Choroidal and Retinal Atrophy of Bietti Crystalline Dystrophy Patients with *CYP4V2* Mutations Compared to Retinitis Pigmentosa Patients with *EYS* Mutations

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Short title:
Choroidal and retinal atrophy of BCD

Conflict of Interest:
None declared.

Funding/Support:
This work was supported in part by the Innovative Techno-Hub for Integrated Medical Bio-Imaging of the Project for Developing Innovation Systems, from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

Financial Disclosures:
NY: Topcon Corporation (Financial Support), Nidek (Financial Support, Consultant), Canon (Financial Support).

KEY WORDS

Bietti crystalline dystrophy; choroid; CYP4V2; EYS; optical coherence tomography; retinitis pigmentosa

BRIEF SUMMARY STATEMENT

We found severe atrophy in all layers of the retina, retinal pigment epithelium/Bruch's membrane, and choroid in the macular region in Bietti crystalline dystrophy patients with *CYP4V2* mutations, while only the retina was severely atrophic in retinitis pigmentosa patients with *EYS* mutations.

ABSTRACT

Purpose

To compare atrophy of the choroid and retina between Bietti crystalline dystrophy (BCD) patients and *EYS*-related retinitis pigmentosa (RP) patients with a similar degree of central visual field defects, age, and axial length (AL).

Methods

Nine eyes of nine BCD patients with *CYP4V2* mutations (BCD group) were examined. Moreover, we selected ten eyes of ten RP patients with *EYS* mutations matched for age, AL, and mean deviation (measured with the 10-2 SITA standard program; *EYS*-RP group), and ten eyes of ten normal volunteers matched for age and AL (control group). Macular thicknesses of the choroid and retina were measured via swept-source optical coherence tomography.

Results

The macular choroid was significantly thinner in the BCD group than in the *EYS*-RP and control groups, although the thickness did not significantly differ between the *EYS*-RP and control groups. The macular retina was significantly thinner in the BCD and *EYS*-RP groups than in the control group, although the thickness did not significantly differ between the BCD and *EYS*-RP groups at most sites.

Conclusion

BCD patients with *CYP4V2* mutations showed more severe macular choroid atrophy as compared to *EYS*-related RP patients. These different damage patterns suggest differences in choroidal expression between *CYP4V2* and *EYS*.

INTRODUCTION

Bietti crystalline dystrophy (BCD) is a rare retinal dystrophy characterized by the presence of yellow and shiny deposits on the cornea and posterior pole of the retina, along with progressive atrophy of the retina, choriocapillaris, and choroid.[1] A long-term follow-up study showed that the formation of retinal crystals is followed by gradual atrophy of the retinal pigment epithelium (RPE) and constriction of the visual field.[2] BCD is caused by mutations of the *CYP4V2*.[3] The phenotype of *CYP4V2*-related BCD is highly variable[4] and the mechanisms underlying the pathological changes in BCD remain unclear because of the rarity of the disease; therefore, no efficacious treatment is currently available.

The symptoms of BCD, including night blindness and progressive constriction of the visual field, are similar to those of retinitis pigmentosa (RP). However, gene expression patterns of *CYP4V2* and the causative genes for RP are reportedly different. In particular, *CYP4V2* is expressed in the retina and RPE in the eyes.[3] Although the RPE is generally thought to be the site of the primary pathological abnormality in BCD patients with *CYP4V2* mutations, Nakano et al. showed weak staining of ganglion cells and internal/external nuclear layers in the retina by immunohistochemical staining.[5] Their figure also suggested *CYP4V2* expression in the choroid. In contrast, the human gene *EYS*, which is the most frequently mutated gene in Japanese RP patients,[6, 7] encodes a protein that is localized in the outer segments of photoreceptors.[8] Thus, the difference in expression patterns of the causative genes is responsible for the degree and the timing of the damage to each neuroretinal and choroidal layer.

Although the degenerative changes in RP mainly occur in photoreceptor cells, Dhoot et al. reported that the macular choroidal thickness was significantly decreased in patients with RP.[9] The precise mechanism of choroidal thinning in RP is unclear. However, several studies have shown that the death of RPE cells secondary to the death of photoreceptor cells is implicated in choriocapillary atrophy.[10, 11] In fact, vascular endothelial growth factor (VEGF) is produced by the RPE and is necessary for choroidal maintenance[12]; therefore, a lack of VEGF due to an RPE disorder may play a role in choroidal thinning. Recently, Saatoci et al. demonstrated the thinning of the retina and choroid in BCD.[13] Considering the differences in the sites of expression of the causative gene between BCD and *EYS*-related RP, we believe that the degree and timing of the damage to each neuroretinal layer and choroid layer may differ between these two diseases. In the present study, we compared the presence of macular atrophy of the retina, RPE/Bruch's membrane (BM), and choroid between BCD patients with *CYP4V2* mutations and *EYS*-RP patients at a similar disease stage.

METHODS

This study was approved by the ethics committee of Kyoto University, Graduate School of Medicine (Kyoto, Japan) and adhered to the tenets of the Declaration of Helsinki. The nature of the study and the possible consequences of participation were explained to all candidates, and written informed consent was obtained from all participants.

Subjects

Consecutive patients with retinal degenerative disease or retinal dystrophy who visited the Department of Ophthalmology and Visual Sciences at Kyoto University Graduate School of Medicine (Kyoto, Japan) between January 2011 and October 2015 were recruited to participate in the study. All patients provided blood samples for the detection of gene mutations. All participants also underwent a comprehensive ophthalmologic examination, including measurement of best-corrected visual acuity (BCVA) with a Landolt C chart and axial length (AL) with an IOL Master (Carl Zeiss Meditec). All BCVA data were converted to the logarithm of the minimal angle of resolution (logMAR) for statistical analysis. Slit-lamp biomicroscopy, indirect ophthalmoscopy, color fundus photography (TRC-NW8F; Topcon Corp, Tokyo, Japan), fundus autofluorescence (FAF) with an Optos device (Optos PLC, Scotland, United Kingdom), spectral domain optical coherence tomography (SD-OCT, Spectralis; Heidelberg Engineering, Dossenheim, Germany), swept source optical coherence tomography (SS-OCT, Topcon Corp., Tokyo, Japan), mean deviation (MD) measurements with a Humphrey field analyzer (HFA; Carl Zeiss Meditec, Dublin, CA) with the 10-2 Swedish Interactive Threshold Algorithm standard program for the evaluation of macular sensitivity, and 30-Hz flicker electroretinography (ERG) were also performed. ERG results were recorded according to the International Society for Clinical Electrophysiology of Vision standard protocol recommended in 2008 with the LS-C (Mayo Co., Nagoya, Japan) and Neuropack MEB-2204 systems (Nihon Kohden, Tokyo, Japan).[14] With regard to inclusion criteria, we enrolled BCD patients with *CYP4V2* mutations and with clear SD-OCT and SS-OCT images available. A clinical diagnosis of BCD was made based on the presence of characteristic fundus crystalline deposits, retino-choroidal dystrophy, and patchy RPE atrophy, whereas *CYP4V2* mutations were detected via Sanger sequencing. The exclusion criteria were as follows: missing necessary data and the presence of other eye diseases except for refractive errors, cataract, and pseudophakia. When both eyes in one patient met the criteria, one eye was randomly selected for analysis. We also examined Japanese RP patients with *EYS* mutations detected by next-generation

sequencing[6] who were selected to be matched for age, AL, and MD among the consecutive patients, as well as Japanese volunteers with no eye disease except for refractive errors who were matched for age and AL. The BCVA of the volunteers was 20/20 or better.

