Heritability of Optic Disc and Cup Measured by the Heidelberg Retinal Tomography in Chinese: The Guangzhou Twin Eye Study

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Purpose. To assess the heritability of disc area (DA), cup area (CA), and cup–disc area ratio (CDAR) as intermediate phenotypes for glaucoma in Chinese subjects in a classic twin study.

Methods. Twins (n = 1160) aged 7 to 15 years were identified in the Guangzhou Twin Registry. Optic disc parameters were measured with a Heidelberg Retina Tomograph (HRT; Heidelberg Engineering GmbH, Heidelberg, Germany) by the same examiner and grader. Zygosity was confirmed by genotyping with 16 polymorphic markers in all same-sex twin pairs. The DA, CA, and CDAR of the right eyes were chosen as the traits of interest in the analysis. Heritability was assessed by structural variance component genetic modeling, with Mx quantitative genetic modeling software, after adjustment for age and gender.

Results. Of those recruited, 1114 twins were identified in the analysis, including 355 monozygotic (MZ) and 202 dizygotic (DZ) twin pairs. The intraclass correlation coefficients were 0.79 for DA, 0.83 for CA, and 0.80 for CDAR in MZ pairs and 0.30, 0.37, and 0.35, respectively, in DZ pairs. The age- and sex-adjusted variance component model identified additive genetic and unshared environmental effects (AE model) being best fit for DA, CA, and CDAR. This best-fitting model yielded 77.3% additive genetic (95%CI: 73.0%–80.8%) and 22.7% unshared environment (95% CI: 19.2%–27.0%) for DA, 82.7% (95% CI:79.4%–85.5%) and 17.3% (95% CI: 14.5%–20.6%) for CA, 78.6% (95% CI: 74.5%–82.0%) and 21.4% (95% CI: 18.0%–25.4%) for CDAR.

Conclusions. The variance of optic nerve head parameters (DA, CA, and CDAR) appears to be attributable to additive genetic and unshared environmental effects. Approximately 80% of these phenotypic variances are genetically determined. (*Invest Ophthalmol Vis Sci.* 2008;49:1350–1355) DOI:10.1167/iovs.07-1146

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Population-based studies consistently suggested that the prevalence of primary open angle glaucoma (POAG) in African-origin people is at least four times greater than in European and Asians.[1] On the other hand, familial aggregation of glaucoma has been known for many years.[2,3] A population-based, extended-family study confirmed an increased risk for POAG in those with affected family members, and enlarged cup–disc ratio was the earliest and most prominent feature of familial aggregation.[4] Ethnic variation and familial aggregation appear to suggest a genetic tendency of POAG. So far, almost all known pattern of inheritance have been suggested for glaucoma, including sex-linked recessive, autosomal recessive, autosomal dominant, and multifactorial.[5] Efforts on gene identification have been fruitful, *MYOC*,[6] *OPTN*,[7] and *WDR36*[8] gene mutations were identified for adult-onset POAG, whereas *CYP1B1* was thought to be associated with congenital glaucoma but later was found to contribute to adult-onset POAG.[9] However, the genetic mechanism of adult-onset POAG is far more complicated than expected. Mutations of *MYOC* genes were found in only 3% to 5% sporadic adult-onset cases of POAG.[10,11] This finding underscores that the genetic mechanism of most of those with glaucoma is largely unknown. Instead of complying with Mendelian inheritance patterns, adult-onset POAG likely results from multiple genes and probably their interaction, as well as the gene environmental interactions.[10,11]

POAG is an uncommon, age-dependent, and dichotomous phenotype. It is difficult to define clinically, in part due to its clinical heterogeneity and lack of uniform diagnostic criteria. These difficulties pose challenges on accurate phenotyping across generations and further gene searching efforts in traditional pedigree studies. Using a quantitative trait as an intermediate phenotype (also called "endophenotype") has been increasingly adopted for familial aggregation and gene mapping in complex diseases,[12] such as psychiatric disorder,[13] hypertension,[14] and cancer.[15] Optic disc damage (i.e., commonly measured as cup–disc ratio), is an important quantitative marker for the diagnosis of POAG, as an evidence of existing retinal ganglion cells damage. Based on extended pedigrees from population-based participants, heritability of vertical cup–disc ratio (VCDR) was estimated as 0.48 in the Beaver Dam Eye Study[16] and 0.56 in the Salisbury Eye Evaluation project.[17] Both studies involved adult participants aged mainly 60 years and older: One study used stereoscopic fundus photography[16] and the other one used a +90-D lens for disc evaluation.[17] However, evidence of familial aggregation itself may or may not imply genetic effects.[18] Measured or unmeasured nongenetic risk factors are often difficult to exclude. On the other hand, subjective measurement of the optic disc may introduce systematic observer bias.

