# The Clinical Features and Genetic Spectrum of a Large Cohort of Chinese Patients With Vitelliform Macular Dystrophies

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• PURPOSE: To provide the clinical and genetic characteristics of a large cohort of Chinese patients with vitelliform macular dystrophies.
• DESIGN: Cross-sectional study.
• METHODS: One hundred and thirty-four unrelated Chinese patients diagnosed with Best vitelliform macular dystrophy (BVMD), autosomal recessive bestrophinopathy (ARB), or adult vitelliform macular dystrophy (AVMD) were enrolled. Detailed ophthalmic examinations and genetic testing on vitelliform macular dystrophy–related genes were performed. Genotype and phenotype association were analyzed among different diagnostic groups.
• RESULTS: In total, 87 BVMD, 30 AVMD, and 17 ARB patients were enrolled in this study. Genetic analysis identified 37 *BEST1* mutations in 53 patients with BVMD and ARB. Of these, 5 variants (c.254A>C, c.291C>G, c.722C>G, c.848_850del, c.1740-2A>C) were novel. The variant c.898G>A was a hotspot mutation, which was identified in 13 patients with BVMD and 1 patient with ARB. There were significant differences of ocular biometric parameters among patients with homozygous or compound heterozygous mutations, heterozygous mutations, and those without mutations of *BEST1*. Homozygous or compound heterozygous patients had shortest axial length (AL), shallowest anterior chamber depth (ACD), and highest intraocular pressure (IOP); patients without mutations had longest AL, deepest ACD, and lowest IOP; and heterozygous patients were in between. Moreover, 7 patients harboring heterozygous mutations in *BEST1* and 3 patients without *BEST1* mutations showed similar clinical appearance to ARB in our cohort.
• CONCLUSIONS: This is the largest sample size study of Chinese vitelliform macular dystrophy patients. Our results indicated that assessment of angle-closure risk is a necessary consideration for all types of *BEST1*-related vitelliform macular dystrophies. The study expanded both the clinical and genetic findings of 3 common types of vitelliform macular dystrophies in a Chinese population. (Am J Ophthalmol 2020;216:69–79. © 2020 Elsevier Inc. All rights reserved.)

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MUTATIONS IN THE *BEST1* GENE ARE ASSOCIATED with a group of clinical phenotypes known as vitelliform macular dystrophies, with Best vitelliform macular dystrophy (BVMD) being the first described and the most common phenotype.[1] Autosomal recessive bestrophinopathy (ARB)[2] and adult vitelliform macular dystrophy (AVMD)[3] are another 2 major types of the phenotypic spectrum. BVMD (OMIM 153700), also known as Best disease, initially described in 1905 by Friedrich Best,[4] is by far the second most common cause of inherited maculopathy.[5] It is ophthalmoscopically characterized by an elevated macular lesion filled with large deposits of lipofuscin-like material, which creates a yellowish lesion resembling an egg yolk. BVMD was first linked to the *BEST1* gene in 1998,[6] and the majority of BVMD is inherited in an autosomal dominant pattern with variable penetrance. Like BVMD, AVMD (OMIM 608161) can also be characterized by autosomal dominant inheritance and the appearance of vitelliform lesions in the fovea usually one-third to 1 disc diameter in size, which is typically smaller than that of BVMD.[7] AVMD is less frequent than BVMD, and represents a late-onset form with a mean age in the fifth decade. Another distinct *BEST1*-associated retinal disorder, ARB (OMIM 611809), was first described by Burgess and associates in 2008[8] and is inherited as an autosomal recessive pattern with either homozygous or compound heterozygous mutations of the *BEST1* gene. ARB typically involves extramacular subretinal vitelliform deposits and an accumulation of fluid within and/or beneath the retina in the macula, with severe reduction or absence of electro-oculogram (EOG) light rise.

Except for macular lesions, patients with vitelliform macular dystrophies are often reported to have crowded

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**TABLE 1.** Demographic and Clinical Information of All Patients

| | BVMD | ARB/ARB-like | AVMD |
|---|---|---|---|
| Patients | 87 | 17 | 30 |
| Eyes | 174 | 34 | 60 |
| Age of diagnosis (y) | 37.45 ± 17.53 | 32.82 ± 15.96 | 59.77 ± 8.34 |
| Age of onset (y) | 31.77 ± 13.86 | 25.82 ± 12.13 | 56.57 ± 8.45 |
| Sex (female:male) | 28:59 | 12:5 | 9:21 |
| EOG (Arden ratio) | 1.26 ± 0.17 | 1.12 ± 0.19 | 2.27 ± 0.07 |
| BCVA (logMAR) | 0.42 ± 0.39 | 0.59 ± 0.45 | 0.48 ± 0.39 |

ARB = autosomal recessive bestrophinopathy; AVMD = adult vitelliform macular dystrophy; BCVA = best-corrected visual acuity; BVMD = Best vitelliform macular dystrophy; EOG = electro-oculograms.

anterior segment. ARB was reported to be often accompanied by hyperopia and shallow anterior chamber. Approximately up to 50% of ARB patients suffer from angle-closure glaucoma (ACG).[2] However, few reports suggested that hyperopia and reduced axial length (AL) may also exist in BVMD patients,[9–11] and no studies were published showing the relationship of AVMD and ACG so far.

As the main causative gene of vitelliform macular dystrophies, *BEST1* has, to date, been identified in more than 300 pathologic mutations in the Human Gene Mutation Database (HGMD; professional version 2019.3),[12] which account for approximately 50% of BVMD patients and very few cases of AVMD patients.[13,14] However, so far only limited numbers of Chinese cases have been documented. Besides the *BEST1* gene, peripherin-2 (*PRPH2*) and interphotoreceptor matrix proteoglycan (*IMPG1* and *IMPG2*) have also been reported in AVMD and BVMD in western populations,[15–18] but no related data were available in Chinese patients as well.

