Genetics

Investigating Microperimetric Features in Bietti Crystalline Dystrophy Patients: A Cross-Sectional Longitudinal Study in a Large Cohort

Yufei Xu,[1,2] Xiao Liu,[1–3] Nan Wu,[1,2] Yanling Long,[1,2] Jiayun Ren,[1,2] Yu Wang,[1,2] Xinyi Su,[4–7] Zengping Liu,[4,5,7] Yu Fujinami-Yokokawa,[8–10] Kaoru Fujinami,[8,9,11,12] Fang Chen,[13] Xiaohong Meng,[1–3] and Yong Liu[1–3]

[1]Southwest Hospital/Southwest Eye Hospital, Third Military Medical University (Army Medical University), Chongqing, China
[2]Key Lab of Visual Damage and Regeneration & Restoration of Chongqing, Chongqing, China
[3]Jinfeng Laboratory, Chongqing, China
[4]Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research, Singapore, Singapore
[5]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[6]Department of Ophthalmology, National University Hospital, Singapore, Singapore
[7]Singapore Eye Research Institute (SERI), Singapore, Singapore
[8]UCL Institute of Ophthalmology, University College London, United Kingdom
[9]Laboratory of Visual Physiology, Division of Vision Research, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan
[10]Department of Health Policy and Management, Keio University School of Medicine, Tokyo, Japan
[11]Moorfields Eye Hospital, United Kingdom
[12]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan
[13]Department of Medical Technology, Chongqing Medical and Pharmaceutical College, Chongqing, China

Correspondence: Xiaohong Meng, Southwest Hospital/Southwest Eye Hospital, Third Military Medical University (Army Medical University), No. 30 Gaotanyan St., Shapingba District, Chongqing 400038, China;
cqwmwm@163.com.
Yong Liu, Hospital/Southwest Eye Hospital, Third Military Medical University (Army Medical University), No. 30 Gaotanyan St., Shapingba District, Chongqing 400038, China;
liuyong@tmmu.edu.cn.

YX and XL contributed equally to this article as co-first authors.

PURPOSE. To assess the clinical and genetic characteristics of patients with Bietti crystalline dystrophy (BCD) with a focus on potential of microperimetry in monitoring macular function.

METHODS. A total of 208 genetically-confirmed BCD patients were enrolled in this retrospective study. The patients were categorized into subgroups based on their fundus characteristics (fovea sparing and fovea involved), optical coherence tomography (OCT) findings (presence/absence of retinal pigment epithelium [RPE] or ellipsoid zone [EZ] line at the fovea/parafovea), and genetic profiles (Mis/Mis, Tru/Mis, Tru/Tru). Fixation patterns were analyzed, and macular sensitivity (MS) parameters were compared among different groups. Longitudinal analysis was performed to calculate the annual changes in MS parameters. Correlation between genotype and phenotype were further investigated by analyzing cumulative incidence of vision impairment among different genotypic groups.

RESULTS. Patients with well-preserved RPE or EZ at the foveal/parafoveal region exhibited higher MS. Notably, there was a decline in sensitivity parameters, with a decrease of -2.193 dB/year (95% confidence interval [CI] -4.292 to -0.095, $P = 0.041$) at the fovea and -1.353 dB/year (95% CI -2.047 to -0.659, $P < 0.001$) in average sensitivity. An age-adjusted comparison of sensitivity among genotypic groups and cumulative incidence analyses showed no association between genotypic groups and vision loss.

CONCLUSIONS. Microperimetry proves to be one of a credible tool for detecting macular functional changes in BCD patients. BCD patients with different genotypes may have similar disease progression.

Keywords: Bietti crystalline dystrophy, *CYP4V2*, microperimetry

Bietti crystalline dystrophy (BCD, MIM 210370), an autosomal recessive inherited disease, was first described by G.B. Bietti in 1937.[1] It is characterized by yellow-white crystalline deposits dispersed throughout the retina, combined with thinning of retinal pigment epithelium (RPE) and choroid.[2,3] BCD is responsible for 10% of non-syndromic retinitis pigmentosa (RP) with higher prevalence in Asians, especially in Chinese populations.[4,5] In 2004, *CYP4V2*, a member of the cytochrome P450 superfamily, was identified as the BCD disease-causing gene.[5] Functional deteriora-

tion of the gene carries a diminished transformation of fatty acid precursors into ω-3 poly-unsaturated fatty acids and an increased accumulation of intracellular deposits of fatty acids.[6,7] RPE was primarily damaged, followed by degeneration of photoreceptors.[8] However, the exact mechanisms of disease progression in BCD remain largely unknown.

Although there is no efficient treatment, the apparent clinical characteristics of BCD, together with its clear genetic background, make it an ideal candidate for gene therapy.[9,10] Best-corrected visual acuity (BCVA), fundus photography, fundus autofluorescence (FAF), and spectral domain-optical coherence tomography (SD-OCT) have been used to monitor disease progression of BCD.[2,11] However, there was no consensus on ideal evaluation modality to track treatment response. Clinical trials need to review the response of gene therapy over relatively short time periods. Although BCVA remains the gold standard tool for measuring visual function,[12] largely stable BCVA in BCD patients limited the accuracy of BCVA in tracking functional changes.[13] A useful tool assisting BCVA in monitoring treatment effectiveness have not been determined.

Microperimetry (MP), has been widely used in tracking retinal disease progression,[14–16] or adopted as an endpoint in clinical trials of interventional inherited retinal diseases such as Stargardt disease[17,18] and RP.[19,20] However, no studies have evaluated whether MP was useful for adequately detecting functional changes in BCD patients. This study aimed to investigate the potential of MP in tracking disease progression in BCD patients. In addition, we analyzed the genotype-phenotype association using the largest cohort to date to draw a conclusion regarding the more detailed correlation.