SS-OCT measurement of choroidal and retinal thicknesses

For choroidal and retinal thickness measurements, we acquired horizontal and vertical B-scan images through the fovea with SS-OCT. The light source was a wavelength-sweeping laser with a tuning range of approximately 100 nm centered at 1050 nm, which yielded an axial resolution in tissue of 8 μm. Trained examiners performed the SS-OCT examinations after pupil dilation. They achieved pupil centration during the scan by using an internal fixation target, which was confirmed through a built-in camera within the SS-OCT system. Choroidal and retinal thicknesses were measured with a built-in caliper tool. The outermost highly reflective retinal band comprised the RPE and BM.[15] Retinal thickness was defined as the distance between the vitreoretinal interface and the outer border of the RPE/BM complex. Choroidal thickness was defined as the distance between the outer border of the RPE/BM complex and the chorioscleral interface (Figure 1). Each thickness was manually measured at the subfovea and at 0.5, 1.0, and 1.5 mm from the center of the fovea in the superior, temporal, inferior, and nasal quadrants (a total of thirteen points) with built-in caliper tool software and AL adjustment system. Central retinal thickness and subfoveal choroidal thickness measurements were made on horizontal and vertical scans, and the mean of the two measurements was used as a representative value.

SD-OCT measurement of the RPE/BM thickness

By using a previously described method,[16] we measured the thickness of the RPE/BM complex on horizontal and vertical B-scan images through the fovea, obtained with SD-OCT, as the axial resolution of SS-OCT (8 μm) is higher than that of SD-OCT(4–6 μm), which is adequate to measure a thin RPE/BM complex.[17] At each location of interest on the retina, we acquired 100 SD-OCT images and averaged them to reduce speckle noise. Thereafter, we manually measured the thickness of the RPE/BM complex at the same subfoveal and macular sites described above with the built-in caliper tool software and AL adjustment system. The representative subfoveal value was calculated as the mean of the horizontal and vertical measurements.

Statistical analysis

Data are presented as the mean ± standard deviation where applicable. One-way

analysis of variance was performed to compare the three groups. Corrections for multiple comparisons were made with a Tukey test. Comparisons of differences between the two groups, including age, AL, and MD matching, were performed using *t*-tests. We analyzed the correlations of the thickness values of the choroid, RPE/BM complex, and retina with age by using Pearson's rank correlation coefficient. The above statistical analyses were performed using SPSS version 21 (IBM, New York, NJ, USA). The cut-off value for subfoveal choroidal thickness, the best sensitivity–specificity balance, and the area under the receiver operating characteristic curve (AUROC) were calculated using MedCalc version 12 (MedCalc Software, Ostend, Belgium). A *P*-value of < .05 was considered statistically significant.

RESULTS

A total of nine eyes of nine Japanese BCD patients with *CYP4V2* mutations (five women and four men; BCD group); 10 eyes of 10 Japanese RP patients with *EYS* mutations who were matched for age, AL, and MD (four women and six men; *EYS*-RP group); and 10 healthy eyes of 10 Japanese volunteers (seven women and three men; control group) were included in this study. The characteristics of the study population are shown in Table 1 and clinical characteristics of the BCD group and *EYS*-RP group are shown in Table 2. The mean ages of the BCD group, *EYS*-RP group, and control group were not significantly different (BCD group vs *EYS*-RP group, $P = .31$; BCD group vs control group, $P = .36$; and *EYS*-RP group vs control group, $P = .82$). The mean AL was not significantly different between the groups (BCD group vs *EYS*-RP group, $P = .92$; BCD group vs control group, $P = .80$; and *EYS*-RP group vs control group, $P = .93$). The mean MD was not significantly different between the BCD group and *EYS*-RP group ($P = .17$).

Choroidal thickness

The mean subfoveal choroidal thicknesses in the BCD, *EYS*-RP, and control groups were 109.6 ± 30.3, 251.8 ± 55.2, and 285.7 ± 91.7 μm, respectively (Table 3). The macular choroid in the BCD group was significantly thinner than that in the control group at all sites and that in the *EYS*-RP group at all sites, except for the nasal site, 1.5 mm from the fovea. In contrast, there were no significant differences in the macular thickness of the choroid between the *EYS*-RP group and control group at all sites.

RPE/BM complex thickness

The mean subfoveal RPE/BM complex thicknesses in the BCD, *EYS*-RP, and control groups were 11.3 ± 1.6, 17.9 ± 3.6, and 23.5 ± 2.0 μm, respectively (Table 4). There

were significant differences in complex thickness among the three groups at all thirteen sites. The RPE/BM complex was thinner in the BCD group than in the *EYS*-RP group and thinner in *EYS*-RP than in the control group.

Retinal thickness

The mean central retinal thicknesses in the BCD, *EYS*-RP, and control groups were 117.9 ± 52.6, 180.3 ± 44.5, and 228.7 ± 22.4 μm, respectively (Table 5). Compared to the control group, the macular retina was significantly thinner in the BCD and *EYS*-RP groups at all sites. In contrast, the retinal thickness in the macular area of the BCD and *EYS*-RP groups was significantly different at only three sites.

Choroidal thickness uniformity in the BCD and *EYS*-RP groups

In the *EYS*-RP group, the choroid was significantly thinner at the nasal site, 1.5 mm from the fovea, than at the subfovea ($P < .01$) (Figure 2), and the choroid was significantly thinner nasal to the fovea than temporal to the fovea. However, no such difference was observed in the BCD group ($P = .29$). The choroidal thickness at 1.5 mm temporal to the fovea was not significantly different compared to the subfoveal choroidal thickness both in the BCD and *EYS*-RP groups ($P = .77$ and $P = .66$, respectively).

Differentiation of the BCD and *EYS*-RP groups by thickness analysis

When the cut-off value for the subfoveal choroidal, subfoveal RPE/BM complex, and central retinal thicknesses were set at 156.5, 12, and 166 μm, respectively, we could distinguish between the BCD and *EYS*-RP groups with a sensitivity of 100% and specificity of 90%, with a sensitivity of 89% and specificity of 100%, and with a sensitivity of 89% and specificity of 70%, respectively. With regard to the AUROC, the best sensitivity–specificity balance achieved was 0.978, 0.967, and 0.833, respectively.

Correlation between age and thickness

There were no significant correlations between age and the thickness of the subfoveal choroid, subfoveal RPE/BM complex, and central retina in the BCD group (choroid, $P = .60$; RPE/BM, .66; and retina, .91, respectively) or in the *EYS* group (choroid, $P = .51$; RPE/BM, .09; and retina, .34, respectively).

Differentiation of homozygotes and heterozygotes in the BCD group

The mutations detected in the study patients are listed in Table 2. Among the nine BCD patients, four had homozygous mutations and five had compound heterozygous

mutations. All patients had at least one c.802-8_810del17inGC mutation which is known to cause deletion of exon 7. There were no significant differences in age (57.5 ± 6.6 vs 58.2 ± 4.7 years), MD (-30.9 ± 2.7 vs -28.8 ± 4.9 dB), subfoveal choroidal thickness (96.4 ± 19.6 vs 120.1 ± 35.1 μm), subfoveal RPE/BM complex thickness (10.5 ± 0.4 vs 12.0 ± 1.9 μm), and central retinal thickness (103.9 ± 40.2 vs 129.2 ± 62.9 μm) between the cases with homozygous and compound heterozygous mutations (*P* = .22, .09, .15, .37, and .20, respectively). However, there was a significant difference in AL between them (24.4 ± 1.0 vs 24.1 ± 0.5 mm, *P* = .003).

