Iris Volume Change With Physiologic Mydriasis To Identify Development of Angle Closure: The Zhongshan Angle Closure Prevention Trial

Running head: Iris volume change to identify angle closure progression

Synopsis: This prospective assessment demonstrates that lower loss of iris volume with physiologic pupil dilation is an additive risk factor for angle closure disease progression.

Authors

Chimei Liao, MD, PhD,[1]

Harry A. Quigley, MD,[2]

Yuzhen Jiang, MD, PhD,[3]

Shengsong Huang, MD, PhD,[1]

Wenyong Huang, MD, PhD,[1]

David S. Friedman, MD, PhD,[4]

Paul J. Foster, PhD, FRCS(Ed),[3]

Mingguang He, MD, PhD [1,5,6]

Affiliations

1. State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou, China
2. Glaucoma Center of Excellence, Wilmer Institute, Johns Hopkins, Baltimore, Maryland, USA
3. NIHR Biomedical Research Centre at Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, England
4. Glaucoma Center of Excellence, Massachusetts Eye and Ear, Harvard University, Boston, Massachusetts.
5. Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Victoria, Australia
6. Ophthalmology, Department of Surgery, University of Melbourne, Melbourne, Victoria, Australia

Corresponding Author: Chimei Liao, ccy0755@163.com.

Contribution

Research design was conducted by Paul Foster, David Friedman and Mingguang He. Data acquisition and research execution was carried out by Yuzhen Jiang, Shengsong Huang and Wenyong Huang. Study idea was developed by Harry Quigley. Data analysis and interpretation was performed by Chimei Liao. The manuscript was finished by Harry Quigley and Chimei Liao, proofread and revised by Paul Foster, David Friedman and Mingguang He.

Financial Support: This work is supported by the Fight for Sight (grant 1655; UK), the Sun Yat-sen University 5010 Project Fund (grant 2007033; China), the National Natural Science Foundation of China (grant 81420108008; China), Fundamental Research Funds of the State Key Laboratory in Ophthalmology (China) and Moorfields Eye Charity (previously Special Trustees of Moorfields Eye Hospital, no award/grant no.).The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. CL, YJ, SH, WH, HAQ, MH, PJF, and DSF had access to all the data in the study. MH, PJF, HAQ, and DSF had final responsibility for the decision to submit for publication.

Conflict of Interest: The authors have no financial or other conflicts of interest concerning this study.

Patient consent: Obtained.

Ethics approval: Ethical Review Board of Sun Yat-Sen University, the Ethical Committee of Zhongshan Ophthalmic Center ([2007]12),, and the Moorfields Eye Hospital (via the London School of Hygiene & Tropical Medicine) and Johns Hopkins University institutional review boards.

Tables and Figures: 5 Tables, 2 Supplementary tables and 5 Supplementary Figures

ABSTRACT

Aims: To assess dynamic change of iris area (Iarea) and volume (VOL) with physiologic pupil dilation for progression of primary angle closure suspects.

Methods: Participants underwent baseline examinations including gonioscopy and anterior segment OCT (AS-OCT) as part of the Zhongshan Angle Closure Prevention Trial. The AS-OCT images were obtained both in the dark and light. Progression was defined as development of primary angle closure or an acute angle closure attack. Static ocular biometrics and dynamic changes were compared between progressors and non-progressors and multivariable logistic regression was developed to assess risk factors for progression.

Results: A mean 16.8% decrease in Iarea and a mean 6.26% decrease in VOL occurred with pupil dilation, while 22.96% non-progressors and 40% progressors presented VOL increases with pupil dilation. Iarea in light and dark and VOL in light were significantly smaller in progressors. In a multivariable logistic model, older age (P=0.008), narrower horizontal angle opening distance 250μm from the scleral spur (AOD250, P=0.001), flatter iris curvature (IC, P=0.006), and lower loss of iris volume (ΔVOL, P=0.04) were significantly associated with progression. With receiver operating characteristic analysis, the area under the curve for ΔVOL alone was 0.621, while that for the combined index (age, AOD250, IC and ΔVOL) was 0.824. Eyes with elevated intraocular pressure had less iris volume loss compared with progressors developing peripheral anterior synechiae alone (P=0.055 for ΔVOL adjusted for pupil enlargement).

Conclusion: A smaller change in ΔVOL is an additive risk factor to identify eyes more likely to develop angle closure disease.

What is already known on this topic - Various measures have been assessed from anterior segment optical coherence tomography data to identify primary angle closure suspects eyes that will develop disease, but most of them have focused on static anatomic measurements. There is now considerable evidence that the dynamic behaviors of the iris are contributing features to primary angle closure disease (PACD). However, the role of dynamic change of the iris in the development of angle closure has not been investigated in longitudinal study.

What this study adds - we provided the first prospective assessment of the value of iris area and volume change with pupil dilation as predictive parameters for incident PACD disease and found that lower loss of iris volume is an additive risk factor to identify eyes more likely to develop angle closure disease.

How this study might affect research, practice or policy - Dynamic change in ocular tissues should be considered at least as important as the static anatomy of anterior structures for the prediction of angle closure development.

Key Words: angle closure glaucoma, iris, volume change, optical coherence tomography

Clinical trial registry: ISRCTN45213099.

INTRODUCTION

It is well established that eyes developing primary angle closure disease (PACD) in its various forms have shorter axial length and more crowded angle parameters.[1-3] However, there are many more persons who have smaller eyes and narrower chamber angles than those who will develop PACD. The Zhongshan Angle Closure Prevention (ZAP) randomized clinical trial showed that a small number of untreated primary angle closure suspects (PACS) subjects develop PACD in 6 years.[4] While laser iridotomy has minimal complications, carrying out the procedure in all PACS eyes would treat many eyes would likely ever develop PACD. The methods that have been used to estimate subjectively or to measure anatomically the features of PACS fail to differentiate the more likely candidates for PACD with reasonable predictive power. Various measures have been assessed from anterior segment optical coherence tomography (ASOCT) data to identify PACS eyes that will develop disease,[5 6] but most ASOCT research has focused on static anatomic measurements.

