Genetics

Longitudinal Natural History Study of Visual Function in Bietti Crystalline Dystrophy: Implications for Early Intervention

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Purpose. To delineate the natural history of visual function parameters over time in individuals with Bietti crystalline dystrophy.

Methods. This was a single-center retrospective longitudinal cohort study. Participants ($n = 29$) with a clinical diagnosis of Bietti crystalline dystrophy who harbored two alleles of disease-causing variants of the cytochrome P450 family 4 subfamily V member 2 gene (*CYP4V2*) were enrolled. Best-corrected visual acuity (BCVA), visual field (VF), and full-field ERG (ffERG) at baseline and their changes during the follow-up period were evaluated. Annual progression rates were calculated using three methods.

Results. The mean age at the initial visit was 34.2 ± 7.5 years, with 5.9 ± 3.1 years follow-up. The annual progression rate from the longitudinal analysis using averaged individual progression rates was 0.079 logMAR units for BCVA, 1.14 dB for mean defect (MD) value of VF, and −18.06 µV and −5.45 µV for the b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG, respectively. Mixed-model linear regression revealed annual progression rates of 0.068 logMAR units, 0.86 dB, −13.29 µV, and −3.75 µV, respectively. Cross-sectional progression rates from visual function versus age at baseline were 0.011 logMAR units, 0.47 dB, −1.85 µV, and −1.07 µV, respectively, which were significantly slower than those from the longitudinal data. Interocular symmetries for the MD values of VF and ffERG were good.

Conclusions. Annual BCVA, VF, and ffERG progression rates were rapid, emphasizing the need for regular follow-up and early intervention. The progression rate cannot be inferred accurately from cross-sectional data from patients of different ages.

Keywords: bietti crystalline dystrophy, longitudinal natural history, visual acuity, visual field, electroretinography

Bietti crystalline dystrophy (BCD, OMIM#210370) is an autosomal recessive inherited chorioretinal dystrophy characterized by progressive loss of the RPE, choroid, and photoreceptors, with the presence of fine crystalline deposits in the retina and corneal limbus.[1] The disease is rare in Caucasian countries, but relatively common in East Asian countries, particularly among the Chinese and Japanese populations.[1] The cytochrome P450 family 4 subfamily V member 2 gene (*CYP4V2*)[2] that is responsible for BCD is located at 4q35 and consists of 11 exons (GenBank NM_000390.2), spanning approximately 21.7 kilobases of genomic DNA. This gene encodes a novel member of the cytochrome P450 enzyme family, which is a ubiquitously expressed selective hydroxylase for saturated and polyunsaturated medium-chain fatty acids.

Patients with BCD typically experience nyctalopia during the second and third decades of life, followed by progressive peripheral vision loss, which can eventually lead to tunnel vision and legal blindness.[3] However, information on the age of onset, severity, rate of disease progression, and clinical presentation is rarely and variably reported.[1,4–7] Given the recent success of gene therapy for inherited retinal diseases (IRDs), it is important to obtain information on the natural course of these diseases. This information will help to select eligible candidates, establish appropriate clinical endpoints, and compare the evolution of disease after treatment. In addition, it is important to consider the interocular symmetry of visual function in treatment trials, because the untreated eye can serve as the control for the treated eye. To the best of our knowledge, studies on the longitudinal natural history and interocular symmetry of visual function in large cohorts with BCD are rare.

When conducting natural history studies, it is more appropriate to undertake prospective, large-scale, and long-term follow-up studies. However, IRDs are rare and often accompanied by a high rate of misdiagnosis and underdiagnosis. Additionally, owing to visual impairment, there is significant loss to follow-up, making it challenging to

achieve large-scale, long-term, longitudinal, natural history studies. In many cases, researchers rely on cross-sectional data from patients of varying ages to infer the progression rate of the disease. Nevertheless, the disparities between progression rates derived from longitudinal and cross-sectional data are seldom addressed. Furthermore, the methodology used to calculate the progression rate warrants discussion.

In the current study, we presented a detailed longitudinal characterization of visual functional changes and interocular symmetry of visual functional parameters in a large cohort of patients with BCD. Moreover, we used three methods to calculate the progression rate of visual functional parameters. We aimed to characterize disease progression and to answer whether the progression rate could be inferred from cross-sectional data from patients of different ages. This is the largest longitudinal natural history study on a cohort of patients with molecularly confirmed BCD.

Methods

Study Participants

This single-center retrospective cohort study enrolled patients who met the following criteria: (1) male or female patients older than 16 years at the initial visit, (2) genetic diagnosis consistent with autosomal recessive mutations in the *CYP4V2* gene, (3) a minimum of two visits during the follow-up period, and (4) a minimum duration between the initial and final visit of 1 year. Patients with other retinal disorders, ocular disorders affecting retinal function, or systemic diseases associated with mutations in other retinal genes were excluded from the study. The study protocol was approved by the Institutional Review Board of Peking Union Medical College Hospital, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Visual Function Measurement and Analysis

Visual function measurements, including best-corrected visual acuity (BCVA) using the Snellen VA test, visual field (VF) using the Perimeter Octopus 101 or Octopus 900 (Haag-Streit AG, Koeniz, Switzerland), and full-field ERG (ffERG) using the RetiPort ERG system (Roland Consult, Wiesbaden, Germany), were performed and analyzed. Criteria review and data collection were performed by the central principal investigator (XXH) to avoid bias and improve consistency.

Snellen VA test results were converted to decimal and logMAR unit value using the following formula: logMAR = −log (decimal acuity) for subsequent analysis. Patients' abilities to count fingers, detect hand movements, and detect light perception were assigned logMAR values of 2.6, 2.7, and 2.8, respectively.[8] Data were collected from participants of different ages. For BCVA analysis, only patients who underwent bilateral BCVA measurements at a minimum of two visits over a minimum period of 1 year were included.

