

Genetic variations in *Bestrophin-1* and associated clinical findings in two Chinese patients with juvenile-onset and adult-onset best vitelliform macular dystrophy

YING LIN^{1*}, TAO LI^{1*}, CHENGHONG MA^{2*}, HONGBIN GAO^{3,4*}, CHUAN CHEN^{1,5},
YI ZHU^{1,5}, BINGQIAN LIU¹, YU LIAN¹, YING HUANG¹, HAICHUN LI¹, QINGXIU WU¹,
XIAOLING LIANG¹, CHENJIN JIN¹, XINHUA HUANG¹, JIANHUA YE² and LIN LU¹

¹State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, Guangdong 510060; ²Department of Endocrine, College of Clinical Medicine, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong 510080; ³Guangdong Laboratory Animals Monitoring Institute, Key Laboratory of Guangdong Laboratory Animals, Guangzhou, Guangdong 510640; ⁴Department of Toxicology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong 510515, P.R. China; ⁵Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami, FL 33136, USA

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Abstract. Best vitelliform macular dystrophy (BVMD) is a hereditary retinal disease characterized by the bilateral accumulation of large egg yolk-like lesions in the sub-retinal and sub-retinal pigment epithelium spaces. Macular degeneration in BVMD can begin in childhood or adulthood. The variation in the age of onset is not clearly understood. The present study characterized the clinical characteristics of two Chinese patients with either juvenile-onset BVMD or adult-onset BVMD and investigated the underlying genetic variations. A 16-year-old male (Patient 1) was diagnosed with juvenile-onset BVMD and a 43-year-old female (Patient 2) was diagnosed with adult-onset BVMD. Comprehensive ophthalmic examinations were performed, including best-corrected visual acuity, intraocular pressure, slit-lamp examination, fundus photography, optical coherence tomography, fundus fluorescein angiography

imaging and Espion electrophysiology. Genomic DNA was extracted from peripheral blood leukocytes collected from these patients, their family members, and 200 unrelated subjects within in the same population. The 11 exons of the *bestrophin-1* (*BEST1*) gene were amplified by polymerase chain reaction and directly sequenced. Both patients presented lesions in the macular area. In Patient 1, a heterozygous mutation c.903T>G (p.D301E) in exon 8 of the *BEST1* gene was identified. This mutation was not present in any of the unaffected family members or the normal controls. Polymorphism phenotyping and the sorting intolerant from tolerant algorithm predicted that the amino acid substitution D301E in bestrophin-1 protein was damaging. In Patient 2, a single nucleotide polymorphism c.1608C>T (p.T536T) in exon 10 of the *BEST1* gene was identified. These findings expand the spectrum of *BEST1* genetic variation and will be valuable for genetic counseling and the development of therapeutic interventions for patients with BVMD.

Correspondence to: Dr Jianhua Ye, Department of Endocrine, College of Clinical Medicine, The First Affiliated Hospital of Guangdong Pharmaceutical University, 19 Nonglinxia Road, Guangzhou, Guangdong 510080, P.R. China
E-mail: jhyee2016@sina.com

Dr Lin Lu, State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 South Xianlie Road, Guangzhou, Guangdong 510060, P.R. China
E-mail: drlulinocular@126.com

*Contributed equally

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Introduction

Best vitelliform macular dystrophy (BVMD), also known as Best disease, is a hereditary retinal disease characterized by the bilateral accumulation of large egg yolk-like lesions in the sub-retinal and sub-retinal pigment epithelium (RPE) spaces (1,2). This lesion may eventually break up and spread throughout the macular area, leading to central vision reduction and retinal detachment (3). The clinical manifestation of BVMD varies substantially in different disease stages and among individual patients (4). For example, retinal lesions in BVMD typically occur bilaterally and symmetrically; however, rarely, patients can exhibit unilateral maculopathy (5,6). Patients with atypical BVMD presentation can have multifocal macular and extramacular involvement, including

retinitis pigmentosa, microcornea, retinal dystrophy, cataract, and posterior staphyloma syndrome (6-13). Macular degeneration in BVMD can begin from childhood or adulthood, and is classified as juvenile-onset BVMD or adult-onset BVMD, respectively (14). The variation in the age of onset is not clearly understood, and few studies have compared the differences between these two classifications (13,15). Currently, there is no effective treatment for BVMD.