METHODS

Participants

Patients from the First Hospital Affiliated to the Army Medical University were recruited into this retrospective study from January 2005 to December 2022. BCD was clinically diagnosed by three senior consultants based on typical clinical findings with identified CYP4V2 mutations. The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the First Hospital Affiliated to the Army Medical University (approval number: (B) KY2021151). The requirement for informed consent was waived by the ethics committee.

Clinical Investigations

Comprehensive ophthalmological examinations were conducted, including BCVA, fundus photography (nonmyd WX 3D, Kowa, Tokyo, Japan), FAF imaging (Spectralis, Heidelberg Engineering, Heidelberg, Germany), and OCT (Spectralis, Heidelberg Engineering; and ZEISS CIRRUS, Carl Zeiss Meditec AG, Oberkochen, Germany). Medical records were reviewed, and concomitant diseases were collected. BCVA was converted to the equivalent value in the logarithm of the minimum angle of resolution (LogMAR) unit, and low visual categories, including counting finger and hand movement, were valued at 1.98 and 2.28.[21]

Microperimetry Examinations

All tests were performed using macular analyzer integrity assessment microperimetry (Centervue, Padova, Italy). A standard Goldmann III stimulus size was used, with the background luminance set at 4 asb and the maximum luminance set at 1000 asb. The dynamic range of the device stimulus was 36 dB. Sensitivity measures were obtained by the 4-2 staircase strategy, and the grid consisted of 37 test loci distributed in a radial pattern, sampling retinal locations at 0°, 2°, 6°, and 10° eccentricity from the fovea. Fixation stability and location were automatically recorded by the device. Fixation location was classified into three categories: predominantly central fixation (>50% of fixation points within 2° of the foveal center), poor central fixation (between 25% and 50% of fixation points within 2° of the foveal center), and predominantly eccentric fixation (<25% of fixation points within 2° of the foveal center). Fixation stability was documented in three categories: stable (>75% of fixation points within a 2° diameter circle), relatively unstable (<75% of fixation points within a 2° circle but >75% within a 4° circle), and unstable (<75% of fixation points within a 4° circle).[22]

Variant Detection

A total of 129 patients underwent either eye gene enrichment (from 36 to 450 target genes) panel-based next-generation sequencing or whole exome sequencing, and Sanger bidirectional sequencing was conducted for confirmation. Direct Sanger sequencing of *CYP4V2* was performed in the remaining 79 patients to confirm the variants. Novel *CYP4V2* mutations were analyzed using the following databases and prediction software: GnomAD (http://gnomad.broadinstitute.org/), 1000Genomes (https://www.internationalgenome.org/data/), MutationTaster (http://www.mutationtaster.org/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), and Mutationassessor (http://mutationassessor.org/r3/). In accordance with the American College of Medical Genetics guidelines, novel variants were classified as pathogenic, likely pathogenic, uncertain significance, likely benign, or benign.[23]

Groupings

Yuzawa's staging gives a centrifugal impression of disease progression.[13,24] However, certain patients presented with widespread disease that did not involve the fovea and were categorized as the fovea-sparing group (Fig. 1). The functional differences between the fovea-sparing group and the fovea-involved group were analyzed. Based on the ellipsoid zone (EZ) line or RPE integrity at the fovea/parafovea in OCT, patients were divided into EZ-/RPE- preserved group or the EZ-/RPE- atrophy group. Patients harboring two *CYP4V2* mutations were classified into three genotype groups. Group Tru/Tru: patients with two truncation variants, Group Tru/Mis: patients with one truncation and one missense variant, and Group Mis/Mis: patients with two missense variants.