VF testing was conducted using either a Perimeter Octopus 101 or Octopus 900 instrument. The TOP strategy with a G pattern, consisting of 59 points within a central circle of 30°, was used for all VF tests included in this analysis. The background luminance was set at four apostilbs. White stimuli with a standard Goldmann size III of 1000-apostilb maximum luminance and 100-ms duration were used. Before the examination, a short training program was conducted and standard test rules were followed by the examiners and participants.[9] To assess test reliability, the reliability factor, which is the standard output of the perimeter, was used. Results from tests with reliability factor scores of greater than 25% were excluded from the analysis to avoid significant positive or negative bias. The mean defect (MD), also a standard output of the Octopus, was used to determine the extent of VF defects. Only patients who had reliable bilateral VF measurements over 1 year, with measurements collected at least two time points, were included in the VF analysis.

ffERG recordings were obtained at this center using corneal ERG jet contact lens electrodes following the standards set by the International Society for Clinical Electrophysiology of Vision. Patients with undetectable ffERG responses were not considered for this test for the subsequent visits. For inclusion in the ffERG analysis, patients underwent at least two bilateral ffERG measurements with a 1-year follow-up duration. In this study, the b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG responses were selected for ffERG analysis.

Statistical Methods

For descriptive analysis, continuous variables are presented as means ± SDs or medians and quartiles, as appropriate. Progression rates of the four visual parameters were calculated using three methods.

Method 1: Longitudinal Analysis Using Averaged Individual Progression Rates. The progression rates of each eye were calculated as the individual progression rates. For eyes that had more than two visit data points, the progression rate was obtained by fitting a linear regression model with visual function as the dependent variable and follow-up time as the independent variable. The slope of the regression line represents the progression rate. For eyes that had only two visit data points, the progression rates were obtained by dividing the visual function changes by the follow-up time. Progression rates reported as unit per year of the four visual parameters were obtained by averaging progression rates from each eye. Progression rates reported as percent per year were calculated by dividing the yearly progression rate by the corresponding baseline value. The results were presented for all eyes as well as by laterality.

Method 2: Longitudinal Analysis Using Mixed-Effect Linear Regression. To handle the cluster effect of repeat measurements of each eye's parameters and two counterpart eyes from the same patient, we used the mixed-effect linear regression model with random intercept and random slope to deduce the overall rates of progression for each of the four visual parameters. For correlation structure, we used completely general (unstructured) covariance matrix parameterized directly in terms of variances and covariances both for intereye correlation and longitudinal correlation. In the analyses, we treated patient age as a fixed effects quantitative explanatory variable. Each eye of each patient was selected as a random effects' variable. Each parameter was analyzed in turn as the dependent variable. The coefficient of slope for age is the overall rate of progression.

Method 3: Cross-sectional Analysis Using Baseline Data From Patients of Different Ages. The progression rate of the four visual parameters was obtained

by fitting a linear regression model with visual function and age at the first visit as the dependent and independent variables, respectively. The slope of the regression line represents the progression rate. The results were presented for all eyes as well as by laterality. We used the Bland–Altman method and intraclass correlation coefficient (ICC) to assess interocular symmetry in baseline data, overall data, and progression rates for each study parameter. In Bland–Altman analyses, the proportion of individuals with interocular differences within the 95% confidence interval for 95% limits of agreement was calculated.[10,11]. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA), and the SAS codes for mixed-effect model are included in the Appendix 1. Statistical significance was set at a two-sided P value of less than 0.05.

Results

Study Participants

Of the 29 unrelated patients of Han Chinese ethnicity who participated in this study, 19 were females and 10 were males (Table 1; Fig. 1). The mean ± SD age of participants at baseline was 34.2 ± 7.5 years (median, 32.2 years; range, 21.3–48.7 years), with a mean of 2.7 ± 1.1 visits (median, 2; range, 2–7) and a mean follow-up period of 5.9 ± 3.1 years (median, 5.8 years; range, 1.0–16.0 years).

Visual Function

For the statistical analysis of visual function, 29, 16, and 14 patients were included in the BCVA, VF, and ffERG analyses, respectively (Fig. 2). Bilateral visual function measurements were performed over 1 year, with measurements collected at least at two time points. The reliability factor was required to be less than 25% for VF measurements. The age at baseline, number of visits, and follow-up periods for these visual function measurements are shown in Table 2.

Baseline Values for Four Visual Functional Parameters

At baseline, four visual function parameters were investigated: BCVA, MD values of VF, and b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG. Table 3 presents the baseline values of the four parameters. The mean BCVA in logMAR at baseline equivalents was 0.37 ± 0.44 or approximately 0.43, decimal VA. The mean MD of VF at baseline was 15.75 ± 7.57. The mean b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG at baseline were 156.86 ± 131.65 µV and 50.14 ± 37.51 µV, respectively.

Progression Rates From Longitudinal Analysis Using Averaged Individual Progression Rates

Table 4 presents the progression rates obtained from longitudinal analysis using averaged individual progression rates for all four parameters. The mean ± SE BCVA annual progression rate based on bilateral data was 18.1% ± 22.99%, which was equivalent to an annual increase of 0.079 ± 0.016 logMAR. The mean ± SE MD progression rate based on bilateral data was 1.14 ± 0.23 dB/year. Additionally, the mean ± SE decline rates based on bilateral data of b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG were 18.06 ± 3.06 µV and 5.45 ± 1.08 µV, respectively.