Unfortunately, metrics such as trabecular iris space area (TISA), angle opening distance (AOD) and local iris thickness are significantly altered by small differences in the identified position of the scleral spur, which is typically marked manually and is therefore subject to observer bias.[7 8] By contrast, the iris area is minimally affected by typical variability in scleral spur marking. In addition, the peripheral angle metrics in ASOCT are most often determined without consideration of pupil size that can change dramatically with physiologic pupil dilation.[9] Yet, past literature does not routinely consider pupil size

change in these measurements. Change in angle parameters and in their dynamic change with pupil size is related to subject age.[10]

There is now considerable evidence that the dynamic behavior of the iris and choroid are contributing features to PACD.[11-13] In all eyes, the iris loses volume by aqueous humor transfer from the iris stroma to the anterior chamber immediately upon pupil dilation. This feature has been confirmed in persons whose derivation is Europe, Africa, India, and China. In cross-sectional studies, the tendency to have less reduction in iris area or calculated iris volume on pupil dilation has been shown to be a risk factor for development of PACD and is even more strongly correlated with acute angle closure (AAC).[14-18] Modeling of iris behavior suggests that lower iris stromal permeability could contribute to development of PACD.[19] The ZAP studied 889 PACS subjects who underwent unilateral laser peripheral iridotomy in a randomized trial. In this report, we present data from the untreated fellow eyes of the ZAP trial, providing the first prospective assessment of the value of iris area and volume change with pupil dilation as predictive parameters for incident PACD disease.

Methods

The ZAP Trial was a single-center, randomized interventional controlled trial performed at Zhongshan Ophthalmic Center, Guangzhou, China. Full details of the study design were previously published.[20] The trial was approved by the Ethical Review Board of Sun Yat-Sen University, the Ethical Committee of Zhongshan Ophthalmic Center ([2007]12), and the Moorfields Eye Hospital (via the London School of Hygiene & Tropical Medicine) and Johns Hopkins University Institutional Review Boards. This trial was done in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolling. The International Standard Randomized Controlled Trial Number (ISRCTN) was issued on May 6, 2008 (ISRCTN45213099) by the ISRCTN registry.

Participants aged 50–70 years received a screening examination to identify PACS persons without prior laser iridotomy having gonioscopic angle closure for ≥6 clock hours of angle circumference. Subjects at baseline had no peripheral anterior synechiae (PAS) on gonioscopic examination and their intraocular pressure (IOP) was ≤ 21 mm Hg. No clinical damage was seen on optic disc examination and automated visual field testing was within normal limits or borderline.

Patients were examined after baseline exams and laser treatment in one eye at 2 weeks, 6 months, 18 months, 36 months, 54 months, and 72 months. The primary outcome was the incidence of primary angle closure by 72 months, defined as the composite of three study endpoints: (1) IOP measurements above 24 mm Hg on two separate occasions, or (2) development of at least one clock hour of PAS in any quadrant, or (3) an episode of AAC with IOP ≥ 40 mm Hg associated with specific symptoms. Subjects satisfying one or more of these criteria were denoted progressors.

For ASOCT, one horizontal and one vertical scan (both limbus to limbus) were obtained in the dark (illumination <5 Lux) and another horizontal scan was obtained in the light for each eye using the Visante ASOCT (Carl Zeiss Meditec, Dublin, CA, USA). The bright conditions were created by overhead LED lighting with ample natural light to ensure sufficient illumination. The dark OCT image were collected after dark adaptation for 5 minutes. Data in this report was only from the horizontal scans obtained at baseline. ASOCT images were analyzed using custom semiautomated software (Zhongshan Angle Assessment Program [ZAAP], Guangzhou, China). The iris area was calculated as the cumulative cross-sectional area of the full length (from scleral spur to pupil) of the iris. In this analysis, iris area (Iarea) was calculated as the average of right and left sides of iris in the image. Iris volume (VOL) calculation was based on the principles of the centroid theorem, in which the geometric center of mass of the iris is called the centroid (Supplementary Figure 1). The formula used for VOL was: $V = A*2\pi r$ where V is VOL, A is the average iris area, r is the distance from centroid to the optical axis, and $2\pi r$ is the circumference of the iris at its centroid. The various ASOCT parameters that were calculated are summarized in Supplementary Table S1.

To compare changes in parameters with change in illumination, the values in the light condition were subtracted from that in the dark condition. Thus, a value <0 indicates the parameter decreased with pupil dilation, while changes >0 denote increases after dilation. The data in this report are from the one untreated eye per subject. From 889 total subject eyes completing the study, 761 are included in this report, including 36 that met study criteria as progressors. A total of 128/761 participants were excluded, including 11 untreated progressors. Among these exclusions, 41 (including 1 progressor) were excluded for poor quality ASOCT horizontal scans. Fifty (including 8 progressors) were excluded for unrecognized iris contour in ASOCT scans. Thirty-seven (including 2 progressors) were excluded for inadequate pupil dilation from light to dark (<0.5 mm). Thus, the study population for analysis consisted of 736 non-progressors and 25 progressors. The excluded subjects were significantly older (by about 1

year), more often male, had shallower anterior chamber depth and greater lens thickness, and proportionately more progressors than non-progressors were excluded (Table 1).

Statistical Methods

Measurements of biometric parameters were calculated by averaging corresponding measurements from the right and left sides of horizontal image, except for VOL which was calculated by the sum from the right and left sides. Means and standard deviations were presented for all continuous variables. Normality of data was assessed using the Shapiro-Wilk test and by plotting histograms of measurement distributions. Means of continuous variables were compared between light and dark using paired t-test or Wilcoxon matched-pairs signed-ranks test, and between progressors and non-progressors using the unpaired t test or Wilcoxon rank-sum test according to their distribution, respectively. Proportions of categorical variables were compared using the Pearson's chi-square test.