Novel mutations in *CYP4V2*

We found two novel mutations (c.710C>A and c.1378T>C) in *CYP4V2* by identifying the pathogenicity of the variants. These two variants were predicted as damaging by silico prediction programs (SIFT and Polyphen2), and were not found in the Japanese exome database (Human Genetic Variation Database, http://www.genome.med.kyoto-u.ac.jp/SnpDB/ [in the public domain]).

DISCUSSION

This study clearly showed that the macular choroid in the eyes of BCD patients with *CYP4V2* mutations was significantly thinner than that in the eyes of RP patients with *EYS* mutations at a similar stage of visual field damage. The BCD group also had a thinner macular RPE/BM thickness as compared to the *EYS*-RP group. In contrast, the thickness of the macular retina did not markedly differ between the eyes of BCD patients with *CYP4V2* mutations and the eyes of RP patients with *EYS* mutations, although the macular retina was significantly thinner in both the BCD group and *EYS*-RP group than in the control group. These findings suggest that pathological atrophy occurs simultaneously in the retina, RPE/BM, and choroid in BCD patients with *CYP4V2* mutations. In the patients with *EYS*-RP, pathological atrophy occurs initially at the retina, followed by the RPE/BM and choroid. We matched participants according to MD values in the BCD and *EYS*-RP groups, and according to age and AL, as these parameters are associated with choroidal thickness in normal eyes.[18]

Li et al. reported that *CYP4V2* is expressed in the retina and RPE.[3] Another previous report also suggested expression of *CYP4V2* in the choroid in their immunohistochemistry figure.[5] In contrast, *EYS* is expressed in the outer segments of photoreceptors.[8] The expression of *CYP4V2* and *EYS* in the retina would cause retinal atrophy in the BCD and *EYS*-RP groups, respectively, consistent with our results; in particular, retinal thickness was decreased in the BCD and *EYS*-RP groups as compared to the control group. The expression of *CYP4V2* in the RPE/BM and

choroid could have led to the severe damage of the RPE/BM and choroid observed in the BCD group as compared to the *EYS*-RP and control groups.

A previous study reported that macular choroidal thickness was significantly decreased in patients with RP[9], inconsistent with our findings. The mean subfoveal choroidal thickness was 251.8 ± 55.2 μm in the *EYS*-RP group in the present study and 245.6 ± 103 μm in the previous study, whereas the thickness of the controls was 285.7 ± 91.7 μm in the present study and 337.8 ± 109 μm in the previous study. The differences in mean age (54.0 ± 11.2 versus 40.6 ± 12.9 years) and the possible difference in AL (mean spherical equivalent of −1.45 ± 2.61 diopters in the previous study and mean AL of 24.33 ± 1.26 mm in the present study) could explain these differences. The clinical findings of RP are variable because of the variability in the causative genes.[19] This study focused on *EYS* mutations because *EYS* is a major causative gene of RP not only in Japanese,[6, 7] but also in Spanish,[20] British and Chinese,[21] and Israeli and Palestinian populations.[22] At present, the choroidal thickness in cases of RP with *EYS* mutations has not been thoroughly investigated. Hence, further studies investigating the choroidal thickness in *EYS*-RP at various disease stages are warranted.

Although BCD can be classified into early, intermediate, and advanced stages,[23, 24] the choroidal thickness would not differ according to the stage and the choroid would become atrophic during the early stage. In the present study, the enrolled patients were at the early and intermediate stages and the mean subfoveal choroidal thickness of the BCD group was 107.5 ± 29.3 μm. A previous study reported that the mean subfoveal choroidal thickness was 95.37 ± 55.93 μm in intermediate- and late-stage BCD patients; however, the authors did not confirm the presence of *CYP4V2* mutations.[13] These values for the subfoveal choroidal thickness are substantially less than those in the controls. Furthermore, in the present study, case 4 (early stage) had a subfoveal choroidal thickness of 75 μm and case 7 (intermediate stage) had a subfoveal choroidal thickness of 81 μm. These values are similar to the mean subfoveal thickness values of patients in the previous study at the intermediate and late stage (95.37 μm). In the present study, the cut-off value for the subfoveal choroidal thickness was set at 156.5 μm, which enabled the discrimination between BCD with *CYP4V2* mutations and RP with *EYS* mutations (sensitivity of 100% and specificity of 90%). Therefore, it is important to monitor choroidal thickness, as it can be used an index for distinguishing BCD patients from *EYS*-RP patients even during the early stages.

Halford et al. reported that the phenotype was highly variable in BCD patients, and that deletions of exon 7 were associated with more severe disease.[4] In the present study, all BCD patients carried at least one mutation that caused the deletion

of exon 7; however, there was no significant difference in subfoveal choroidal atrophy, subfoveal RPE/BM complex atrophy, and central retinal atrophy between homozygotes and compound heterozygotes. The severity of the atrophy of the choroid, RPE/BM complex, and retina was similar between the patients who carried the c.802-8_810del17inGC mutation in the homozygous state and in the compound heterozygous state. However, we could not compare the severity between patients with deletion of exon 7 and with other mutations in the present study.

The distribution of the choroidal thickness may also help in distinguishing between BCD and RP with *EYS* mutations. The choroid was thinner in regions nasal to the fovea compared to regions temporal to the fovea in RP with *EYS* mutations; however, in BCD patients with *CYP4V2* mutations, the choroidal thickness became uniformly atrophic. Hence, longitudinal studies on the progression of choroidal atrophy in these patients are needed to investigate the causes.

Retinal and RPE sheet transplantation is being suggested as a potential treatment method for degenerative diseases such as age-related macular degeneration and RP.[25, 26] However, choroidal degeneration develops at the early stage of BCD and sheet transplantation of the choroid, along with the retina and RPE, would be necessary in those cases. Although retinal atrophy was confirmed in the BCD group, we recently reported that the cone photoreceptor cell density remained for visual dysfunction in BCD.[27] Hence, sheet transplantation of the choroid and RPE might be sufficient for resolving BCD. Gene therapy is another option for BCD. In fact, gene therapy for severe retinal dystrophy has previously been reported,[28-30] and would be beneficial for patients with *CYP4V2* mutations at any stage of BCD.

This study has some limitations, including the cross-sectional design. The choroid, RPE/BM, and retina were significantly thinner even in the early-stage BCD patients than in the controls in this study. However, the choroid was not significantly thinner in the *EYS*-PR group than in the control group. In the *EYS*-RP group, retinal atrophy is the primary change observed, followed by atrophy of the photoreceptor cells and RPE/BM; finally, atrophy of the choroid occurs. A longitudinal study evaluating the choroidal, RPE/BM complex, and retinal thicknesses will aid in understanding the time course of structural changes in BCD patients with *CYP4V2* mutations and RP patients with *EYS* mutations. Moreover, the thicknesses were manually measured, which could have introduced errors. However, most previous studies have used manual measurements, and the reliability of these methods is generally accepted. Third, all BCD patients carried the c.802-8_810del17inGC mutation, which was reported to be associated with more severe phenotype, although we did not select the BCD patients. This mutation is most commonly found in Japanese BCD patients.[31] Hence, further study should be performed in BCD patients

without this specific mutation.

