Heritability of the Iridotrabecular Angle Width Measured by Optical Coherence Tomography in Chinese Children: The Guangzhou Twin Eye Study

Mingguang He,1,2 Jian Ge,1 Dandan Wang,1 Jian Zhang,1 Alex W. Hewitt,3 Yoon-Mi Hur,4 David A. Mackey,5 and Paul J. Foster2

PURPOSE. To estimate the heritability of the iridotrabecular angle width measured by anterior segment optical coherence tomography (ASOCT) in a classic twin study.

METHODS. Twins aged 8 to 16 years were identified from the Guangzhou Twin Registry. ASOCT was used to obtain one horizontal scan, and the images were analyzed with custom software. Angle width was represented by the angle opening distance (AOD) at the 500-μm anterior-to-scleral spur, as well as the angle recess area (ARA) and trabecular-iris space area (TISA) located 750 μm anterior to the scleral spur. Zygosity was confirmed by genotyping with 16 polymorphic markers in all same-sex twin pairs. Heritability was assessed by observed variance component genetic modeling after adjustment for age and sex, with the Mx program.

RESULTS. The mean age of the participants was 11.7 ± 2.6 years in 305 monozygotic (MZ) and 11.8 ± 2.4 years in 157 dizygotic (DZ) pairs. The intraclass correlation coefficients for the AOD, ARA, and TISA were 0.70, 0.75, and 0.72 in the MZ pairs and 0.54, 0.52, and 0.33 in the DZ pairs, respectively. A model with additive genetic (69.7%; 95% CI: 63.9%–74.6%) and unique environmental effects (30.3%; 95% CI: 25.4%–36.1%) was the most parsimonious for AOD. Similar results were identified for ARA and TISA.

CONCLUSIONS. The variance in drainage angle width in Chinese children appears to be largely attributable to genetic effects, with a heritability of approximately 70%. (Invest Ophthalmol Vis Sci. 2008;49:1356–1361) DOI:10.1167/iovs.07-1397

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Supported by National Natural Science Foundation of China Grant 30772395; the Program for New Century Excellent Talents in University; the National Ministry of Education Grant NCET-06-0720; Sino-Australia NSFC-DEST (the Chinese State National Science Fund Committee–Australian Department of Education, Science and Training) Special Fund 30571513; and the Guangzhou Science and Technology Development Fund 200623-E0061. DAM is a Pfizer Australia Research Fellow.

Submitted for publication October 28, 2007; revised December 9 and 29, 2007; accepted February 21, 2008.

Disclosure: M. He, None; J. Ge, None; D. Wang, None; J. Zhang, None; A.W. Hewitt, None; Y.-M. Hur, None; D.A. Mackey, None; P.J. Foster, Carl Zeiss Meditec (R)

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Population-based studies suggest that the prevalence of primary angle-closure glaucoma (PACG) is approximately 1.5% in the Chinese people, a statistic that is markedly higher than that of European or African populations.1,2 The observed ethnic differences in the prevalence of PAC have led to considerable interest in identifying the genetic cause of the disease or its predisposing anatomic traits. Familial aggregation of anterior chamber depth, a PAC-related anatomic trait or "intermediate phenotype"3 for angle closure, has been reported in extended family studies.4–6 Furthermore, in our recent population-based twin study, we concluded that the variation in anterior chamber depth is predominately attributable to additive genetic effects (90.1%), with only a small proportion (9.9%) of the variation due to unique environmental influences.7

Appositional contact or permanent synechiae of the drainage angle is the most common mechanistic feature for the development of angle-closure damage. Previous cross-sectional studies have demonstrated, based on gonioscopic findings, a "dose-response" increase in the rates of peripheral anterior synechiae (PAS) in eyes with narrow drainage angle widths.8,9 Although longitudinal data are needed for confirmation, these findings underscore the fact that persons with narrow drainage angles are at a greater risk for the development of PAC-related problems.

Traditionally, angle width has been measured by gonioscopy, with various types of grading systems.10–12 Despite much work validating these schemes, gonioscopic grading is a subjective measurement that usually depends on the examiner’s experience and skill, being particularly subject to measurement errors introduced by manipulation and variation of illumination.13

Recent modifications of optical coherence tomography (OCT) have provided a fast, noninvasive, and objective means of imaging the anterior chamber and in particular the iridotrabecular angle.14,15 A single image at a particular meridian can capture the entire cornea, both angles, and the anterior portion of the lens. In addition, the anterior segment OCT (ASOCT) obtains superior definition of the iris surface and angle structures such as the scleral spur.15 With the aid of custom software, ASOCT has now become an optimal method of quantifying the ITA. Such accurate quantification facilitates the investigation of determinants of ocular structure.