Univariable and multivariable logistic regression models were developed to investigate the association between baseline horizontal parameter measurements and progression. Multivariable model was developed using the best subset selection method to maximize the adjusted R2 value. Units for biometric parameters were modified for physiologic significance and interpretability of odds ratios (ORs). Area under the receiver operating characteristic curve (AUC) metrics were calculated for univariable and multivariable models to assess predictive performance. All statistical analysis was performed using Stata SE V.12.0 (Stata Corp, College Station, TX, USA) with a statistical significance set at 0.05.

RESULTS

1. Overall change in iris area and volume

Across all included subjects, VOL decreased with pupil dilation, with a mean 6.26% decrease in VOL and mean decrease in Iarea of 16.8% (not adjusted for pupil size change) (Supplementary Figures 2, 3). The mean pupil size in light was 2.71 ± 0.51 mm, increasing to a mean of 4.46 ± 0.69 mm in the dark, for an absolute increase in pupil size of 1.74 ± 0.54 mm. The relationships between VOL or Iarea change with pupil enlargement were highly significant (correlation coefficient for VOL and pupil enlargement = -0.15, $P<0.001$; correlation coefficient for Iarea and pupil enlargement=-0.58, $P<0.001$), with greater loss as the

pupil enlarged in Iarea (9.82%/mm pupil enlargement) and VOL (3.68%/mm pupil enlargement; Supplementary Figure 4).

Interestingly, though VOL decreased with pupil dilation in most subjects, a significant part of subjects showed iris volume increase with pupil dilation. Specifically, VOL increase in 179 (23.52%) subjects, including 169 (22.96%) non-progressors and 10 (40%) progressors. (Supplementary Table S2) Among those with VOL increase, 153 (85.47%) subjects had a <10% increase while 4 (2.23%) subjects had VOL increase >20%, up to 8.56mm^3.

2. Differences between non-progressors and progressors

The measured parameters were compared between non-progressors and progressors in the light and dark condition (Table 2). In a previous publication, the static measurements in the dark condition for the ZAP study population were included; however, since a modest number of subjects were excluded in the present study as indicated in Methods, we repeat the Dark condition as well as the Light condition data here for those subjects included in this study. In general, progressors had narrower AOD, and smaller TISA in each condition. The IT750 was thinner in the light in progressors ($p = 0.01$), but nearly equally as thick in the dark to that in non-progressors ($p = 0.44$). Interestingly, Iarea in light and dark and VOL in the light were smaller in progressors than non-progressors. The centroid position relative to the central image axis (CENT) was not significantly different either in light or dark between groups.

We compared the change from light to dark in measured OCT parameters between non-progressors and progressors. Progressors had significantly lower ΔAOD500 (less extent of angle narrowing with pupil enlargement), greater ΔIT750 (more pronounced iris thickening with pupil enlargement), and lower ΔVOL (less decrease in VOL with pupil enlargement) ($p = 0.04$, 0.004, and 0.045, respectively; Table 3). In a logistic regression model (Table 4), the variables significantly associated with progression were narrower AOD250, older age, flatter iris curvature, and lower loss of VOL. The mean change in centroid length was not significantly different in the 2 groups. With receiver operating characteristic (ROC) analysis (Supplementary Figure 5), the area under the curve for ΔVOL alone was 0.621, while for the combined index of variables, which included ΔVOL, significantly related to progression status, it was 0.824. If we use ΔVOL alone, the optimal cutoff point to separate progressors from non-progressors was -1.16, with a sensitivity of 0.60 and specificity of 0.64 according to the Youden index. For the combined index, at a sensitivity of 84%, the specificity was 77%.

Iarea in the dark decreased in 99% of non-progressors and in 96% of progressors, with the small remainder actually increasing area (change value ≥ zero) at larger pupil size (p = 0.29, Fisher's exact test; Supplementary Table S2). By contrast, VOL in the dark increased in 23% of non-progressors and in 40% of progressors (difference between patient groups, p = 0.048).

3. Differences among progressors

Among 25 progressors with available ASOCT data, 1 had acute attack and PAS, 3 had IOP elevation and PAS, 2 had IOP elevation only and 19 had PAS only. We grouped progressors into two groups: one included eyes that had elevated IOP alone with those who had both elevated IOP and concurrent PAS and a second group that had PAS alone (Table 5). The only parameter that was significantly different between the groups was a greater lens vault in the dark in the elevated IOP group (lens more anterior to the scleral spur to scleral spur line). The ΔVOL and ΔVOL/ΔPD were marginally significant, with the elevated IOP eyes having less volume loss or even a gain in volume.

DISCUSSION

Using ASOCT measured both in light and dark, we found iris area and volume decreased with pupil dilation in most PACS patients. Over 72 months of observation, patients presenting lower loss of iris volume with physiologic pupil dilation at baseline had higher risk of progression of angle closure.

Our data confirm that iris area and volume change when the pupil dilates. This feature of iris dynamic behavior is present in most eyes and may have evolved to avoid angle obstruction in the eye. How fluid moves into and out of the iris is an important area for study. It may relate to presence of macroscopic open areas (crypts) or more likely due to microscopic interactions between fluid in the stroma and its matrix molecular structure. Glycosaminoglycans retain water in tissue and their composition and distribution may explain the relative increase/decrease in iris volume. Differences in collagens[21] and genome wide associations with collagen 11 and PLEKHA7,[22] coding for a junctional protein thought to have a role in maintenance of the blood aqueous barrier, have been reported in PACD eyes.

Several cross-sectional studies have shown that eyes with PACD lose less iris area and volume than non-PACD controls.[13 18 23] This is particularly true of eyes that develop AAC of PACD.[18 24] The association has been seen in persons derived from every major region of the world, so it is not unique to Chinese

persons, though the trait of losing less volume may be more common among Chinese persons. The present study is the first, to our knowledge, to show that less iris volume loss (or even a calculated gain in volume) on pupil dilation is significantly associated with incident PACD. Baskaran et al. found a low rate of conversion to PACD in PACS in Singapore Chinese persons in a randomized trial of iridotomy, but did not report iris area/volume changes in their cohort.[25] While our number of progressors was small, there was nearly a significant difference between progressors that developed high IOP and those that were identified due to development of PAS—with the high IOP group having the lower volume loss/mm pupil enlargement. The percent iris area loss per mm pupil enlargement here was similar in magnitude to that reported by Seager et al.[13] The progressors did not have thicker iris nor larger iris areas at baseline—in fact, their iris area and iris thickness at baseline in the light was significantly smaller than that of the non-progressors, but became equal to that of non-progressors in the dark condition. Dynamic change in ocular tissues is at least as important as the static anatomy of anterior structures.

