Early-Onset Posterior Capsule Opacification: Incidence, Severity, and Risk Factors

Xiaoxun Gu · Xiaoyun Chen · Guangming Jin · Lanhua Wang · Enen Zhang · Wei Wang · Zhenzhen Liu · Lixia Luo

ABSTRACT

Introduction: To evaluate the incidence, severity, and risk factors of early-onset posterior capsule opacification (PCO) following uneventful phacoemulsification and intraocular lens (IOL) implantation.

Methods: Patients with cataracts who underwent phacoemulsification and IOL implantation surgery for 3 months from September 2019 to April 2020 were enrolled. All the subjects completed a comprehensive ocular examination. Retroillumination images of the posterior capsule were obtained using a slit lamp with imaging system, and PCO was graded by two ophthalmologists. Univariate and multivariate logistic regression analyses were performed to assess the risk factors for PCO.

Results: A total of 1039 subjects were enrolled, with mean age 66.68 ± 11.43 years and 42.06% were male. The incidence of early-onset PCO in the 3 months after cataract surgery was 29.93%, and PCO of grade 3 and grade 4 was present in 31 patients (2.98%). Patients with complicated cataract had a higher incidence of PCO than age-related cataract, especially for patients with previous pars plana vitrectomy (PPV) surgery (P < 0.001). Moreover, the incidence of PCO increased with the deficiency of capsulorhexis–IOL overlap (P < 0.001). Multivariate logistic regression also showed that previous PPV surgery (OR 2.664, P = 0.003) and incomplete capsulorhexis–IOL overlap were risk factors for PCO (180–360° overlap: OR 2.058, P < 0.001; < 180° overlap: OR 5.403, P < 0.001).

Conclusions: Larger capsulorhexis and PPV surgery history contribute to the occurrence of early-onset PCO, indicating that primary posterior continuous curvilinear capsulorhexis can be considered during cataract surgery for patients with PPV history.

Keywords: Capsulorhexis; Cataract surgery; Early-onset posterior capsule opacification; Intraocular lens; Risk factors
Posterior capsule opacification (PCO) is the most common postoperative complication with the prevalence about 50% in the 5 years after cataract surgery [1]. However, if PCO occurs early after surgery it dramatically reduces patient satisfaction because of the increase of light scattering and the reduction of visual performance [2, 3]. Neodymium–yttrium aluminum garnet (Nd:YAG) capsulotomy is commonly used for treatment for PCO. Nevertheless, it may induce vision-related complications such as ocular inflammation, cystoid macular edema, and retinal detachment [4–6], and gives rise to a large financial burden on the health care system [7, 8].

Previous studies have reported that primary posterior continuous curvilinear capsulorhexis (PCCC) during cataract surgery is a valid and safe way to prevent PCO [9–11]. It not only significantly reduces PCO formation and the need for Nd:YAG laser capsulotomy but also has a low rate of complications such as retinal detachment and endophthalmitis [9–11]. Although surgery age, intraocular lens (IOL) material and design, and surgical technique are associated with long-term PCO, the incidence of early-onset PCO and its risk factor are still unknown [1, 12–14]. Therefore, figuring out the risk factors for early-onset PCO and performing PCCC combined with phacoemulsification for such patients may help to reduce the need of Nd:YAG treatment in the early postoperative period and improve patient satisfaction.

In this cross-sectional study, we focused on early-onset PCO 3 months after cataract surgery, analyzed its characteristics, and identified the main risk factors for it in a large sample.
quality affecting capsulorhexis and PCO evaluation; (5) failure to finish all the examinations leading to incomplete data. The procedure of how the participants enrolled in this study is shown in Fig. 1.

**Study Procedures**

All the patients fulfilled a structured questionnaire that contained demographic data (age, gender), medical history (ophthalmic and systemic history), and lifestyle (smoking, drinking, and body mass index) preoperatively. Ocular examinations performed before surgery and the surgical procedures were reported in Chen’s previous study [15]. Briefly, lens thickness (LT), axial length (AL), and anterior chamber depth (ACD) were measured by IOL Master 700 (Carl Zeiss, Meditec, Dublin, CA, USA) before the surgery. Afterwards, all the patients received standard phacoemulsification for cataract extraction and an in-the-bag folded IOL implantation surgery by experienced cataract surgeons in 1 week. Electronic medical records were checked retrospectively and IOL types were recorded.