Twin studies have been used widely to determine the relative genetic and environmental contributions made to a particular disease or trait. In a classic twin design, heritability—that is, the proportion of the total phenotypic variation attributable to genetic variance—can be estimated.16 By definition, monozygotic (MZ) twins have identical copies of their genes, whereas dizygotic (DZ) or fraternal twins, on average, have 50% of their genes in common. Assuming that the two types of twins share the same extent of environmental factors,17 greater similarities in phenotypes of MZ than in DZ twins can be attributed to the additional gene sharing. The purpose of this analysis was to estimate the heritability of ITA.
The study participants were recruited from the Guangzhou Twin Registry, which is described in depth elsewhere. In brief, this registry was established in Guangzhou City, China. More than 9700 pairs of twins born between 1987 and 2000 were identified by compilation of an official Household Register of Guangzhou and with a follow-up door-to-door verification. Since 2006, we have invited for annual examination all twins aged 7 to 15 years (at July 1, 2006) living in two neighboring districts of the Zhongshan Ophthalmic Center, where the examination station was established. The participation rate was approximately 82.3%.

ASOCT imaging was performed in all twins who participated in our second annual examination between July and August 2007. The twins ranged between 8 and 16 years of age at the time of the examination. Twin pairs were excluded from this present analysis if one or both twins had pathologic changes (10 retinopathy of prematurity, 1 congenital cataract, 1 optic nerve atrophy).

Zygosity of all same-sex twin pairs (466 pairs of same-sex twins) was determined by 16 multiplex short tandem repeats (STRs; PowerPlex 16 system; Promega, Madison, WI) at the Forensic Medicine Department of Sun Yat-Sen University after the first examination in 2006. Zygosity in opposite-sex twin pairs was considered to be DZ, thereby not requiring genotyping.

Written, informed consent was obtained for all participants from either their parents or legal guardians. Ethical approval was obtained from the Zhongshan University Ethics Review Board and Ethics Committee of Zhongshan Ophthalmic Center, and this study was conducted in accordance with the tenets of the World Medical Association’s Declaration of Helsinki.

ASOCT imaging (Visante; Carl Zeiss Meditec, Dublin, CA) was performed in a standard dark room (<5 lux illuminations) before pharmacologic dilation of the pupils. Refractive correction was used to adjust the internal fixation target, to achieve a nonaccommodated state. One scan, centered over the pupil, was taken on the horizontal meridian (between 0° and 180°). The scan orientation was directionally aligned until an anterior corneal reflex was achieved (indicated by an interference flare on the axis of anterior chamber). The fixation angle was adjusted to align and obtain a horizontal image. The image of each eye of each person that had the best quality (as judged by visibility of the scleral spur and maximum interference flare) was selected and saved for analysis.

The ASOCT images were extracted from the device’s output function as 816 × 636 pixel JPEG (lossless data compression) files. Custom software, the Zhongshan Angle Assessment Program (ZAAP, Guangzhou, China), was used for automatic extraction of the 300 × 600 8-bit grayscale (intensities from 0 to 255) valid image portion of the output file. This program performed noise and contrast conditioning. Algorithms automatically defined the borders of the corneal epithelium and endothelium, anterior and posterior surface of the iris, and anterior surface of the lens. A line-smoothing algorithm that was explicitly defined by the edge-finding algorithms used the derivative data to repair poor resolution of the borders (Fig. 1). Angle opening distance (AOD), angle recess area (ARA), and trabecular-iris space area (TISA) were selected to represent the iridotrabeular angle width. AOD was defined as the length of a line extending from the anterior iris to the corneal endothelium perpendicular to a line along the trabecular meshwork at a given distance from the scleral spur. AOD calculated at a distance of 500 μm from the scleral spur was used for analysis. ARA was defined by the area bordered by the anterior iris surface, corneal endothelium and a line perpendicular to the corneal endothelium drawn to the iris surface from a point 750 μm anterior to the scleral spur. TISA further modified this measurement by not including the area below a line drawn from the scleral spur to the anterior iris perpendicular to the corneal endothelium.

