Heritability of Central Corneal Thickness in Chinese: The Guangzhou Twin Eye Study

Yingfeng Zheng,1 Jian Ge,1 Guofu Huang,1 Jian Zhang,1 Bin Liu,1 Yoon-Mi Hur,2 and Mingguang He1,3,5

PURPOSE. To assess the heritability of central corneal thickness (CCT) in Chinese children in a classic twin study.

METHODS. Twins aged 8 to 16 years were recruited from the Guangzhou Twin Registry. Pachymetry data were obtained by one operator using the same imaging system. Zygosity was confirmed by genotyping with 16 polymorphic markers in all same-sex twin pairs. The CCT of the right eyes was chosen as the trait of interest in the analysis. Heritability was assessed by a general sex-limitation model, using Mx software (University of Richmond, Virginia).

RESULTS. Four hundred forty-nine twin pairs were available for data analyses, including 131 pairs of monozygotic boys (MZM), 44 pairs of dizygotic boys (DZM), 166 pairs of monozygotic girls (MZF), 31 pairs of dizygotic girls (DZF), and 77 pairs of opposite-sex dizygotic (OSDZ) twins. Twin correlations for CCT were 0.90 for MZM, 0.92 for MZF, 0.56 for DZM, 0.61 for DZF, and 0.44 for OSDZ twins. A sex-limitation model combining additive genetic and unique environmental factors produced the best fit for the data. Heritability estimates for CCT were 0.88 (95% confidence interval [CI]: 0.84–0.91) in the boys and 0.91 (95% CI: 0.89–0.93) in the girls. Unique environmental effects explained only 0.12 (95% CI: 0.09–0.16) and 0.09 (95% CI: 0.07–0.11) of the variance in CCT in the boys and the girls, respectively.

CONCLUSIONS. Additive genetic effects appear to be the major contributor to the variation of CCT in Chinese population. Heritability of CCT appears to be slightly greater in the girls than in the boys. (Invest Ophthalmol Vis Sci. 2008;49:4303–4307) DOI:10.1167/iovs.08-1934

Glaucoma is the second leading cause of blindness in the world, affecting nearly 70 million people.1 Despite its being such a common condition, the etiology of glaucoma remains unclear. Juvenile-onset glaucoma shows mode of Mendelian inheritance, whereas adult-onset primary open angle glaucoma (POAG), the most common form of glaucoma, has been shown to be inherited as a complex trait.2 Family-based linkage analysis has been useful for Mendelian inherited diseases, but this method had not been very tractable for complex diseases.3 Although several glaucoma-causing genes and chromosome regions have been identified in large families,1–7 these genes accounted for only a small proportion of adult-onset cases of POAG. This finding may be attributable to many factors associated with common diseases, such as nonuniform definition of phenotypes, genetic heterogeneity, and insufficient statistical power in the study design.2 These difficulties highlight a need for adoption of alternative strategies. Endophenotypes are continuous traits along the pathways between disease and distal genotype.9 They may provide more information than simple description of affected or unaffected status and therefore could make it easier to map disease-susceptible genes. This endophenotype strategy has been successfully applied to the linkage analyses of many complex disorders.6,9 To be functional as a validated endophenotype, the trait should be heritable.8 In the context of POAG, heritability estimates of several quantitative traits have been reported, such as intraocular pressure (IOP),10,11 optic disc parameters,10 and retinal nerve fiber layer (RNFL) thickness.11 Central corneal thickness (CCT) has been considered to be a powerful risk factor and thus has been hypothesized to be another quantitative trait (endophenotype) for POAG.12,13 To our knowledge, the heritability of CCT in European people has been reported in only one study.12 Population studies have consistently suggested ethnic differences in CCT: The cornea tends to be thicker in Caucasians, thinner in Africans, and between the two in East Asians.13,15 Ethnic differences in prevalence and pattern of glaucoma between East Asian and Caucasian populations have been noted as well.16,17 Therefore, it would be interesting to confirm the heritability findings of CCT in East Asian populations. In the present investigation, we investigated the heritability estimate for CCT in a twin cohort aged from 8 to 16 years of age recruited through a population-based twin registry in southern China. All twins were of homogeneous Han ethnicity and were healthy without corneal disease and elevated IOP. We used a single imaging system (Pentacam; Oculus, Wetzlar, Germany), which is a noncontact imaging technique, to obtain objective measurements.