BVMD is linked to the 15 kb *bestrophin-1* (*BEST1*) gene, located on chromosome 11q12-q13. The *BEST1* gene contains 11 exons, of which 10 are protein-coding (1). More than 250 disease-causing mutations in *BEST1* have been reported to be associated with BVMD (16,17). The 68 kDa bestrophin-1 protein, encoded by *BEST1*, is localized to the basolateral plasma membrane of RPE cells and contains several domains with a high degree of evolutionary conservation (18). The function of bestrophin-1 remains unclear. It is proposed to act as a chloride channel, activated by intracellular Ca^{2+} and/or as a channel regulator (19). It may regulate both the transepithelial electrical properties and the Ca^{2+} signaling of RPE (20). RPE cells with mutant *BEST1* are unable to effectively metabolize photoreceptor outer segments, leading to the anomalous accumulation of lipofuscin (4). Bestrophin-1 also promotes epithelial-to-mesenchymal transition of renal collecting duct cells under the regulation of transforming growth factor $\beta 1$, which has a critical role during cell growth and differentiation (21-26). Furthermore, under pathological conditions such as neuroinflammation and neurodegeneration, bestrophin-1 can redistribute in reactive astrocytes from perisynaptic microdomains to the soma, promoting the release of γ -aminobutyric acid (27). Identification of the genetic variations in BVMD may improve the understanding of the etiology and pathogenesis of the disease, and provide cues for the development of potential therapeutic interventions.

This study aimed to characterize the clinical manifestations and investigate the underlying genetic variations of a 16-year-old male and 43-year-old female with juvenile-onset and adult-onset BVMD, respectively.

Patients and methods

Study subjects and clinical ophthalmic examinations. A 16-year-old male with juvenile-onset BVMD (Patient 1) and a 43-year-old female with adult-onset BVMD (Patient 2), both from southern China, were diagnosed at Zhongshan Ophthalmic Center (Guangzhou, P.R. China). Visual acuity was examined using the Early Treatment Diabetic Retinopathy Study chart (Precision Vision, La Salle, IL, USA) (28). Images of the anterior segment were captured using a BX 900 Slit Lamp (Haag-Streit, Bern, Switzerland). Measurements of the anterior segment were recorded with Pentacam HR version 70700 (Oculus VR, LLC, Wetzlar, Germany). Optical Coherence Tomography (OCT) was performed by Cirrus HD-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Fundus photography and fundus fluorescein angiography (FFA) imaging was performed using a Heidelberg Retina Angiograph (Heidelberg Engineering, Heidelberg, Germany). Multifocal electroretinography (mfERG) was performed to assess the amplitudes of the rod and cone responses using the Espion electrophysiology system (Diagnosys LLC, Littleton,

MA, USA). Physical examinations were performed to exclude systemic diseases.

Sample collection and mutation screening. Venous blood samples were collected from the study subjects, their family members and 200 subjects without BVMD within the same population (15-48 years old; sex ratio: Male/female=108/92). Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. Briefly, a total amount of 1 ml of blood sample was collected from each subject, lysed by red blood cell lysis buffer (Sigma Aldrich, Merck KGaA, Darmstadt, Germany), and centrifuged at $2,000 \times g$ for 5 min at room temperature. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (Qiagen GmbH, Hilden, Germany) (29,30). Exons of the *BEST1* gene were amplified by polymerase chain reaction (PCR) with primers as previously described (31-34). The primer sequences are listed in Table I. PCR was conducted in a 50 μl reaction system, using the PCR amplification kit (Takara Bio, Inc., Otsu, Japan). The amplification included a single 5 min step at 94°C , followed by 40 cycles of 94°C for 45 sec, $58-61^{\circ}\text{C}$ for 45 sec, 72°C for 45 sec and a final 10 min step at 72°C . The PCR products were sequenced from both directions with an ABI3730 Automated Sequencer (PE Biosystems Inc., Foster City, CA). Sequenced products were analyzed using Seqman (version 2.3; Technelysium Pty Ltd., Brisbane, Australia), and compared with reference sequences in the database at the National Center for Biotechnology Information (NC_000011.10).

To analyze the effect of missense variants, polymorphism phenotyping (PolyPhen) and the sorting intolerant from tolerant (SIFT) algorithms were used to predict the possible impact of an amino acid substitution on the protein structure and function, using straightforward physical and comparative considerations (35-39). Variants were considered to be pathogenic when at least one of the two programs predicted a deleterious effect of the amino acid substitution on the protein structure and function. The Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/index.php>) was used to screen mutations reported in published studies. HomoloGene (<https://www.ncbi.nlm.nih.gov/homologene>) was used to check whether the mutated amino acid residues were conserved across different species.

Ethics. All experimental protocols and the methods were carried out in accordance with the guidelines approved by the ethics committee of Zhongshan Ophthalmic Center of Sun Yat-sen University (Guangzhou, P.R. China). Written informed consent was obtained from each subject in accordance with The Declaration of Helsinki. All participants provided informed consent for the publication of their data, including images and examination results.

