<sup>[36]</sup>. There are efforts to correlate type of mutation or its location with *CRB1*-related disorders and their severities. Studies attempting to connect specific mutations in *CRB1* to specific phenotypes have not reached remarkable success<sup>[27,31]</sup>. However, it seems that null alleles (i.e., nonsense, frameshift deletions/duplication/insertions, and canonical splice site mutations) are mostly common among more severe retinal dystrophies and LCA patients<sup>[32,37,38]</sup>, with the same as our cases. Kuniyoshi *et al.*<sup>[39]</sup> have reported three novel null mutations in affected individuals with milder severity, slow progression, and without nystagmus. Another study have shown the combination of two null alleles in 40% of LCA cases, but not in early-onset RP patients<sup>[40]</sup>.

To establish phenotype-genotype correlation regarding the location of missense mutations, Beryozkin *et al.*<sup>[27]</sup> have studied the phenotype of patients with homozygous missense mutations in *CRB1*. They found that patients with mutations within the Ca-binding EGF-like domains have more severe disease (LCA or early RP) compared to patients with homozygous missense mutations in the laminin AG-like domains. Detected variants in this study are located in EGF-like domain 12 and expected to have severe effects. Several other missense or stop-gained mutations, mostly resulting in LCA, have been reported in this domain<sup>[12,31,41]</sup>.

In summary, there is no straightforward association between type of mutations and their clinical

consequences; variability of *CRB1*-related phenotype may correlate with genetic background and modifier loci and even environmental factors rather than type of mutation in *CRB1*<sup>[32,42]</sup>. Intrafamilial variability in LCA families has been reported since 1960s<sup>[43-45]</sup>. Our proband in pedigree LC3288 did not show nystagmus; it was the first symptom detected in his affected aunt. The same variability has been reported in a Chinese family; one sib was affected by *CRB1*-LCA presented with nystagmus, whereas his affected sister did not<sup>[46]</sup>. Of 300 reported mutations, 50 were nonsense variants. Besides, 27 nonsense and 33 small indels, which are located in the downstream of our detected variant and lead to premature termination codons, have previously been reported in HGMD<sup>[36]</sup>.

Evidence supports the causative effects of the detected variants: absence in population databases, deleterious effects in computational predictive tools; loss of function as a known mechanism of the disease, co-segregation of the phenotype in three affected members of pedigree LC3288, and specificity of patients' phenotypes for *CRB1*-related disorders. Therefore, these variants can be classified as pathogenic variants based on American College of Medical Genetics and Genomics guideline for the interpretation of sequence variants<sup>[47]</sup>.

Consanguineous marriages are prevalent in Iranian population with the rate of 38.6%, which first cousin marriages are the most common form<sup>[8]</sup>. Therefore, higher prevalence of single gene disorders such as LCA than Western countries could be expected. As a result, the implementation of high-throughput methods such as WES to detect disease-causing variants in diseases with high clinical and genetic heterogeneities in this kind of populations can be important in the early diagnosis of the patients and making informed decision of their relatives in premarriage or preconception genetic counseling.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST.

None declared.

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