All the eligible participants were followed up at 3 months after cataract surgery. After completing the general ophthalmic examinations (visual acuity, intraocular pressure, and slit lamp biomicroscopy), all the patients accepted a mixture of 0.5% tropicamide and 0.5% phenylephrine hydrochloride eye drops (Santen, Suzhou, China) every 5 min three times for mydriasis. Retroillumination images of the posterior capsule were collected with a Haag-Streit BQ-900 slit lamp with EyeCap imaging system (Haag-Streit International, Koeniz, Switzerland). Fundus examination was conducted with Kowa nonmyd WX 3D camera (2D/3D non-mydriatic retinal camera, Kowa, Japan) after pupil dilation.

**Posterior Capsule Opacification Grading and Classification of Capsulorhexis–Intraocular Lens Overlap**

Retroillumination images of the posterior capsules obtained from all patients were graded by two ophthalmologists (XX-G and XY-C) according to Congdon’s study: grade 0 (without PCO), no opacity or opacity appeared only on peripheral capsule; grade 1, wrinkling or opacity of the capsule limited in a circle 4 mm in diameter and centered on the visual axis, but the posterior polar retina could be viewed clearly; grade 2, central/paracentral opacity worse than grade 1 that affected the detailed observation of macula slightly, but had no effects on the observation of cup/disc ratio; grade 3, central/paracentral opacity worse than grade 2, and making the cup/disc ratio difficult to ascertain; grade 4, central/paracentral opacity as defined above, but making fundus observation difficult or impossible [16]. According to Congdon’s study, we defined PCO grade 3 or above as PCO with visual impact [16]. Controversial grading images were reassessed by a senior ophthalmologist. Representative images of different PCO grades are shown in Fig. 2.

The classification of capsulorhexis–IOL overlap was evaluated as follow: 360° overlap, capsulorhexis overlapping IOL edge continuously for four quadrants; 180–360° overlap,
capsulorhexis overlapping IOL edge at least two quadrants but fewer than four quadrants; < 180° overlap, capsulorhexis overlapping IOL edge less than two quadrants.

**Statistical Analysis**

Statistical analysis was performed using StataSE15 (version 15.0, Stata Corp LP, TX, USA). All data were presented as means (standard deviations) or counts and percentages according to data type. Trends in corrected distance visual acuity (CDVA) of different PCO grades were assessed using the Wilcoxon-type non-parametric test for trend across different PCO groups. Differences between with PCO group and without PCO group were compared by independent t test and Pearson chi-square test. Univariate and multivariate logistic regression analyses were performed to determine the risk factors of PCO. A P value less than 0.05 was considered to be statistically significant.

**RESULTS**

A total of 1039 eyes of 1039 participants (mean [SD] age, 66.68 [11.43] years; 437 [42.06%] male) were enrolled in this study. The main diagnosis (74.98%) was age-related cataract, and 25.02% were complicated cataract. There were 798 patients who had hydrophobic acrylic IOL implanted, of which 83.96% were square-edge IOL. Of 241 patients with implanted hydrophilic acrylic IOL, 229 patients (95.02%) chose square-edge IOL with 360° posterior barrier. Table 1 shows the basic characteristics of the patients.

As illustrated in Table 2, the prevalence of early-onset PCO was 29.93% (311/1039) in all the patients at 3 months after cataract surgery, while the prevalence was higher in patients with complicated cataracts than that in patients with age-related cataracts (40.38% [105/260] vs 26.44% [206/779]). There were statistically significant differences in the distribution of different levels of PCO between age-related cataract and complicated cataract (P < 0.001). In particular, the incidence of grade 3+ PCO in complicated cataract was obviously higher than that in age-related cataract (8.08% [21/260] vs 1.28% [10/779]).

In order to assess visual impact caused by PCO, participants with previous ocular diseases or surgery that might affect visual acuity, such as high myopia (AL ≥ 26 mm), fundus diseases, glaucoma, and previous PPV surgery, were excluded from the analysis. There were 147 eyes from 147 patients with age-related cataracts with PCO eligible for this analysis. As shown in Table 3, corrected distance visual acuity (CDVA) declined with the severity of PCO (P trend = 0.011), and PCO mainly induced slight visual impairment (P trend = 0.015).