When static and dynamic attributes are both used, a substantial improvement in the number needed to treat would be achieved. For example, over the 6 years of the study, 889 eyes were untreated in the study and 36 eyes were confirmed as progressors. If all eyes had received iridotomy, the ratio of treated to protected eyes would be 24.7. If the combined index maximum sensitivity/specificity (84%/77%) were used to select those receiving treatment, 30 eyes of progressors would be identified and treated, while 196 non-progressors would be treated, for a ratio of 7.5. This would miss 6 eyes of progressors whose combined index did not reach the standard, but could dramatically reduce the number needed to treat. We should also note that 19 treated eyes met criteria for progression despite iridotomy, most by development of PAS.

Past research has indicated some parameters of ASOCT that indicate the likelihood of PACD.[26-31] Some of these biometric parameters are prospectively predictive of incident gonioscopic angle closure or increased angle narrowing.[10 32 33] Xu et al have reported the other ASOCT parameters associated with progressive development of PACD in the ZAP study population.[5] In a multivariable model, narrower AOD500, flatter iris curvature, and older age were associated significantly with progression. There have been attempts to construct algorithms of multiple risk factors to identify better those more likely to develop PACD[34]; however, these did not function at a level sufficient for population screening. In part, this is due to parameters such as AOD, TISA, and iris thickness being very sensitive to the marking of the

scleral spur position. Lens vault and iris curvature are much less or not at all dependent on marked spur position, and, in several studies these seem to better identify those with developed PACD.

It may seem puzzling that iris volume change, as calculated, is less than area change, and even that volume can seem to have mildly increased when area decreased. The two variables determining iris volume, and its change, are iris area change and centroid position change. Zhang et.al[16] found that the increase in centroid-to-centroid distance in the dark was significantly greater in PAC/PACG subjects than PACS or normal subjects. In the present data, centroid length increased by approximately 0.5 mm on each side of the iris in the dark, and, on average, was not statistically different between progressors and non-progressors. But, more progressors had a calculated volume increase. This suggests that the modestly greater centroid movement toward the peripheral angle contributed to the risk for progression. As pointed out by Seager et al., the greater peripheral movement of the centroid corresponds to relatively more iris stroma nearer to the angle than in the light, making closure of the angle more likely.

There are limitations in the present investigation that merit mention. The number of progressors was small, due to the low incidence of development of PACD in PACS and the inability to include some progressors in the data. We didn't collect topical and systemic medications at follow-up examinations, thus subjects having medications that could affect the iris or angle configuration could not be excluded from the analysis. All subjects were Chinese, but the feature of iris volume loss has been demonstrated in persons from every continent. It would be an improvement to measure iris volume using many cross-sectional areas and to integrate them, such as with swept source OCT (SSOCT). However, one study utilizing this methodology found less significant differences between normal eyes and those with PACD and OAG.[35] With the many images available from SSOCT, the need to identify the scleral spur becomes relatively impractical. Furthermore, superior areas of the anterior segment eye are difficult to image by ASOCT, limiting the value to methods with images in this area.

CONCLUSIONS

In summary, a smaller change in iris volume with physiological pupil size change has been shown to be an additive risk factor to identify eyes more likely to develop PACD among PACS.

REFERENCES

1. Foster PJ, Alsbirk PH, Baasanhu J, et al. Anterior chamber depth in Mongolians: variation with age, sex, and method of measurement. *Am J Ophthalmol* 1997;124(1):53-60. doi: 10.1016/s0002-9394(14)71644-7 [published Online First: 1997/07/01]
2. He M, Huang W, Zheng Y, et al. Anterior chamber depth in elderly Chinese: the Liwan eye study. *Ophthalmology* 2008;115(8):1286-90, 90 e1-2. doi: 10.1016/j.ophtha.2007.12.003 [published Online First: 2008/05/13]
3. Nolan WP, See JL, Chew PT, et al. Detection of primary angle closure using anterior segment optical coherence tomography in Asian eyes. *Ophthalmology* 2007;114(1):33-9. doi: 10.1016/j.ophtha.2006.05.073 [published Online First: 2006/10/31]
4. He M, Jiang Y, Huang S, et al. Laser peripheral iridotomy for the prevention of angle closure: a single-centre, randomised controlled trial. *Lancet* 2019;393(10181):1609-18. doi: 10.1016/S0140-6736(18)32607-2 [published Online First: 2019/03/18]
5. Xu BY, Friedman DS, Foster PJ, et al. Ocular Biometric Risk Factors for Progression of Primary Angle Closure Disease: The Zhongshan Angle Closure Prevention Trial. *Ophthalmology* 2022;129(3):267-75. doi: 10.1016/j.ophtha.2021.10.003 [published Online First: 2021/10/12]
6. Jiang Y, Wang W, Wang L, et al. Association of anterior segment parameters and 5-year incident narrow angles: findings from an older Chinese population. *Br J Ophthalmol* 2021;105(7):970-76. doi: 10.1136/bjophthalmol-2020-315852 [published Online First: 2020/07/24]
7. Seager FE, Wang J, Arora KS, et al. The effect of scleral spur identification methods on structural measurements by anterior segment optical coherence tomography. *J Glaucoma* 2014;23(1):e29-38. doi: 10.1097/IJG.0b013e31829e55ae [published Online First: 2013/06/29]
8. Leung CK, Cheung CY, Li H, et al. Dynamic analysis of dark-light changes of the anterior chamber angle with anterior segment OCT. *Invest Ophthalmol Vis Sci* 2007;48(9):4116-22. doi: 10.1167/iovs.07-0010 [published Online First: 2007/08/29]
9. Barkana Y, Dorairaj SK, Gerber Y, et al. Agreement between gonioscopy and ultrasound biomicroscopy in detecting iridotrabecular apposition. *Arch Ophthalmol* 2007;125(10):1331-5. doi: 10.1001/archopht.125.10.1331 [published Online First: 2007/10/10]
10. Jiang Y, Wang D, Wang W, et al. Five-year changes in anterior segment parameters in an older population in urban southern China: the Liwan Eye Study. *Br J Ophthalmol* 2020;104(4):582-87. doi: 10.1136/bjophthalmol-2019-313827 [published Online First: 2019/09/06]
11. Quigley HA, Friedman DS, Congdon NG. Possible mechanisms of primary angle-closure and malignant glaucoma. *J Glaucoma* 2003;12(2):167-80. doi: 10.1097/00061198-200304000-00013 [published Online First: 2003/04/03]
12. Silver DM, Quigley HA. Aqueous flow through the iris-lens channel: estimates of differential pressure between the anterior and posterior chambers. *J Glaucoma* 2004;13(2):100-7. doi: 10.1097/00061198-200404000-00004 [published Online First: 2004/04/21]
13. Seager FE, Jefferys JL, Quigley HA. Comparison of dynamic changes in anterior ocular structures examined with anterior segment optical coherence tomography in a cohort of various origins. *Invest Ophthalmol Vis Sci* 2014;55(3):1672-83. doi: 10.1167/iovs.13-13641 [published Online First: 2014/02/22]
14. Quigley HA, Silver DM, Friedman DS, et al. Iris cross-sectional area decreases with pupil dilation and its dynamic behavior is a risk factor in angle closure. *J Glaucoma* 2009;18(3):173-9. doi: 10.1097/IJG.0b013e31818624ce [published Online First: 2009/03/20]
15. Aptel F, Denis P. Optical coherence tomography quantitative analysis of iris volume changes after pharmacologic mydriasis. *Ophthalmology* 2010;117(1):3-10. doi: 10.1016/j.ophtha.2009.10.030 [published Online First: 2009/11/20]