Forty consecutive participants from the twin cohort were selected to assess reproducibility. ASOCT images were captured on two separate occasions, 5 to 10 minutes apart. The images were analyzed later by the same masked grader, using the ZAAP software. The agreement between two independent image acquisitions was assessed by mean ± SD of the difference, coefficient of variance (CV = mean) and 95% limits of agreement. The test-retest differences for AOD, ARA, and TISA were 0.017 ± 0.087 mm (paired t-test, P = 0.243), 0.005 ± 0.078 mm² (paired t-test, P = 0.714), and 0.002 ± 0.058 mm² (paired t-test, P = 0.870). The intersession CVs for AOD, ARA, and TISA were 13.2%, 16.3%, and 13.2%. Limit of agreements were −0.012 to 0.045 mm for AOD, −0.021 to 0.030 mm² for ARA, and −0.017 to 0.020 mm² for TISA.

Data Analysis and Genetic Modeling

The right eye was arbitrarily selected to represent the phenotypic characteristics of the specific individual in the data analysis. The estimation of heritability in our study was based on model-fitting analyses as well as on the comparison of correlations of IITA between MZ and DZ twins.

As outlined earlier, three quantitative traits (AOD, ARA, and TISA) were used to represent the IITA. The average of the measurements on temporal and nasal quadrants on one meridian scan was used for the analysis. AOD and TISA parameters were approximately normally distributed, with skewness and kurtosis values being <1.0 (skewness = 0.67, kurtosis = 0.28 for AOD; skewness = 0.70, kurtosis = 0.46 for TISA). However, ARA showed skewness of 1.00 and a kurtosis of 1.68. To achieve a normal distribution, we log transformed ARA before model-fitting analyses.

The Mx program was used for model-fitting variance component analyses. The total phenotypic variance was decomposed into
Table 1. Phenotypic Characteristics of Twin Pairs by Zygosity and Sex

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>n (pairs)</th>
<th>Age (years)</th>
<th>AOD (mm)</th>
<th>ARA (mm²)</th>
<th>TISA (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dizygotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male–male</td>
<td>45</td>
<td>11.4 (2.5)</td>
<td>0.661 (0.191)</td>
<td>0.551 (0.155)</td>
<td>0.442 (0.118)</td>
</tr>
<tr>
<td>Female–female</td>
<td>32</td>
<td>12.1 (2.4)</td>
<td>0.687 (0.235)</td>
<td>0.556 (0.199)</td>
<td>0.457 (0.143)</td>
</tr>
<tr>
<td>Opposite sex</td>
<td>80</td>
<td>11.8 (2.3)</td>
<td>0.695 (0.234)</td>
<td>0.551 (0.183)</td>
<td>0.465 (0.146)</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>11.8 (2.4)</td>
<td>0.684 (0.222)</td>
<td>0.547 (0.178)</td>
<td>0.457 (0.138)</td>
</tr>
<tr>
<td>Monozygotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male–male</td>
<td>142</td>
<td>11.5 (2.7)</td>
<td>0.662 (0.232)</td>
<td>0.556 (0.211)</td>
<td>0.444 (0.151)</td>
</tr>
<tr>
<td>Female–female</td>
<td>163</td>
<td>11.8 (2.5)</td>
<td>0.651 (0.245)</td>
<td>0.520 (0.202)</td>
<td>0.437 (0.155)</td>
</tr>
<tr>
<td>Total</td>
<td>305</td>
<td>11.7 (2.6)</td>
<td>0.656 (0.239)</td>
<td>0.527 (0.206)</td>
<td>0.440 (0.153)</td>
</tr>
</tbody>
</table>

Descriptive data (mean ± SD) are presented for the right eyes of first-born twins.

Results

After the exclusion of 22 pairs with missing ASOCT data (as a consequence of inability to obtain OCT scanning or data that were not analyzable because of poor image quality), a total of 462 twin pairs (305 MZ, 157 DZ) aged between 8 and 16 years were available for analysis. Table 1 summarizes the demographic and phenotypes of interest in our study cohort. The ages were available for analysis. The ages were not significantly different (t-test, P = 0.833). No significant differences between MZ and DZ twins were identified in AOD (0.656 ± 0.239 mm for MZ, 0.684 ± 0.222 mm for DZ, P = 0.228), ARA (0.527 ± 0.206 mm² vs. 0.547 ± 0.178 mm², P = 0.336), or TISA (0.440 ± 0.153 mm² vs. 0.457 ± 0.138 mm², P = 0.253).