MATERIALS AND METHODS

Subjects
All subjects were recruited from the Guangzhou Twin Registry, which is population based and has been described elsewhere.18 Twins aged 7 to 15 years (defined at the date of July 1, 2006) living in two districts neighboring the examination station were recruited and examined annually. The images were collected for all the twins participating in our second wave data collection in July and August 2007. At the time of the examination, the twins were 8 to 16 years of age. Twins with history or sign of pathologic changes, contact lenses correction, or previous refractive surgery were excluded from our analysis. Written

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informed consents were obtained for all participants from either their parents or legal guardians after a comprehensive explanation of the study. The study was approved from the Zhongshan University Ethics Review Board and Ethics Committee of Zhongshan Ophthalmic Center and adhered to the tenets of the World Medical Association’s Declaration of Helsinki.

Zygosity of all same-sex twin pairs was determined by 16 multiplex STRs (PowerPlex 16 System; Promega, Madison, WI) at the Forensic Medicine Department of Sun Yat-Sen University in 2006. Opposite-sex twin pairs were deemed dizygotic and did not require genotyping.

**Examination and Measurement**

The imaging system (Pentacam; Oculus) captured the image of the anterior segment by rotating Scheimpflug camera and a monochromatic slit light source (light-emitting diode at 475 nm). Using the displayed arrows, one experienced examiner (GH) focused and aligned the real-time image of the subject’s eye. The 25 images per scan option was selected and automatic-release mode was used. The measurements were made from 9 to 12 AM and from 2:30 to 5:30 PM. The pachymetric results were automatically calculated by the device and output to a data sheet (Excel; Microsoft, Redmond, WA). The corneal thickness defined as a measurement at the real-time image of the subject’s eye. The 25 images per scan option was arbitrarily chosen and automatic-release mode was used. The measurement automatically started only when correct alignment and focus of the eyes were achieved. All measurements were made from 9 to 12 AM and from 2:30 to 5:30 PM. The pachymetric results were automatically calculated by the device and output to a data sheet (Excel; Microsoft, Redmond, WA). The corneal thickness defined as a measurement at the apex point was taken to be the CCT in the present study. Only the measurements with a quality factor (QS) of >95% were considered valid and were included in the analysis. No eye drop was used before the imaging examination. The CCT results on the right and left eyes were similar (correlation coefficient = 0.95; P < 0.0001). Thus, we arbitrarily chose the right eyes of each twin in the data analysis.

**Data Analysis and Genetic Modeling**

Maximum likelihood correlations were calculated for the five groups of twins (monzygotic male [MZM], dizygotic male [DZM], monzygotic female [MZF], dizygotic female [DZF], and opposite sex dizygotic [OSDZ] twins) and model-fitting analyses were performed with a specific software package (Mx; Statistical Modeling, Richmond, VA).

In classic twin studies, variation of a trait can be decomposed into sources of additive genetic (A), common environmental (C), and unique environmental (E) effects. MZ twins are derived from one fertilized egg and share 100% of their genes. DZ twins are derived from two distinct fertilized eggs and share, on average, 50% of their genes. Because the twins in our sample were reared together, both MZ and DZ twins shared 100% of common family environmental effects. Heritability is defined as the proportion of the total variance attributable to genetic variance.

The use of opposite-sex DZ twin pairs in twin studies provides an opportunity to detect sex-specific effects as well as gender difference in genetic and environmental influences on the phenotype under study. In the present study, opposite-sex DZ correlation was lower than same-sex DZ correlation, suggesting that sex-specific effects may play a role in CCT variation. Thus, sex-limitation models were fit to the data using the model-fitting analysis software (Mx; Statistical Modeling).

Figure 1 depicts the general sex-limitation model we used for the analysis. A significant change in degree of freedom between the two models. The selection of the best-fitting model was made using the log-likelihood ratio test (LRT): A significant change in χ² between the full and reduced models suggested that the reduction was not acceptable, whereas a nonsignificant change in χ² indicated that the reduced model was better than the full model.

**RESULTS**

Four hundred forty-nine healthy twin pairs aged 8 to 16 years (131 MZM, 44 DZM, 166 MZF, 31 DZF, and 77 OSDZ twins) were included in this study after exclusion of 25 pairs with missing or poor-quality pachymetry images in either or both twins. Table 1 shows demographic characteristics as well as correlations in CCT in the five groups of twins. The mean age of all the subjects was 11.8 ± 2.6 years and was not significantly different across the MZ and DZ twins (P = 0.44, unpaired t-test). No significant difference was found in CCT between the MZ and DZ twins (551.9 μm for the MZ and 551.7 μm for the DZ; P = 0.93). The CCT was normally distributed (Kolmogorov-Smirnov test, P > 0.15) in the present sample (Fig. 2).