16. Zhang Y, Li SZ, Li L, et al. Dynamic Iris Changes as a Risk Factor in Primary Angle Closure Disease. *Invest Ophthalmol Vis Sci* 2016;57(1):218-26. doi: 10.1167/iovs.15-17651 [published Online First: 2016/01/25]
17. Lin J, Wang Z, Chung C, et al. Dynamic changes of anterior segment in patients with different stages of primary angle-closure in both eyes and normal subjects. *PLoS One* 2017;12(5):e0177769. doi: 10.1371/journal.pone.0177769 [published Online First: 2017/05/26]
18. Narayanaswamy A, Zheng C, Perera SA, et al. Variations in iris volume with physiologic mydriasis in subtypes of primary angle closure glaucoma. *Invest Ophthalmol Vis Sci* 2013;54(1):708-13. doi: 10.1167/iovs.12-10844 [published Online First: 2013/01/10]
19. Panda SK, Tan RKY, Tun TA, et al. Changes in Iris Stiffness and Permeability in Primary Angle Closure Glaucoma. *Invest Ophthalmol Vis Sci* 2021;62(13):29. doi: 10.1167/iovs.62.13.29 [published Online First: 2021/10/30]
20. Jiang Y, Friedman DS, He M, et al. Design and methodology of a randomized controlled trial of laser iridotomy for the prevention of angle closure in southern China: the Zhongshan angle Closure Prevention trial. *Ophthalmic Epidemiol* 2010;17(5):321-32. doi: 10.3109/09286586.2010.508353 [published Online First: 2010/09/28]
21. He M, Lu Y, Liu X, et al. Histologic changes of the iris in the development of angle closure in Chinese eyes. *J Glaucoma* 2008;17(5):386-92. doi: 10.1097/IJG.0b013e31815c5f69 [published Online First: 2008/08/16]
22. Vithana EN, Khor CC, Qiao C, et al. Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. *Nat Genet* 2012;44(10):1142-46. doi: 10.1038/ng.2390 [published Online First: 2012/08/28]
23. Zhang Y, Li SZ, Li L, et al. Quantitative analysis of iris changes after physiologic and pharmacologic mydriasis in a rural Chinese population. *Invest Ophthalmol Vis Sci* 2014;55(7):4405-12. doi: 10.1167/iovs.13-13782 [published Online First: 2014/04/26]
24. Aptel F, Chiquet C, Beccat S, et al. Biometric evaluation of anterior chamber changes after physiologic pupil dilation using Pentacam and anterior segment optical coherence tomography. *Invest Ophthalmol Vis Sci* 2012;53(7):4005-10. doi: 10.1167/iovs.11-9387 [published Online First: 2012/05/24]
25. Baskaran M, Kumar RS, Friedman DS, et al. The Singapore Asymptomatic Narrow Angles Laser Iridotomy Study: Five-Year Results of a Randomized Controlled Trial. *Ophthalmology* 2022;129(2):147-58. doi: 10.1016/j.ophtha.2021.08.017 [published Online First: 2021/08/29]
26. Nongpiur ME, He M, Amerasinghe N, et al. Lens vault, thickness, and position in Chinese subjects with angle closure. *Ophthalmology* 2011;118(3):474-9. doi: 10.1016/j.ophtha.2010.07.025 [published Online First: 2010/11/03]
27. Aung T, Nolan WP, Machin D, et al. Anterior chamber depth and the risk of primary angle closure in 2 East Asian populations. *Arch Ophthalmol* 2005;123(4):527-32. doi: 10.1001/archopht.123.4.527 [published Online First: 2005/04/13]
28. Nongpiur ME, Haaland BA, Friedman DS, et al. Classification algorithms based on anterior segment optical coherence tomography measurements for detection of angle closure. *Ophthalmology* 2013;120(1):48-54. doi: 10.1016/j.ophtha.2012.07.005 [published Online First: 2012/09/27]
29. Guzman CP, Gong T, Nongpiur ME, et al. Anterior segment optical coherence tomography parameters in subtypes of primary angle closure. *Invest Ophthalmol Vis Sci* 2013;54(8):5281-6. doi: 10.1167/iovs.13-12285 [published Online First: 2013/06/22]
30. Xu BY, Liang S, Pardeshi AA, et al. Differences in Ocular Biometric Measurements among Subtypes of Primary Angle Closure Disease: The Chinese American Eye Study. *Ophthalmol Glaucoma* 2021;4(2):224-31. doi: 10.1016/j.ogla.2020.09.008 [published Online First: 2020/09/18]