Results

Clinical findings. Patient 1 had no known familial history of ocular disease. Refractive error was +4.50 diopter sphere (DS) in both eyes, with best corrected visual acuity (BVCA) at 1.0 in the right eye and 0.1 in the left eye. The cornea and the lens were transparent. Fundus examination revealed that the right eye had prominent yellow-white sub-retinal scarring with

Table I. Summary of the primers and products length used for the amplification of the exons of *bestrophin-1*.

Exon	Forward (5'-3')	Reverse (5'-3')	Product size (bp)	Annealing temperature (°C)
2	AGTCTCAGCCATCTCCTCGC	TGGCCTGTCTGGAGCCTG	212	61
3	GGGACAGTCTCAGCC ATCTC	CAGCTCCTCGTGATCCTCC	238	58
4	AGAAAGCTGGAGGAGCCG	GCGGCAGCCCTGTCTGTAC	1408	59
5	GGGGCAGGTGGTGTTCAGA	GGCAGCCTCACCAGCCTAG	150	59
6	GGGCAGGTGGTGTTCAGA	CCTTGGTCCTTCTAGCCTCAG	181	59
7	CATCCTGATTTCAAGGTTCC	CTCTGGCCATGCCTCCAG	257	59
8	AGCTGAGGTTTAAAGGGGGA	TCTCTTTGGGTCCACTTTGG	215	59
9	ACATACAAGGTCCTGCCTGG	GCATTAAGTAGTGCTATTCTAAGTTCC	298	59
10A	GGTGTTGGTCCTTTGTCCAC	CTCTGGCATATCCGTCAGGT	591	59
10B	CTTCAAGTCTGCCCCACTGT	TAGGCTCAGAGCAAGGGAAG	457	59
11	CATTTTGGTATTTGAAATGAAGG	CCATTTGATTCAGGCTGTTG	216	59

Bp, base pairs.

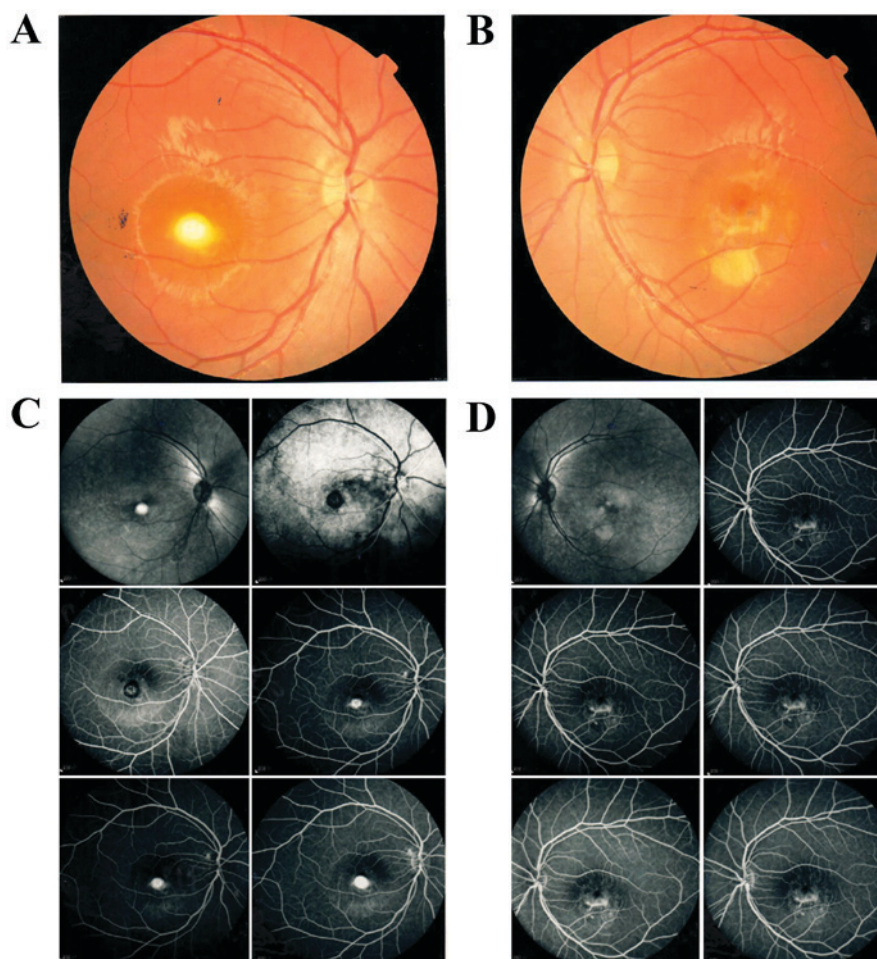


Figure 1. Fundus examination and FFA of Patient 1. (A and B) Fundus examination revealed the right eye had prominent yellow-white sub-retinal scarring with pigmented borders, surrounded by a serous retinal detachment. The left eye had fragmented vitelliform lesions. (C and D) FFA identified slight hyperfluorescence with mild leakage in the fovea of the macula in both eyes. FFA, fundus fluorescein angiography. (A and C) right eye; (B and D) left eye.