In conclusion, BCD patients with *CYP4V2* mutations showed severe atrophy of the macular choroid, compared to the macular choroidal atrophy in RP patients with *EYS* mutations. These different patterns in damage suggested differences in the choroidal expression between *CYP4V2* and *EYS*.

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FIGURE LEGENDS

Figure 1. Vertical swept source optical coherence tomography (SS-OCT) images and Heidelberg retina angiography images of eyes in Bietti crystalline dystrophy patients with *CYP4V2* mutations, retinitis pigmentosa patients with *EYS* mutations, and control subjects.

Left Top. Representative 30° Heidelberg retina angiography (HRA) infrared images of an eye of a patient with Bietti crystalline dystrophy with *CYP4V2* mutations (Case 2) Sporadic crystalline deposits can be observed. The dots represent the measurement points at different distances from the fovea.

Red, subfovea; green, 0.5 mm; yellow, 1.0 mm; blue, 1.5 mm

Left Bottom. Representative 30° HRA autofluorescence images of a Bietti crystalline dystrophy patient with *CYP4V2* mutations (Case 2)

Patchy atrophy of the retinal pigment epithelium has spread.

Right Top. Representative swept source optical coherence tomography (SS-OCT) images of an eye of a Bietti crystalline dystrophy patient with *CYP4V2* mutations (Case 2)

The thicknesses of the subfoveal choroid, subfoveal retinal pigment epithelium-Bruch's membrane (RPE-BM) complex, and central retina are 94, 12, and 166 μm, respectively. The left side is superior and right-side is inferior.

The summits of the triangles represent the measurement points at different distances from the fovea.

Red, subfovea; green, 0.5 mm; yellow, 1.0 mm; blue, 1.5 mm

Right Middle. Representative SS-OCT images of an eye of a retinitis pigmentosa patient with *EYS* mutations (Case 9)

The thicknesses of the subfoveal choroid, subfoveal RPE-BM complex, and central retina are 273, 14, and 195 μm, respectively.

Right Bottom. Representative SS-OCT images of a normal eye (56 year-old man; axial length, 24.69 mm)

The thicknesses of the subfoveal choroid, subfoveal RPE-BM complex, and central retina are 363, 23, and 227 μm, respectively.

Figure 2. Horizontal and vertical choroidal thickness differences according to specific sites between Bietti crystalline dystrophy patients with *CYP4V2* mutations and retinitis pigmentosa patients with *EYS* mutations

BCD group = Bietti crystalline dystrophy with *CYP4V2* mutations group

EYS-RP group = retinitis pigmentosa with *EYS* mutations group

The bars indicate standard errors.

Top: Horizontal changes in the choroidal thickness

Although the choroidal thickness at each site is comparable in Bietti crystalline dystrophy patients with *CYP4V2* mutations, the choroid in retinitis pigmentosa patients with *EYS* mutations is thinner in regions nasal to the fovea than in regions temporal to the fovea.

Bottom: Vertical changes in choroidal thickness

There is almost no difference in vertical choroidal thickness across the sites in these groups.

Figure1LeftTop

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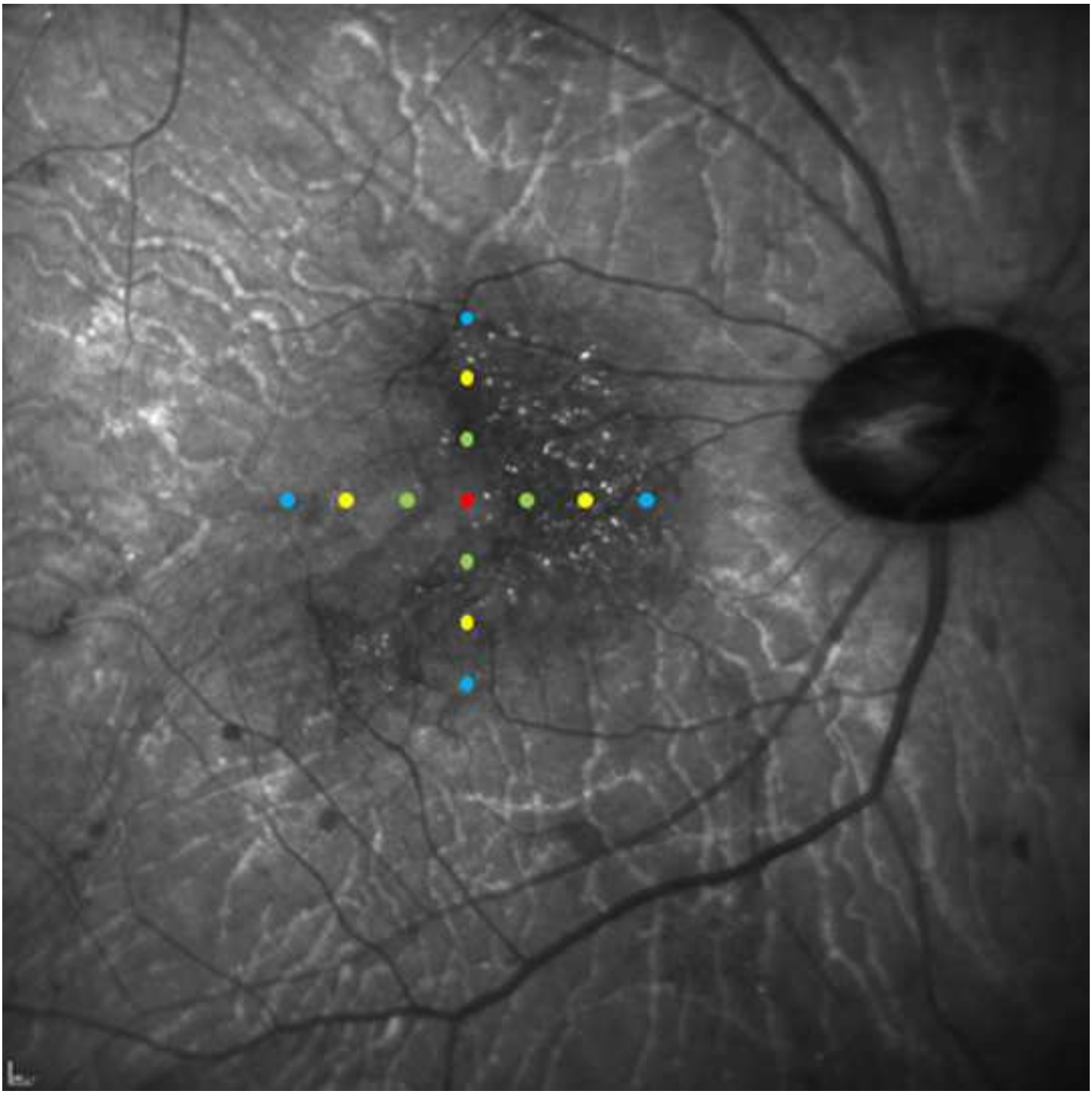

