A novel compound heterozygous BEST1 gene mutation in two siblings causing autosomal recessive bestrophinopathy

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Abstract

Purpose: To describe the clinical features, imaging characteristics, and genetic test results associated with a novel compound heterozygous mutation of BEST1 gene resulting in an autosomal recessive bestrophinopathy (ARB).

Methods: Two siblings underwent a complete ophthalmic examination, including dilated fundus examination, fundus photography, fundus autofluorescence imaging, spectral-domain optical coherence tomography (OCT), fluorescein angiography (FA), electroretinography (ERG), and electrooculography (EOG). A clinical diagnosis of ARB was established based on multimodal retinal imaging. Subsequently, clinical exome sequencing, consisting of a panel of 6670 genes, was carried out to assess genetic alterations in the protein-coding region of the genome of the patients.

Results: Two siblings (17-year-old female and 15-year-old male) presented with reduced visual acuity and bilateral symmetrical subretinal deposits of hyperautofluorescent materials in the posterior pole. Spectral-domain OCT demonstrated subretinal fluid accumulation. Clinical exome sequencing revealed two mutations in the protein-coding region of the BEST1 gene, including a novel mutation (R105S), creating aberrant BEST1 variants p.Glu35Lys (E35K) and p.Arg105Ser (R105S) expressing from the canonical BEST1 transcript.

Conclusions: We have identified and described the phenotype of a novel BEST1 mutation (R105S) in a heterozygous state along with a previously reported mutation of BEST1 (E35K) causing autosomal recessive bestrophinopathy (ARB).

Introduction

The BEST1 (alternatively VMD2, RP50, BMD) gene located on chromosome 11q12.3 (genomic coordinates: 11:61,946,721-61,965,514) encodes a transmembrane pentameric protein consisting of 585 amino acids that is predominantly expressed in the basolateral plasma membrane of the retinal pigment epithelium (RPE).^[1-4] It functions as a calcium-activated chloride channel (CaCC) which regulates the flow of chloride and other monovalent anions across cellular membranes in response to intracellular calcium levels.^[4-6] Mutation of this gene has been associated with a wide range of ocular phenotypes that may be influenced by age, gender, environment, epigenetic factors, and presence of modifier genes and are collectively termed as bestrophinopathies.^[1,7,8] ARB may result from a total absence (null phenotype) of functional BEST1 protein in the RPE,^[9,10] improper localization to the cell membrane with intact anion channel activity^[11] or lack of channel activity specifically.^[12]

Schatz et al.^[13] in 2006, were the first to report two related patients with multifocal vitelliform dystrophy with compound BEST1 heterozygous variants. Two years later, Burgess et al.^[9] coined the term autosomal recessive bestrophinopathy (ARB) and concluded that this condition was the fourth phenotype associated with BEST1 gene mutations. Phenotypes associated with pathogenic variants of BEST1 include **(1)** conditions that predominantly affect the macula, including autosomal recessive bestrophinopathy (ARB, OMIM 611809)^[9,12]; Best disease (OMIM, 153700)^[14,15] and adult vitelliform macular dystrophy (OMIM 153840); **(2)** those with generalized retinal involvement, including autosomal dominant vitreoretinchoroidopathy (OMIM 193220)^[16,17]; rod-cone dystrophy and retinitis pigmentosa (OMIM 613194)^[1,18]; and **(3)** diseases with retinal and anterior segment involvement, including autosomal dominant microcornea, rod-cone dystrophy, early-onset cataract, and posterior staphyloma.^[1] In contrast to Best vitelliform macular dystrophy (BVMD), which is results from autosomal dominant BEST1 mutations^{[19][20,21]}, autosomal recessive bestrophinopathy (ARB) is associated with biallelic mutations in the BEST1 gene^[9].

The clinical features of ARB include a gradual and progressive visual loss, hyperopia, predominantly peri-macular sub-retinal vitelliform deposits of lipofuscin in the retinal pigment epithelium (RPE), evident as hyperautofluorescent areas at the posterior pole, accumulation of subretinal and/or intraretinal fluid, absence of light peak in EOG, normal or reduced ERG, and in some, shallow anterior chambers, predisposing the affected patients to angle-closure glaucoma.^[9,22,23] The reduction in electrooculogram light peak-to-dark trough ratio can be explained by the severe generalized RPE dysfunction. Full-field electroretinography typically normal early on in the disease and shows abnormal results from late childhood or adolescence, indicating generalized rod and cone dysfunction. In addition, pattern electroretinography evidence of macular dysfunction is also seen.^[9] ARB usually manifests in the first two decades of life but may remain asymptomatic as late as the fifth decade.^[9,12,24] In this article, we present the results of a clinical, electrophysiological, and genetic investigation of two siblings with ARB.