pigmented borders, surrounded by a serous retinal detachment. The left eye exhibited fragmented vitelliform lesions (Fig. 1A and B). FFA revealed a mild hyperfluorescence

with moderate leakage in the fovea of the macula in both eyes (Fig. 1C and D). OCT scans revealed that the foveal region in both eyes was abnormally thick, due to neuroretinal

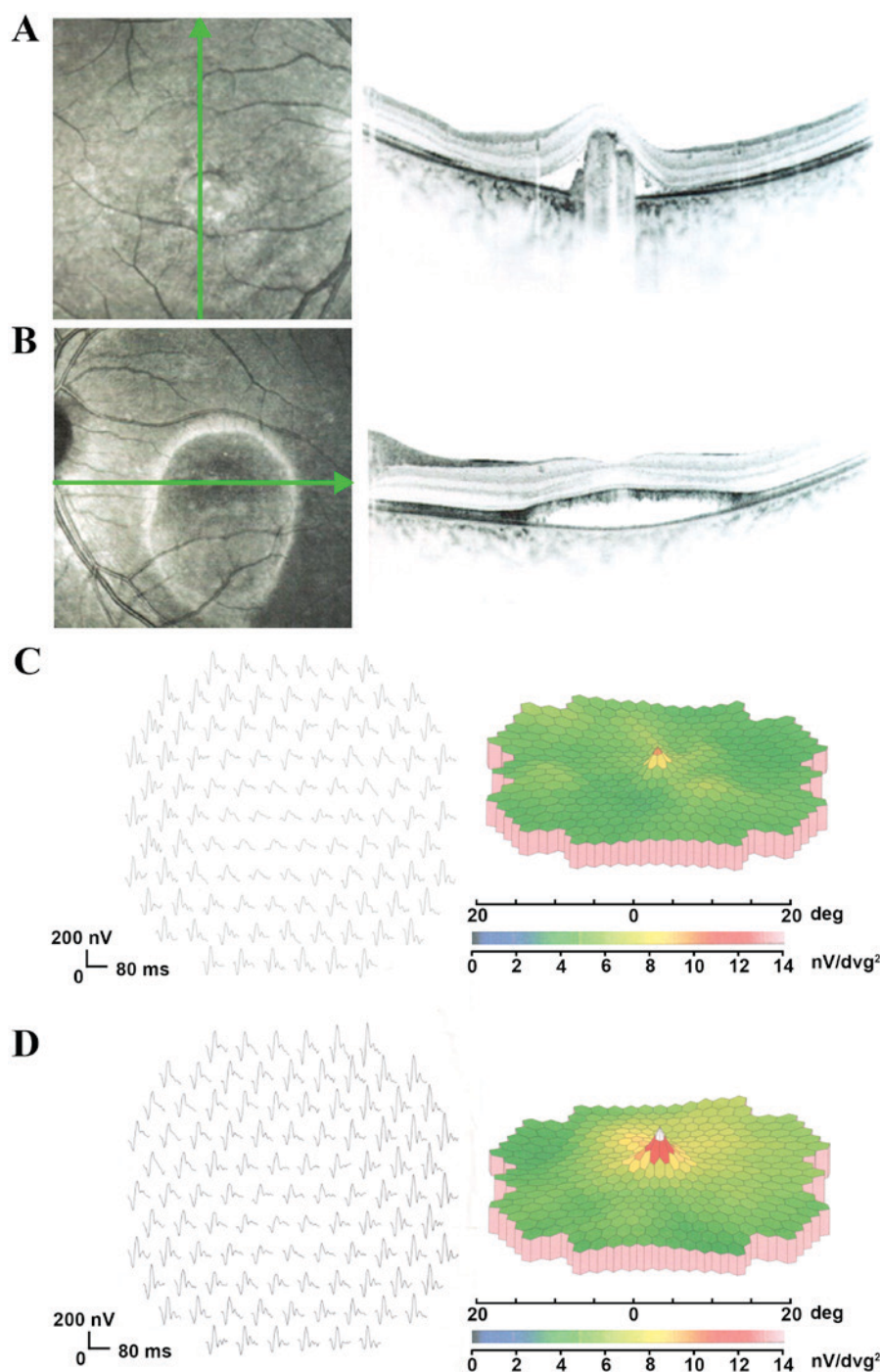


Figure 2. OCT scans and mfERG of Patient 1. (A and B) OCT scans revealed that the foveal region of the both eyes were abnormally thick due to neuroretinal detachment from the retinal pigment epithelium. (C and D) mfERGs (response of the posterior fundus) identified a mild decrease in the amplitude of the foveal response for each eye, while the peripheral mfERG amplitudes were within normal limits. OCT, optical coherence tomography; mfERG, multifocal electroretinography. (A and C) right eye; (B and D) left eye.