In this study, we analyzed the spectrum of gene mutations in a large Chinese cohort with vitelliform macular dystrophies including different subtypes of BVMD, ARB, and AVMD. Genotype-phenotype correlations were analyzed; especially, the potential association between *BEST1* mutations and ocular biometric parameters were evaluated.

## METHODS

• **PATIENTS:** This cross-sectional study was approved by the Institutional Review Board of Eye and ENT Hospital of Fudan University, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Informed consents were obtained from all participants or their legal guardians.

One hundred and thirty-four independent patients diagnosed with vitelliform macular dystrophies, including BVMD, ARB, or AVMD, were recruited from April 2012 to March 2019. The diagnoses were made based on the clinical characteristics including fundus appearance, EOG, age of onset, and family history. All BVMD patients met the following criteria: juvenile-to-adult onset; macular vitelliform lesions showing vitelliform, vitelliruptive, pseudohypopyon, or atrophic and cicatricial changes of at least 1 eye; abnormal EOG with Arden ratio below 1.55; at least 1 family member had a typical vitelliform lesion.[19] ARB or ARB-like patients were characterized by bilateral multifocal vitelliform lesions presenting scattered lipofuscin deposits or flecks in the posterior pole with subretinal fluid or intraretinal cystoid edema, and reduced light rise in EOG.[8,20] In this group of patients, those with homozygous or compound heterozygous mutations were defined as ARB while those with single heterozygous mutations or without muations were defined as ARB-like. The clinical diagnosis criteria for AVMD were as follows: age of onset older than 45 years; a vitelliform foveal lesion between the ellipsoid layer and the retinal pigment epithelium at various stages of at least 1 eye; and normal or slightly subnormal EOG findings.[3,21]

**TABLE 2.** Numbers of Patients With Specific Phenotypes of Vitelliform Macular Dystrophies and Genotypes of *BEST1* Mutations

| | *BEST1*(+/+) | *BEST1*(+/-) | *BEST1*(-/-) |
|---|---|---|---|
| BVMD | 0 | 39 | 48 |
| ARB/ARB-like | 7 | 7 | 3 |
| AVMD | 0 | 0 | 30 |

ARB = autosomal recessive bestrophinopathy; AVMD = adult vitelliform macular dystrophy; BVMD = Best vitelliform macular dystrophy.

• **CLINICAL EVALUATIONS:** Demographic information was recorded at the time of diagnosis, including age of onset, age of diagnosis, sex, and ethnicity. Detailed ophthalmic examinations were conducted on all subjects,

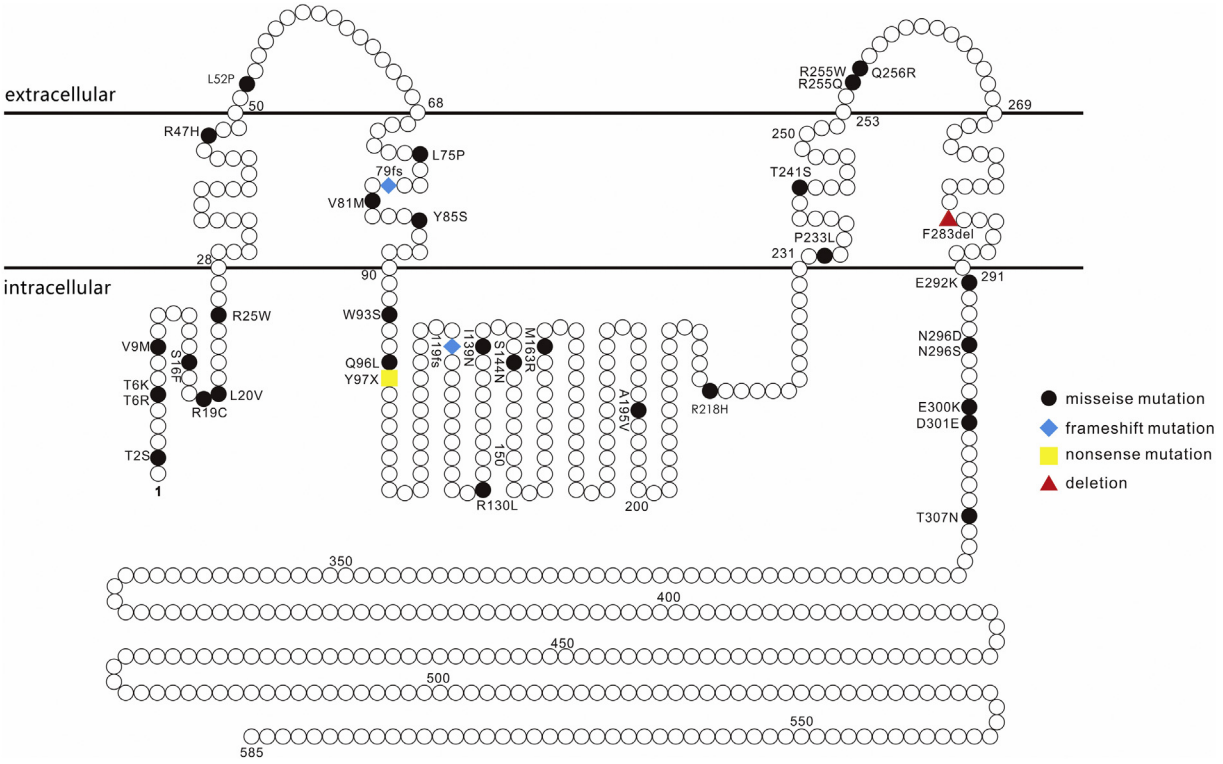


**FIGURE 1. Diagram of the mutations in *BEST1*. The diagram is a predicted structure of the bestropohin-1 gene with 585 amino acids.[1,22] The missense mutations are shown as black circles, the frameshift mutations are shown as blue diamonds, the nonsense mutation is shown as a yellow square, and the deletion is shown as a red triangle.**