Figure 3 presents correlations for all MZ and DZ twin pairs. Higher MZ than DZ twin correlations suggest the significance of genetic influences on CCT. We also compared maximum-likelihood correlations in the five types of twins. Same-sex DZ twin correlations were somewhat greater than DZ twin correlations (r = 0.38, unpaired t-test). No significant difference was found in CCT between the MZ and DZ twins (551.9 μm for the MZ and 551.7 μm for the DZ; P = 0.93). The CCT was normally distributed (Kolmogorov-Smirnov test, P > 0.15) in the present sample (Fig. 2).

Table 2 presents model-fitting results. The difference in χ² between the saturated model and the full general sex-limitation model (model 1) was not significant (Δχ² = 21.45, for 16 df; P = 0.162), indicating that the general sex-limitation model was acceptable. Sex and age effects in twin analyses can bias estimates of genetic and environmental influences on the trait under study. Therefore, we treated sex and age as covariates.
in the models, to control their main effects. Fixing the additive genetic correlation for OS/DS twins ($\rho_{AB}$) at 0.5 did not worsen the model fit (model 2), indicating that the same genes may have been active in the boys and the girls. When we equated A, C, and E across sexes, the resultant change in $\chi^2$ attained a borderline significance (model 3). Eliminating the C parameter from both sexes in model 2 did not significantly worsen the fit (model 4), whereas removing the A parameter did (model 5). Thus, model 4 was chosen as the best-fitting, most parsimonious model. In this model, additive genetic factors were 87.8% (95% CI: 83.9%–90.7%) for the boys and 91.4% (95% CI: 88.8%–93.3%) for the girls. The remaining unique environmental influences including measurement error were 12.2% (95% CI: 83.9%–90.7%) for the boys, and 8.6% (95% CI: 6.7%–11.2%) for the girls.

During the pilot study, the right eyes of 37 consecutive children (aged 8–16 years) from the refraction clinic were measured twice by a single experienced examiner (GH) on two separate occasions, to determine intraobserver variability. Mean test–retest differences were 0.89 μm for CCT (paired $t$-test, $P = 0.44$) and 95% limits of agreement were $-13.13$ to $14.91$ μm.

**DISCUSSION**

The Guangzhou Twin Eye Study is population based, and the twins were ascertained independent of zygosity and eye disease status, reducing the problem of selection-bias commonly observed in volunteer-based data. In the present study, we estimated the genetic contribution to CCT variation in a young Chinese population of both sexes. Maximum-likelihood correlations and model-fitting analyses provided evidence of substantial genetic effects on variation in CCT. Furthermore, the sex-limitation model suggested differences between the sexes (female > male) in CCT heritability.

Previous studies on the heritability estimates of CCT are scarce. Based on 86 families and 187 sibs, Alsbirk et al. suggested familial aggregation of CCT with heritability estimates of 0.6 to 0.7. However, this estimate was based on simple correlations rather than on genetic modeling. Moreover, family studies cannot distinguish between genetic factors and shared environmental factors within families. The heritability was substantially lower in the Alsbirk study than in the present study. This finding is explicable on the ground that Alsbirk’s study included both adults and children, while our study included only youths. Heterogeneity in age in the Alsbirk sample may have resulted in a low heritability estimate for CCT. Another possible reason is that Alsbirk used a simple and nonautomated optical pachymetry, which may to some extent lower the within-pair correlations of CCT.

To our knowledge, only one study has been conducted to estimate the heritability of CCT in a twin design: In 256 twin pairs (131 MZ and 125 DZ) from Australia and the United Kingdom, Toh et al. reported a heritability estimate of 0.95 (95% CI: 0.93–0.96) and near 0 common environmental effects for CCT (intraclass correlation was 0.95 for the MZ and 0.52 for the DZ twins). Similarly, our study yielded heritability estimates of 0.88 (95% CI: 0.84–0.91) in the boys and 0.91 (95% CI: 0.89–0.93) in the girls with a substantially larger sample size (297 pairs of MZ twins and 152 pairs of DZ twins). Our study demonstrates that the heritability of CCT is similar in Chinese and Europeans, suggesting consistency, with the possibility of a central role of genetic factors. It would be interesting to explore further and compare the effects and number of CCT-determining genes among ethnic groups, given that CCT variations have been shown in different ethnic populations.