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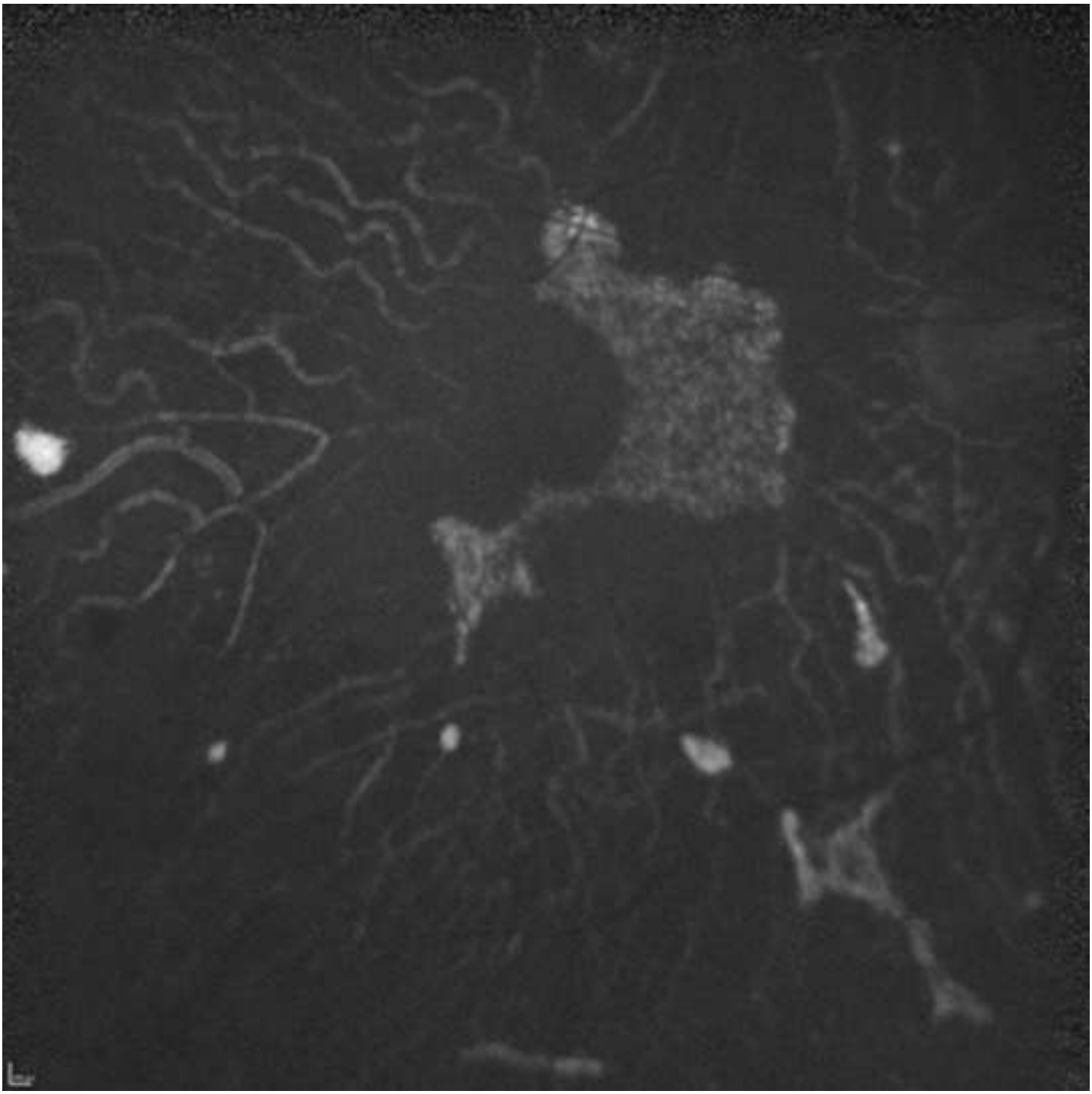

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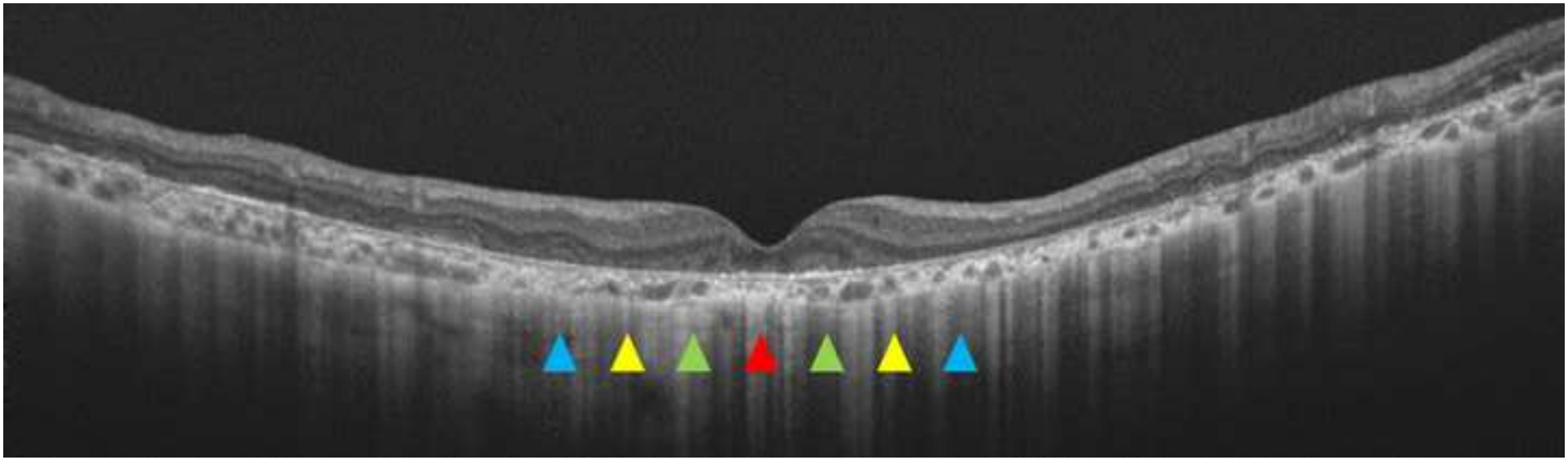

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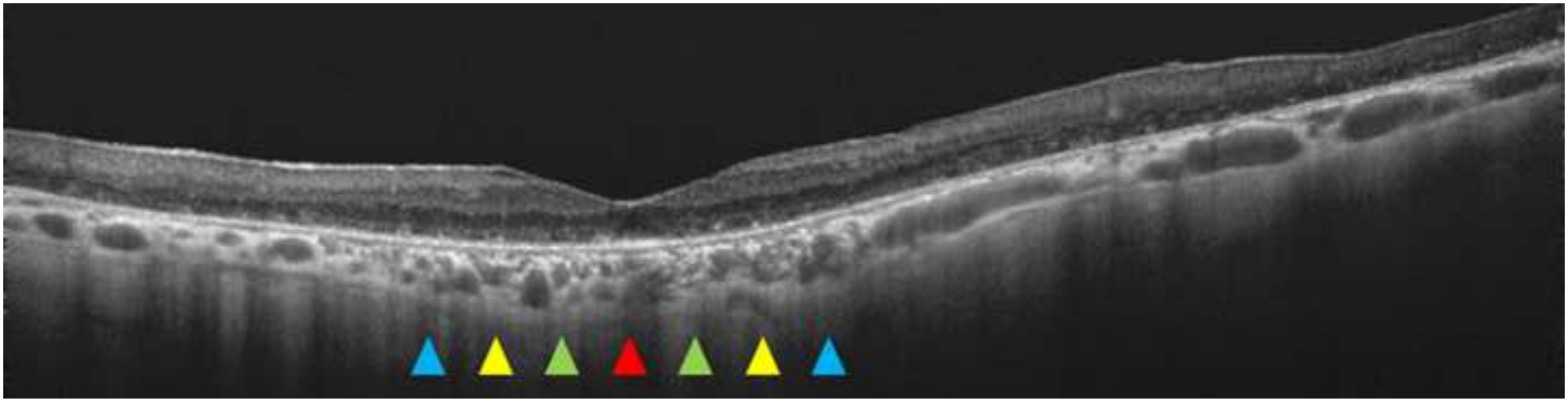