Twin studies are widely used as a "perfect natural experiment" to determine heritability.[19] A comparison of similarities of phenotypes between monozygotic (MZ) and dizygotic (DZ) twins allows for the estimation of heritability when the pair-

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wise variation of environmental factors is assumed to be very similar between MZ and DZ groups.

The purpose of this analysis was to estimate the heritability of optic disc parameters measured by HRT (Heidelberg Retina Tomograph; Heidelberg Engineering GmbH, Dossenheim, Germany), a device shown to be objective and reproducible,[20] in a Chinese young twin cohort identified from a population-based twin registry.

Materials and Methods

Participants

The study subjects were recruited from our Guangzhou Twin Registry, which has been described elsewhere.[21] All twins were aged 7 to 15 years (at July 1, 2006) and lived in two districts neighboring the Zhongshan Ophthalmic Center, where the examination station was set up for baseline data collection. Written informed consent was obtained for all participants from either parents or guardians of the participating children after a careful, detailed explanation of the study. Ethics approval was obtained from the Zhongshan University Ethical Review Board and Ethics Committee of Zhongshan Ophthalmic Center. The study was conducted in accordance with the tenets of the World Medical Association's Declaration of Helsinki.

The zygosity of all same-sex twin pairs was ascertained by a 16 multiplex short-tandem repeat (STR) DNA typing system (PowerPlex 16 system; Promega, Madison, WI)[22] at the forensic medical department of Sun Yat-sen University. Zygosity in opposite-sex twin pairs was considered as dizygotic without a need for genotyping.

Examination

An HRT 3 confocal scanning ophthalmoscope (Heidelberg Engineering, Heidelberg, Germany) was used to collect optic disc imaging data by an experienced examiner (BL) before any contact on the cornea (in the present study, Tonopen; Medtronics Ophthalmics, Jacksonville, FL) and pupil dilation. An artificial tear drop was used if the tear film was not intact. The keratometry data (measured by an IOLMaster; Carl Zeiss Meditec, Oberkochen, Germany) were entered into the software to adjust the settings in accordance with the refractive error. An astigmatic lens was used and indicated in the software's "enter eye data" section if the participant presented with astigmatism greater than 1 D. HRT optic nerve head (ONH) module was used for image acquisition. Images with a mean standard deviation greater than 50 μm were excluded and recollected. Image analysis was performed later after the image collection session was completed. It was performed after the process of topographic computation by a single observer (BL) who was masked to the zygosity results. The optic disc margin (contour line) was manually marked at the inner edge of Elschnig's ring. The contour line was placed and further verified by using the interactive topography display. The stereometric parameters were then automatically calculated by the software. We chose to use only three area-based parameters (global cup area, disc area, and cup–disc area ratio) in the study, because these parameters are less affected by the variation of placement of the contour line than are the depth-related parameters (such as rim volume, cup shape, mean RNFL and so on).[20] This effort to minimize the variation due to measurement errors helps to attain a more accurate estimation of heritability, which is based on the assessment of intrapaired similarities in MZ and DZ pairs.

The twin pairs in which one or both twins had pathologic changes (e.g., retinopathy of prematurity, congenital cataract, or any other optical media problems) or recent orthokeratology contact lenses correction and previous myopic laser treatment were excluded from the present analysis.

Data Analysis and Genetic Modeling

The right eye was selected to represent the phenotypic characteristics of the specific individual in the data analysis, given that the results on right and left eyes were similar (correlation coefficient for disc area = 0.81; cup area = 0.82; cup disc–area ratio = 0.79). These three optic disc parameters were treated as quantitative traits and analyzed using quantitative genetic modeling. The estimation of heritability in twin studies is based on concordance comparison of phenotypes between MZ and DZ twins. MZ twins share 100% of their genes, whereas DZ twins, on average, share only half. Assuming that two types of twins share the same similarities of environmental factors, greater similarities in phenotypes of MZ represent additional gene sharing.[23]