In our analysis, the most parsimonious model was the one with higher CCT heritability in the girls than in the boys. This finding must be treated with caution, as the confidence intervals of heritability in boys and girls widely overlapped, reflecting an insufficient power in the present sample. There was no previous evidence supporting a difference between the sexes in CCT, and it is difficult to provide biological explanations of this finding. Previous studies of sex variations of CCT have been controversial. Patterns of male over female, female over male, and no sex difference have been reported. Although hormonal change in women is noted as having a possible impact, this effect may not be applicable to our young twins (aged, 8–16 years). Future research with a larger

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**TABLE 1. Baseline Characteristics and Maximum-Likelihood Correlations of Twin Pairs by Zygosity and Sex**

<table>
<thead>
<tr>
<th></th>
<th>Pairs (n)</th>
<th>Age (y)</th>
<th>Central Corneal Thickness (μm)*</th>
<th>Maximum-Likelihood Correlation†</th>
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<tbody>
<tr>
<td></td>
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<td></td>
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<tr>
<td><strong>MZ twins</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male–male</td>
<td>131</td>
<td>11.6 (2.7)</td>
<td>554.7 (36.4)</td>
<td>0.90 (0.86–0.93)</td>
</tr>
<tr>
<td>Female–female</td>
<td>166</td>
<td>11.8 (2.6)</td>
<td>549.7 (31.9)</td>
<td>0.92 (0.89–0.94)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>297</td>
<td>11.7 (2.6)</td>
<td>551.9 (34.0)</td>
<td></td>
</tr>
<tr>
<td><strong>DZ twins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male–male</td>
<td>44</td>
<td>11.8 (2.6)</td>
<td>562.2 (31.7)</td>
<td>0.56 (0.31–0.74)</td>
</tr>
<tr>
<td>Female–female</td>
<td>31</td>
<td>12.0 (2.6)</td>
<td>547.4 (30.5)</td>
<td>0.61 (0.30–0.79)</td>
</tr>
<tr>
<td>Opposite sex</td>
<td>77</td>
<td>11.9 (2.3)</td>
<td>550.1 (25.7)</td>
<td>0.44 (0.24–0.61)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>152</td>
<td>11.9 (2.4)</td>
<td>551.7 (30.3)</td>
<td></td>
</tr>
</tbody>
</table>

* Descriptive data are expressed as the mean (SD), based on the right eyes of the twins.
† Comparison based on the right eye, with adjustment for sex and age. The 95% CIs are given in parentheses.
sample would help to clarify whether the current result can be replicated. For a complex trait with sex-specific genetic architecture, one broad implication of the present finding is that genetic models incorporating sex effects (for example, a sex-limited linkage model) can increase the ability to detect signals of susceptibility loci during a genome-wide screening.

There is not a simple explanation of the genetic effect of CCT, given that its biological determinants remain unknown. No specific genes or chromosomal regions have been reported to be linked to CCT. An embryonic study showed that development of the cornea starts as early as 5 weeks in utero. Ehlers et al. suggested that CCT in children could reach adult levels before 2 years of age. Some rare disorders have been found to have a distinct CCT value in clinical studies. It is noteworthy that some of them are glaucoma-associated developmental syndromes, such as iris hypoplasia, aniridia, dysgenetic lens, pseudoexfoliation syndrome, Peters anomaly, and Axenfeld-Rieger Syndrome.

The use of objective measurement for phenotyping is an important issue in conducting twin studies. Twins, especially MZ twins tend to participate together in the study examination, often dressing alike. Examiners who use a subjective instrument (such as optical pachymetry) can be unintentionally affected by the appearance of the twins and may give similar readings for those who look alike. In our study, we were able to collect CCT data objectively based on an imaging system (Pentacam; Oculus) that includes a rotating Scheimpflug camera that yields CCT readings with high repeatability and interoperator reproducibility. This instrument provides measurements comparable or even exchangeable with those obtained by ultrasound pachymetry. Moreover, the noncontact feature of the system makes it more feasible for making measurements in the children.