The AOD, ARA, and TISA correlated highly (Pearson correlation coefficients: AOD versus ARA = 0.92, AOD versus TISA = 0.98, ARA versus TISA = 0.96). Multiple linear regression of AOD with age and sex (R² = 0.08, P < 0.0001) suggested that the mean AOD increased by 0.026 mm per year of age (P < 0.0001) and was not significantly different between boys and girls (P = 0.640, adjusted for age). The associations of ARA and TISA with age and sex were similar: Mean ARA increased by 0.021 mm² per year (P < 0.0001), and TISA by 0.016 mm² (P < 0.0001). ARA and TISA were not significantly different between the boys and girls (P = 0.523 for ARA and 0.725 for TISA).

The sex- and age-adjusted intraclass correlations (ICCs, equivalent to the pair-wise correlation coefficient) were 0.73 and 0.36 for AOD in MZ and DZ pairs, respectively. The ICCs for MZ and DZ pairs in ARA and TISA were similar (Table 2). In general, ICCs were consistently high in all MZ twin pairs and were greater in the girls than in the boys. The variation in ICCs was significantly greater in DZ pairs (indicated by 95% CI). The pair-wise correlation in MZ and DZ are also demonstrated in Figure 2.

A series of model comparisons suggested that one with AE (additive and unique environmental) components for all three measures was the most parsimonious (Table 3). Domi

Table 2. Intraclass (Pair-Wise) Correlation Coefficients for Iridotrabecular Angle (95% CI)

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>n (pairs)</th>
<th>AOD</th>
<th>ARA</th>
<th>TISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ Pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male–male</td>
<td>142</td>
<td>0.68 (0.58–0.76)</td>
<td>0.77 (0.69–0.82)</td>
<td>0.70 (0.60–0.77)</td>
</tr>
<tr>
<td>Female–female</td>
<td>163</td>
<td>0.77 (0.70–0.83)</td>
<td>0.78 (0.71–0.83)</td>
<td>0.78 (0.71–0.83)</td>
</tr>
<tr>
<td>By age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–10 years</td>
<td>119</td>
<td>0.76 (0.67–0.83)</td>
<td>0.78 (0.70–0.84)</td>
<td>0.78 (0.70–0.84)</td>
</tr>
<tr>
<td>11–13 years</td>
<td>100</td>
<td>0.69 (0.56–0.78)</td>
<td>0.78 (0.68–0.84)</td>
<td>0.71 (0.60–0.80)</td>
</tr>
<tr>
<td>14–16 years</td>
<td>86</td>
<td>0.70 (0.57–0.79)</td>
<td>0.73 (0.61–0.81)</td>
<td>0.69 (0.56–0.79)</td>
</tr>
<tr>
<td>Total</td>
<td>305</td>
<td>0.73 (0.67–0.78)</td>
<td>0.77 (0.72–0.81)</td>
<td>0.74 (0.69–0.79)</td>
</tr>
<tr>
<td>DZ Pairs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male–male</td>
<td>45</td>
<td>0.27 (–0.03–0.52)</td>
<td>0.18 (–0.12–0.45)</td>
<td>0.25 (–0.05–0.51)</td>
</tr>
<tr>
<td>Female–female</td>
<td>52</td>
<td>0.53 (0.21–0.73)</td>
<td>0.46 (0.12–0.69)</td>
<td>0.55 (0.24–0.75)</td>
</tr>
<tr>
<td>Opposite sex</td>
<td>80</td>
<td>0.36 (0.16–0.54)</td>
<td>0.35 (0.14–0.53)</td>
<td>0.35 (0.14–0.53)</td>
</tr>
<tr>
<td>By age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–10 years</td>
<td>57</td>
<td>0.34 (0.08–0.55)</td>
<td>0.42 (0.17–0.61)</td>
<td>0.37 (0.12–0.57)</td>
</tr>
<tr>
<td>11–13 years</td>
<td>60</td>
<td>0.47 (0.24–0.64)</td>
<td>0.48 (0.25–0.65)</td>
<td>0.48 (0.26–0.65)</td>
</tr>
<tr>
<td>14–16 years</td>
<td>40</td>
<td>0.19 (–0.13–0.48)</td>
<td>−0.03 (–0.33–0.29)</td>
<td>0.09 (−0.23–0.39)</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>0.36 (0.22–0.49)</td>
<td>0.33 (0.18–0.46)</td>
<td>0.36 (0.21–0.49)</td>
</tr>
</tbody>
</table>

Comparison between twin pairs was based on the right eyes. The 95% CI was calculated based on the Fisher transformation.
nant genetic effects (D) were not significant for any of the three study variables and were consequently dropped. Table 3 also shows the parameter estimates in the best-fitting models for AOD, ARA, and TISA.