Statistical Analysis

Statistical analyses were performed using SPSS (Version 20.0; IBM Corp., Armonk, NY, USA). Data were tested for normality using the Kolmogorov-Smirnov or Shapiro-Wilk test. One-way analysis of variance or Kruskal-Wallis H test was used to compare age at symptom onset and mean macular sensitivity (MS) among the genotypic groups. Student's *t* test or Mann Whitney test was used to compare mean MS and BCVA among the fundus and OCT groups. MS trajectories were
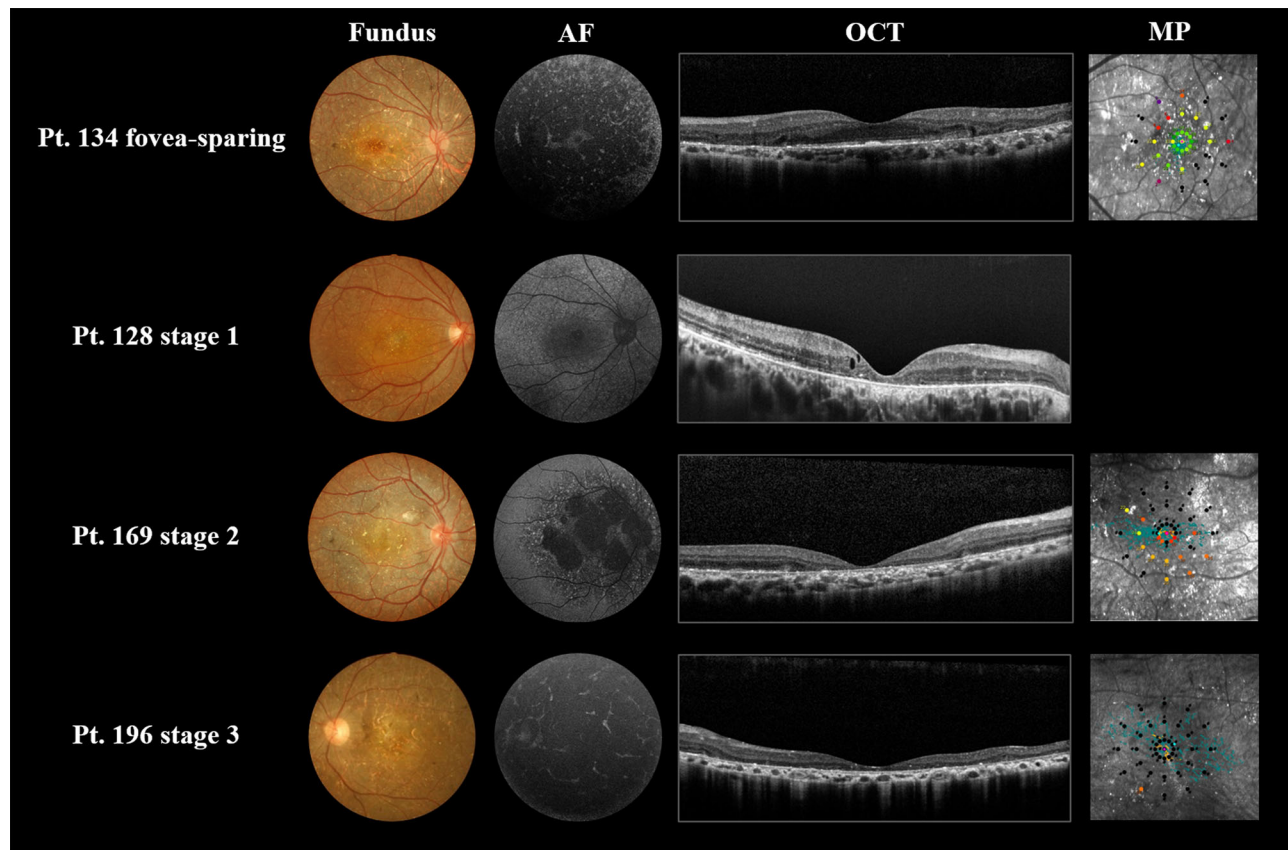
FIGURE 1. Fundus appearance, FAF, macular OCT, and MP images of four representative cases of Bietti crystalline dystrophy. Patient 134: onset at 32 years; BCVA of 0.4 in the logMAR unit for the left eye; Fundus: fovea sparing group; SD-OCT: EZ-preserved group; Genotype Tru/Mis: [c.802-8_810del17insGC]; [c.992A>C p.H331P]: multiple glistening deposits scattered in the posterior pole of the left eye, associated with hypofluorescent atrophic patch with partial preservation of the foveal region in FAF. SD-OCT image showed some hyperreflective deposits at the central macula and outer retinal tubulations confining to the out nuclear layer. The EZ line was preserved at the center macular area and severely disrupted outside the center. MP exhibited stable fixation and predominantly central fixation. Patient 128: onset at 30 years; BCVA of 0.5 in the logMAR unit for the right eye, Fundus: stage 1; SD-OCT: EZ atrophy group; Genotype Tru/Mis: [c.802-8_810del17insGC]; [c.992A>C p.H331P]: macular dystrophy with multiple glistening deposits scattered in the posterior pole of the right eye. FAF image showed a hypofluorescent signal at the macula with numerous hyperfluorescent signals outside. SD-OCT image demonstrated outside retinal extensive loss at macular area with some hyperreflective deposits throughout the retina and edema at the inner layers. MP result was unavailable. Patient 169: onset at 30 years; BCVA of 0.15 in the logMAR unit for the right eye, Fundus: stage 2; OCT: EZ atrophy group; Genotype Tru/Tru: [c.802-8_810del17insGC]; [c.1091-2A>G]: some crystalline confluent deposits at the posterior pole, with multiple patchy coalescent hypofluorescent areas beyond the posterior pole in FAF. OCT showed outside retinal extensive loss in the macular area. MP result exhibited unstable fixation and predominantly eccentric fixation. Patient 196: onset at 40 years; BCVA of 0.4 in the logMAR unit for the left eye, Fundus: stage 3; OCT: EZ atrophy group; Genotype Tru/Mis: [c.810delT]; [c.992A>C p.H331P]: a small number of residual crystalline deposits throughout the fundus, extensive atrophy of RPE-choriocapillaris complex, and nearly entire hypofluorescent areas located throughout the degenerative lesions in FAF. OCT showed outside retinal extensive loss in the macular area. MP result exhibited unstable fixation and predominantly eccentric fixation.

estimated using generalized linear mixed-effects models, with random eye-within-patient intercepts and independent random slopes-within-eyes, and fixed effects for baseline age and time. In survival analysis, low vision and legal blindness were defined as $0.05 \leq BCVA < 0.3$ and $BCVA < 0.05$.[25] The log-rank test was used to compare the cumulative incidence of vision impairment, and the right eye was selected for analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Demographics and Mutation Spectrum

A total of 208 genetically-confirmed BCD patients were enrolled (85 participants have been published in our previous study)[26] and all patients had the typical BCD phenotype (Table 1). Corneal involvement was noticed in 136

patients out of 168 individuals who had detailed description of cornea and no corneal clarity was noticed. Other ocular diseases were found in 28 of the 208 patients and prevalence of high myopia was high (Table 2). Hyperlipemia and thyroid nodules were the most common concomitant nonocular diseases. No eyes with concomitant eye disease underwent MP examination and four patients were excluded out the survival analysis of vision impairment for concomitant eye diseases.