Several limitations of this study should be noted. First, previous studies have shown that results from twin studies can be generalized to the singleton population for many complex traits and mortality. However, whether findings on CCT from twin studies can be extrapolated to the singleton population is uncertain. To date, no study has been undertaken to compare CCT between twins and singletons in a uniform methodologic protocol. Nevertheless, in comparison findings in the present study (CCT = 551.8 ± 32.8 µm), investigators in a study identified a very similar CCT distribution (553 ± 32.7 µm) in 1233 Chinese children in rural China (age: 14.7 ± 0.8 years), and a similar level of CCT (546.0 ± 31.8 µm) was also found in Singaporean Chinese children (age: 9–11 years) when slit lamp optical pachymetry was used. Second, a heritability estimate is population-specific. Our results obtained in Chinese children

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**Table 2. Results of Fitting the ACE Sex-Limitation Model to CCT**

<table>
<thead>
<tr>
<th></th>
<th>Model 1 (Full Model)</th>
<th>Model 2 (r_0 fixed)</th>
<th>Model 3 (f = m)</th>
<th>Model 4* (AE)</th>
<th>Model 5 (CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_f</td>
<td>61.2 (31.6–91.9)</td>
<td>63.7 (31.6–90.2)</td>
<td>66.3 (47.9–90.1)</td>
<td>91.4 (88.8–93.3)</td>
<td>86.2 (81.6–89.5)</td>
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<tr>
<td>C_f</td>
<td>30.4 (0–60.05)</td>
<td>27.9 (0–60.1)</td>
<td>23.6 (0–42.1)</td>
<td>10.1 (8.3–12.3)</td>
<td>8.6 (6.7–11.2)</td>
</tr>
<tr>
<td>E_f</td>
<td>8.4 (6.5–11.0)</td>
<td>8.4 (6.5–11.0)</td>
<td>10.1 (8.3–12.3)</td>
<td>8.6 (6.7–11.2)</td>
<td>13.8 (10.5–18.4)</td>
</tr>
<tr>
<td>A_m</td>
<td>63.3 (35.6–88.7)</td>
<td>66.8 (34.3–88.8)</td>
<td>66.3 (47.9–90.1)</td>
<td>87.8 (83.9–90.7)</td>
<td>72.5 (65.4–78.5)</td>
</tr>
<tr>
<td>C_m</td>
<td>24.6 (0–54.0)</td>
<td>21.1 (0–53.4)</td>
<td>23.6 (0–42.2)</td>
<td>10.1 (8.3–12.3)</td>
<td>12.2 (9.3–16.1)</td>
</tr>
<tr>
<td>E_m</td>
<td>12.1 (9.2–16.1)</td>
<td>12.1 (9.2–16.1)</td>
<td>10.1 (8.3–12.3)</td>
<td>12.2 (9.3–16.1)</td>
<td>27.5 (21.5–34.6)</td>
</tr>
<tr>
<td>−2LL</td>
<td>8226.763</td>
<td>8226.866</td>
<td>8236.358</td>
<td>8230.691</td>
<td>8334.085</td>
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<tr>
<td>df</td>
<td>888</td>
<td>892</td>
<td>892</td>
<td>892</td>
<td>891</td>
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<tr>
<td>AIC</td>
<td>6450.763</td>
<td>6448.866</td>
<td>6452.358</td>
<td>6469.691</td>
<td>6552.085</td>
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<tr>
<td>Δχ²</td>
<td>0.103</td>
<td>9.595</td>
<td>3.928</td>
<td>107.322</td>
<td></td>
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<tr>
<td>Δdf</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.748</td>
<td>0.048</td>
<td>0.269</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Model 1: r_0 fixed; A_f ≠ A_m; C_f ≠ C_m; E_f ≠ E_m. Model 2: r_0 fixed to 0.5; A_f ≠ A_m; C_f ≠ C_m; E_f ≠ E_m. Model 3: r_0 fixed to 0.5; A_f = A_m; C_f = C_m; E_f = E_m. Model 4: same as model 2, but omit C_f and C_m. Model 5: same as model 2; but omit A_f and A_m. The 95% CIs are given in parentheses.

A, additive genetic; C, common environment; E, unique environment; f, female; m, male; r_0, additive genetic correlation for opposite sex twins; −2LL, twice the negative log-likelihood of the data; Δχ², difference in −2LL; Δdf, difference in degree of freedom; P, probability that Δχ² is zero; AIC, Akaike information criterion.

* Best-fit model.
may not be applicable to other populations or adults. Third, classic twin studies are based on an assumption of equivalent environment, which suggests that both types of twins share broadly the same environment. Although it could be difficult to determine whether this assumption is true, it is unlikely that twin studies on such ocular biometry measurement as CCT may have violated this assumption.

In this study, we have demonstrated that genetic effects play an important role in determining CCT variation in healthy Chinese children, with heritability estimates of 0.88 and 0.91 in the boys and the girls, respectively. We believe that our results will help guide molecular genetic investigations to search genes for CCT and enhance understanding of the etiology of glaucoma.

References