detachment from the RPE. In the right eye, the neuroretinal detachment was likely triggered by the abnormal accumulation of hyperreflective materials beneath the retina (Fig. 2A), whereas in the left eye, it was likely due to the accumulation of sub-retinal fluid (Fig. 2B). mfERGs (response of the posterior fundus) revealed a mild decrease in the amplitude of the foveal response in both eyes, although the peripheral mfERG amplitudes were within normal limits (Fig. 2C and D).

Patient 2 had myopia and their decline in vision had occurred over the last 3 years. Refractive error was -10.0 DS

in the right eye and -9.0 DS in the left eye. The BVCA was 0.4 in the right eye and counting fingers at 50 cm away from the left eye. The cornea and the lens were transparent. Fundus examination revealed atrophic lesions in both eyes (Fig. 3A and B). FFA revealed significant early hyperfluorescence that had increased intensity at the late stage of the angiographic sequence, with mild leakage in the right eye (Fig. 3C). The macular lesions exhibited a dystrophic pattern in the left eye (Fig. 3D). OCT revealed that the foveal regions in both eyes were abnormally thin due to atrophy of the retina and RPE

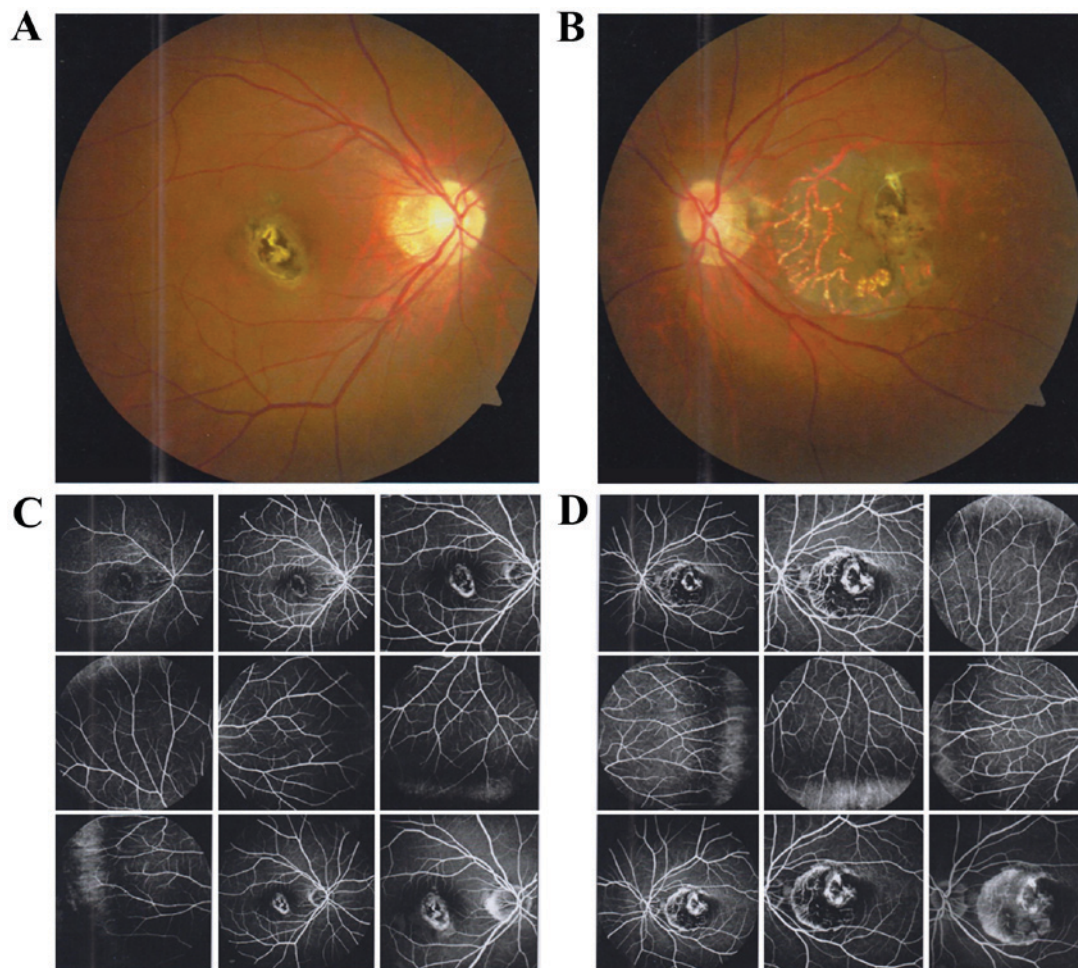


Figure 3. Fundus examination and FFA of Patient 2. (A and B) Fundus examination identified atrophic lesions in both eyes. (C and D) FFA revealed significant early hyperfluorescence with increased intensity at the late stage of the angiographic sequence. FFA, fundus fluorescein angiography. (A and C) right eye; (B and D) left eye.