Progression Rates From Longitudinal Analysis Using Mixed-Effect Linear Regression

A mixed-model linear regression method was used to determine the overall progression rate for each parameter. The mean ± SE annual progression of BCVA, MD value of VF, and b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG were 0.068 ± 0.012 logMAR units ($P < 0.001$), 0.86 ± 0.11 dB ($P < 0.001$), −13.29 ± 2.16 µV ($P < 0.001$), and −3.75 ± 0.59 µV ($P < 0.001$), respectively (Table 5).

Progression Rates From Cross-Sectional Analysis Using Baseline Data

To obtain the cross-sectional progression rate, we analyzed data on visual function versus age at baseline and calculated the annual progression rates of BCVA, MD values of VF, and b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG (Table 6). The results showed that the mean ± SE annual progression rates based on bilateral data were 0.011 ± 0.008 logMAR units, 0.47 ± 0.18 dB, −1.85 ± 3.22 µV, and −1.07 ± 0.90 µV, respectively, which were much slower than those obtained from the longitudinal data.

Interocular Symmetry

We used the Bland–Altman method and ICC to assess interocular symmetry in baseline data, overall data, and progression rates for each study parameter. In the Bland–Altman analysis, the cases within the 95% confidence interval for 95% limits of agreement was exceeded 90% for all four parameters in baseline data, overall data, and progression rates, implying that the interocular symmetry was good for all these parameters (Table 7). Additionally, the interocular symmetry in baseline data and overall data, assessed using ICC, was moderate for BCVA (ICCs = 0.692) and very high for the MD values of VF (ICCs > 0.95), scotopic ERG (ICCs > 0.95), and photopic ERG (ICCs > 0.95). The interocular symmetry in progression rates assessed using ICC, was moderate for both BCVA (ICC = 0.696) and the MD values of VF (ICC = 0.727), and was high or very high for both scotopic ERG (ICC = 0.867) and photopic ERG (ICC = 0.975) (Table 8).

Discussion

The development of clinical trials on gene therapy in IRDs necessitates a comprehensive understanding of the natural rate of disease progression and the establishment of reliable functional outcome measures. Meanwhile, it is essential to gain insight into the interocular symmetry of visual function to guide the design of clinical trials. This study aimed to contribute to the existing knowledge on this topic by conducting the largest natural history study to date to evaluate longitudinal visual functional characteristics and interocular symmetry in a cohort of patients with molecularly confirmed BCD.

Although several case and cross-sectional studies have investigated VA follow-up and characteristics in patients with BCD, no longitudinal studies with large BCD cohorts have been conducted. In a 20-year follow-up study of one patient with BCD, Lockhart et al.[4] reported a pooled slope of +0.024 logMAR per year for BCVA. In a cross-sectional study of 208 patients with BCD, Li et al.[12] predicted an annual progression rate of 0.049 logMAR units in the group aged more

Table 1. General Characteristics of the Patients With BCD

			BCVA		VF-MD		3.0 Scotopic ERG		3.0 Photopic ERG	
Patient	**Sex**	**Age at Visit, Years**	**RE**	**LE**	**RE**	**LE**	**RE**	**LE**	**RE**	**LE**
1	Female	43.2	0.05	0.30	23.3	24.0	263	251	73	87
		54.0	0.60	2.70	27.9	27.9	0	0	0	0
2	Male	26.3	−0.18	−0.18	/	/	371	400	106	131
		28.4	−0.08	−0.08	3.2	3.5	/	/	/	/
		30.9	0.00	0.00	4.9	6.6	256	269	88	98
		36.5	0.10	0.40	15.5	14.4	93	103	23	28
		40.0	0.22	0.40	21.9	20.9	/	/	/	/
		41.4	0.40	0.60	22.7	23.6	0	0	19	17
		42.3	0.82	0.82	/	/	/	/	/	/
3	Male	41.2	0.40	0.70	/	/	0	0	12	8
		46.0	0.60	1.10	/	/	/	/	/	/
		49.4	0.82	0.92	/	/	0	0	0	0
4	Male	26.2	0.00	0.22	12.8	9.9	/	/	/	/
		32.3	0.00	0.30	23.0	24.6	/	/	/	/
5	Male	42.6	0.40	0.40	/	/	/	/	/	/
		47.6	0.70	0.70	/	/	/	/	/	/
		50.3	0.82	1.00	/	/	/	/	/	/
6	Female	39.0	−0.08	−0.08	/	/	/	/	/	/
		44.2	−0.08	−0.08	/	/	/	/	/	/
		46.7	0.10	0.22	/	/	/	/	/	/
7	Female	48.7	0.70	0.40	/	/	254	264	58	53
		55.2	0.70	0.40	/	/	131	75	24	16
8	Female	25.5	0.10	1.00	3.3	1.7	/	/	/	/
		32.6	0.10	1.40	8.9	14.7	/	/	/	/
		34.5	0.22	1.40	/	/	/	/	/	/
9	Female	44.3	0.92	0.92	/	/	50	44	19	14
		51.5	2.70	2.60	/	/	0	0	0	0
10	Female	31.3	0.40	0.30	18.1	21.1	0	0	0	0
		32.7	0.40	0.22	/	/	/	/	/	/
		35.7	0.52	0.92	21.8	25.8	/	/	/	/
		36.7	0.60	0.82	/	/	/	/	/	/
		37.9	0.70	1.22	/	/	/	/	/	/
11	Female	31.4	0.10	0.22	22.0	21.1	122	114	22	20
		38.1	0.22	2.70	28.1	26.2	0	0	0	0
12	Female	21.3	0.30	0.40	22.7	20.7	/	/	/	/
		30.1	0.70	1.00	24.7	24.5	/	/	/	/
13	Female	41.8	0.10	0.10	17.3	13.8	/	/	/	/
		45.6	0.30	0.22	17.5	15.5	/	/	/	/
14	Female	26.3	−0.08	0.00	2.8	3.7	429	405	121	119
		32.0	0.00	0.00	7.1	10.1	423	365	108	111
		34.7	0.10	0.10	11.5	13.6	160	124	48	39
15	Female	25.0	0.82	−0.08	/	/	/	/	/	/
		29.1	2.70	0.92	/	/	/	/	/	/
16	Female	37.0	0.70	0.92	/	/	/	/	/	/
		44.6	1.40	1.70	/	/	/	/	/	/
17	Male	34.1	0.30	0.30	/	/	60	53	21	17
		38.4	0.60	0.70	/	/	15	15	8	5
18	Male	29.6	0.22	0.22	21.8	23.1	162	174	64	56
		30.7	0.22	0.30	/	/	/	/	/	/
		33.4	0.30	0.52	23.4	25.0	93	75	47	37
		34.9	0.30	0.70	20.4	25.2	64	36	39	24
19	Female	34.4	0.10	0.10	11.4	14.7	174	170	40	36
		37.5	0.30	0.30	19.4	26.7	68	62	15	13
20	Male	42.0	0.52	0.52	/	/	/	/	/	/
		44.6	0.70	0.70	/	/	/	/	/	/
21	Male	27.4	0.82	1.00	9.6	15.6	/	/	/	/
		28.4	0.82	1.00	13.4	16.2	/	/	/	/
22	Male	31.0	0.10	0.22	12.8	14	120	115	80	77
		33.4	0.22	0.22	18.1	19.5	40	50	38	39
		36.0	0.52	0.22	/	/	/	/	/	/
23	Female	39.1	0.92	2.70	/	/	0	0	0	0
		40.3	0.70	2.70	/	/	/	/	/	/
		41.7	0.70	2.70	/	/	/	/	/	/
		43.4	0.92	2.70	/	/	/	/	/	/