including the best-corrected visual acuity (BCVA); EOG; slit-lamp biomicroscopy; fundus examination with color fundus photography (Topcon TRC50LX; Topcon, Tokyo, Japan); spectral-domain optical coherence tomography (SDOCT), fundus autofluorescence, fluorescein angiography, or indocyanine green angiography simultaneously by Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany); intraocular pressure (IOP) measured by Goldmann applanation tonometer; anterior chamber depth (ACD) and anterior chamber angles measured by ultrasound biomicroscopy; AL; white-to-white distance; lens thickness; and K1, K2 keratometry measured by Lenstar (LS900; Haag-Streit, Koniz, Switzerland).

• **GENETIC ANALYSIS:** Genomic DNA was extracted from peripheral blood of all patients with the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). Five hundred and two retinal disease–associated genes (including *BEST1*, *PRPH2*, *IMPG1*, and *IMPG2*) were sequenced in all patients by next-generation sequence using the Illumina HiSeq 2000 sequencing system (Illumina, Inc, San Diego, California, USA). The reference sequence of NM_004183.4 (*BEST1*) was used to detect variants, which were then annotated by ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), 1000 Genomes Project ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp (https://www.internationalgenome.org/), and Exome Variant Server (https://evs.gs.washington.edu/EVS/). Sanger sequencing was used for verification of the detected variants within the family members.

• **STATISTICAL ANALYSIS:** One-way analysis of variance was used to analyze the differences in the age, BCVA, and ocular parameters among different groups of patients with homozygous or compound heterozygous mutations, patients with heterozygous mutations, and those without mutations in *BEST1* gene. The $\chi 2$ test was used to calculate the significance in sex (male vs female) and lesion location (single lesion vs multiple lesions) among 3 groups. Statistical analysis was performed using SPSS statistical software (version 17; SPSS Inc, Chicago, Illinois, USA). *P* values of less than .05 were considered statistically significant.

## RESULTS

IN TOTAL, 87 PATIENTS WERE DIAGNOSED AS BVMD, 17 PAtients were diagnosed as ARB or ARB-like bestrophinopathy, and 30 patients were diagnosed as AVMD. The demographic and clinical information of all patients is summarized in Table 1. All 87 BVMD patients (174 eyes)

**TABLE 3.** Identified *BEST1* Mutations in Cohort of Patients With Vitelliform Macular Dystrophies

| Nucleotide Changes | Amino Acid Changes | Numbers Of Patients | Type Of Mutations | References |
|---|---|---|---|---|
| c.5C>G | p.Thr2Ser | 2 | Missense | 23 |
| c.17C>A | p.Thr6Lys | 1 | Missense | 24 |
| c.17C>G | p.Thr6Arg | 1 | Missense | 25 |
| c.25G>A | p.Val9Met | 1 | Missense | 6 |
| c.47C>T | p.Ser16Phe | 1 | Missense | 25,26 |
| c.55C>T | p.Arg19Cys | 1 | Missense | 25 |
| c.58C>G | p.Leu20Val | 1 | Missense | 27 |
| c.73C>T | p.Arg25Trp | 1 | Missense | 25,28 |
| c.140G>A | p.Arg47His | 3 | Missense | 25,29 |
| c.155T>C | p.Leu52Pro | 1 | Missense | 30 |
| c.224T>C | p.Leu75Pro | 1 | Missense | 23 |
| c.232_233insT | p.Ser79PhefsX153 | 1 | Frameshift mutation | 12 |
| c.241G>A | p.Val81Met | 1 | Missense | 31 |
| c.254A>C | p.Tyr85Ser | 1 | Missense | None (novel) |
| c.278G>C | p.Trp93Ser | 1 | Missense | 25 |
| c.287A>T | p.Gln96Leu | 1 | Missense | 12 |
| c.291C>G | p.Tyr97X | 1 | Nonsense mutation | None (novel) |
| c.346_355dup | p.Glu119Glyfs*116 | 1 | Frameshift mutation | 23 |
| c.389G>T | p.Arg130Leu | 1 | Missense | 14,32 |
| c.416T>A | p.Ile139Asn | 1 | Missense | 12 |
| c.431G>A | p.Ser144Asn | 2 | Missense | 33 |
| c.488T>G | p.Met163Arg | 1 | Missense | 14,25,32 |
| c.584C>T | p.Ala195Val | 1 | Missense | 24,34,35 |
| c.653G>A | p.Arg218His | 1 | Missense | 12 |
| c.698C>T | p.Pro233Leu | 1 | Missense | 24 |
| c.722C>G | p.Thr241Ser | 1 | Missense | None (novel) |
| c.763C>T | p.Arg255Trp | 2 | Missense | 25,30,32,33,36 |
| c.764G>A | p.Arg255Gln | 2 | Missense | 32,36 |
| c.767A>G | p.Gln256Arg | 1 | Missense | 12 |
| c.848_850del | p.Phe283del | 1 | Deletion | None (novel) |
| c.874G>A | p.Glu292Lys | 1 | Missense | 37 |
| c.886A>G | p.Asn296Asp | 1 | Missense | 38 |
| c.887A>G | p.Asn296Ser | 1 | Missense | 25–27 |
| c.898G>A | p.Glu300Lys | 14 | Missense | 23,29,39,40 |
| c.903T>G | p.Asp301Glu | 2 | Missense | 30,41 |
| c.920C>A | p.Thr307Asn | 1 | Missense | 10,23 |
| c.1740-2A>C | _ | 1 | Splice site | None (novel) |