All patients were verified through genetic testing: 79 patients (37.98%) were homozygous, 126 patients (60.58%) had compound heterozygous mutations, and in three cases (1.44%), only one *CYP4V2* mutation was identified. A total of 38 *CYP4V2* disease-causing variants were detected. Consistent with previous studies,[26] c.802-8_810del17insGC (46.39%), c.992A>C p.H331P(17.07%), and c.1091-2A>G (14.18%) ranked the top three prevalent variants. Addition-

TABLE 1. Demographics and Baseline Characteristics of Patients With Bietti Crystalline Dystrophy

Variables	Data
Subjects	208
Sex	
Males	98 (47.12%)
Females	110 (52.88%)
First symptoms*	
Vision declined	109 (52.40%)
Night blindness	88 (42.31%)
Vision distorted	3 (1.44%)
Photophobia	1 (0.48%)
Narrowed visual field	1 (0.48%)
Age at consultation** (y)	41.0 (36.0-47.0)
Age of symptom onset† (y)	33.0 (27.0-39.0)
LogMAR BCVA§	0.65 (0.33-1.30)
Right eye	0.52 (0.30-1.00)
Left eye	0.70 (0.37-1.30)
Genotype‖	
Homozygous	79 (37.98%)
Compound heterozygous	126 (60.58%)
Heterozygous	3 (1.44%)

LogMAR, logarithm of minimum angle of resolution.

Continuous data was presented as median and interquartile range and categorical data was presented as number or percentage.

* First symptom was available in 202 subjects.
** Age at consultation was available in 203 subjects.
† Age of symptom onset were available in 197 subjects.
§ LogMAR BCVA was available in 179 subjects.
‖ Genetic data was obtained from 208 subjects.

TABLE 2. Concomitant Diseases Identified in Patients With Bietti Crystalline Dystrophy

Diseases	
Concomitant ocular diseases*	
High myopia	23 (11.06%)
Macular hole	2 (0.96%)
Retinal detachment	1 (0.48%)
Glaucoma	1 (0.48%)
Choroidal neovascularization	1 (0.48%)
Concomitant nonocular diseases†	
Hyperlipidemia	30 (30.30%)
Thyroid nodule	19 (19.19%)
Subcutaneous lipoma	4 (4.04%)
Gallstone	1 (1.01%)
Pituitary adenoma	1 (1.01%)

* Record of concomitant eye diseases was collected from 208 patients.
† Record of concomitant diseases in nonocular systems was collected from 99 patients.

ally, four novel variants including c.1226-1G>A, c.698G>A p.R233K, c.386delinsAT p. P129Hfs*41, and c.1382_1383insC p. A462Cfs*50 were noticed (Supplementary Table S1 and Supplementary Table S2).

Patients With Well-Preserved Macular Structure and Function Are More Likely To Exhibit Favorable Fixation Patterns

A total of 114 eyes from 58 patients underwent MP examinations. Among these, 47 eyes exhibited stable fixation, whereas nine eyes showed relatively unstable fixation, and 58 eyes displayed unstable fixation. The majority of eyes

TABLE 3. Preservation of Fovea Detected by Fundus Images Was Associated With Better Visual Function in Patients With Bietti Crystalline Dystrophy

Parameters	Fovea Sparing	Fovea Involved	*P* Value
Macular sensitivities (dB)			
Fovea	21.87 ± 3.27	18.52 ± 4.69	0.019
Average sensitivity	15.93 ± 3.17	6.24 ± 2.55	<0.001
BCVA	0.30 (0.10, 0.52)	0.70 (0.40, 1.30)	<0.001

Data of macular sensitivities were presented as mean ± standard deviation. There were 15 eyes (41.67%) in the fovea-sparing group and 21 eyes (58.33%) in the fovea-involved group in the analysis for macular sensitivities. In the analysis of BCVA divided by fundus stages, BCVA of the right eye was selected for analysis. The distribution of eyes across groups were as follows: 35 in the fovea-sparing group and 130 in the fovea-involved group. *P* < 0.05 was considered of significant differences.

with stable fixation (31 out of 47) had Snellen BCVA ≥ 20/50, whereas most of eyes with unstable fixation (43 out of 58) had Snellen BCVA ≤ 20/60. The mean BCVA in patients with stable fixation (0.30 [0.10, 0.40]) was better than that in patients with relative unstable fixation (0.70 [0.30, 1.00], *P* = 0.009) and unstable fixation (0.70 [0.40, 1.30], *P* < 0.001). Patients of fovea-sparing were more likely to have stable fixation (23 out of 26), whereas the majority of patients of stage 3 had unstable fixation (40 out of 54). In addition, patients with better preservation of EZ or RPE at the fovea/parafovea tended to exhibit stable fixation compared to those without.

Of the analyzed eyes, 48 exhibited predominantly central fixation, three had poor central fixation, and 63 displayed predominantly eccentric fixation. Patients with predominantly central fixation exhibited better BCVA (0.30 [0.10, 0.40]) compared to those with predominantly eccentric fixation (0.70 [0.52, 1.30], *P* < 0.001). Patients with preservation of EZ or RPE were more inclined to exhibit predominantly central fixation. However, no significant association between fixation location and fundus stage was observed.

Some eyes with predominantly eccentric fixation presented with stable or relatively unstable fixation. Among patients with predominantly eccentric fixation, 15 individuals had Snellen BCVA ≥ 20/50, and eight subjects had Snellen BCVA ≥ 20/40. However, all these patients exhibited unstable fixation.