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Table 1. Continued

Patient	Sex	Age at Visit, Years	BCVA		VF-MD		3.0 Scotopic ERG		3.0 Photopic ERG	
			RE	LE	RE	LE	RE	LE	RE	LE
24	Female	32.2	0.22	0.10	/	/	/	/	/	/
		33.9	0.22	0.22	/	/	/	/	/	/
25	Male	30.3	0.40	0.22	/	/	/	/	/	/
		31.6	0.40	0.22	/	/	/	/	/	/
26	Female	37.1	0.10	0.10	23.2	22.8	/	/	/	/
		38.1	0.22	0.10	/	/	/	/	/	/
		41.0	0.22	0.10	23.0	21.8	/	/	/	/
27	Female	24.8	0.52	0.30	/	/	18	16	18	16
		30.6	0.40	0.22	/	/	0	0	20	10
28	Female	47.3	0.30	0.22	20.4	22.0	215	125	59	54
		50.2	0.70	0.70	24.2	25.6	103	91	37	39
29	Female	31.4	0.40	0.40	23.3	24.3	7	16	16	7
		36.1	1.00	1.00	26.8	26.9	0	0	0	0

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography.

than 40 years ($P < 0.01$), using a linear regression model. In a cross-sectional study involving 21 patients with BCD, Chan et al.[7] reported that logMAR VA increased with age between VA = 0.0018 age^2 – 0.0793 age + 0.3079 and VA = 0.0002 age^2 + 0.0157 age − 0.7738. In our study, we found that the BCVA progression rate from longitudinal analysis using averaged individual progression rates was 0.079 logMAR units/year. This rate was faster than those reported by Lockhart et al. and Li et al. and falls within the range reported by Chan et al. However, the results of our study may be more representative, given the cross-sectional nature of the studies by Li et al.[12] and Chan et al.,[7] and the small sample size ($n = 1$) in the study by Lockhart et al.[4]

In contrast with kinetic perimetry, static automated perimetry can measure retinal sensitivity and MD and contribute to the longitudinal assessment of IRD over time, either because of disease progression or in response to novel therapies. The Octopus 900 device has also demonstrated good repeatability and has been used in important prospective studies on IRD.[13,14] Tee et al.[14] previously reported an annual decline of 0.69 dB/year in mean sensitivity using the Octopus 900 device with Goldmann size V stimulus in a prospective study of *RPGR*-related patients with RP. However, there are no reports about the annual progression of the mean sensitivity or MD of VF in patients with BCD. In this study, we reported for the first time that the mean annual increase in MD progression rate was 1.135 dB (17.2%) in such patients. Although limited literature has been published on the progression of VF in static automated Octopus Perimetry with a size III stimulus in patients with RP or BCD, Xu et al.[15] reported annual rates of decline in the VF area using a Humphrey device for V4e, III4e, and I4e targets of 7.5%, 10.7%, and 12.5%, respectively, based on data from 52 patients with RP. Moreover, Nagy et al.[16] estimated that the annual progression of VF loss for target III4e was approximately 14.5% for RP. Although the progression rate of MD values used in the current study cannot be directly compared with the progression rate of VF area used in the aforementioned studies, our results demonstrate a relatively rapid rate of VF progression.