showed macular lesions with different stages, including previtelliform lesions in 23 eyes, vitelliform lesions in 33 eyes, pseudohypopyon lesions in 11 eyes, vitelliruptive lesions in 71 eyes, and choroidal neovascularization/fibrotic lesions in 30 eyes. Besides, an atypical appearance of bilateral macular holes was found in 2 eyes, and additional extramacular vitelliform lesions were presented in 4 eyes. Among all BVMD patients, 2 patients underwent surgery for ACG. Seventeen ARB or ARB-like patients (34 eyes) displayed subretinal yellowish deposits or flecks in the macula as well as along the retinal vascular arcades with foveal cystoid macular edema and extensive serous subretinal fluid with an elongated photoreceptor outer segment layer. Fourteen of these ARB or ARB-like patients had bilateral ACG or diagnosis of angle-closure suspect, among whom 4 underwent trabeculectomy and 2 had laser peripheral iridotomy. All 30 AVMD patients (60 eyes) exhibited macular yellowish deposits, among whom 4 had additional extramacular lesions with various numbers and sizes. All these AVMD patients had normal AL, IOP, ACD, and open anterior chamber angles.

In this cohort of 134 patients with bestrophinopathy, *BEST1* mutations were found in 53 patients. Among BVMD patients, 39 patients carried heterozygous mutations with a mutation detection rate of 45% (39/87). Among ARB–ARB-like patients, 14 patients carried *BEST1* variants, including 5 with compound heterozygous mutations, 2 with homozygous mutations, and 7 with heterozygous mutations, with a mutation detection rate of 82% (14/17). And no mutations were detected in all of the 30 AVMD patients (Table 2). In total, 37 *BEST1* mutations were detected, including 32 missense mutations, 2

**TABLE 4.** Comparison of the Ocular Parameters and Clinical Features in Patients Grouped by *BEST1* Mutations

| Features | *BEST1*(+/+) | *BEST1*(+/-) | *BEST1*(-/-) | *P* Values |
|---|---|---|---|---|
| Patients | 7 | 46 | 81 | - |
| Eyes | 14 | 92 | 162 | - |
| Age of onset (y) | 27.43 ± 10.89 | 29.54 ± 13.79 | 41.35 ± 16.93 | .000 |
| Sex (female:male) | 5:2 | 18:28 | 26:55 | .106 |
| BCVA | 0.69 ± 0.41 | 0.47 ± 0.38 | 0.43 ± 0.40 | .067 |
| Lesion (isolated: multi, eyes) | 0:14 | 76:16 | 142:20 | .000 |
| AL (mm) | 21.59 ± 0.39 | 22.02 ± 0.98 | 23.10 ± 1.39 | .000 |
| ACD (mm) | 1.84 ± 0.25 | 2.43 ± 0.43 | 2.67 ± 0.43 | .000 |
| IOP (mm Hg) | 28.78 ± 11.73 | 17.05 ± 5.21 | 15.46 ± 4.80 | .000 |
| W to W (mm) | 11.63 ± 0.35 | 11.75 ± 0.52 | 11.82 ± 0.46 | .388 |
| K1 | 43.01 ± 1.80 | 43.45 ± 1.59 | 43.30 ± 1.47 | .646 |
| K2 | 43.94 ± 1.97 | 44.33 ± 1.99 | 44.14 ± 1.50 | .712 |
| LT (mm) | 4.31 ± 0.18 | 4.08 ± 0.49 | 4.13 ± 0.42 | .359 |

ACD = anterior chamber depth; AL = axial length; BCVA = best-corrected visual acuity; IOP = intraocular pressure; K1 = flat keratometry; K2 = steep keratometry; LT = lens thickness; W to W = white-to-white distance.
*P* values represent the overall differences among 3 groups.

frameshift mutations, 1 splicing mutation, 1 nonsense mutation and 1 deletion mutation (Figure 1, Table 3). Five of them—c.254A>C (p.Tyr85Ser), c.291C>G (p.Tyr97X), c.722C>G (p.Thr241Ser), c.848_850del (p.Phe283del), and c.1740-2A>C—were novel mutations, while the other 32 mutations had been previously reported. Interestingly, there was 1 mutation of c.898G>A (p.Glu300Lys) detected in 14 patients (13 were heterozygous and 1 was compound heterozygous). The second most frequent mutation was c.140G>A (p.Arg47His), which was detected in 3 patients in our cohort. Additionally, c.5C>G (p.Thr2Ser), c.431G>A (p.Ser144Asn), c.763C>T (p.Arg255Trp), c.764G>A (p.Arg255Gln), and c.903T>G (p.Asp301Glu) were detected in 2 patients, respectively. No mutations in *PRPH2*, *IMPG1*, and *IMPG2* genes were identified in any of our patients.

Furthermore, we analyzed the clinical characteristics, including ocular parameters, among different groups of patients with homozygous or compound heterozygous mutations, with heterozygous mutations, and without mutations in the *BEST1* gene. The comparison results showed there were statistically significant differences in AL, ACD, IOP, age of onset, and lesion locations among patients with homozygous or compound heterozygous mutations, heterozygous mutations, and those without identified mutations (all $P < .05$) (Table 4). Interestingly, patients with homozygous or compound heterozygous mutations had shortest AL, shallowest ACD, and highest IOP; and patients without mutations had longest AL, deepest ACD, and lowest IOP among the 3 groups. There was a trend observed that the anterior segment tends to be more crowded with more mutations in the *BEST1* gene.