Preservation of Fovea Detected by Fundus Imaging Associates With Higher Macular Sensitivities

Excluding eyes with relatively unstable fixation or unstable fixation and blurry fundus images, 36 eyes of 23 patients were enrolled in the analysis of MS. Fovea-sparing group tend to have higher MS than Fovea-involved group at the fovea (*P* = 0.019, Table 3) and in average sensitivity (*P* < 0.001). Notably, the mean BCVA in the fovea-sparing group was better than that in the fovea-involved group (*P* < 0.001), further indicating that preservation of fovea was associated with better function.

Patients With Well-Preserved Macular Structure Exhibit Higher Macular Sensitivities

The EZ band serves as a widely recognized biomarker correlated strongly with visual acuity and retinal function.[27] We
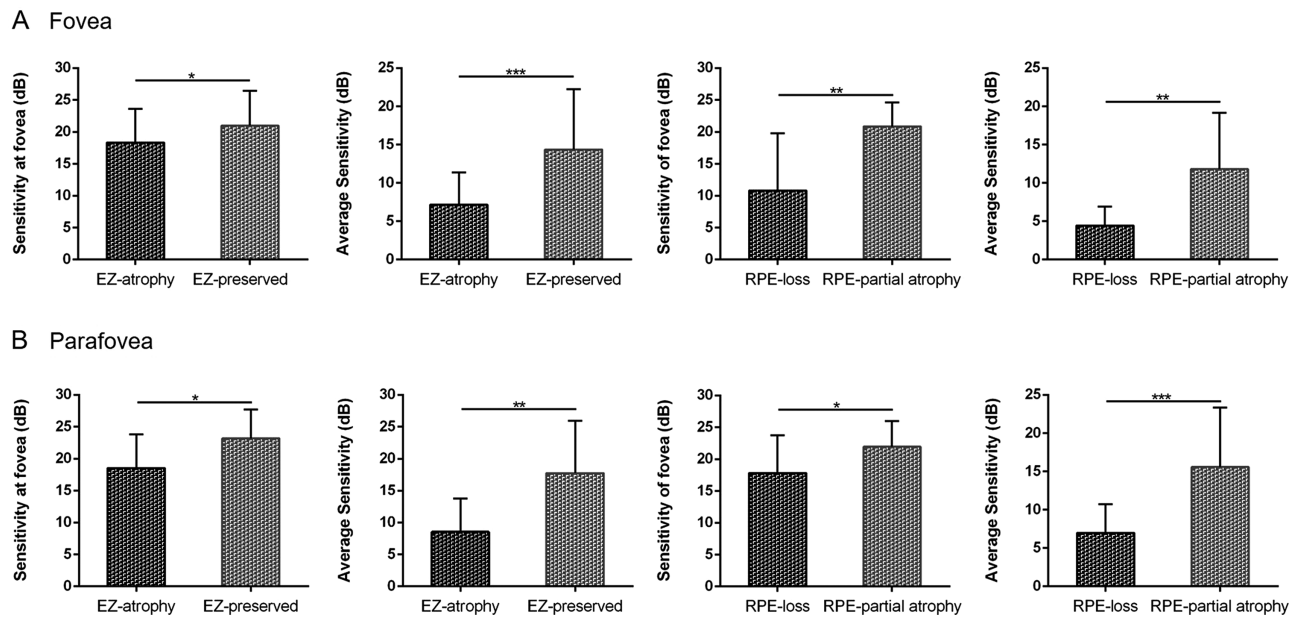
FIGURE 2. Well-preserved macular structure was associated with higher MS in patients with Bietti crystalline dystrophy. The mean sensitivity threshold of the macula detected by microperimetry was compared among optical coherence tomography groups. Patients were divided based on absence or presence of RPE or EZ line in the location of fovea or parafovea. Patients with presence of RPE or EZ line either at the fovea or at the parafovea demonstrated higher MS at the fovea or average sensitivity than those not (all $P \leq 0.043$).

recorded MS of OCT groups from 45 eyes with stable fixation of 29 patients (Fig. 2). Foveal EZ-atrophy group had lower sensitivity (18.33 ± 5.26 dB, n = 21) compared to foveal EZ-preserved group (21.00 ± 5.45 dB, n = 24) at the fovea ($P = 0.043$) and similar difference was observed in average sensitivity (7.16 ± 4.20 vs. 14.37 ± 7.86 dB; $P < 0.001$). In addition, parafoveal EZ-atrophy group (n = 33) demonstrated lower MS than parafoveal EZ-preserved group (n = 12) at the fovea (18.52 ± 5.30 dB vs. 23.17 ± 4.55 dB; $P = 0.010$) and in average sensitivity (8.55 ± 5.23 dB vs. 17.77 ± 8.18 dB; $P = 0.001$).

RPE is widely recognized as the primary pathology site in BCD patients,[28] and patients were divided based on preservation of RPE in the foveal/parafoveal region. Mean MS at the fovea in the foveal RPE-loss group (10.80 ± 9.01 dB; n = 5) was lower than that in the foveal-partial atrophy group (20.88 ± 3.72 dB, n = 40; $P = 0.002$) and similar difference was observed in average sensitivity (4.44 ± 2.48 dB vs. 1.83 ± 7.32 dB; $P = 0.006$). There were differences of MS at the fovea (17.83 ± 5.90 dB vs. 21.95 ± 4.04 dB; $P = 0.019$) and in average sensitivity (6.98 ± 3.76 vs 15.60 ± 7.74 dB; $P < 0.001$) between parafoveal RPE-loss group (n = 21) and parafoveal RPE-partial atrophy group (n = 24). Notably, patients with well-preserved RPE and EZ in the fovea or parafovea region tend to exhibited higher sensitivity and better BCVA (Fig. 2, Supplementary Fig. S1, and Supplementary Fig. S2).