Our results from ffERG testing showed that impairment of rod function occurs before impairment of cone function, supporting the idea that the progression of BCD may follow a rod–cone dystrophy pattern, which is consistent with previous reports.[17,18] Although previous studies have reported attenuated amplitudes in the b-waves of scotopic and photopic responses,[18–20] the longitudinal assessments of ffERG worsening are limited. For instance, Lockhart et al.[4] reported that ffERGs were initially normal in both eyes of a 27-year-old patient with BCD, slowly declined over time, and were significantly below normal when the patient was 39 years old. Similarly, Yanagi et al.[21] found that one patient had no obvious progression on ERG during a 3-year follow-up, and the other two had progression during a 2-year follow-up, but the progression rate was not specified further. To fill this gap, we conducted a longitudinal study of ffERG progression rate in a large BCD cohort. Our findings revealed that the mean b-wave amplitudes at baseline were severely reduced for both scotopic 3.0 (156.86 µV) and photopic 3.0 (50.14 µV) ERG responses according to the normal value of our center, indicating that severe impairment of rod- and cone-mediated retinal function had already occurred at baseline. Moreover, our results showed that the b-wave amplitude decline rate was −18.06 µV/year (−16.48%) for scotopic 3.0 ERG and −5.45 µV/year (−14.22%) for photopic 3.0 ERG responses, suggesting that ERG worsens at a very rapid rate. Among 15 patients, 7, whose mean age was 43.02 ± 8.50 years, presented with nonrecordable scotopic/scotopic and photopic responses at the final visit, suggesting that severe panretinal dysfunction occurred at approximately 43 years of age. This finding implies that ffERG testing may lose its value in patients in advanced stages, and other tests such as full-field stimulus testing may be required to monitor the progression of retinal visual function. However, ffERG testing is valuable for the early diagnosis and evaluation of disease progression in patients with early stage BCD.

Previous studies have examined the interocular symmetry of fundus crystals and the absent autofluorescence area in patients with BCD.[22] However, the interocular symmetry of visual function parameters has not been investigated. This study is the first to analyze interocular symmetry for visual function parameters (including BCVA, VF, and ffERG) in patients with BCD in baseline data, overall data, and progression rates. Our analysis found that the interocular

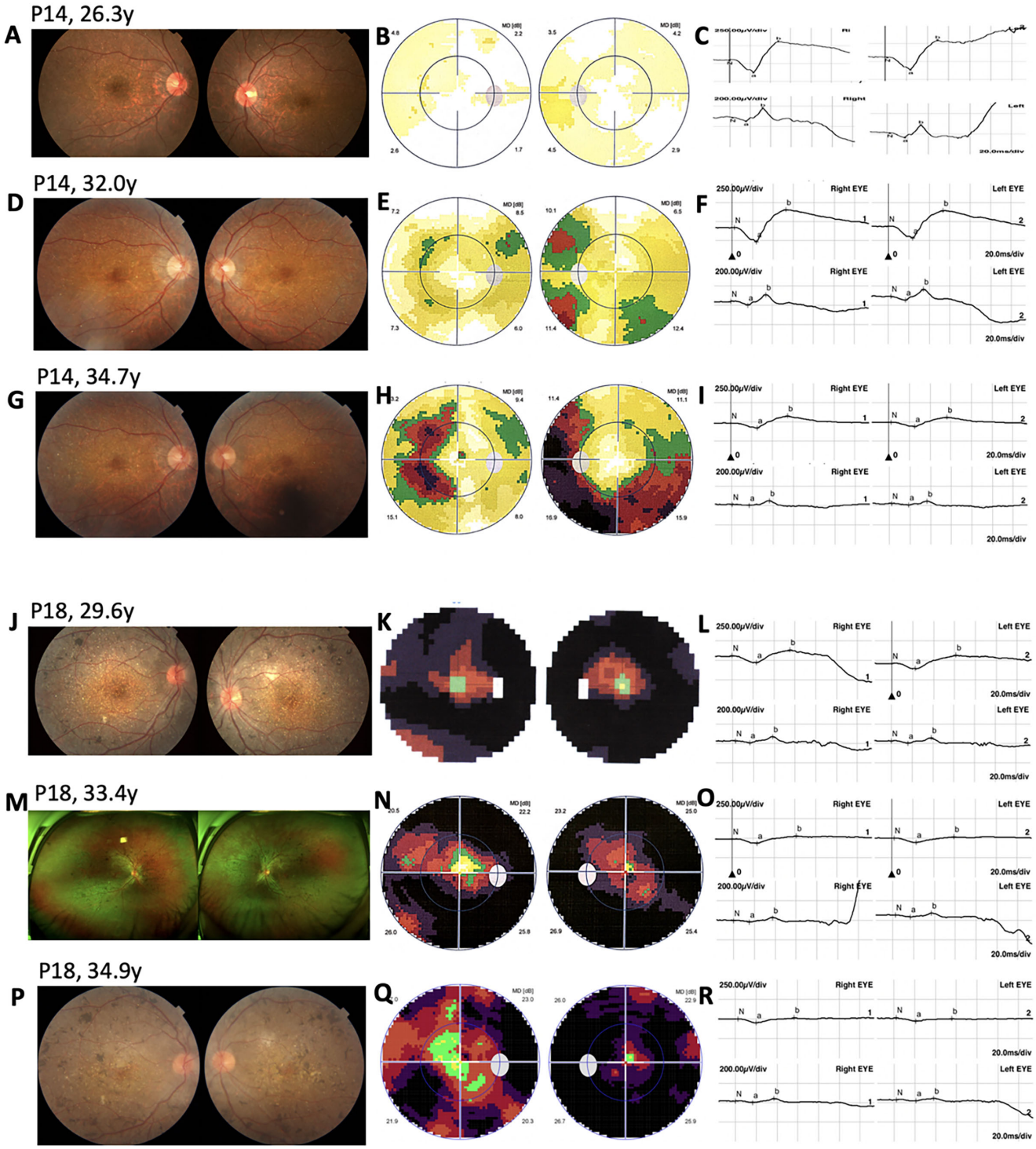

Figure 1. Color fundus photographs (CFPs), VFs, and ffERGs examples from two patients with BCD (P14 and P18) during the follow-up. The CFPs (**A**, **D**, **G**, **J**, **M**, **P**) show the variable extent of small yellow-white crystalline deposits dispersed throughout the posterior pole and variation in chorioretinal atrophy. The VF results (**B**, **E**, **H**, **K**, **N**, **Q**) show a progression in VF loss. The ffERG results (**C**, **F**, **I**, **L**, **O**, **R**) show a decline in b-wave amplitudes of scotopic 3.0 ERG and photopic 3.0 ERG responses during the follow-up.