Methods

Clinical Investigation

This study was approved by the Institutional Ethics Committee of the Jawaharlal Nehru Medical College and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from the parents of the minor subjects prior to conducting investigations.

Clinical investigations in patients included detailed history and physical examination, best-corrected visual acuity, slit-lamp examination, indirect ophthalmoscopy, fundus photography, fundus autofluorescence imaging, spectral-domain optical coherence tomography (OCT), fluorescein angiography, full-field ERG and EOG. The ERG and EOG were performed in accordance to the guidelines of the International Society for Clinical Electrophysiology of Vision (www.iscev.org).

Genetic analysis

DNA was isolated from the patient's blood sample using QIAamp DNA Blood Mini Kit and was subjected to targeted gene capture using a custom capture kit, which captures a panel of 6670 protein-coding genes. The libraries thus generated were sequenced to mean coverage of >80-100X coverage on the Illumina sequencing platform. The GATK best practices framework was followed to identify the variants in the sample using Sentieon (v201808.07).

The reads were aligned to the human reference genome (GRCh38.p13) using Sentieon aligner and analyzed to remove duplicates, recalibrate, and re-align indels^[25]. Sentieon haplotype caller was used to identify variants that are relevant to the clinical indication. Gene annotation of the variants was performed using the VEP program against the Ensembl release 99 human gene model^[26,27]. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM (updated on 11th May 2020), GWAS, HGMD (v2020.2) and SwissVar^[28-32].

Bioinformatics Analysis

The potential functional impact of all the candidate variants was investigated using three programs, including PolyPhen2 (<http://genetics.bwh.harvard.edu/pph/>, in the public domain), Mutation Taster (<http://www.mutationtaster.org/>, in the public domain), and SIFT (<http://sift.jcvi.org/>, in the public domain).

Results

Clinical findings

Patient A, a 17-year-old female, reported blurred distance vision in both eyes for three years. Her best-corrected visual acuity was found to be 6/9 in both eyes, which did not change during the follow-up of 1 year. Patient B, 15-year old male, reported a unilateral decrease in visual acuity and inward deviation of the left eye since four years of age. On examination, he was found to have left esotropia of 15 prism diopters with prescribed correction and 25 prism diopters without correction for distance. His best-corrected visual acuity was 6/6 in his right eye and 5/60 in his left eye, with an accommodative-convergence over accommodation (AC/A) ratio of 2:1. The clinical characteristics of the two affected family members are summarized in *Table 1*.

Table 1

Clinical profile of the patients

Patient	Age/sex	Axial length/ AC depth (mm)		BCVA, spherical equivalents		FAF	OCT	ERG	EOG
		OD	OS	OD	OS				
A	17/F	21.80/ 2.65	21.64/ 2.71	6/9 (-1.25)	6/9 (-1.50)	Hyper autofluorescence	Intra-retinal and sub-retinal deposits with subretinal fluid	Normal	Absent light peak
B	15/M	21.61/ 3.48	21.54/ 3.53	6/6 (+5.00)	5/60 (+5.00)	Hyper autofluorescence	Sub-retinal fluid and deposit	Normal	Absent light peak
AC, Anterior Chamber; BCVA, Best-corrected visual acuity; FAF, Fundus autofluorescence; OCT, Optical Coherence Tomography; ERG, electroretinogram; EOG, Electrooculogram									

Slit-lamp examination of the anterior segment of both patients was unremarkable. Dilated fundus examination revealed yellowish sub-retinal deposits in both patients (*Figure 1 A, B and Figure 2 A, B*). Fundus autofluorescence (FAF) images for both patients demonstrated areas of hyper autofluorescence (*Figure 1 C, D and Figure 2 C, D*). Fluorescein angiography (FA) showed late staining of vitelliform materials without any leakage in the macula (*Figure 1 E, F and Figure 2 E, F*). Electroretinogram (ERG) was normal and an absent light peak on EOG was noted in both the patients. Optical coherence tomography (OCT) revealed subretinal fluid and intra-retinal fluid along with subretinal deposits and schitic changes predominantly in inner and outer nuclear layers of the retina of patient A (*Figure 1 G, H*). OCT in the affected sibling demonstrated subretinal fluid and deposits, but intra-retinal fluid and schitic changes were not seen (*Figure 2 G, H*).

Both of the affected siblings were treated with topical carbonic anhydrase inhibitors and followed up for one year. The intraretinal fluid in the macular region did not improve after one year of treatment. The unaffected parent and other siblings were examined; however, no ocular or systemic abnormalities were observed. (*Figure 3*).