Table 4 compares the characteristics of patients with or without PCO. Patients with PCO were younger than those without PCO (mean [SD], 64.79 [12.20] years vs 67.50 [11.01], P = 0.001). Additionally, the incidence of PCO

![Fig. 2](image)

Fig. 2 Representative images of different grades of posterior capsule opacification
was much higher in patients with PPV surgery history (53.70% [29/54] vs 28.63% [282/985], \( P < 0.001 \)) and incomplete capsulorhexis–IOL overlap (<180° overlap: 56.06% [74/132]; 180–360° overlap: 32.17% [157/488]; 360° overlap: 19.09% [80/419]; \( P < 0.001 \)). Moreover, patients with PCO had longer AL than those without PCO (mean [SD], 24.56 [2.48] mm vs 24.24 [2.22] mm, \( P = 0.042 \)). However, the incidence of PCO showed no statistically significant differences in gender (\( P = 0.804 \)), smoking (\( P = 0.818 \)), drinking (\( P = 0.419 \)), BMI (\( P = 0.863 \)), hypertension (\( P = 0.225 \)), diabetes (\( P = 0.208 \)), and IOL types (\( P = 0.074 \)).

The results of univariate and multivariate logistic regression analysis are presented in Table 5. The univariate logistic regression analysis demonstrated that previous PPV surgery (OR 2.892, 95% CI 1.664–5.025; \( P < 0.001 \)) and the deficiency of capsulorhexis–IOL overlap were risk factors of PCO (180–360° overlap: OR 2.010, 95% CI 1.476–2.737, \( P < 0.001 \); <180° overlap: OR 5.406, 95% CI 3.548–8.240, \( P < 0.001 \)). However, age was a protective factor of PCO (OR 0.980, 95% CI 0.969–0.991; \( P = 0.001 \)).

Multivariate logistic regression analysis showed that previous PPV surgery and incomplete capsulorhexis–IOL overlap were positively associated with PCO (PPV: OR 2.664, 95% CI 1.409–5.037, \( P = 0.003 \); 180–360° overlap: OR 2.058, 95% CI 1.486–2.850, \( P < 0.001 \); <180° overlap: OR 5.403, 95% CI 3.436–8.496, \( P < 0.001 \)). Although hydrophilic IOL was negatively associated with PCO (OR 0.610, 95% CI 0.427–0.875; \( P = 0.007 \)), the rates of PCO were not significantly different between hydrophilic IOL and hydrophobic IOL groups (\( P = 0.074 \), Table 4). Nevertheless, age, gender, smoking, drinking, BMI, hypertension, and diabetes showed no association with PCO. Taken together, these results suggested that previous PPV surgery and larger capsulorhexis with insufficient IOL overlap are the significant risk factors for early-onset PCO.

### DISCUSSION

Even though several studies focusing on the effects of specific factors on the incidence of long-term PCO have been performed, such as capsulotomy sizes and IOL types, there was scarce evidence on how multiple factors affect incidence of early-onset PCO. In this study, we found that PCO could occur a short time after cataract surgery. Approximately 2.98% of patients had grade 3+ PCO 3 months after...
surgery, and the incidence was even higher in patients with complicated cataract (8.08%). Moreover, we also determined that previous PPV surgery and incomplete capsulorhexis–IOL overlap were risk factors for early-onset PCO. Patients with previous PPV surgery and insufficient capsulorhexis–IOL overlap had much higher rates of PCO.

The incidence of early-onset PCO has been reported to range from 11% to 40.7% in patients with cataracts with silicone IOL implantation at 6 weeks to 3 months after cataract surgery in previous studies [17, 18]. However, no study reported the incidence of short-term PCO for acrylic acid IOL implantation. In our study, 29.93% of patients developed early-onset PCO 3 months after cataract surgery, of which 66.88% with PCO presented grade 1 that only involved the peripheral posterior capsule and did not affect visual acuity. The incidence of grade 2+ PCO in our study was 9.91%, including 2.98% of grade 3 and grade 4 PCO affecting vision. These results implied that the incidence of early-onset PCO should not be ignored.