Microperimetry Can Track Subtle Changes in Visual Function, Whereas BCVA Remains Largely Stable

BCD patients tend to show near-normal BCVA that can last up to four decades despite severe or extinguished morphological and functional ophthalmological examinations. To date, no effective tools can precisely assess the disease progression, and we found that MP can track the disease progression even in some patients with unstable fixation for the first time (Fig. 3). A longitudinal analysis of visual function was performed in 16 eyes with stable fixation of nine patients covering all kinds of fundus stages. The mean age of patients was 35.67 ± 8.29 years old. Annual change of mean BCVA was not significant ($P = 0.805$), whereas the rate of decline in MS at the fovea and in average sensitivity were −2.193 dB/year (95% CI, −4.292 to −0.095, $P = 0.041$), and −1.353 dB/year (95% CI, −2.047 to −0.659, $P < 0.001$).

Patients With Different Genotypes May Have Similar Disease Progression

Nineteen (9.27%), 77 (37.56%), and 109 (53.17%) patients were classified in the Mis/Mis group, the Tru/Mis group, and the Tru/Tru group, respectively. A total of 29 eyes with stable MS were recruited, and no significant difference was observed in the mean MS in the entire MP-tested area (Fig. 4A). Eighteen (9.23%), 70 (35.90%), and 107 patients (54.87%) with available age of onset were divided into the three groups as listed, showing no significant difference (Fig. 4B). Additionally, 17 (9.55%), 67 (37.64%), and 94 patients (52.81%) were divided into the three groups as listed to compare the cumulative incidence of low vision or legal blindness and no significant differences were observed (Fig. 4B).