31. Moghimi S, Vahedian Z, Fakhraie G, et al. Ocular biometry in the subtypes of angle closure: an anterior segment optical coherence tomography study. *Am J Ophthalmol* 2013;155(4):664-73, 73 e1. doi: 10.1016/j.ajo.2012.10.014 [published Online First: 2012/12/19]
32. Baskaran M, Iyer JV, Narayanaswamy AK, et al. Anterior Segment Imaging Predicts Incident Gonioscopic Angle Closure. *Ophthalmology* 2015;122(12):2380-4. doi: 10.1016/j.ophtha.2015.07.030 [published Online First: 2015/09/12]
33. Nongpiur ME, Aboobakar IF, Baskaran M, et al. Association of Baseline Anterior Segment Parameters With the Development of Incident Gonioscopic Angle Closure. *JAMA Ophthalmol* 2017;135(3):252-58. doi: 10.1001/jamaophthalmol.2016.5847 [published Online First: 2017/02/15]
34. Zhang Y, Zhang Q, Li L, et al. Establishment and Comparison of Algorithms for Detection of Primary Angle Closure Suspect Based on Static and Dynamic Anterior Segment Parameters. *Transl Vis Sci Technol* 2020;9(5):16. doi: 10.1167/tvst.9.5.16 [published Online First: 2020/08/22]
35. Mak H, Xu G, Leung CK. Imaging the iris with swept-source optical coherence tomography: relationship between iris volume and primary angle closure. *Ophthalmology* 2013;120(12):2517-24. doi: 10.1016/j.ophtha.2013.05.009 [published Online First: 2013/07/16]

Table 1. Baseline characteristics of eligible participants included in and excluded from analysis.

	Inclusion (n=761)	Exclusion (n=128)	P Value
Age, years	59.17 ± 4.97	60.30 ± 5.14	**0.02**
Female, n%	639 (83.97%)	98 (76.56%)	**0.04**
IOP, mmHg	14.78 ± 2.80	15.32 ± 2.83	0.14
AL, mm	22.48 ± 0.72	22.53 ± 0.77	0.63
ACD, mm	2.56 ± 0.21	2.47 ± 0.23	**<0.001**
LT, mm	4.87 ± 0.32	4.98 ± 0.29	**<0.001**
Progressor, n%	25 (3.29%)	11 (8.59%)	**0.01**

IOP, intraocular pressure; AL, axial length; ACD, anterior chamber depth; LT, lens thickness. AL, ACD and LT measurements here were derived from A-scan.

Boldface values indicate significance at $P < 0.05$.

Table 2. Differences among Horizontal Biometric Measurements at Baseline between Non-progressors and Progressors.

	Light			Dark		
	Non-progressors (n=736)	Progressors (n=25)	P Value	Non-progressors (n=736)	Progressors (n=25)	P Value
AOD250 (mm)	0.120 ± 0.047	0.072 ± 0.042	**<0.001**[b]	0.073 ± 0.052	0.030 ± 0.029	**<0.001**[b]
AOD500 (mm)	0.155 ± 0.048	0.113 ± 0.055	**0.001**[b]	0.089 ± 0.053	0.061 ± 0.053	**0.01**[b]
AOD750 (mm)	0.216 ± 0.062	0.189 ± 0.066	0.06[b]	0.133 ± 0.063	0.117 ± 0.069	0.25[b]
TISA500 (mm^2)	0.075 ± 0.022	0.051 ± 0.019	**<0.001**[b]	0.051 ± 0.026	0.030 ± 0.021	**0.001**[b]
TISA750 (mm^2)	0.129 ± 0.032	0.096 ± 0.031	**<0.001**[b]	0.086 ± 0.037	0.057 ± 0.034	**<0.001**[b]
ARA (mm^2)	0.158 ± 0.048	0.112 ± 0.041	**<0.001**[b]	0.103 ± 0.052	0.063 ± 0.039	**<0.001**[b]
IT750 (mm)	0.447 ± 0.077	0.408 ± 0.062	**0.01**[b]	0.488 ± 0.074	0.485 ± 0.066	0.44[a]
IT2000 (mm)	0.465 ± 0.058	0.439 ± 0.062	0.05[b]	0.494 ± 0.062	0.486 ± 0.070	0.47[a]
Iarea (mm^2)	1.890 ± 0.261	1.771 ± 0.202	**0.02**[b]	1.565 ± 0.207	1.482 ± 0.154	**0.049**[b]
Icurv (mm)	0.436 ± 0.105	0.395 ± 0.116	0.17[b]	0.437 ± 0.093	0.374 ± 0.095	**0.001**[b]
ITCM (mm)	0.589 ± 0.065	0.562 ± 0.070	**0.02**[a]	0.620 ± 0.064	0.598 ± 0.070	0.053[a]
CENT (mm)	3.555 ± 0.189	3.562 ± 0.228	0.97[b]	4.006 ± 0.219	4.017 ± 0.244	0.79[b]
VOL (mm^3)	35.942 ± 5.333	33.355 ± 4.279	**0.02**[b]	33.464 ± 4.740	32.422 ± 4.780	0.34[b]
LV (μm)	757.946 ± 173.499	787.880 ± 182.569	0.20[a]	738.285 ± 169.760	742.773 ± 190.659	0.45[a]
ACD (mm)	2.211 ± 0.197	2.162 ± 0.234	0.13[b]	2.213 ± 0.195	2.164 ± 0.233	0.12[b]
PD (mm)	2.714 ± 0.510	2.740 ± 0.559	0.95[b]	4.458 ± 0.686	4.479 ± 0.730	0.44[a]