It is known that age is a lingering factor associated with PCO. The pathogenesis of PCO is caused by the residual LEC proliferation, migration, and differentiation after cataract surgery [19]. Sundelin et al. reported that the process of PCO formation was more active in younger patients in a vitro study [20]. Tokko and colleagues also demonstrated that age was negatively associated with the need for YAG capsulotomy [13]. Though there was no significant correlation between age and PCO in multivariate analysis in our study, patients with PCO were significantly younger than those without PCO. Therefore, age may be associated with PCO, particularly for long-term PCO, but is not a major risk factor for early-onset PCO.

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### Table 2 Distribution of early-onset posterior capsule opacification in age-related cataract and complicated cataract

| PCO grades     | Total, (n, %) | Age-related cataract, n (%) | Complicated cataract, n (%) | P value |
|----------------|--------------|-----------------------------|-----------------------------|---------|
| Without PCO    | 728 (70.07)  | 573 (73.56)                 | 155 (59.62)                 |         |
| With PCO       | 311 (29.93)  | 206 (26.44)                 | 105 (40.38)                 |         |
| Grade 1        | 208 (20.02)  | 157 (20.15)                 | 51 (19.61)                  |         |
| Grade 2        | 72 (6.93)    | 39 (5.01)                   | 33 (12.69)                  |         |
| Grade 3 + grade 4 | 31 (2.98)  | 10 (1.28)                   | 21 (8.08)                   |         |
| Total          | 1039         | 779                         | 260                         | <0.001  |

**PCO** posterior capsule opacification

### Table 3 Influences of posterior capsule opacification on visual impairment in age-related cataract

| CDVA (logMAR) | Grade 1 | Grade 2 | Grade 3 + grade 4 | P trend |
|---------------|---------|---------|-------------------|---------|
| Total, mean (SD) | 0.12 (0.21) | 0.18 (0.18) | 0.3 (0.41) | 0.011   |
| CDVA ≤ 0.1(20/25), n (%) | 90 (78.95) | 16 (59.26) | 3 (50.00) | 0.537   |
| 0.1 (20/25) < CDVA ≤ 0.3 (20/40), n (%) | 16 (14.04) | 6 (22.22) | 2 (33.33) | 0.015   |
| CDVA > 0.3 (20/40), n (%) | 8 (7.01) | 5 (18.52) | 1 (16.67) | 0.957   |

**PCO** posterior capsule opacification, **CDVA** corrected distance visual acuity
### Table 4 Characteristics of patients with and without posterior capsule opacification

|                                      | Total     | Without PCO | With PCO | P value |
|--------------------------------------|-----------|-------------|----------|---------|
| **Patients, n (%)**                  | 1039      | 728 (70.07) | 311 (29.93) | –       |
| **Age (years), mean (SD)**           | 1039      | 67.50 (11.01)| 64.79 (12.20) | 0.001   |
| **BMI, mean (SD)**                   | 1039      | 23.78 (3.67) | 23.82 (3.17) | 0.863   |
| **Axial length (mm), mean (SD)**     | 1039      | 24.24 (2.22) | 24.56 (2.48) | 0.042   |
| **Gender, n (%)**                    |          |             |          | 0.804   |
| Male                                 | 437      | 308 (70.48) | 129 (29.52) |         |
| Female                               | 602      | 420 (69.77) | 182 (30.23) |         |
| **Smoking, n (%)**                   |          |             |          | 0.818   |
| Yes                                  | 97       | 69 (71.13)  | 28 (28.87)  |         |
| No                                   | 942      | 659 (69.69) | 283 (30.04) |         |
| **Drinking, n (%)**                  |          |             |          | 0.419   |
| Yes                                  | 125      | 91 (72.80)  | 34 (27.20)  |         |
| No                                   | 914      | 637 (69.69) | 277 (30.31) |         |
| **Hypertension (yes)**               |          |             |          | 0.225   |
| Yes                                  | 349      | 253 (72.49) | 96 (27.51)  |         |
| No                                   | 690      | 475 (68.84) | 215 (31.16) |         |
| **Diabetes (yes)**                   |          |             |          | 0.208   |
| Yes                                  | 170      | 126 (74.12) | 44 (25.88)  |         |
| No                                   | 869      | 602 (69.28) | 267 (30.72) |         |
| **Previous PPV surgery, n (%)**      |          |             |          | 0.208   |
| Yes                                  | 54       | 25 (46.30)  | 29 (53.70)  |         |
| No                                   | 985      | 703 (71.37) | 282 (28.63) |         |
| **IOL types**                        |          |             |          | < 0.001 |
| Hydrophilic, n (%)                   | 241      | 180 (74.69) | 61 (25.31)  |         |
| Hydrophobic, n (%)                   | 798      | 548 (68.67) | 250 (31.33) | 0.074   |