DISCUSSION

Determining indicators to monitor therapeutic effect is essential in clinical trials. Choroidal thinning, crystalline deposits number, EZ band length, and foveolar thickness have been used to quantify progression with limitations of
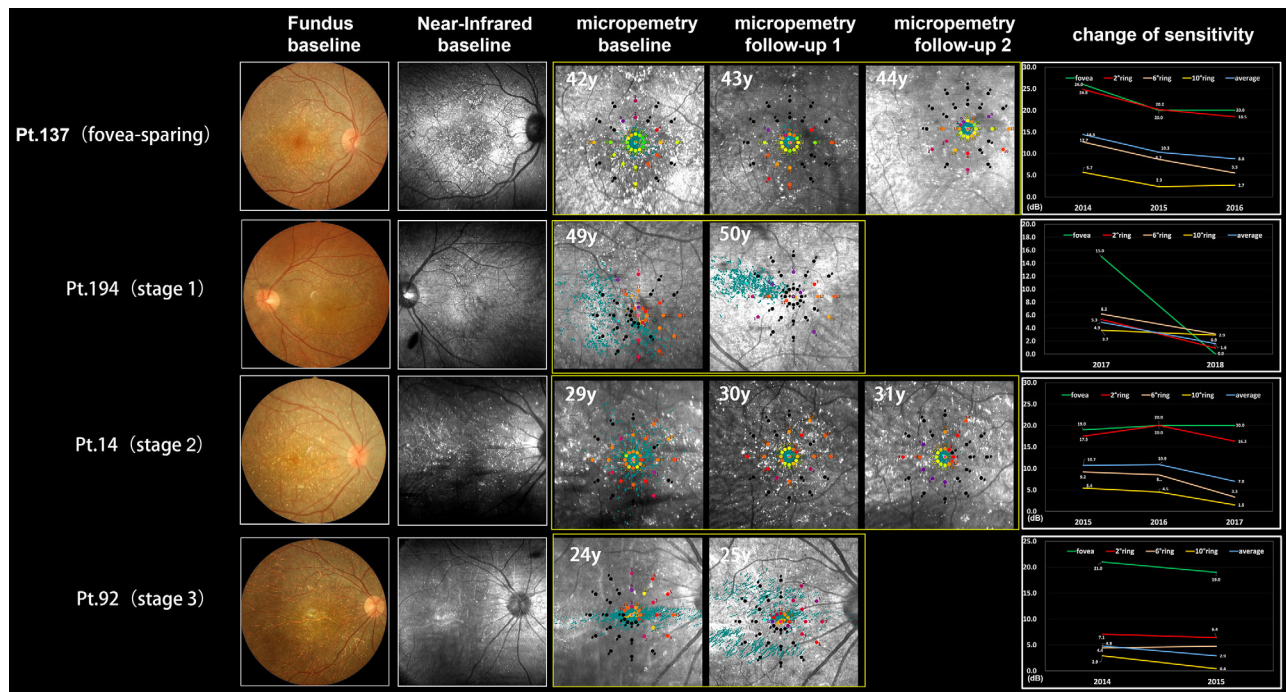
FIGURE 3. Fundus appearance, NIR-FAF, the liner chart, and MP map of sensitivity changes at the follow-up year of four representative cases with Bietti crystalline dystrophy. Patient 137: onset at 41 years; BCVA of 0 in the logMAR unit for the right eye; Fundus: fovea-sparing group; OCT: unavailable, Genotype Tru/Tru: [c.802-8_810del17insGC]; [c.802-8_810del17insGC]: BCVA was maintained at 0 for a three-year follow; fundus image showed multiple crystal deposits scattered in the posterior pole of the right eye corresponding to hyperreflective spots in the NIR-FAF. MP maps exhibited stable fixation during a three-year follow. Patient 194: onset at 39 years; BCVA of 0.7 in the logMAR unit for the right eye; Fundus: stage 1; OCT: EZ atrophy group; Genotype Mis/Mis: [c.992A>C p.H331P]; [c.1199G>A p.R400H]: BCVA was maintained at 0.7 for a three-year follow, fundus image showed some crystalline deposits extending to midperiphery. NIR-FAF demonstrated hyperreflective spots at the posterior pole. MP maps exhibited unstable fixation at the two-year follow-up. Patient 14: onset at 27 years; BCVA of 0.3 in the logMAR unit for the left eye; Fundus: stage 2; OCT: EZ atrophy group; Genotype Tru/Mis: [c.1091-2A>G]; [c.1384G>C p.A462P]: BCVA was maintained at 0.3 for a two-year follow, fundus image showed macular dystrophy with multiple glistening deposits scattered in the posterior pole of the left eye. NIR-FAF exhibited numerous hyperreflective spots around the macula, and MP maps manifested relatively stable fixation at the three-year follow. Patient 92: onset at 19 years; BCVA of 0.82 in the logMAR unit for the right eye; Fundus: stage 3; SD-OCT: EZ atrophy group, Genotype Tru/Tru: [c.802-8_810del17insGC]; [c.802-8_810del17insGC]: BCVA was maintained at 0.82 for a two-year follow; Fundus image and NIR-FAF showed few retinal crystalline deposits (hyperinflation in NIR-FAF) with atrophy of retinal pigment epithelium and choroid. MP maps demonstrated that the fixation was relatively stable at 24 years and unstable at 25 years.
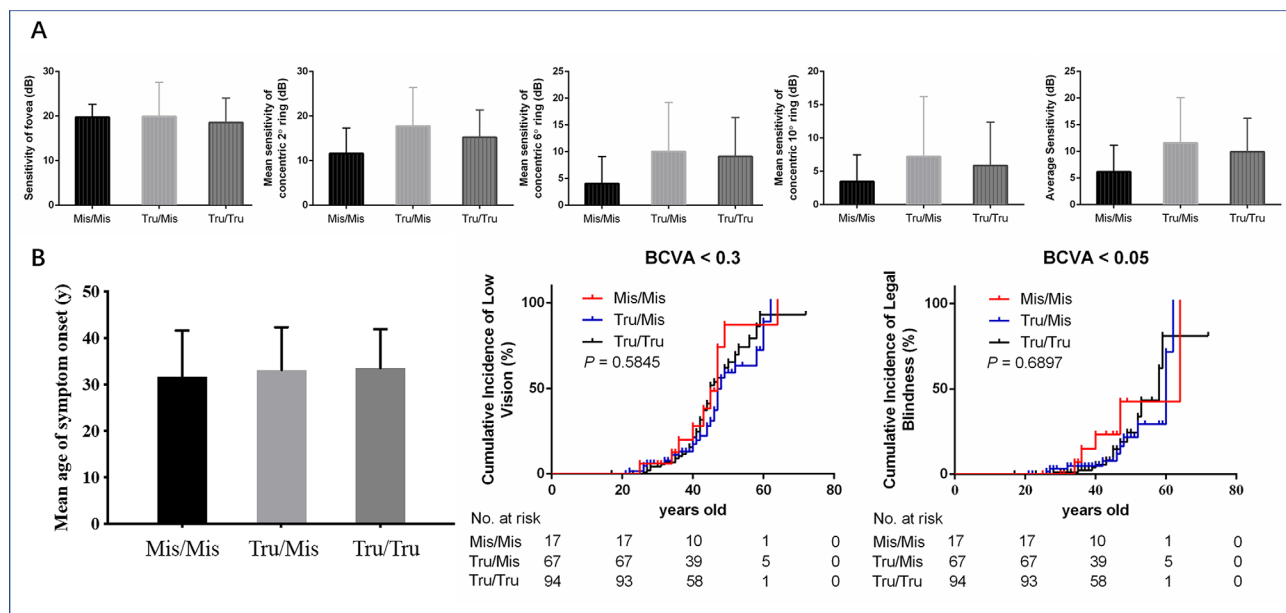

FIGURE 4. Patients with different genotypes may have similar disease progression. (**A**) No significant difference was observed in the mean macular sensitivity of each eccentric ring or average sensitivity threshold among different age-matched genotypic groups. (**B**) There was no significant difference in the comparison of the age of onset among the three genotypic groups, and further comparisons of the cumulative incidence of low vision or legal blindness among the genotypic groups showed no statistically significant differences.

inaccuracy or complex operation.[27,29,30] Long-term stability of BCVA and possibility of being influenced by chance involvement of certain areas of retina limited accuracy of BCVA in tracking treatment response. A more comprehensive approach to evaluate visual function should be encouraged.

Over the previous two decades, MP has been widely used to characterize functional vision under various retinal conditions.[31,32] Anikina et al.[31] characterized retinal function in patients with *RPGR*-associated RP using MP-derived metrics and found that MP had good test-retest reliability for the disease and could track the progression of vision loss. Iftikhar et al.[32] evaluated the yearly progression of RP and showed a significant annual decline in retinal sensitivity. Only a few case reports have described the MP features in BCD patients.[29,33] In this study, most eyes with stable fixation had Snellen BCVA $\geq$ 20/50 and a majority of the eyes with unstable fixation had Snellen BCVA $\leq$ 20/60, for which the two points may serve as cutoff value for determining fixation stability before conducting MP examination. Although the mean BCVA in patients with predominantly central fixation was higher than in those with predominantly eccentric fixation, there was no applicable cutoff value to distinguish fixation location. Consistent with the findings reported by Midena et al.,[34] some eyes with predominantly central fixation exhibited stable or relatively unstable fixation. Learning process of patients with bilateral disease that develop increased ability to use their eccentric preferred fixation location may account for the phenomenon. In patients with predominantly eccentric fixation, eight subjects were with Snellen BCVA $\geq$ 20/40. However, the patients were all of unstable fixation and may thus undergo sharp decline in BCVA. For BCD patients, MP may help predicting change of BCVA.