Fourteen patients in our study carried the same mutation, c.898G>A (p.Glu300Lys), in the *BEST1* gene. Thirteen of them showed typical macular lesions from previtelliform to typical vitelliform changes and macular choroidal neovascularization/scarring, except that 1 patient had additional extramacular vitelliform lesions (Figure 2). Among them, focal choroidal excavation was found in 1 patient, ACG was found in 1 patient (Figure 3), and short AL was found in 2 patients in both eyes. Another patient with compound heterozygous mutations (p.Glu300Lys/p.Ala195Val) demonstrated bilateral multiple vitelliform lesions in the posterior pole of the retina and accumulation of subretinal and intraretinal fluid with narrow anterior chamber angle.

There were 7 patients harboring heterozygous mutations in *BEST1* (4 missense mutations: p.Arg47His, p.Tyr85Ser, p.Met163Arg, p.Leu52Pro; 2 frameshift mutations: p.Glu119Glyfs*116, p.Ser79PhefsX153; and 1 splicing mutation: c.1740-2A>C) and 3 patients without mutations in *BEST1* showing similar clinical appearance to autosomal recessive vitelliform in our cohort. They presented as bilateral multifocal hyperautofluorescent vitelliform deposits with shallow subretinal fluid throughout the posterior pole with intraretinal cysts in the macula and were defined as ARB-like phenotype (Figure 4). The mean age at onset was 20.63 ± 11.64 years (range 5-40 years), and the mean BCVA was 0.58 ± 0.48 (range 0.01-1.7). Among them, 2 patients underwent filtration surgery because of ACG and 1 patient accepted laser peripheral iridotomy for diagnosis of angle-closure suspect.

## DISCUSSION

IN THIS STUDY, WE PERFORMED GENETIC TESTING IN 134 UNrelated Chinese patients with BVMD, ARB, and AVMD,

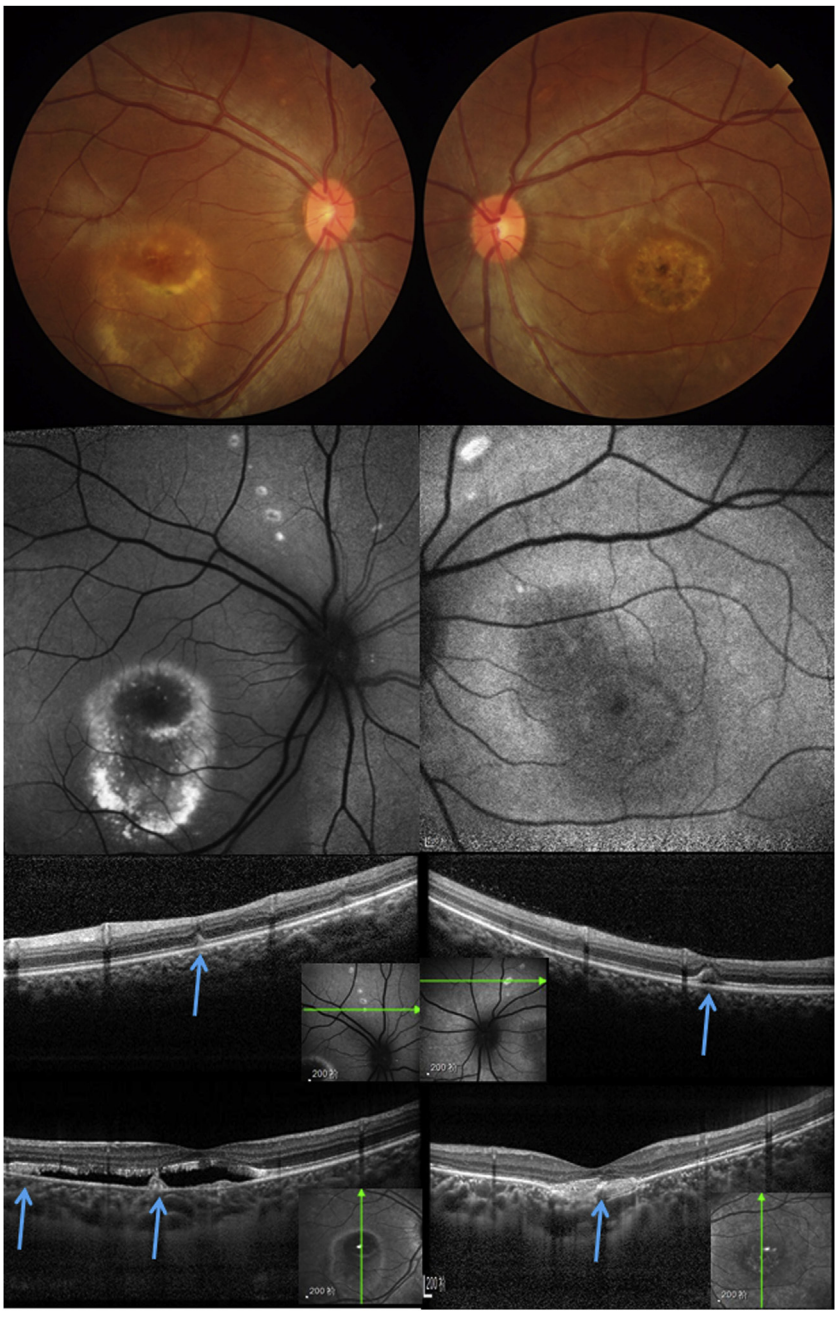

**FIGURE 2. Fundus images of a 25-year-old female patient harboring the *BEST1* mutation p.Glu300Lys. Top row. Fundus photographs showed vitelliruptive lesions in fovea with extramacular yellow deposits scattered adjacent to the temporal vascular arcades in both eyes. Middle row. Fundus autofluorescence images showed the macular and extramacular vitelliform lesions with focal hyperautofluorescence. Bottom row. Spectral-domain optical coherence tomography images showed subretinal fluid at the macula and diffused hyperreflective subretinal accumulations illustrated by blue arrows.**