symmetries for the MD values of VF and ffERG were good, indicating that they are valuable parameters for comparing treated and control eyes in clinical trials. However, BCVA showed a moderate interocular symmetry, as determined by ICCs, and good interocular symmetry was determined using the Bland–Altman method. Therefore, it may not be a perfect parameter for internal control. Nonetheless, considering its impact on daily living, VA should be included as a parameter for identifying suitable candidates and quantifying changes in treatment studies. Although there are no previous studies

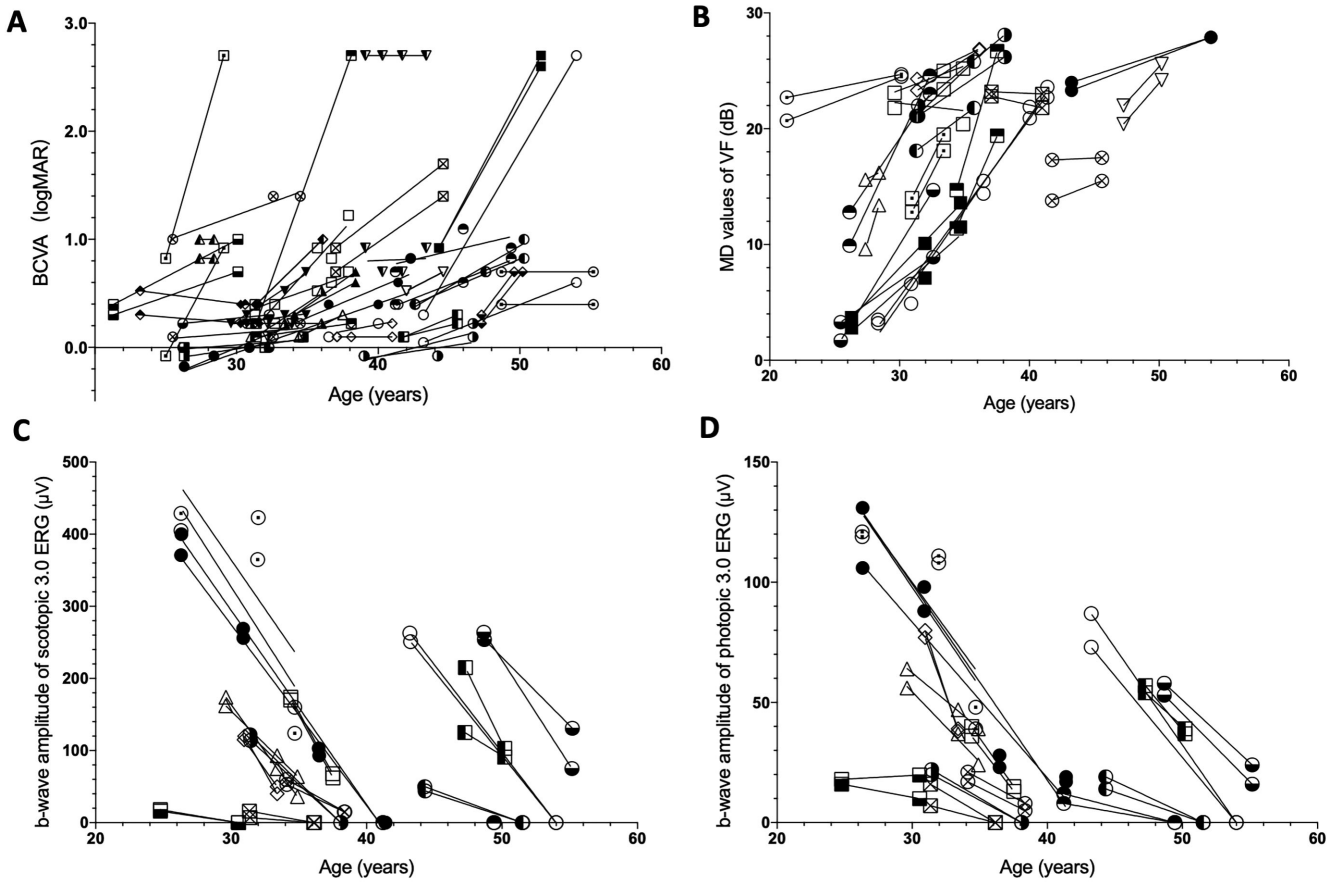

Figure 2. Scatter plots of the correlations between parameters and age for each patient with BCD at each visit. (**A**) BCVA (logMAR) and age, (**B**) MD values of VF and age, (**C**) b-wave amplitude of scotopic 3.0 ERG and age, and (**D**) b-wave amplitude of photopic 3.0 ERG and age. The spots of the *left* and *right eyes* of the same patient are represented by the same icon in each picture, and the straight line represents the progression rate of each eye.