Classification of capsulorhexis overlap IOL edges

△ Adis
Another important finding in our study was that previous PPV surgery was a significant risk factor for early-onset PCO. Previous studies also reported that the incidence of PCO was 33.3–50% in vitrectomized eyes at about 2–3 years after cataract surgery [21, 22]. Pinter and Sugar further pointed out that PCO was more common in vitrectomized eyes than in control eyes (51% vs 21%) for about 2 years follow-up [21]. In our study, 29 of 54 patients

Table 4 continued

|                | Total | Without PCO       | With PCO       | P value     |
|----------------|-------|-------------------|----------------|-------------|
| 360° overlap    | 419   | 339 (80.91)       | 80 (19.09)     |             |
| 180–360° overlap| 488   | 331 (67.83)       | 157 (32.17)    |             |
| < 180° overlap  | 132   | 58 (43.94)        | 74 (56.06)     | < 0.001     |

PCO posterior capsule opacification, BMI body mass index, PPV pars plana vitrectomy, IOL intraocular lens

Table 5 Univariate and multiple logistic regression analysis of risk factors associated with early-onset posterior capsule opacification

| Factors                        | PCO                  | Univariate analysis | Multivariate analysis |
|--------------------------------|----------------------|---------------------|-----------------------|
|                                | Odds ratio (95% CI)  | P                    | Odds ratio (95% CI)  | P          |
| Age (years)                    | 0.980 (0.969–0.991)  | 0.001               | 0.987 (0.973–1.000)  | 0.058      |
| Gender (male)                  | 1.035 (0.790–1.354)  | 0.804               | 1.022 (0.738–1.416)  | 0.896      |
| Smoking (yes)                  | 1.052 (0.683–1.620)  | 0.818               | 1.037 (0.627–1.715)  | 0.887      |
| Drinking (yes)                 | 0.841 (0.553–1.280)  | 0.419               | 0.704 (0.434–1.141)  | 0.155      |
| BMI                            | 1.003 (0.966–1.043)  | 0.863               | 1.000 (0.957–1.044)  | 0.982      |
| Hypertension (yes)             | 0.838 (0.631–1.115)  | 0.225               | 0.883 (0.629–1.240)  | 0.473      |
| Diabetes (yes)                 | 0.787 (0.543–1.142)  | 0.208               | 0.865 (0.573–1.307)  | 0.492      |
| Previous PPV surgery (yes)     | 2.892 (1.664–5.025)  | < 0.001             | 2.664 (1.409–5.037)  | 0.003      |
| Axial length (mm)              | 1.059 (1.002–1.120)  | 0.042               | 1.056 (0.993–1.122)  | 0.081      |
| IOL types (hydrophilic)        | 0.743 (0.536–1.030)  | 0.074               | 0.610 (0.427–0.875)  | 0.007      |

Classification of capsulorhexis–IOL overlap

|                | Ref.       | Ref.       | Ref.       | Ref.       |
|----------------|------------|------------|------------|------------|
| 360° overlap   | Ref.       | Ref.       | Ref.       | Ref.       |
| 180–360° overlap| 2.010 (1.476–2.737) | < 0.001 | 2.058 (1.486–2.850) | < 0.001 |
| < 180° overlap  | 5.406 (3.548–8.240) | < 0.001 | 5.403 (3.436–8.496) | < 0.001 |

PCO posterior capsule opacification, BMI body mass index, PPV pars plana vitrectomy, IOL intraocular lens

Another important finding in our study was that previous PPV surgery was a significant risk factor for early-onset PCO. Previous studies also reported that the incidence of PCO was 33.3–50% in vitrectomized eyes at about 2–3 years after cataract surgery [21, 22]. Pinter and Sugar further pointed out that PCO was more common in vitrectomized eyes than in control eyes (51% vs 21%) for about 2 years follow-up [21]. In our study, 29 of 54 patients