Based on the correlations between MZ and DZ pairs, the variance component model includes the additive (A) genetic, dominant (D) genetic, common (C), and unique (E) environmental variance. The E component also includes measurement errors. The best-fitting model was determined based on a maximum likelihood test and χ^2 test. A significant change in χ^2 values between the full and the reduced model would indicate that the parameter removed from the full model was significant and therefore should be retained in the model. In contrast, a nonsignificant change in χ^2 values suggested that the parameter eliminated from the full model was not significant and therefore, should be dropped to achieve parsimony of the model. Because the intraclass correlation coefficient for optic disc parameters in DZ twins is less than half of the ones in MZ, the model fitting starts from the ADE model. Heritability was then estimated by the ratio of additive (plus dominant) genetic variance to the total phenotypic variance. Because these cup and disc parameters were significantly associated with age and sex, as a control for the main effects of age and sex, the model treated the age and sex variables as covariates. This age-sex–adjusted model is equivalent to using linear regression and adjusting for the effect of age as well as sex and then using the residuals to compute the variance–covariance matrices. Mx was used for the fitting of genetic model fitting.[24]

Results

Among the total 705 pairs of eligible twins who were invited, 557 pairs (355 MZ and 202 DZ pairs) were available for the HRT data analysis after excluding 12 twin pairs with pathologic (10 retinopathy of prematurity, 1 congenital cataract, 1 optic nerve atrophy) and 11 with missing HRT data on either twin. The demography and distribution of optic disc parameters were summarized in Table 1. The ages of MZ (10.8 $\pm$ 2.6 years) and DZ (10.9 $\pm$ 2.4 years) pairs were not significantly different (*t*-test, $P = 0.642$). No significant differences between MZ (1.97 $\pm$ 0.44 mm) and DZ twins (1.99 $\pm$ 0.46 mm) were identified in disc area ($P = 0.51$), cup area (0.50 $\pm$ 0.31 mm for MZ, 0.52 $\pm$ 0.32 mm for DZ; $P = 0.476$) and cup–disc area ratio (CDAR; 0.24 $\pm$ 0.12 arbitrary units for MZ, 0.25 $\pm$ 0.12 arbitrary units for DZ; $P = 0.464$).

CDAR had an approximately normal distribution (skewness and kurtosis test for normality, P for skewness = 0.071, P for kurtosis = 0.326; Fig. 1). The mean CDARs in the boys (0.26 $\pm$ 0.12 units) and girls (0.22 $\pm$ 0.11 units) were significantly different (*t*-test, $P = 0.0002$). Multiple linear regression of CDAR with age and sex ($R^2 = 0.04$, $P < 0.0001$) suggested that mean CDAR decreased by 0.006 unit per year of age ($P = 0.001$) and was 0.03 unit ($P < 0.001$) smaller in the girls (adjusted for age). Both disc area and cup area were smaller in the girls (disc area: 2.03 $\pm$ 0.45 mm, boys; 1.92 $\pm$ 0.44 mm, girls; $P = 0.004$; cup area: 0.56 $\pm$ 0.33 mm, boys; 0.46 $\pm$ 0.29 mm, girls; $P = 0.001$).

Intraclass correlation coefficients (ICC, equivalent to the pair-wise correlation coefficient) between twin pairs are summarized in Table 2. ICCs were approximately 0.80 in MZ pairs and DZ had ICCs slightly less than one-half the ones of MZ (Table 2). The pair-wise correlation on optic disc parameters in MZ and DZ was also demonstrated in scatterplots (Fig. 2).

Because the ICC in DZ is less than one-half of those in MZ twins, the full model started from ADE in maximum-likelihood

TABLE 1. Phenotypic Characteristic of Twin Pairs by Zygosity and Gender

	n	Age	Disc Area	Cup Area	CDAR
Monozygotic twin					
Male-male	167	10.6 (2.7)	2.04 (0.47)	0.57 (0.33)	0.26 (0.12)
Female-female	188	10.9 (2.6)	1.91 (0.41)	0.45 (0.28)	0.22 (0.11)
Subtotal	355	10.8 (2.6)	1.97 (0.44)	0.50 (0.31)	0.24 (0.11)
Dizygotic twin					
Male-male	65	10.6 (2.5)	2.02 (0.47)	0.55 (0.35)	0.25 (0.13)
Female-female	41	11.0 (2.4)	2.05 (0.54)	0.50 (0.36)	0.23 (0.11)
Opposite sex	96	11.0 (2.3)	1.95 (0.41)	0.51 (0.27)	0.25 (0.10)
Subtotal	202	10.9 (2.4)	1.99 (0.46)	0.52 (0.32)	0.25 (0.12)
Total	557	10.8 (2.5)	1.98 (0.15)	0.51 (0.31)	0.24 (0.12)

Descriptive data presented were obtained from the right eyes of the first-born twin and are expressed as the mean (±SD).