Table 2. Summary of Visual Functional Measurements in Patients With BCD

		Age at the Initial Measurement		No. of Visits		Follow-up Periods	
	No. of Subjects	Mean ± SD	Median (Range)	Mean ± SD	Median (Range)	Mean ± SD	Median (Range)
BCVA	29	34.2 ± 7.5	32.2 (21.3–48.7)	2.7 ± 1.1	2 (2–7)	5.9 ± 3.1	5.8 (1.0–16.0)
VF	16	32.1 ± 7.1	31.1 (21.3–47.3)	2.3 ± 0.8	2 (2–5)	5.8 ± 3.2	5.0 (1.0–13.0)
ffERG	14	35.3 ± 8.1	32.8 (24.8–48.7)	2.3 ± 0.6	2 (2–4)	6.5 ± 3.4	6.1 (2.4–15.0)

Table 3. Baseline Values of Visual Functional Parameters

	Baseline Values of Both Eyes		Baseline Values of Right Eyes		Baseline Values of Left Eyes	
	Mean ± SD	Median (p25, p75)	Mean ± SD	Median (p25, p75)	Mean ± SD	Median (p25, p75)
BCVA/logMAR	0.37 ± 0.44	0.30 (0.10, 0.52)	0.33 ± 0.31	0.30 (0.10, 0.52)	0.41 ± 0.54	0.30 (0.10, 0.40)
VF-MD/dB	15.75 ± 7.57	17.70 (10.65, 22.35)	15.50 ± 7.64	17.70 (10.50, 22.35)	16.00 ± 7.74	18.15 (11.85, 22.40)
3.0 scotopic ERG/μV	156.86 ± 131.65	123.50 (47.00, 252.50)	160.36 ± 134.13	142.00 (50.00, 254.00)	153.36 ± 134.09	120.00 (44.00, 251.00)
3.0 photopic ERG/μV	50.14 ± 37.51	46.50 (17.50, 75.00)	50.64 ± 35.36	49.00 (19.00, 73.00)	49.64 ± 40.88	44.50 (16.00, 77.00)

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography.

Table 4. Progression Rate From Longitudinal Analysis Using Averaged Individual Progression Rates

Progression Rates		Progression Rates of Both Eyes			Progression Rates of Right Eyes			Progression Rates of Left Eyes		
		Mean ± SD	SE	Median (p25, p75)	Mean ± SD	SE	Median (p25, p75)	Mean ± SD	SE	Median (p25, p75)
BCVA	logMAR units/y	0.079 ± 0.082	0.016	0.055 (0.029, 0.097)	0.061 ± 0.093	0.017	0.045 (0.010, 0.069)	0.081 ± 0.092	0.017	0.064 (0.010, 0.103)
	%/y	18.13 ± 114.97	22.99	24.33 (10.12, 35.50)	19.44 ± 30.52	5.77	13.24 (0.00, 29.08)	12.75 ± 73.85	13.96	12.24 (0.00, 35.98)
VF-MD	dB/y	1.14 ± 0.91	0.23	1.01 (0.36, 1.79)	1.11 ± 1.04	0.26	0.87 (0.33, 1.63)	1.16 ± 1.02	0.25	0.91 (0.44, 1.68)
	%/y	17.22 ± 21.81	5.45	5.69 (1.74, 25.14)	13.81 ± 16.01	4.00	5.47 (1.42, 23.09)	17.28 ± 27.38	6.85	4.37 (2.16, 25.02)
3.0 scotopic ERG	μV/y	−18.06 ± 11.44	3.06	−23.10 (−26.06, −6.52)	−18.55 ± 12.55	3.35	−18.70 (−27.34, −6.94)	−17.57 ± 11.80	3.15	−20.26 (−26.94, −6.10)
	%/y	−16.48 ± 7.77	2.16	−15.57 (−19.86, −9.53)	−14.67 ± 6.26	1.74	−15.04 (−17.73, −9.31)	−14.32 ± 5.35	1.48	−14.99 (−17.33, −9.31)
3.0 photopic ERG	μV/y	−5.45 ± 4.06	1.08	−5.37 (−7.45, −2.40)	−5.51 ± 4.20	1.12	−4.96 (−7.48, −3.07)	−5.39 ± 4.01	1.07	−5.40 (−8.10, −1.94)
	%/y	−14.22 ± 8.01	2.14	−13.43 (−17.38, −9.39)	−11.94 ± 6.48	1.73	−12.42 (−15.04, −7.31)	−12.78 ± 5.19	1.39	−11.46 (−16.69, −9.31)

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography.

Table 5. Progression Rates From Longitudinal Analysis Using Mixed-Effect Linear Regression

	Progression Rates		
	Mean	SE	*P* Value
BCVA	0.068	0.012	<0.0001
VF-MD	0.86	0.11	<0.0001
3.0 scotopic ERG	−13.29	2.16	<0.0001
3.0 photopic ERG	−3.75	0.59	<0.0001

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography.

on the interocular symmetry of visual function in BCD, our findings are consistent with most studies on other IRDs that found strong interocular symmetry in the VF, but relatively weak interocular symmetry in the VA.[13,15,23,24]

Owing to the limitations of this study, such as being retrospective and having a small sample size, it is currently not possible to provide a definitive conclusion on which method is the most accurate to calculate the progression rate. However, the present results showed that the annual progression rates calculated from the cross-sectional data were significantly slower than those calculated from longitudinal data, which is consistent with a previous study.[25] This finding indicates that the progression rate cannot be inferred accurately from cross-sectional data. The annual progression rates obtained using mixed-effect linear regression models were also closer to those using averaged individual progression rates than those using the cross-sectional data. The longitudinal analysis using mixed-effect linear regression model, although more advanced and complex in its approach, typically requires a larger sample size for robust support. The sample size in this study may not be sufficient to meet the requirements of this model, which leads us to speculate that the longitudinal analysis using averaged individual progression rates is more accurate in this study. These results need to be replicated in future prospective studies with larger sample sizes and longer follow-up duration. Nonetheless, the difference between cross-sectional and longitudinal progression rates indicates that cross-sectional data from patients of different ages should not be used to infer progression rates from longitudinal measurements in natural history studies. Therefore, longitudinal studies are essential to accurately measure the disease