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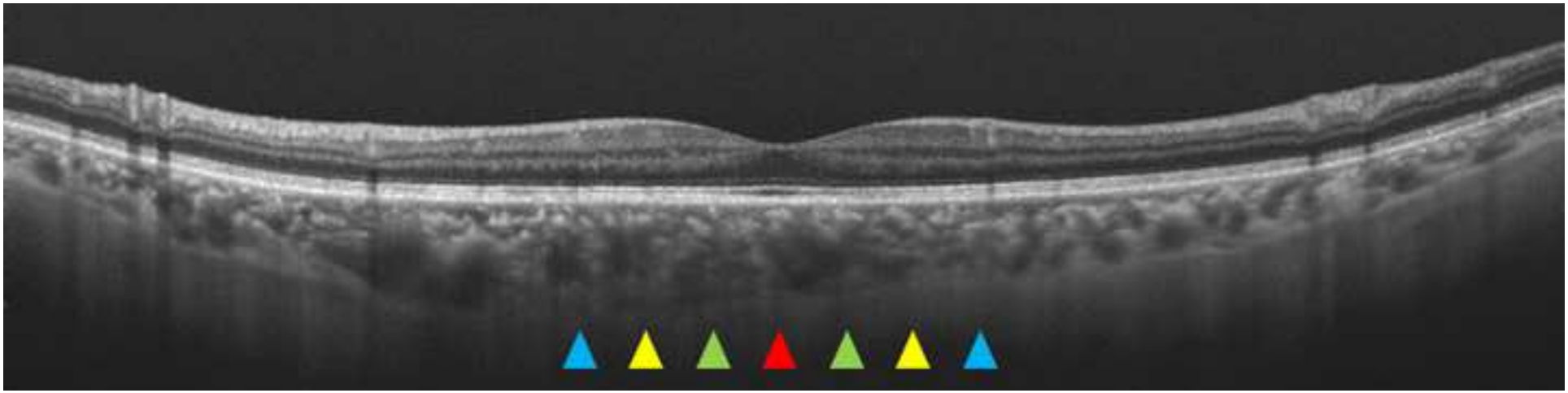

Figure2Top

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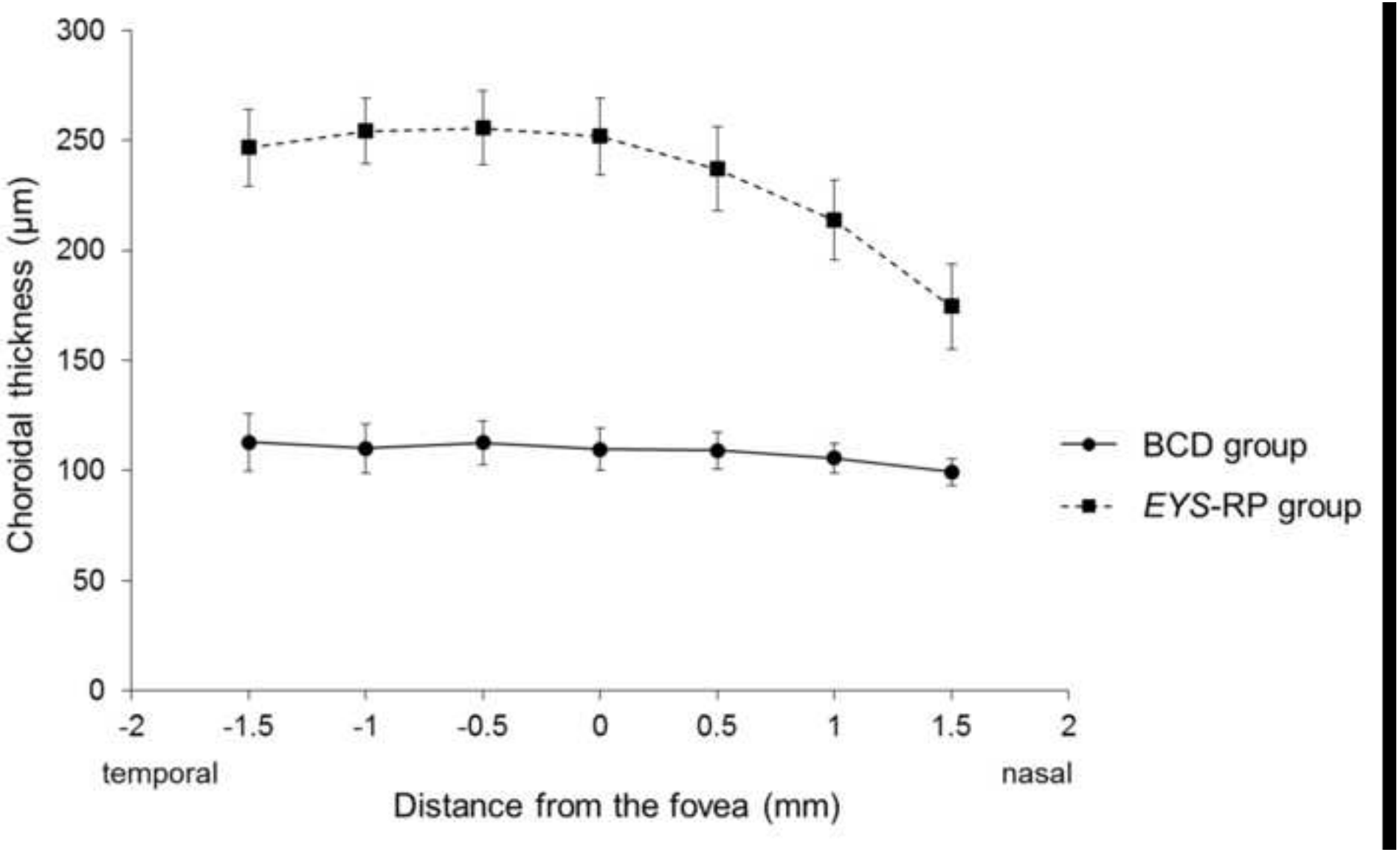