**DISCUSSION**

This is the first study specifically exploring the heritability of angle-width parameters measured by ASOCT in a large population-based cohort. The results of our study are consistent with an important role for genetic factors in variations in angle width in this population of Chinese twins. We found that genetic influences account for approximately 70% of the variation in angle width as determined by the AOD, ARA, and TISA. It was peculiar that we identified values that are generally lower than that previously calculated for other intermediate phenotypes for angle closure.

PAC has been associated in part with anterior chamber depth, and persons who have shallower depths are more prone to the development of PAC. The heritability estimates for anterior chamber depths have ranged between 51% and 88% in Caucasian twin populations, although in our Chinese twin population we have recently reported a heritability of approximately 90%.

Figure 2. Intrapair correlations for AOD, ARA, and TISA in MZ and DZ twin pairs in the Guangzhou Twin Eye Study.
Although there is a close association of PAC with ACD, angle width is probably the most relevant anatomic trait for determining risk of the disease. Angle width reflects proximity of the peripheral iris to the trabecular meshwork. A narrower drainage angle is linked to an increasing frequency of peripheral anterior synechiae (PAS), a hallmark of damage from PAC.1,2 However, angle width is anatomically determined by other biometric traits: lens thickness and position, anterior chamber depth, iris thickness, level of insertion, and probably ciliary body position.26,27 The genetic determinant of angle width, therefore, probably represents the combination of all these associated biometric traits. If the heritability values of these biometric traits are not consistently high, it may in part explain the difference in heritability of 90% for ACD, compared with approximately 70% in ASOCT measurements of angle width. Another possible explanation for the lower heritability in ASOCT angle width is the inherent variability in measurements occurring during image acquisition and analysis. ACD measurements are relatively straightforward and are probably less likely to be prone to measurement error.

The twins in our study were recruited from a population-based twin registry, and as such the selection bias in this study is likely to be minimal.28 Furthermore, the fact that the distribution of refractive error (spherical equivalent) in our twin cohort was comparable to that of the general population suggests that the phenotypic characteristics of this twin cohort may be similar to singletons in general.29 Other biases such as the misclassification of zygosity are extremely unlikely, given the limitations. Our study participants were healthy children in whom angle-closure is extremely uncommon. We did not identify any cases of angle closure (specifically, cases with narrow angles with established iridotrabecular contact) among our participants. Consequently, the results are, strictly speaking, cohort specific and may not be generalizable to the other populations. The relative contributions of pupil block, iris thickness and insertion, lens position, and thickness to measurable angle width are all likely to differ among individuals. Given that these factors have not been accounted for in this study, it is plausible that the heritability of narrow angles in adults differs in magnitude from that calculated in our study. Nonetheless, significant genetic components appear to contribute to angle width.

There are a small number of putative loci and genes implicated in angle width morphogenesis. For example, nanophthal-mos30 has been linked to the short arm of chromosome 11, and mutations in the membrane-type frizzled-related protein on 11q23 have been identified in a small number of persons with this condition.31 In addition, a recent, currently unreplicated study has reported that variants in an extracellular matrix metalloprotease gene are associated with acute PAC.32 We believe that the use of this relatively young twin cohort is more appropriate for this purpose because the intrapair environmental factors are likely to be similar in young people. Adult or elderly twins may incur the disadvantages of diversified intrapair environmental influences, thereby compromising the likelihood of identifying the true underlying genes influencing these traits, as the discordant environmental factors may override these contributions.

In summary, we used ASOCT and custom software to measure iridotrabecular angle biometrics, which are intermediate phenotypes for angle closure. We found substantial heritability, values of approximately 70%, for all traits measured that included the AOD, the TISA, and the ARA. These findings are consistent with an important role of genetic factors in variations in angle width in this population and are therefore consistent with a genetic contribution to the etiology of PAC. The population of DZ twins examined in this study provides an excellent population for determining the genes that may be involved.

References