Table 6. Cross-Sectional Progression Rate From Baseline Measurements

	Progression Rates of Both Eyes				Progression Rates of Right Eyes				Progression Rates of Left Eyes			
	Mean	SE	*P* Value	R^2	Mean	SE	*P* Value	R^2	Mean	SE	*P* Value	R^2
BCVA	0.011	0.008	0.154	0.036	0.009	0.008	0.244	0.050	0.013	0.014	0.343	0.033
VF-MD	0.47	0.18	0.013	0.013	0.47	0.26	0.096	0.186	0.48	0.26	0.089	0.193
3.0 scotopic ERG	−1.85	3.22	0.570	0.013	−1.18	4.76	0.809	0.005	−2.53	4.71	0.602	0.023
3.0 photopic ERG	−1.07	0.90	0.245	0.052	−0.98	1.22	0.441	0.050	−1.16	1.41	0.427	0.053

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography.

Table 7. Interocular Symmetry Using the Bland–Altman Method

	Baseline Data			Overall Data			Progression Rate Obtained Using Averaged Progression Rates From Each Eye		
	Mean ± SD	CI LOA	No. (%) Within CI LOA	Mean ± SD	CI LOA	No. (%) Within CI LOA	Mean ± SD	CI LOA	No. (%) Within CI LOA
BCVA	−0.08 ± 0.43	−1.19 to 1.02	28 (97)	−0.21 ± 0.64	−1.72 to 1.39	70 (91)	−0.02 ± 0.09	−0.25 to 0.21	27 (93)
VF-MD	−0.50 ± 2.42	−7.28 to 6.28	16 (100)	−1.06 ± 2.48	−7.29 to 5.18	36 (97)	−0.05 ± 0.95	−2.71 to 2.61	15 (94)
3.0 scotopic ERG	7.00 ± 27.02	−70.17 to 84.17	13 (93)	8.72 ± 23.56	−51.40 to 68.84	31 (97)	−0.97 ± 8.36	−24.84 to 22.89	13 (93)
3.0 photopic ERG	1.00 ± 9.18	−25.22 to 27.22	14 (100)	1.62 ± 7.51	−17.55 to 20.79	31 (97)	−0.12 ± 1.28	−3.77 to 3.52	14 (100)

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography; CI LOA, 95% confidence interval for 95% limits of agreement.

Table 8. Interocular Symmetry Using the ICC

	Baseline Data			Overall Data			Progression Rate Obtained Using Averaged Progression Rates From Each Eye		
	ICC	95% CI	*P* Value	ICC	95% CI	*P* Value	ICC	95% CI	*P* Value
BCVA	0.692	0.344 to 0.855	0.001	0.692	0.349 to 0.855	0.001	0.696	0.352 to 0.857	0.001
VF-MD	0.975	0.927 to 0.991	0.000	0.970	0.941 to 0.984	0.000	0.727	0.219 to 0.905	0.008
3.0 scotopic ERG	0.990	0.968 to 0.997	0.000	0.991	0.981 to 0.996	0.000	0.867	0.585 to 0.957	0.000
3.0 photopic ERG	0.985	0.954 to 0.995	0.000	0.989	0.977 to 0.995	0.000	0.975	0.923 to 0.992	0.000

3.0 scotopic ERG, b-wave amplitude of scotopic 3.0 electroretinography; 3.0 photopic ERG, b-wave amplitude of photopic 3.0 electroretinography; CI, confidence interval.

course. Given that ascertainment bias can affect the progression rate obtained from cross-sectional data, estimating these parameters over time, as in our longitudinal analysis, is a reliable method to obtain accurate progression rates.

Given that one of the study's limitations is that it is retrospective, a prospective longitudinal study is warranted to confirm our findings. The second limitation of the study was having a limited sample size owing to the low incidence rate of BCD and the strict inclusion criteria used. Future studies with larger sample sizes are required to provide more accurate information regarding the natural history of BCD. Third, this study evaluated the central 30° VF, whereas for a rod–cone dystrophy, a more comprehensive approach is to conduct a wide-field VF testing. It is necessary for future studies to address this aspect. Moreover, we recommend including full-field stimulus testing and microperimetry in future studies to measure the progression of visual function.

In conclusion, our study is the first to describe a longitudinal characterization of visual function in a large cohort of patients with BCD. We used three different methods to assess the annual progression rates of visual function and found that the progression rates from longitudinal analysis using averaged progression rates from each eye and using mixed-effect linear regression were comparable. However, the annual progression rates obtained from cross-sectional data were significantly slower, indicating that progression rates cannot be accurately inferred from cross-sectional data from patients of different ages. We found that the annual progression rates of BCVA, VF, and ffERG were rapid and could provide information on disease progression and identify suitable candidates for future therapeutic trials. VF and ffERG showed significant interocular symmetry, making them suitable parameters for internal controls in treatment trials. These findings expand our limited knowledge of the natural history of BCD and provide insights into the dynamics of disease progression, which could also be useful for providing prognostic information to patients. Finally, we believe that the methods used in this study to assess progression rates and interocular symmetry can be applied to studies involving other IRDs.

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Appendix 1. SAS codes for mixed-effect model

```
"proc mixed data=BCVA_long plots=all;
class id id1;
model BCVA = age/solution cl;
random intercept Age / type=un
sub=id1(id) g;
run;"
"BCVA_long" represents our database,
"id" represents the individual iden-
tifier, "id1" represents to the iden-
tifier for each eye, "BCVA" repre-
sents the logMAR visual acuity values,
and "age" represents the age parame-
ter.
```