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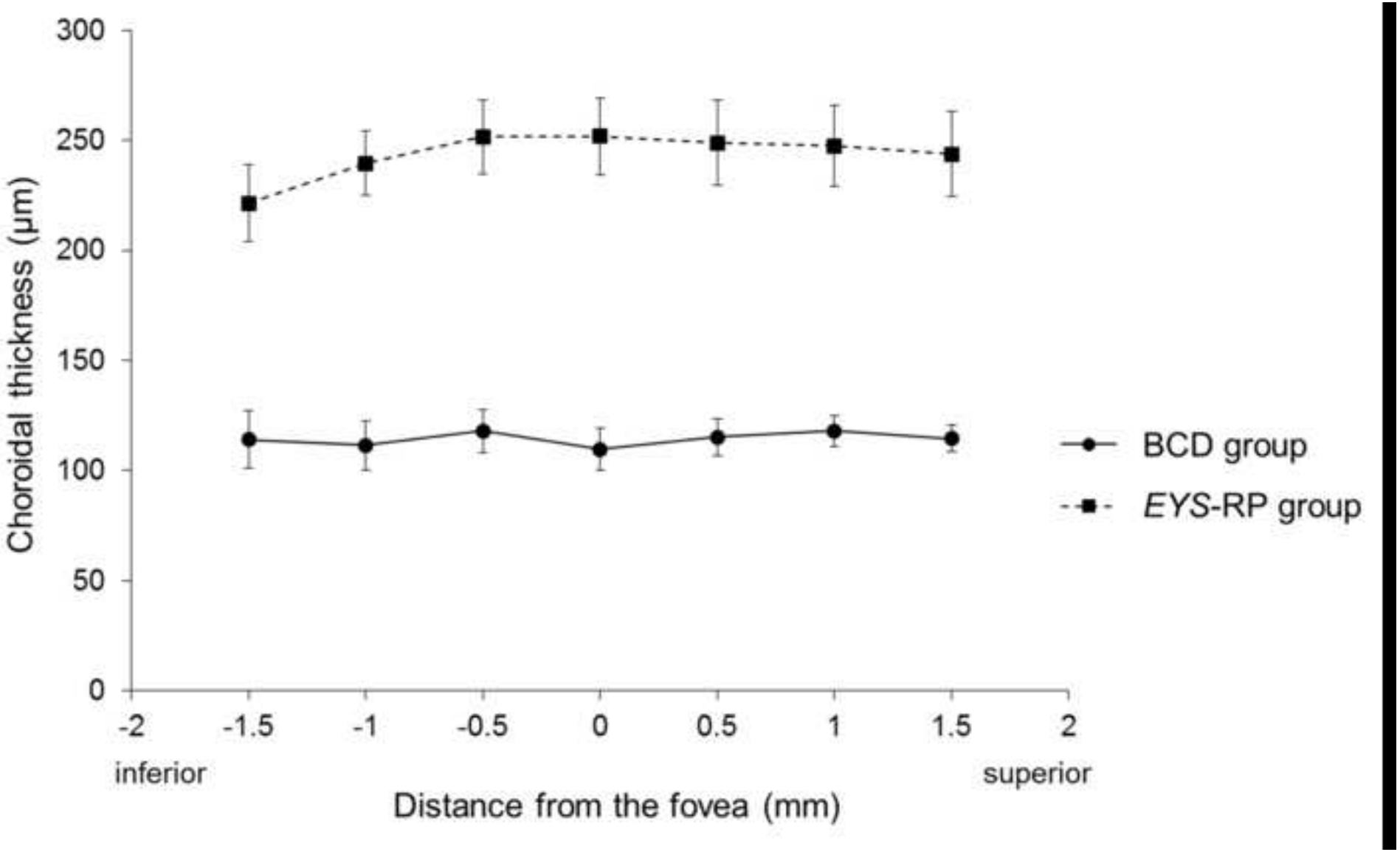

Table1

Table 1. Characteristics of the study population.

	BCD group	*EYS*-RP group	Control group
n, eyes (patients)	9 eyes (9 patients)	10 eyes (10 patients)	10 eyes (10 patients)
Female sex, eyes	5 eyes	4 eyes	7 eyes
Age (years)	57.9 ± 5.3	52.7 ± 14.1	54.0 ± 11.2
Visual acuity, logMAR	0.41 ± 0.37	0.73 ± 0.64	-0.10 ± 0.07
AL (mm)	24.21 ± 0.72	24.27 ± 1.70	24.33 ± 1.26
MD (dB)	-29.77 ± 4.01	-26.13 ± 6.66	

BCD group: Bietti crystalline dystrophy patients with CYP4V2 mutations

EYS-RP group: Retinitis pigmentosa patients with EYS mutations who were matched for age, AL, and MD with the BCD group.

Control group: Japanese volunteers with no eye disease, except for refractive errors, who were matched for age and AL with the BCD group.

Data are presented as mean ± standard deviation where applicable.

AL = axial length; logMAR = logarithm of the minimal angle of resolution; MD = mean deviation measurements using a Humphrey field analyzer (Carl Zeiss Meditec, Dublin, CA) with the 10-2 Swedish Interactive Threshold Algorithm standard program.

Table2

Table 2. Clinical characteristics of Bietti crystalline dystrophy patients with *CYP4V2* mutations and retinitis pigmentosa patients with *EYS* mutations.

Case	Age (years)	Sex	Eye	BCVA	AL (mm)	HFA10-2	ERG (cone flicker)		Mutation
BCD group						MD (dB)	Amplitude (μV)	Latency (ms)	
1	60	F	L	20/20	24.66	−27.59	91.75	25.2	c.802-8_810del17insGC / c.327+1G>A
2	58	F	L	20/25	24.52	−33.18	15.7	29.2	c.802-8_810del17insGC / c.1378T>C*
3	51	M	R	20/250	23.52	−34.82	7.39	34	c.802-8_810del17insGC / c.710C>A*
4	64	F	R	20/40	23.93	−24.25	67.50	30.2	c.802-8_810del17insGC / c.1226-6_1235del16
5	53	M	R	20/100	25.35	−32.26	25.71	28.4	c.802-8_810del17insGC homozygous
6	65	F	L	20/100	23.58	−30.62	extinguished		c.802-8_810del17insGC homozygous
7	51	F	R	20/66	25.12	−33.56	extinguished		c.802-8_810del17insGC homozygous
8	61	M	R	20/20	23.44	−27.21	6.64	37.8	c.802-8_810del17insGC homozygous
9	58	M	R	20/40	23.73	−24.40	39.75	28.0	c.802-8_810del17insGC / c.518T>G
EYS-RP group									
1	66	F	R	20/222	22.55	−33.36	extinguished		c.4957dupA / c.7048delT
2	49	F	R	20/33	24.89	−24.04	extinguished		c.5202_5203del / c.8379_8380insTGCA
3	45	F	R	20/100	23.08	−30.07	extinguished		c.4957dupA / c.8805C>A
4	79	F	R	20/222	23.37	−32.41	N.A.		c.4957dupA / c.8805C>A
5	34	M	R	20/29	28.03	−14.41	extinguished		c.4957dupA homozygous
6	51	M	L	20/200	26.2	−31.06	extinguished		c.9209T>C / c.4957dupA
7	34	M	L	20/13	24.24	−17.53	N.A.		c.4957dupA / c.5014C>T
8	64	M	R	20/222	23.21	−31.77	extinguished		c.7919G>A homozygous
9	56	M	L	20/1000	23.08	−24.78	extinguished		c.8805C>A / c.5014C>T
10	49	M	R	20/22	24.01	−21.91	extinguished		c.4957dupA / c.8805C>A

AL = axial length; BCD group = Bietti crystalline dystrophy with *CYP4V2* mutations group; BCVA = best-corrected visual acuity; ERG = electroretinography; EYS-RP group = retinitis pigmentosa with *EYS* mutations group; F = female; HFA10-2 = Humphrey field analyzer with the 10-2 SITA standard program; L = left eye; N.A.= not available; M = male; MD = mean deviation; R = right eye

* novel mutation

Table3

Table 3. Macular choroidal thickness in Bietti crystalline dystrophy patients with *CYP4V2* mutations and retinitis pigmentosa patients with *EYS* mutations.