(Fig. 4A and B). Similar to Patient 1, mfERGs of Patient 2 revealed a significant decrease in the amplitude of the foveal response in both eyes, although most of the peripheral mfERG amplitudes were within normal limits (Fig. 4C and D).

Mutation screening and bioinformatics analysis. A heterozygous mutation c.903T>G (p.D301E) in exon 8 of the *BEST1* gene was identified in Patient 1, but not in the unaffected family members or the normal controls in the same population. A c.1608C>T (p.T536T) single nucleotide polymorphism (SNP) in exon 10 of the *BEST1* gene was identified in Patient 2 (Fig. 5A). Multiple sequence alignment performed using the HomoloGene database indicated that the residues at position 301 and 536 of bestrophin-1 are highly conserved (Fig. 5B). PolyPhen and SIFT predicted that the D301E amino acid substitution in bestrophin-1 was potentially damaging (Fig. 5C). SIFT predicted that the T536T substitution is tolerated.

Discussion

BVMD is a rare retinal disease with highly variable phenotypic expression (4). In the early stages of BVMD, patients are generally asymptomatic. As the disease progresses, slow

and progressive vision loss occurs. In this study, both patients presented typical BVMD macular phenotypes. However, several extramacular lesions were also present. Hyperopia greater than +3.00 diopters is a recognized feature of juvenile-onset BVMD (31,40). In this study, Patient 1 exhibited hyperopia, whereas Patient 2 exhibited myopia. Other anterior segment abnormalities, including shallow anterior chambers, closed or narrow anterior chamber angles and angle-closure glaucoma may also be present in BVMD (41).

Adult-onset BVMD was first described by Gass in 1974 (42). Compared with juvenile-onset BVMD, adult-onset BVMD is less well-characterized (43). It usually occurs between 30-50 years of age, and is often associated with bilateral macular dystrophy and sub-retinal oval or round yellowish deposition in the macula (44). A definite diagnosis of adult-onset BVMD is often difficult. It is considered as a subtype of pattern dystrophy (PD) (43). Atypical presentations including multifocal vitelliform lesions and the occurrence of choroidal neovascularization (CNV) in various disease stages may confound diagnosis (11,12,45,46). Adult-onset BVMD can also be accompanied by branched retinal vein occlusion (47). Patient 2 was diagnosed with CNV in 2014, and her visual acuity improved following treatment with ranibizumab injection. Although this patient presented