and presented their clinical characteristics including ocular biometric parameters. Thirty-seven *BEST1* mutations were detected in 53 vitelliform macular dystrophy patients in our study. The mutation detection rate was 40% (53/134) in total, 45% in BVMD patients, 82% in ARB or ARB-like patients, and 0% in AVMD patients, which is in accordance with previous reports showing that the mutation rate was around 50%-86% in BVMD patients,[13,29,31,39,42] 100% in ARB patients,[2,36] and 0%-33% in AVMD patients.[29,43] The majority of *BEST1* mutations (32/37) in our patients were missense mutations, which was also consistent with previous studies.[1,12] The *BEST1* gene contains 11 exons and encodes the bestrophin-1 protein, consisting of 585 amino acids. Bestrophin-1 is primarily expressed in the

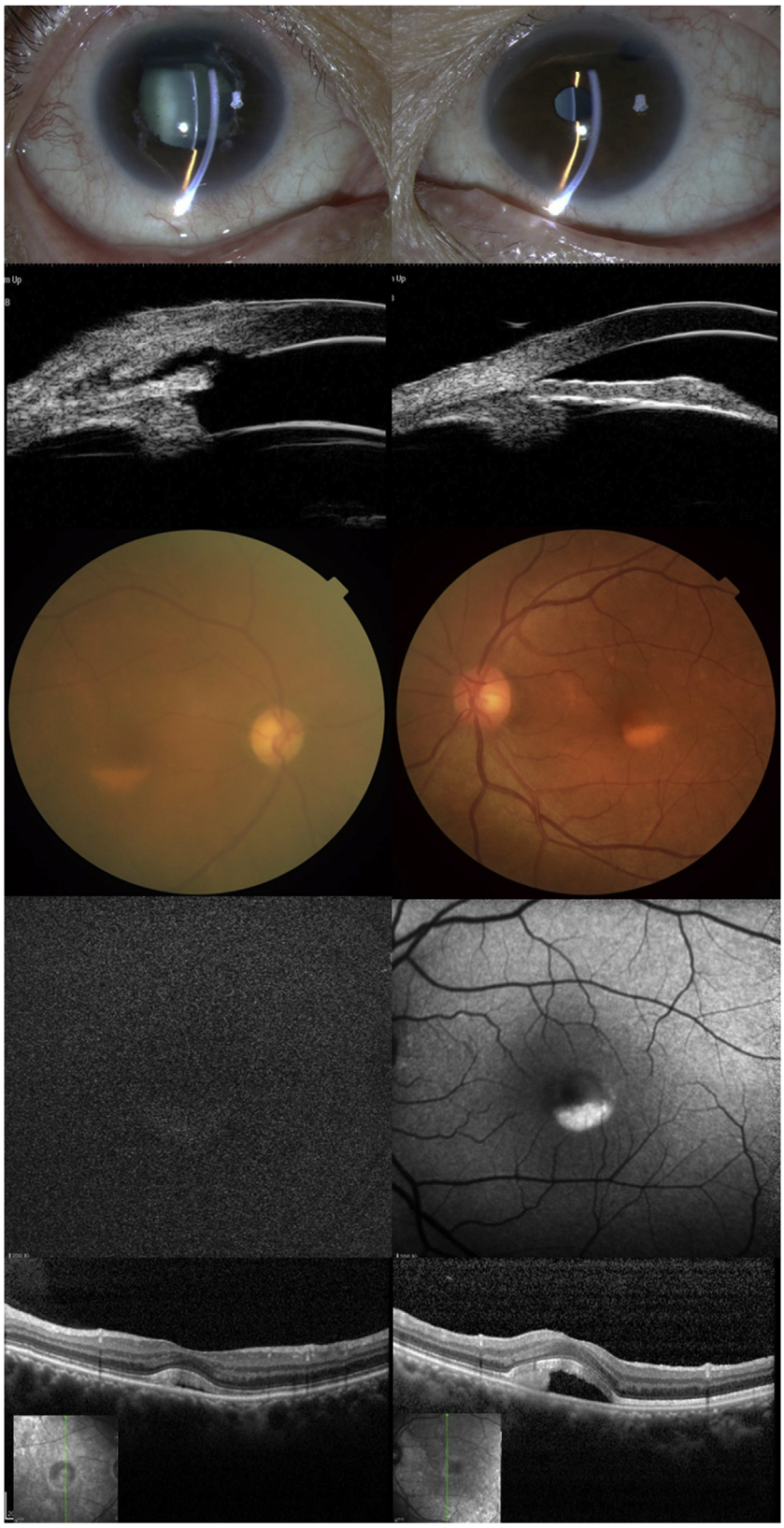

**FIGURE 3. Anterior segment and fundus images of a 58-year-old female patient harboring the *BEST1* mutation p.Glu300Lys. Top row. Color photograph of the anterior segment showed shallow anterior chamber in both eyes and moderately dilated pupil with iris atrophy of the right eye. Second row. Ultrasound biomicroscopy showed angle closure in both eyes and a filtering passage after**