[a] t-test

[b] Wilcoxon rank-sum test

AOD250 = angle opening distance of 250 μm from the scleral spur; AOD500 = angle opening distance of 500 μm from the scleral spur; AOD750 = angle opening distance of 750 μm from the scleral spur; TISA500 = trabecular iris space area bounded by AOD500; TISA750 = trabecular iris space area bounded by AOD750; ARA = angle recess area; IT750 = Iris thickness 750 μm from the scleral spur; IT2000 = Iris thickness 2000 μm from the scleral spur; Iarea = iris area; Icurv = iris curvature; ITCM = the maximum iris thickness at the middle one third of the iris; CENT = the distance from iris centroid to middle line; VOL = iris volume; ACD = anterior chamber depth; PD = pupillary diameter.

Boldface values indicate significance at $P < 0.05$.

Table 3. Differences among Horizontal Biometric Changes with Physiological Pupil Enlargement at Baseline between Non-progressors and Progressors.

Parameters [a]	Non-progressors (n=736)	Progressors (n=25)	P Value
ΔAOD250 (mm)	-0.050 ± 0.041[b]	-0.042 ± 0.033[b]	0.37[d]
ΔAOD500 (mm)	-0.069 ± 0.043[b]	-0.054 ± 0.042[b]	**0.04[c]**
ΔAOD750 (mm)	-0.088 ± 0.053[b]	-0.076 ± 0.055[b]	0.14[c]
ΔTISA500 (mm^2)	-0.026 ± 0.017[b]	-0.022 ± 0.015[b]	0.27[d]
ΔTISA750 (mm^2)	-0.046 ± 0.026[b]	-0.040 ± 0.026[b]	0.39[d]
ΔARA (mm^2)	-0.055 ± 0.038[b]	-0.051 ± 0.038[b]	0.62[d]
ΔIT750 (mm)	0.042 ± 0.071[b]	0.081 ± 0.063[b]	**0.004[c]**
ΔIT2000 (mm)	0.031 ± 0.049[b]	0.046 ± 0.065[b]	0.35[d]
ΔIarea (mm^2)	-0.324 ± 0.157[b]	-0.289 ± 0.136[b]	0.32[d]
ΔIcurv (mm)	0.004 ± 0.087	-0.021 ± 0.090	0.08[c]
ΔITCM (mm)	0.032 ± 0.048[b]	0.035 ± 0.047[b]	0.83[d]
ΔCENT (mm)	0.451 ± 0.157[b]	0.455 ± 0.158[b]	0.71[d]
ΔVOL (mm^3)	-2.478 ± 3.478[b]	-0.933 ± 2.979	**0.045[d]**
ΔLV (μm)	-18.342 ± 68.088[b]	-45.108 ± 87.772[b]	0.06[d]
ΔACD (mm)	0.001 ± 0.014	0.002 ± 0.011	0.73[d]
ΔPD (mm)	1.744 ± 0.542[b]	1.739 ± 0.533[b]	0.94[d]
ΔVOL%/ΔPD (%/mm)	-3.761 ± 5.921	-1.385 ± 7.479	0.14[d]
ΔIarea%/ΔPD (%/mm)	-9.833 ± 4.274	-9.371 ± 4.032	0.99[d]

[a] Parameters change was calculated as dark minus light. Δ<0 means decrease after dilation while Δ>0 means increase after dilation.

[b] Significant change from light to dark using paired t-test or Wilcoxon matched-pairs signed-ranks test

[c] t-test

[d] Wilcoxon rank-sum test

AOD250 = angle opening distance of 250 µm from the scleral spur; AOD500 = angle opening distance of 500 µm from the scleral spur; AOD750 = angle opening distance of 750 µm from the scleral spur; TISA500 = trabecular iris space area bounded by AOD500; TISA750 = trabecular iris space area bounded by AOD750; ARA = angle recess area; IT750 = Iris thickness 750 µm from the scleral spur; IT2000 = Iris thickness 2000 µm from the scleral spur; Iarea = iris area; Icurv = iris curvature; ITCM = the maximum iris thickness at the middle one third of the iris; CENT = the distance from iris centroid to middle line; VOL = iris volume; ACD = anterior chamber depth; PD = pupillary diameter; ΔVOL%/ΔPD = the percent change in iris volume per mm pupil increase; ΔVOL%/ΔPD = the percent change in iris area per mm pupil increase.

Boldface values indicate significance at $P < 0.05$.

Table 4. Logistic Regression Models of the Association between Progression and Biometric Parameters at Dark

	Univariable		Multivariable [a]	
	Odds Ratio (95%CI)	P Value	Odds Ratio (95%CI)	P Value
Age (year)	1.07 (0.99-1.16)	0.07	1.12 (1.03-1.21)	**0.008**
Female	1.41 (0.42-4.80)	0.58		
IOP (mmHg)	1.09 (0.95-1.26)	0.21		
AOD250 (0.01mm)	0.80 (0.71-0.90)	**<0.001**	0.82 (0.73-0.92)	**0.001**
AOD500 (0.01mm)	0.90 (0.83-0.98)	**0.01**		
AOD750 (0.01mm)	0.96 (0.90-1.02)	0.22		
TISA500 ($0.01mm^2$)	0.69 (0.57-0.83)	**<0.001**		
TISA750 ($0.01mm^2$)	0.80 (0.70-0.90)	**<0.001**		
ARA ($0.01mm^2$)	0.82 (0.74-0.91)	**<0.001**		