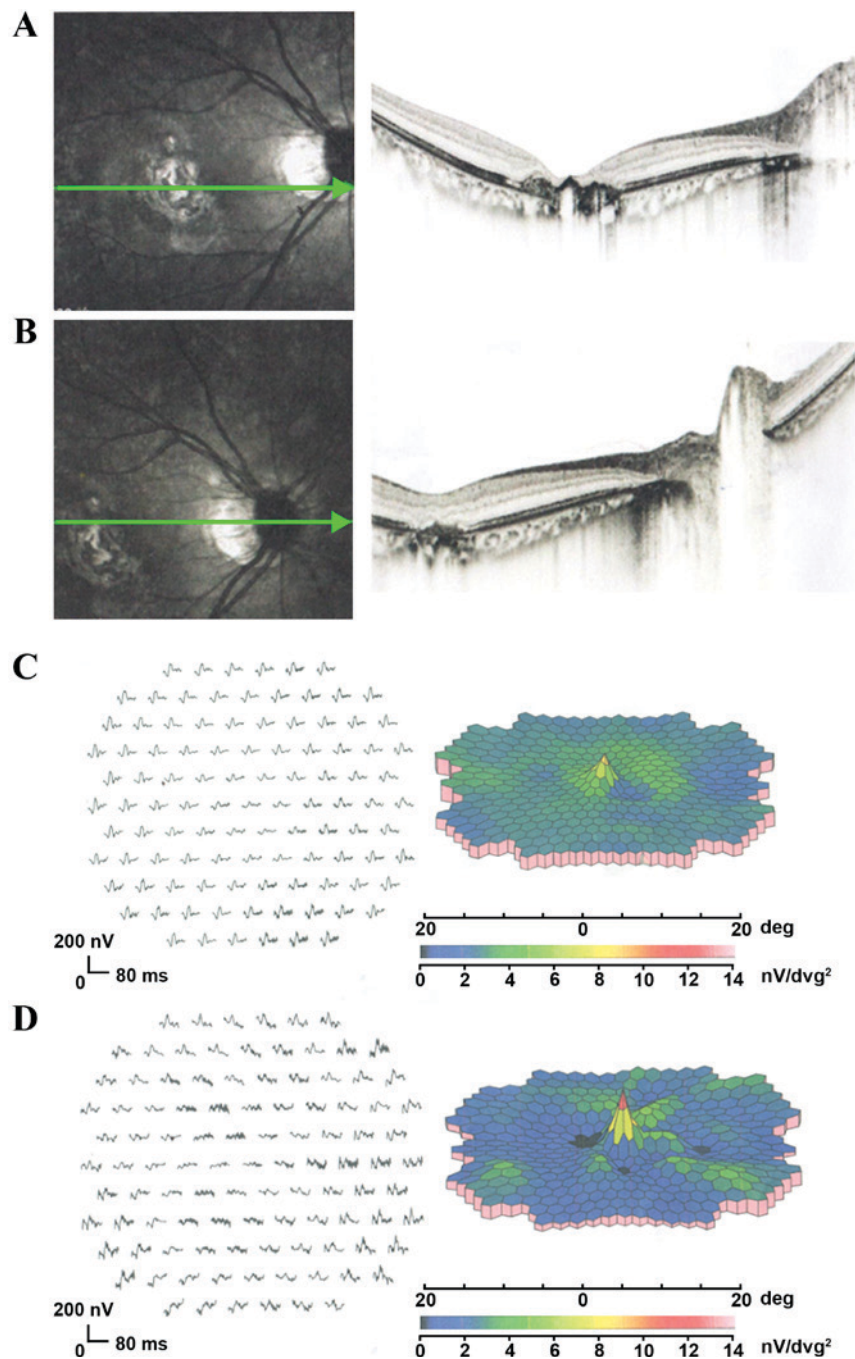


Figure 4. OCT scans and mfERG of Patient 2. (A and B) OCT revealed that the foveal region of both eyes was abnormally thin due to atrophy of retina and the RPE. (C and D) mfERGs identified a significant decrease in the amplitude of the foveal response for each eye, while most of the peripheral mfERG amplitudes were within normal limits. OCT, optical coherence tomography; mfERG, multifocal electroretinography. (A and C) right eye; (B and D) left eye.

typical PD, genetic examination was helpful to confirm the diagnosis following the exclusion of other causes of macular atrophy.

BEST1 gene mutations and polymorphisms have been reported in diverse ethnic groups (15,48-50). In this study, one recurrent mutation, c.903T>G (p.D301E) in exon 8, and one SNP, c.1608C>T (p.T536T), in exon 10 were reported. Caldwell *et al* (48) and Allikmets *et al* (49) also identified several other SNPs in the *BEST1* gene of patients with BVMD, including Y29Y, L37L, A10A, I73I, R218R, P341P, T470T and S519S. As the cellular function of bestrophin-1 protein is not fully elucidated, it is challenging to predict which mutations

are pathological and which are benign. A recent study using patient-derived RPE cells demonstrated that a V235A mutation can lead to the mislocalization of bestrophin-1 from the basolateral membrane to the apical membrane and potentially disrupt the polarized conductance (51). Furthermore, the R218H, L234P and A243T mutations result in defective calcium-activated chloride ion export (52). The D301E mutation identified in this study is located at the C terminus of bestrophin-1. The amino acids between position 292-312 are frequently mutated (Fig. 6). Mutations may disrupt the interaction between the N and C termini, which subsequently alters chloride channel function (53).