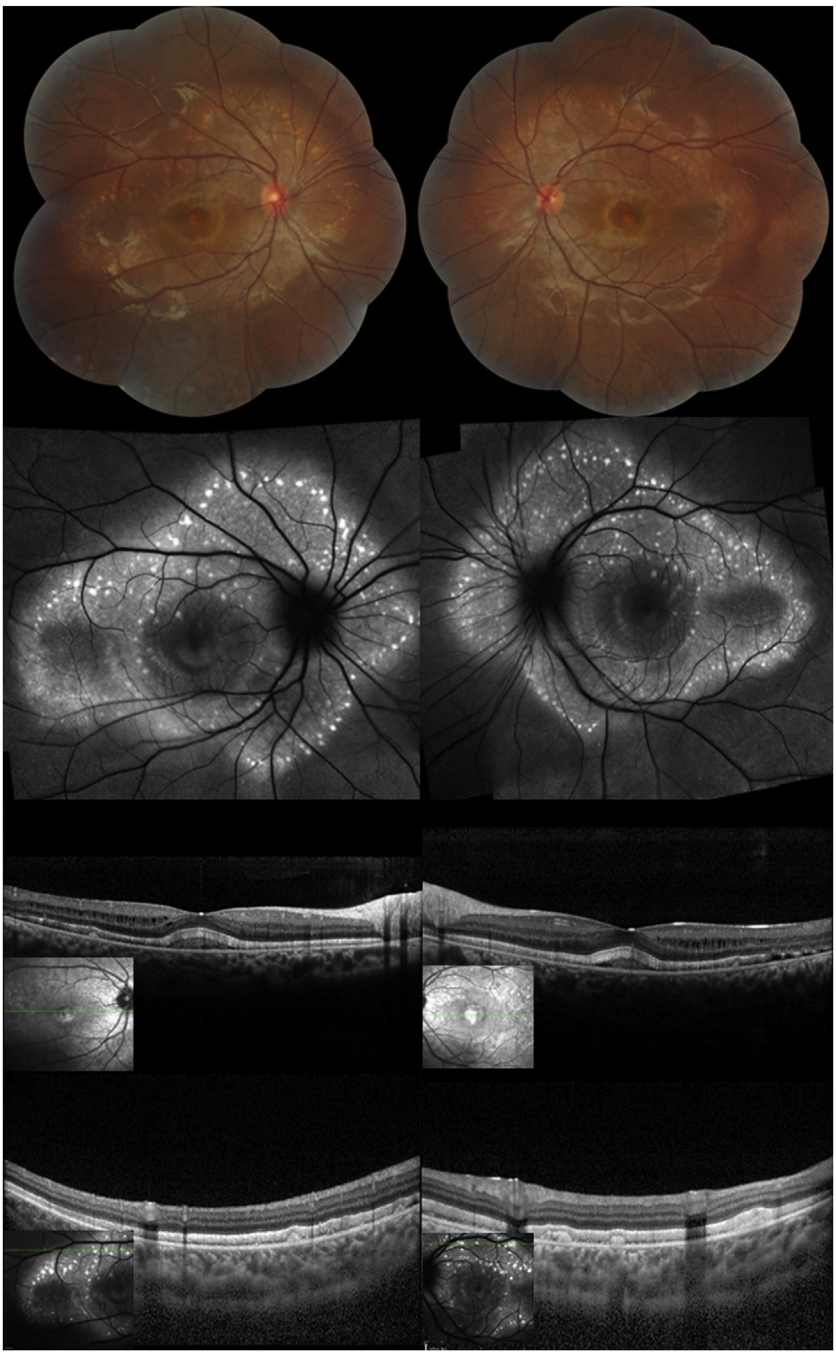

**FIGURE 4. Fundus images of an 11-year-old female patient harboring the *BEST1* mutation p.Glu119Glyfs*116. Top row. Fundus photographs showed numerous yellowish deposits throughout the posterior pole in both eyes. Middle row. Fundus autofluorescence showed corresponding hyperautofluorescence in both eyes. Bottom row. Spectral-domain optical coherence tomography revealed subretinal vitelliform lesions and intraretinal cysts in both eyes.**

basolateral plasma membrane of the retinal pigment epithelium (RPE), and is also presenting intracellularly.[1] As a transmembrane protein, it functions as a $Ca^{2+}$-activated $Cl^-$ and $HCO_3^-$ channel regulator of ion transport. Previous reports showed that missense mutations remarkably clustered in 4 amino acid sequence regions (6-30,

**trabeculectomy in the right eye. Third row. Fundus photographs showed pseudohypopyon-stage macular lesion in both eyes. Fourth row. Fundus autofluorescence showed the macular vitelliform lesion with hyperautofluorescence. Bottom row. Spectral-domain optical coherence tomography revealed the presence of hyperreflective subretinal accumulations with foveal fluid.**

80-104, 221-243, and 293-312) of bestrophin-1.[1] These regions were all located in or close to the RPE plasma membrane, illustrating that the transmembrane domain and their near regions were particularly important for the $Cl^-$ and $HCO_3^-$ channel function of bestrophin-1. Likewise, 4 of our novel heterozygous mutations (p.Tyr85Ser, p.Tyr97X, p.Thr241Ser, p.Phe283del) were all located in or near the RPE plasma membrane. These mutant bestrophin-1 proteins may exert deleterious influence on ion transport and ultimately participate in the pathogenesis of bestrophinopathy. Besides, there was 1 novel splicing mutation (c.1740-2A>C) found in our study. Although only 9 splicing mutations had been identified in the HGMD (professional version 2019.3), the splicing mutations were considered leading to incorrect messenger RNA and therefore no functional *BEST1* protein.[2,24,29,44–46] Moreover, *PRPH2*, *IMPG1*, and *IMPG2* genes have also been previously reported in AVMD and BVMD in western populations,[15–18] but no mutations in *PRPH2*, *IMPG1*, and *IMPG2* were identified in our patients, which might be related with the population differences. Overall, more than 50% of vitelliform macular dystrophy patients in our study were found not to have mutations in known causing genes. Besides novel candidate genes, promoters and other regulatory regions of these causing genes are worth looking at carefully in the future, especially for negative patients.