△ Adis
(53.70%) with a history of PPV surgery had grade 1+ PCO only 3 months after cataract surgery, including 9 patients (16.67%) with grade 3+ PCO affecting vision. A possible explanation for the increasing risk of PCO in vitrectomized eyes is that elevated cytokines caused by inflammation after PPV surgery may accelerate LEC proliferation and migration, which results in PCO development. Additionally, loss of support of the vitreous body may affect the attachment between IOL edge and capsular bag, promoting rapid LEC proliferation and contributing to PCO [23]. As a result of the high incidence of PCO in patients with previous PPV surgery, previous study has proposed that PCCC combined with phacoemulsification may be an alternative to prevent PCO in postvitrectomy eyes with cataract [24].

Previous studies have found capsulorhexis size and its central localization play important roles in PCO [25, 26]. Small capsulorhexis (4.0–5.0 mm) that covers the IOL edge perfectly had a lower PCO rate than large capsulorhexis [25, 26]. In this study, we also found that larger capsulorhexis with incomplete capsulorhexis–IOL overlap was a significant risk factor for early-onset PCO. The complete in-the-bag fixation of IOL provides accurate lens centration and enhances the IOL-optic barrier effect; on the contrary, incomplete capsulorhexis–IOL overlap may provide potential space for the migration and growth of LECs onto the posterior capsule and contributes to PCO formation. Hence, a centered and appropriately sized capsulorhexis that covers the IOL edge completely is critical for cataract surgery.

In the current study, we found that the incidence of PCO was slightly lower in hydrophilic IOL than that in hydrophobic IOL, and hydrophilic IOL was negatively associated with PCO in multivariate analysis. These findings seem to contradict previous research reporting low PCO rates in patients with hydrophobic IOL implantation [27, 28]. The main reason may be that 95.02% of hydrophilic IOLs used in this study were 360° enhanced sharp-edged hydrophilic IOLs. Koshy et al. ascertained that 360° enhanced sharp-edged hydrophilic IOL had a better capsular bag performance, and a lower PCO incidence than sharp-edged hydrophobic IOL in the 2 years after cataract surgery though PCO scoring showed no statistically significant difference between the two groups [29]. These results suggested that the design of IOL may be more important than the material in reducing the incidence of PCO.

This study has several limitations. Since this is a cross-sectional study, the dynamic changes of early-onset PCO and how long grade 1 PCO will need Nd:YAG capsulotomy therapy are unknown. Therefore, a well-designed longitudinal study is necessary to further explore the progression and the associated factors of PCO.

CONCLUSIONS

In the present cross-sectional study, we propose that PCO can occur shortly following uneventful cataract surgery, thus affecting the postoperative visual quality and reducing patient satisfaction. Approximately 1.28% of age-related cataract and 8.08% of patients with complicated cataracts with grade 3+ PCO required Nd:YAG capsulotomy therapy only 3 months after surgery. And CDVA declined with the increase in PCO grades. Moreover, we also demonstrated that previous PPV surgery and incomplete capsulorhexis–IOL overlap were the significant risk factors for early-onset PCO. Therefore, a centered and appropriately sized capsulorhexis that covers the IOL edge perfectly is critical for cataract surgery, especially for patients with previous PPV surgery. Furthermore, for patients with PPV surgery history, prophylactic PCCC can be performed during cataract surgery, so as to avoid the need for Nd:YAG capsulotomy therapy early after surgery and reduce its complications.

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**Author Contributions.** Xiaoxun Gu and Xiaoyun Chen are both first authors, who planned the study, collected the data, analyzed the data and prepared this paper. Guangming Jin and Lanhua Wang planned the study, analyzed the data. Enen Zhang collected the data. Wei Wang reviewed and contributed to the content of this paper. Zhenzhen Liu planned the study, reviewed and contributed to the content of this paper. Lixia Luo planned the study, reviewed and contributed to the content of this paper.

**Disclosures.** Xiaoxun Gu, Xiaoyun Chen, Guangming Jin, Lanhua Wang, Enen Zhang, Wei Wang, Zhenzhen Liu, Lixia Luo declare that they have no conflicts of interest.

**Compliance with Ethics Guidelines.** This cross-sectional study was conducted at Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China. It was approved by the Zhongshan Ophthalmic Center Institutional Review Board (No. 2019 KYPJ033) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant.

**Data Availability.** The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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