IT750 (0.1mm)	0.96 (0.55-1.66)	0.88		
IT2000 (0.1mm)	0.80 (0.42-1.53)	0.51		
Iarea ($0.1mm^2$)	0.81 (0.66-1.00)	**0.047**		
Icurv (0.1mm)	0.46 (0.29-0.73)	**0.001**	0.49 (0.29-0.82)	**0.006**
PD (mm)	1.05 (0.56-1.87)	0.88		
ACD (mm)	0.29 (0.04-2.16)	0.23		
LV (0.1µm)	1.00 (0.99-1.00)	0.90		
VOL (mm^3)	0.95 (0.87-1.04)	0.28		
ΔVOL (mm^3)	1.15 (1.02-1.30)	**0.03**	1.15 (1.01-1.31)	**0.04**
ΔVOL%/ΔPD (%/mm)	519.94 (1.14-242463)	**0.046**		
ΔIarea%/ΔPD (%/mm)	13.50 (0 - 185146)	0.59		

[a]The model was developed using the best subset selection method to maximize the adjusted R^2 value. IOP = intraocular pressure; AOD250 = angle opening distance of 250 µm from the scleral spur; AOD500 = angle opening distance of 500 µm from the scleral spur; AOD750 = angle opening distance of 750 µm from the scleral spur; TISA500 = trabecular iris space area bounded by AOD500; TISA750 = trabecular iris space area bounded by AOD750; ARA = angle recess area; IT750 = Iris thickness 750 µm from the scleral spur; IT2000 = Iris thickness 2000 µm from the scleral spur; Iarea = iris area; Icurv = iris curvature; PD = pupillary diameter; ACD = anterior chamber depth; LV= lens vault; VOL = iris volume; ΔVOL = iris volume change with pupil enlargement; ΔVOL%/ΔPD = the percent change in iris volume per mm pupil increase; ΔVOL%/ΔPD = the percent change in iris area per mm pupil increase.

Boldface values indicate significance at $P < 0.05$.

Table 5. Differences among Horizontal Biometric Measurements at Baseline between Subjects Having Elevated IOP or PAS.

Parameters at dark	Elevated IOP n=6	PAS n=19	P Value
Age (year)	64.66 ± 4.68	61.36 ± 6.34	0.13 [a]
Female	18 (94.74%)	4 (66.67%)	0.06 [a]
IOP (mmHg)	17 ± 3.03	15.37 ± 3.25	0.14 [a]
AOD250 (mm)	0.030 ± 0.027	0.030 ± 0.030	0.97 [b]

AOD500 (mm)	0.051 ± 0.045	0.065 ± 0.056	0.3 [a]
AOD750 (mm)	0.117 ± 0.071	0.119 ± 0.071	0.47 [a]
TISA500 (mm^2)	0.030 ± 0.021	0.029 ± 0.023	0.46 [a]
TISA750 (mm^2)	0.055 ± 0.037	0.058 ± 0.033	0.42 [a]
ARA (mm^2)	0.066 ± 0.046	0.063 ± 0.037	0.95 [b]
IT750 (mm)	0.474 ± 0.060	0.489 ± 0.069	0.33 [a]
IT2000 (mm)	0.459 ± 0.097	0.494 ± 0.060	0.15 [a]
Iarea (mm^2)	1.409 ± 0.143	1.505 ± 0.154	0.1 [a]
Icurv (mm)	0.383 ± 0.133	0.371 ± 0.085	0.4 [a]
PD (mm)	4.366 ± 0.794	4.515 ± 0.727	0.34 [a]
ACD (mm)	2.131 ± 0.212	2.174 ± 0.244	0.35 [a]
LV (µm)	869.687 ± 175.920	702.695 ± 181.126	**0.03** [a]
VOL (mm^3)	30.387 ± 4.240	33.064 ± 4.864	0.12 [a]
ΔVOL (mm^3)	0.630 ± 4.278	-1.427 ± 2.383	0.07 [a]
ΔVOL%/ΔPD (%/mm)	0.029 ± 0.132	-0.027 ± 0.043	0.055 [a]
ΔIarea%/ΔPD (%/mm)	-0.075 ± 0.064	-0.100 ± 0.029	0.10 [a]

[a] t-test

[b] Wilcoxon rank-sum test

IOP = intraocular pressure; PAS = peripheral anterior synechiae; AOD250 = angle opening distance of 250 µm from the scleral spur; AOD500 = angle opening distance of 500 µm from the scleral spur; AOD750 = angle opening distance of 750 µm from the scleral spur; TISA500 = trabecular iris space area bounded by AOD500; TISA750 = trabecular iris space area bounded by AOD750; ARA = angle recess area; IT750 = Iris thickness 750 µm from the scleral spur; IT2000 = Iris thickness 2000 µm from the scleral spur; Iarea = iris area; Icurv = iris curvature; PD = pupillary diameter; ACD = anterior chamber depth; LV= lens vault; VOL = iris volume; ΔVOL = iris volume change with pupil enlargement; ΔVOL%/ΔPD = the percent change in iris volume per mm pupil increase; ΔVOL%/ΔPD = the percent change in iris area per mm pupil increase.

Boldface values indicate significance at $P < 0.05$.

Figure Legends

Supplementary Figure 1. ASOCT images as used in study and processed in ZAAP software. a) The borders of the corneal epithelium, endothelium, the surfaces of the iris and the centroid of the iris are automatically defined by the software's automated segmentation. b) The parameters used in the calculation of iris volume are the iris area (red) and the centroid (CENT) or radius of the center of mass of the iris (yellow dot) from the optical axis (vertical line). Iris volume is calculated from the formula for volume of a torus, e.g. the average iris area of the two sides in the image times the circumference of the iris at its centroid (2π*CENT).

Supplementary Figure 2. Distribution of Iris Volume at Baseline.

Supplementary Figure 3. Distribution of Iris Area at Baseline.

Supplementary Figure 4. Correlation between Iris Volume Change (%) (Left) and Iris Area Change (%) (Right) with Pupil Enlargement at Baseline.

Supplementary Figure 5. Receiving operating characteristic (ROC) curve and area under the curve (AUC) using iris volume change (ΔVOL) and combined index (which included age, angle opening distance of 250 μm from the scleral spur [AOD250], iris curve, and ΔVOL). The values for the best mixture of sensitivity and specificity are indicated on each curve.