Interestingly, there was a hotspot mutation in our cohort. The most common mutation, p.Glu300Lys, which was identified in 13 patients with BVMD and 1 patient with ARB, accounted for 26.4% (14/53) out of the total patients with *BEST1* mutations in our study. The frequency of this hotspot mutation was not detected in the 1000 Genomes Project and Exome Variant Server, which excluded this mutation as a polymorphism in the Chinese population. Previous studies showed that different ethnic population with vitelliform macular dystrophies had variant hotspot mutations, including p.Glu300Lys in Chinese patients,[12] p.Asp302Asn in Danish patients,[27] and p.Arg25Trp in Italian patients.[28] Our study supported the diversity of the genotypes of vitelliform macular dystrophies in different races. Moreover, our study expanded the clinical phenotype of this hotspot, including multifocal extramacular vitelliform lesions, focal choroidal excavation, and ACG, which were not reported previously.[23,29] Thus, we demonstrated that the vitelliform macular dystrophies had heterogeneity in both genotype and phenotype. As were most of our novel mutations, this mutation was also located near the RPE plasma membrane, which may be highly deleterious to the normal structure of bestrophin-1, disturbing the ion transport and consequently leading to the dysfunction of RPE.[1]

Another interesting finding of our study was that the phenotypes of ARB occurred not only in patients with homozygous or compound heterozygous mutations, but also in patients with heterozygous mutations or even patients without *BEST1* mutations. In our study, 7 patients with heterozygous mutations (p.Arg47His, p.Tyr85Ser, p.Met163Arg, p.Leu52Pro, p.Glu119Glyfs*116, p.Ser79PhefsX153, c.1740-2A>C) and 3 patients without *BEST1* mutations showed ARB phenotype. In addition, the previously reported frameshift variant p.Glu119Glyfs*116 was detected in a 45-year-old male patient with bilateral vitelliruptive lesion of BVMD,[23] whereas our 11-year-old female patient carrying the same variant showed ARB-like fundus appearance. By far, the phenotype of ARB was reported as autosomal recessive inherited in most studies. Although our 10 patients do not meet the existing definition of ARB, their manifestations were the same as the phenotypes of ARB and thus were defined as ARB-like phenotype. The ARB-like phenomenon was first reported by Toto and associates,[20] showing 2 young patients with a phenotype resembling ARB caused by 1 heterozygous mutation of a p.I205T variant. Recently, Gattoussi and associates[47] also observed clinical findings like ARB associated with autosomal dominantly inherited mutation of p.F80I in 1 patient and her affected mother and male sibling. Our results indicated that 1 single heterozygous mutation can cause ARB-like phenotype and even *BEST1*-negative patients can have ARB-like phenotype as well. Based on these results, it seems ARB may be considered as a type of clinical phenotype of vitelliform macular dystrophies with a different inheritance model, rather than a type of vitelliform macular dystrophy only caused by homozygous or compound heterozygous mutations of the *BEST1* gene. This hypothesis was also supported by the study of Marmorstein and associates,[48] which established an iPSC model of ARB and illustrated that BVMD and ARB may share a similar etiology. Although the underlying mechanism remains unclear, our finding expands the complex clinical spectrum and heredity of *BEST1*-associated phenotypes, and suggests that the ARB could be redefined with clinical presentations rather than an inheritance model.

ARB has been reported to have high risk for ACG[2,8,36]; however, the relationship between autosomal dominant Best disease and ACG remains unclear. Our study was the first to determine the association between *BEST1* mutations and risk of ACG by analyzing a large vitelliform macular dystrophy cohort. We found patients with homozygous or compound heterozygous mutations had more crowded anterior segment than those with heterozygous mutations and the latter had more crowded anterior segment than those without identified mutations. The cause behind the occurrence of ACG in vitelliform macular dystrophies is yet to be elucidated. There is some evidence supporting that *BEST1* is involved both in ocular development and maintenance of the RPE cells and the photoreceptors in the retina postdevelopmentally, possibly through the transcription factors such as microphthalmia-associated (MITF), orthodenticle

homeobox 2 (OTX2), and cone-rod homeobox (CRX).[49,50] Both MITF and OTX2 are required for the differentiation of the RPE, photoreceptors, and bipolar cells and the development of the anterior segment in the eyes.[51,52] Our study indicates that there is strong association between the numbers of *BEST1* mutations and the crowdedness of the anterior segment. It seems homozygotes or compound heterozygotes carry higher risk for ACG than heterozygotes; and heterozygotes carry higher risk for ACG than patients without *BEST1* mutations. Given the significant risk of ACG, assessment of angle-closure risk is a necessary consideration in all types of *BEST1*-related disease.

In conclusion, we identified 5 novel mutations of the *BEST1* gene and revealed complete clinical phenotypes in a large cohort of Chinese patients with vitelliform macular dystrophies. We found the risk of ACG is increasing with the number of *BEST1* gene mutations and angle-closure risk exists in all types of *BEST1*-related vitelliform macular dystrophies. In addition, a clinical appearance like ARB can also be caused by a single heterozygous mutation in the *BEST1* gene and *BEST1*-negative patient as well. Our study expanded both the genotype and phenotype of vitelliform macular dystrophies in a Chinese population and enhanced the knowledge of vitelliform macular dystrophies.

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