Yuzawa's staging tends to give the impression of a centrifugal expansion. Some studies have noted the fovea-sparing type.[27,29] However, there were no study investigating detailed visual function of patients of fovea-sparing. Notably, 14% of eyes in our study showed a fovea-sparing appearance. We divided patients into fovea-sparing group and fovea-involved group and significant difference of BCVA between the two groups was noticed. In addition, MP had ability to discriminate visual function of patients in the two groups, indicating clinical value of MP in monitoring disease progression. EZ line has been proved to be correlated with visual acuity and RPE is widely recognized as the primary pathology site in BCD patients.[27,28] We thus divided patients into different groups based on OCT findings and significant difference in MS between different OCT groups were observed. The results indicate that macular structure and macular function are well correlated and can be quantified by MP. Additionally, the role of lipid deposition in BCD progression is crucial, as previous research[28] has documented different lipid components accumulating in the RPE, contributing to RPE dysfunction. Importantly, reducing lipid deposits has been shown to rescue BCD phenotypes,[35] suggesting that early intervention could be effective. Our findings highlight the potential of MP as a sensitive tool to monitor disease progression and treatment efficacy, particularly in the early stages of BCD when lipid deposits are present. As lipid levels decline, indicating the onset of RPE and photoreceptor loss, MP results may deteriorate, reinforcing its utility in tracking both the natural history of BCD and identifying optimal windows for therapeutic intervention. However, further study investigating association between MP results and lipid deposits was needed.

Clinical trials need frequent assessment of treatment response, and we thus conducted longitudinal analysis of BCVA and MS. Although annual change of BCVA was not significant, in our study, we proposed significant annual decrease of MS, further confirming the potential of MP in tracking subtle functional changes.

In phase 1 gene therapy trials,[36,37] patients had severe vision loss, and in our study, nearly all eyes with BCVA < 20/200 showed unstable fixation, limiting the clinical utility of MP for assessing disease progression in these cases. However, analyzing fixation patterns could still be valuable for evaluating therapeutic effects. Advanced BCD patients typically demonstrate unstable fixation associated with low vision and may develop eccentric fixation, which involves brain adaptation strategies.[38] For example, in gene therapy studies for choroideremia, shifts in the preferred retinal locus towards treated areas were strong indicators of therapeutic success.[39] In our study, we found that smaller bivariate contour ellipse areas (BCEA 63% and BCEA 95%) were significantly correlated with better visual acuity ($\rho = 0.619$, $P < 0.001$; $\rho = 0.608$, $P < 0.001$), suggesting more stable fixation. Although MP has limitations in advanced cases, it can still monitor treatment effects effectively.

The three most prevalent variants of *CYP4V2*, c.802-8_810del17insGC, c.992A>C p.H331P, and c.1091-2A>G were detected in our cohort. The allele frequency of c.802-8_810del17insGC, a founder mutation in East Asia, was lower than that was previously reported, in which this mutation accounted for frequencies of 62.6% in 125 Chinese patients , 69.4% in 62 Japanese and Korean patients.[40,41] The following two variants showed higher allele frequency in our cohort than in the Chinese cohort of 125 patients: p.H331P (8.7%) and c.1091–2A>G (7.5%).[40,41] The differences between the Chinese cohorts may be due to the higher proportion of homozygous patients in our cohort. The allele frequency of c.1020G>A p.W340X was only 0.75% in this study, which is thought to be another founder mutation in Korean and Japanese BCD patients.[40]

In our study, which involves the largest cohort to date, we did not observe a significant association between genotype and phenotype, a finding that contrasts with earlier studies conducted on smaller cohorts.[2,42] For instance, one study suggested that the p.M66R variant was associated with an earlier onset of symptoms.[2] Conversely, Yin et al.[43] analyzed genetic and phenotypic characteristics in 36 families with BCD and identified no apparent genotype-phenotype correlation and Murakami et al.[40] reported no association between the exon7del homozygote status and the rate of vision loss in a combined Korean and Japanese cohort with 65 patients. The discrepancies between these findings may be explained by the presence of three mutation hotspots—c.802-1918_810del17insGC (frameshift), c.992A>C p.H331P (missense), and c.1091-2A>G (splice site mutation)—all located in highly conserved regions of the CYP4V2 gene. Mutations in these regions are likely to result in severe protein dysfunction, making a negative association plausible. This information will be valuable when designing future clinical trials because BCD is a good target for gene therapy.

This study has several limitations. First, selection bias at recruitment related to disease severity is inherent, because it is difficult to collect data from genetically affected individuals with good visual function who do not visit the hospital.

Second, in the longitudinal MP analysis we excluded patients with unstable fixation, which reduced the number of eyes analyzed. Third, this retrospective case series provided limited information; thus, prospective natural history studies could provide more accurate MP information on the disease severity and progression of BCD.

In conclusion, this study illustrated a spectrum of phenotypes and genotypes in a molecularly confirmed largest BCD cohort thus far. Here, we demonstrated that MP could help provide structure-function relationships and MP was a sensitive test for detecting progression within a relatively short period in BCD patients. In addition, we concluded that the patients in our cohort with deleterious variants were not associated with a severe phenotype.

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