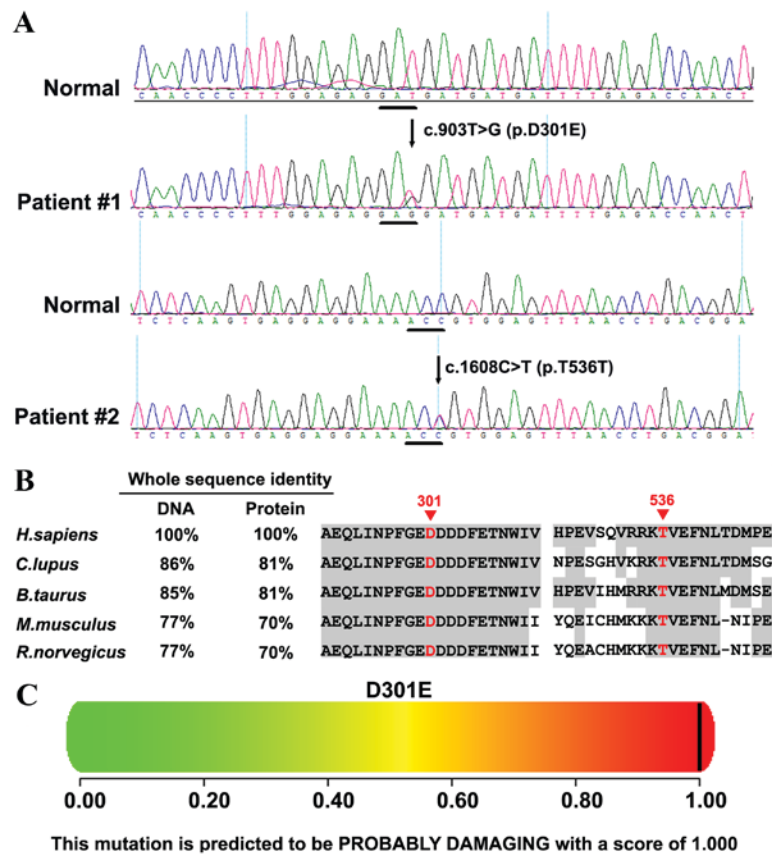


Figure 5. Identification of one recurrent mutation in Patient 1 and one SNP in Patient 2. (A) A heterozygous mutation c.903T>G (p.D301E) in exon 8 of the *BEST1* gene was identified in Patient 1, but not in unaffected family members or the normal controls in the same population. A c.1608C>T (p.T536T) SNP in exon 10 of the *BEST1* gene was identified in Patient 2. (B) Multiple-sequence alignment of the *BEST1* genes and bestrophin-1 proteins from different species. The red triangles indicate the position of the mutation and the SNP. The amino acid residues at these two positions are conserved across species. (C) Polyphen predicted that the amino acid substitution D301E in the protein bestrophin-1 is probably damaging. SNP, single nucleotide polymorphism, *BEST1*, *bestrophin-1* gene.

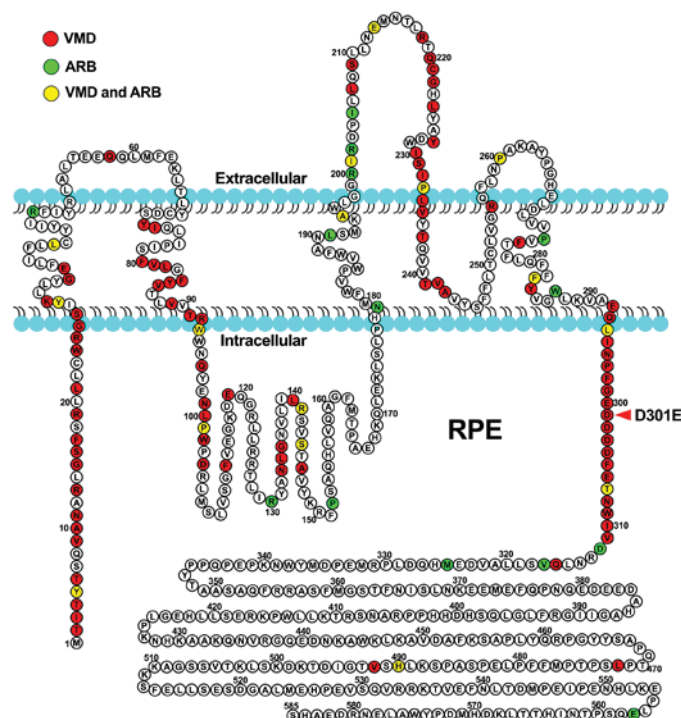


Figure 6. Schematic diagram of the location of identified bestrophin-1 protein mutations. The topology of human bestrophin-1 was drawn according to the model by Tsunenari *et al* (54,55). Red circles indicate mutated amino acids identified in VMD. Green circles indicate mutated amino acids identified in ARB. Yellow circles indicate mutated amino acids identified in both VMD and ARB. Data acquired from UniProt (www.uniprot.org) and <http://www.retina-international.org/mutations-best1-gene>. VMD, vitelliform macular dystrophy; ARB, autosomal recessive bestrophinopathy; RPE, retinal pigmented epithelium.

In conclusion, two recurrent genetic variations of *BEST1* in two Chinese patients with either juvenile-onset BVMD or adult-onset BVMD were identified in this study. These findings expand the mutation spectrum of *BEST1* and may aid in genetic counseling as well as prenatal diagnoses of patients with BVMD. Additionally, characterization of these genetic variations in BVMD provides a basis for future investigation of the underlying disease pathogenesis and the development of therapeutic interventions.

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