Genetic findings

A heterozygous missense mutation was found with a mutation in exon 2 of the BEST1 gene in both patients (chr11: g.61951909G>A; Depth: 119x). It resulted in the amino acid substitution of Lysine for Glutamic acid at codon 35 (p.Glu35Lys; ENST00000378043.9) (*Table 2*). The variant lies in the bestrophin, RFP-TM, chloride channel domain of the BEST1 protein. The in-silico predictions according to PolyPhen-2 is to be probably damaging, and deleterious according to SIFT and MutationTaster2. The reference codon is conserved across species. The p.Glu35Lys variant has not been reported previously in the 1000 genomes and gnomAD databases (minor allele frequency = 0.001%).

Another heterozygous missense mutation was found in exon 4 of the BEST1 gene in both patients (chr11:g.61955783C>A; Depth: 137x) that resulted in the amino acid substitution of Serine for Arginine at codon 105 (p.Arg105Ser; ENST00000378043.9) (*Table 2*). The variant lies in the bestrophin, RFP-TM, chloride channel domain of the BEST1 protein. The in-silico predictions of the variant according to PolyPhen-2 is to be probably damaging, and deleterious according to SIFT and MutationTaster2. The reference codon is conserved across species. The p.Arg105Ser variant has not been reported in the 1000 genomes databases (minor allele frequency = 0.0007%). This mutation has not been previously reported in patients with ARB or VMD. The results of next-gen sequencing are summarized in *Table 2*.

Table 2

Next-gen sequencing (NGS) of BEST-1 gene of patients A and B

Gene (Transcript)	Location	Nucleotide change	Amino Acid change	Inheritance	PolyPhen-2 prediction	ClinVar Classification
BEST1 (+) (ENST00000378043.9)	Exon 2	c.103G>A	p.Glu35Lys	Autosomal recessive	Probably damaging	Uncertain significance
	Exon 4	c.313C>A	p.Arg105Ser (novel)	Autosomal recessive	Probably damaging	Uncertain significance

Discussion

This report analyzed the genetic and clinical characteristics of two siblings with ARB from one family. The clinical diagnosis of ARB was established based on clinical observation and multimodal retinal imaging and further confirmed by genetic testing. The patient A demonstrated good central acuity, consistent with other ARB studies in the first and second decade of life.^[33] However, we noted that the sibling had poor visual acuity in one eye due to amblyopia resulting from uncorrected esotropia. In addition to the retinopathy and amblyopia, it is important to note that abnormal iridocorneal anatomic features, shallow anterior chamber depth, and reduced axial length all predispose patients to an increased prevalence of angle-closure glaucoma that can potentially lead to a further visual decline in those with ARB.^[33]

The exome sequencing revealed a compound heterozygous mutation in BEST1 in both siblings that likely led to ARB. Out of the two copies of BEST1 present in the diploid genome, one of the copies carried a missense mutation in exon 2 (chr11: g.61951909 G>A; Depth: 119x). The missense mutation at exon 2 (E35K) results in the amino acid substitution from Glutamic acid to Lysine at 35th amino acid residue (p.Glu35Lys; ENST00000378043.9). The p.Glu35Lys (E35K)

variant has previously been reported by Tian et al. and Habibi et al., albeit in a homozygous state.^[34,35] To the best of our knowledge, ours is the first study to report this variant in a heterozygous state.

The other copy of BEST1 was observed to have a novel missense mutation in exon 4 (chr11:g.61955783C>A; Depth: 137x) that resulted in the amino acid substitution from Arginine to Serine at 105th amino acid (p.Arg105Ser; ENST00000378043.9) (*Table 2*). To the best of our knowledge, the variant p.Arg105Ser (R105S) has not been reported previously in patients with either ARB or VMD. This mutation is likely pathogenic. Functional analysis needs to be done to prove it and bigger cohorts need to be screened. No genetic analysis was carried out in the parents.