modeling. The genetic modeling consistently suggested that the AE model (additive genes and unique environment) was the best fit for all three optic disc parameters of interest. The effect of D (dominant genetic effect) was dropped (reduced-model χ^2 test: $P = 0.19$ for DA, $P = 0.189$ for CA, and $P = 0.260$ for CDAR). Table 3 shows the detailed goodness-of-fit parameters in an age- and sex-adjusted model. Additive genetic effect (A) explained 77.3% (95% CI: 73.0%–80.8%), equivalent to heritability here, and unshared environment (E) explained the rest 22.7% (95% CI: 19.2%–27.0%) of the variance of DA phenotype. The heritability estimation in CA and CDAR were similar. Additive genetic effects accounted for 82.7% (95% CI:79.4%–85.5%) for CA and 78.6% (95% CI: 74.5%–82.0%) for CDAR, and unshared environmental effects contributed 17.3% (95% CI: 14.5%–20.6%) for CA and 21.4% (95% CI: 18.0%–25.4%) for CDAR, of the total phenotypic variance.

Sixty-five participants aged 7 to 15 years from the twin clinic were measured on two separate occasions on the same day during the pilot study. For the right eye measurement, mean test–retest differences for CDAR were 0.003 ± 0.047 (paired *t*-test, $P = 0.580$). The 95% limits of agreement were −0.015 to +0.008.

DISCUSSION

This study confirms the high heritability of optic disc area, cup area, and CDAR measured by HRT in a population-based, large sample of Chinese twins. The variance of optic disc and cup parameters appears to be attributable to additive genetic and unshared environmental effects. Approximately 80% of these phenotypic variances are genetically determined. To our best knowledge, this is the first twin study to report heritability of optic cup and disc, using HRT measurement in a large sample of twin cohort.

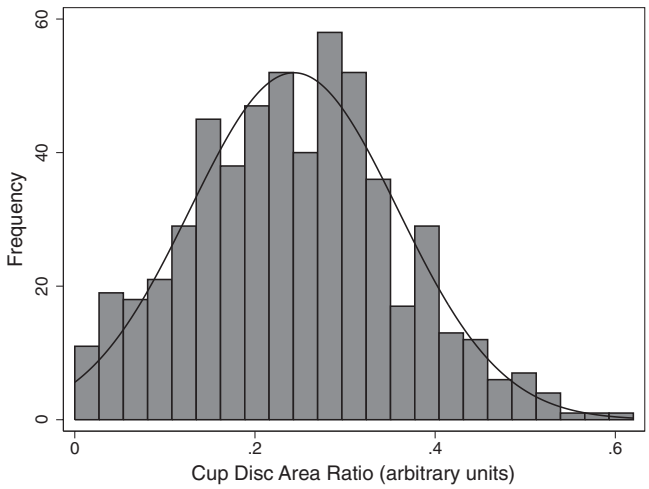

FIGURE 1. Distribution of CDAR in the first-born twin.

This twin cohort was enrolled from a population-based twin registry. Therefore, we avoided the so-called concordance-dependent selection bias introduced by the volunteer-based attendance in a twin study—the MZ twins probably participated for examination more than DZ twins and therefore probably biased the heritability estimation.[23] It is not possible to compare the distribution of optic disc parameters in our twin cohort with that in the general population because of the lack of relevant literature. The fact that the refractive error (spherical equivalent) distribution in our twin cohort was similar to the general child population of the same age[25] appears to suggest that the study cohort is representative and that the data may be generalizable. Furthermore, the twin subjects involved in the study were all aged 7 to 15 years. The intrapair environmental variations are likely to be similar when the pairs of twins are all raised in the same family. In contrast, elderly twins tend to have a greater pair-wise difference in environmental exposure, either measured or unmeasured, in that their surroundings and occupation tend to be diversified in adulthood.

Optic disc parameters were measured by HRT, an objective quantitative tool that may minimize the measurement errors and observer bias. HRT measurements have been shown to be highly reproducible, particularly for the area parameters that are less affected by placement of contour lines.[20] All HRT images were analyzed by the same grader, while refractive error and astigmatism were adjusted during the image acquisition, all of which helps to minimize measurement errors. Furthermore, the estimation of heritability in twin studies is based on the comparison of the pair-wise similarities between MZ and DZ. It is possible that the observers tended to read similar values for MZ twin pairs when they looked very much alike if the cup–disc ratio (CDR) was measured subjectively (direct or indirect ophthalmoscope) and the twin pairs presented themselves together for the examination. HRT is able to avoid this problem, in that the least operator input is involved during the

TABLE 2. ICCs for Disc Area, Cup Area, and CDAR

Twin Pairs	Disc Area	Cup Area	CDAR
MZ male	0.81	0.84	0.77
MZ female	0.75	0.82	0.81
MZ all	0.79	0.83	0.80
DZ male	0.35	0.36	0.32
DZ female	0.45	0.40	0.38
DZ opposite	0.26	0.37	0.39
DZ all	0.30	0.37	0.35