Measurement site	BCD group (μm)	*EYS*-RP group (μm)	Control group (μm)	*P* value[a]		
				BCD group vs *EYS*-RP group	BCD group vs Control group	EYS-RP group vs Control group
Subfovea	109.6 ± 30.3	251.8 ± 55.2	285.7 ± 91.7	< .001*	< .001*	.49
Superior						
0.5 mm	115.2 ± 25.1	248.9 ± 70.4	306.9 ± 99.7	.001*	< .001*	.20
1.0 mm	118.0 ± 28.7	247.5 ± 48.5	306.9 ± 96.8	.001*	< .001*	.13
1.5 mm	114.6 ± 27.6	243.7 ± 47.3	317.2 ± 104.1	.001*	< .001*	.06
Temporal						
0.5 mm	112.6 ± 31.2	255.6 ± 53.1	291.0 ± 88.9	< .001*	< .001*	.44
1.0 mm	110.0 ± 35.0	254.2 ± 46.6	286.5 ± 75.6	< .001*	< .001*	.41
1.5 mm	112.8 ± 41.8	246.6 ± 54.7	289.2 ± 60.1	< .001*	< .001*	.19
Inferior						
0.5 mm	117.9 ± 31.8	251.6 ± 59.9	280.2 ± 87.9	< .001*	< .001*	.59
1.0 mm	111.4 ± 35.8	239.5 ± 59.0	273.1 ± 89.9	.001*	< .001*	.50
1.5 mm	114.1 ± 35.7	221.4 ± 59.8	261.1 ± 78.1	.002*	< .001*	.33
Nasal						
0.5 mm	109.1 ± 26.3	237.0 ± 60.5	267.9 ± 97.8	.001*	< .001*	.59
1.0 mm	105.6 ± 22.0	213.7 ± 57.8	273.8 ± 115.1	.01*	< .001*	.21
1.5 mm	99.3 ± 18.9	174.5 ± 61.3	258.2 ± 125.8	.14	.001*	.08

BCD group: Bietti crystalline dystrophy patients with CYP4V2 mutations

EYS-RP group: Retinitis pigmentosa patients with EYS mutations who were matched for age, AL, and MD with the BCD group.

Control group: Japanese volunteers with no eye disease except refractive errors who were matched for age and AL with the BCD group.

Data are presented as mean ± standard deviation (μm) where applicable.

AL = axial length

[a]Asterisk indicates statistical significance.

Table4

Table 4. Macular retinal pigment epithelium/Bruch's membrane complex thickness in Bietti crystalline dystrophy patients with *CYP4V2* mutations and retinitis pigmentosa patients with *EYS* mutations.

Measurement site	BCD group (μm)	*EYS*-RP group (μm)	Control group (μm)	*P* value[a]		
				BCD group vs *EYS*-RP group	BCD group vs Control group	EYS-RP group vs Control group
Subfovea	11.3 ± 1.6	17.9 ± 3.6	23.5 ± 2.0	< .001*	< .001*	< .001*
Superior						
0.5 mm	10.2 ± 2.1	15.1 ± 5.1	23.5 ± 3.0	.02*	< .001*	< .001*
1.0 mm	9.1 ± 1.5	15.3 ± 5.0	22.5 ± 1.6	.001*	< .001*	< .001*
1.5 mm	9.3 ± 1.0	14.6 ± 5.7	23.3 ± 2.7	.01*	< .001*	< .001*
Temporal						
0.5 mm	9.6 ± 1.3	16.4 ± 4.9	23.0 ± 2.4	< .001*	< .001*	< .001*
1.0 mm	9.8 ± 1.9	13.7 ± 4.5	22.6 ± 2.2	.02*	< .001*	< .001*
1.5 mm	9.3 ± 1.0	13.1 ± 5.2	23.3 ± 1.6	.04*	< .001*	< .001*
Inferior						
0.5 mm	10.4 ± 1.3	15.5 ± 5.7	23.4 ± 2.8	.02*	< .001*	< .001*
1.0 mm	9.8 ± 1.6	14.1 ± 5.0	22.8 ± 1.8	.02*	< .001*	< .001*
1.5 mm	9.6 ± 1.7	13.6 ± 4.5	22.3 ± 2.1	.02*	< .001*	< .001*
Nasal						
0.5 mm	10.6 ± 0.9	17.8 ± 4.7	25.1 ± 2.4	< .001*	< .001*	< .001*
1.0 mm	9.8 ± 1.2	14.6 ± 5.4	24.3 ± 2.2	.02*	< .001*	< .001*
1.5 mm	9.6 ± 1.3	14.1 ± 5.0	23.1 ± 1.5	.01*	< .001*	< .001*

BCD group: Bietti crystalline dystrophy patients with CYP4V2 mutations

EYS-RP group: Retinitis pigmentosa patients with EYS mutations who were matched for age, AL, and MD with the BCD group.

Control group: Japanese volunteers with no eye disease except refractive errors who were matched for age and AL with the BCD group.

Data are presented as mean ± standard deviation (μm) where applicable.

AL = axial length

[a]Asterisk indicates statistical significance.

Table5

Table 5. Macular retinal thickness in Bietti crystalline dystrophy patients with *CYP4V2* mutations and retinitis pigmentosa patients with *EYS* mutations.

Measurement site	BCD group (μm)	*EYS*-RP group (μm)	Control group (μm)	*P* value[a]		
				BCD group vs *EYS*-RP group	BCD group vs Control group	EYS-RP group vs Control group
Center	117.9 ± 52.6	180.3 ± 44.5	228.7 ± 22.4	.01*	< .001*	.04*
Superior						
0.5 mm	219.6 ± 57.0	241.4 ± 34.6	298.3 ± 20.5	.46	.001*	.009*
1.0 mm	256.6 ± 45.8	285.6 ± 25.1	339.6 ± 16.7	.12	< .001*	.002*
1.5 mm	243.0 ± 48.0	282.7 ± 45.0	330.0 ± 16.3	.09	< .001*	.03*
Temporal						
0.5 mm	193.8 ± 59.4	226.2 ± 30.2	289.5 ± 20.7	.19	< .001*	.004*
1.0 mm	228.7 ± 62.6	246.1 ± 19.7	317.4 ± 16.8	.58	< .001*	.001*
1.5 mm	204.6 ± 61.3	240.2 ± 26.1	308.0 ± 17.5	.13	< .001*	.002*
Inferior						
0.5 mm	191.8 ± 61.1	224.5 ± 27.9	289.2 ± 22.3	.19	< .001*	.003*
1.0 mm	214.9 ± 54.3	257.6 ± 21.2	333.2 ± 16.6	.03*	< .001*	< .001*
1.5 mm	201.9 ± 51.8	243.6 ± 28.9	322.0 ± 19.4	.04*	< .001*	< .001*
Nasal						
0.5 mm	204.4 ± 51.3	233.6 ± 42.8	299.2 ± 28.2	.29	< .001*	.004*
1.0 mm	267.4 ± 36.0	272.1 ± 32.0	335.0 ± 15.9	.94	< .001*	< .001*
1.5 mm	276.9 ± 43.9	274.4 ± 31.3	336.9 ± 16.9	.98	.001*	.001*

BCD group: Bietti crystalline dystrophy patients with CYP4V2 mutations

EYS-RP group: Retinitis pigmentosa patients with EYS mutations who were matched for age, AL, and MD with the BCD group.

Control group: Japanese volunteers with no eye disease except refractive errors who were matched for age and AL with the BCD group.

Data are presented as mean ± standard deviation (μm) where applicable.

AL = axial length

[a]Asterisk indicates statistical significance.