The BEST1 is a 585 amino acid long protein containing a highly conserved N-terminal region followed by four transmembrane domains (amino acids 1–390) and carboxy-terminal region (amino acids 391–585).^[36] Structural models of the BEST1 propose the N- and C-termini as being cytosolic with the presence of four transmembrane domains (domains 1, 2, 5, and 6), while domains 3 and 4 are cytoplasmic^[37,38] (*Figure 4 B*). It is noteworthy that the mutations discovered in this study are localized to the N-terminal region (*Figure 4 A-C*). The mutation E35K localizes to the first transmembrane domain, while the R105S mutation alters an amino acid in the cytoplasmic region distal to the second transmembrane domain (*Figure 4 B*). The amino acids at these positions are conserved among mammals (*Figure 4 D*). Among the roughly 270 mutations reported in BEST1 thus far, only about 40 compound heterozygous and homozygous mutations are associated with ARB.^[9,22,23]

Although the detailed pathophysiology that leads to the disease is poorly understood, most of the characterized BEST1 mutations alter electrophysiological properties of the calcium-activated chloride channel (CaCC), which is thought to be determined by the N-terminus portion of BEST1, affecting the flow to chloride across the RPE^[4,36]. Crystallographic studies of wild-type and mutated proteins suggest that BEST1 variants alter the cytoplasmic pore structure, which affects the permeability of anions or anion-cation selectivity (*Figure 4 C*).^[39]

Abbreviations

AC: Anterior Chamber

ARB: Autosomal recessive bestrophinopathy

BCVA: Best-corrected visual acuity

BVMD: Best vitelliform macular dystrophy

EOG: electroretinogram

ERG: Electrooculogram

FA: Fluorescein angiography

FAF: Fundus autofluorescence

IRF: Intraretinal fluid

NGS: Next-gen sequencing

OCT: Optical coherence tomography

OMIM: Online Mendelian Inheritance in Man

RPE: Retinal pigment epithelium

SIFT: Sorting Intolerant From Tolerant

SRF: Subretinal fluid

VMD: Vitelliform macular dystrophy

Declarations

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Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

Consent for study:

Written informed consent for the study was obtained from the parents as the participants were minors.

Ethics approval:

The study was approved by the Jawaharlal Nehru Medical College Institutional Ethics Committee and was performed as per institutional ethics guidelines and in accordance with the tenets of the Declaration of Helsinki.

Competing interests:

The authors declare that they have no competing interests.

Consent for publication:

Written informed consent was obtained from the parents of the patient for publication of this research article.

Authors' contributions:

OIH: involved in the patient's care, diagnostic evaluation, draft preparation, editing and submission of the manuscript;
FN: involved in reviewing the draft, analyzing genetic test results and preparing the topological BEST1 protein model;

OA: involved in reviewing and editing the manuscript, analyzing genetic test and acquiring figures. JPM: involved in the diagnostic evaluation, reviewing and editing of the manuscript.

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Figures

Figure 1

Clinical features of patient A. A 17-year-old otherwise healthy female presented with blurred distance vision in both eyes, which she first noticed when she was 14 years old. Colour fundus photographs (A, B) show bilateral and symmetrical, multifocal subretinal yellowish deposits in the posterior pole and upper nasal region, with notable peripapillary sparing. The yellowish deposits are hyperautofluorescent on blue light fundus autofluorescence (B, C) and circumscribe areas of hypoautofluorescence, where mild leakage is observed in the late phase of fluorescein angiography (E, F). Horizontal spectral-domain optical coherence tomography images through fovea (G, H) show centre-involving subretinal fluid and thickening of the ellipsoid zone, with elongation of the photoreceptor outer segments. Additionally, intraretinal hyporeflective spaces predominantly located in outer nuclear layer reflect intraretinal fluid accumulation.

Figure 2

Clinical features of patient B. A 15-year-old otherwise healthy male presented with a unilateral decrease in visual acuity and inward deviation of the left eye, which was first noticed by his parents when he was four years old. On colour fundus photographs (A, B), bilateral and symmetrical, multifocal subretinal yellowish deposits are seen in the posterior pole and upper nasal region, with notable peripapillary sparing. On blue light fundus autofluorescence (C, D), the yellowish deposits are hyperautofluorescent and circumscribe areas of hypoautofluorescence, where mild leakage is observed in the late phase of fluorescein angiography (E, F). Corresponding lesions demonstrated diffuse hyperfluorescence. Horizontal spectral-domain optical coherence tomography scans through the fovea (G, H) show centre-involving subretinal fluid and thickening of the ellipsoid zone, with elongation of the photoreceptor outer segments.

Figure 3

Pedigree of the family with two affected members. Patient A, 17-year old female (black arrow), and Patient B, 15-year old male (red arrow).

Figure 4

(A) Schematic representation of BEST1 (ENSG00000167995) transcript. Grey boxes represent exons, and lines connecting them represent introns. Green boxes represent the translation start site, while red boxes represent the translation termination site. (B) Topological representation of BEST1 (Milenkovic et al.) representing mutation sites denoted by red stars. One mutation occurs in the transmembrane motif, while the other occurs in the region connecting two motifs. (C) Generated model structure of BEST1 (WT), BEST1 (E35K) & BEST1 (R105S).