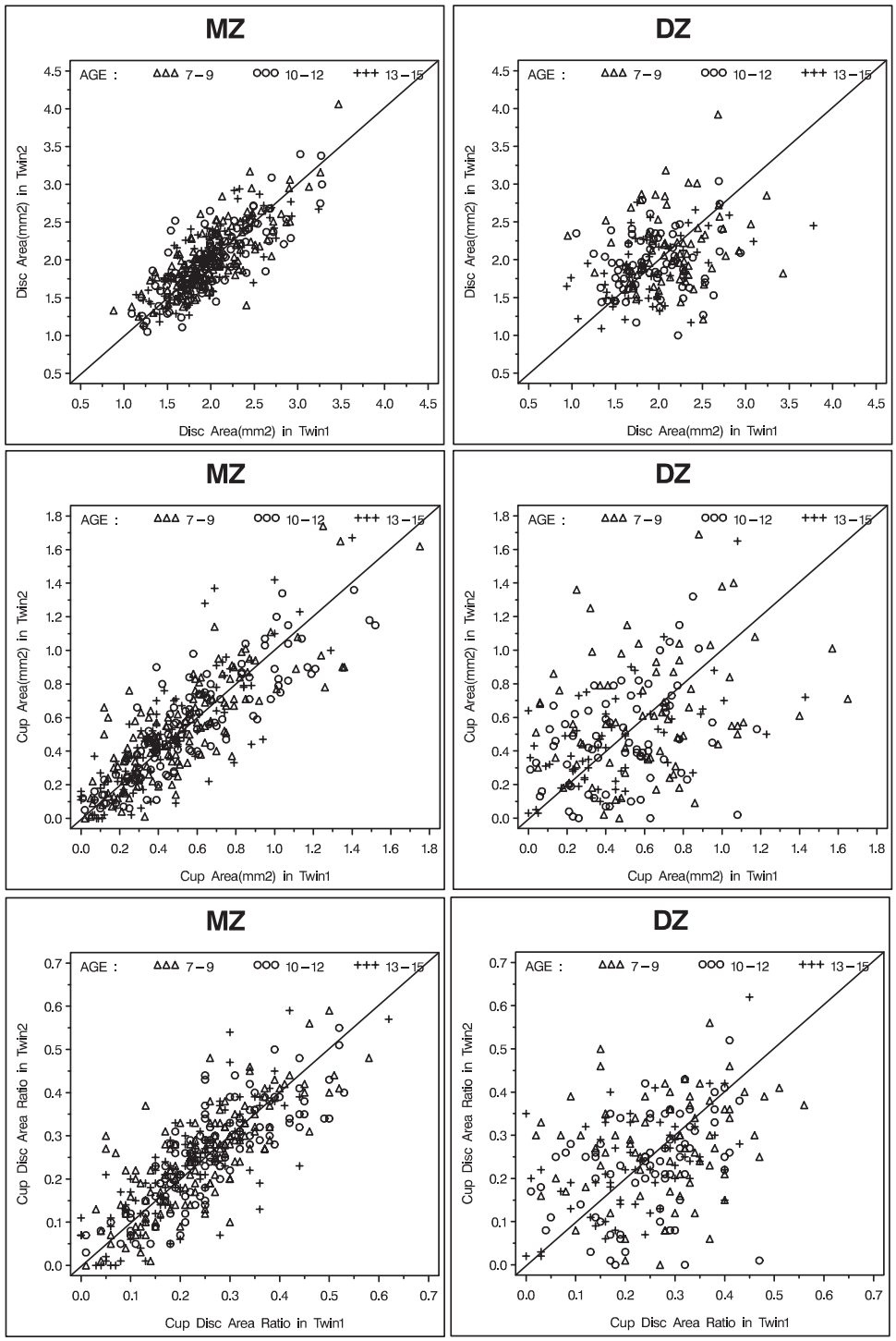

FIGURE 2. Intrapair correlation for HRT optic disc parameters in MZ and DZ twin pairs in the Guangzhou Twin Eye Study.

measurement. However, the measurement errors due to refractive error, particularly in highly myopic eyes,[26] may compromise the accuracy of similarity estimation between twin pairs, therefore, the estimation of heritability may be influenced by the heritability of refractive error.

The estimation of heritability of optic CDR has been attempted in extended-family studies. Armaly,[27] for the first time, described the familial aggregation phenomenon of CDR by family studies. The CDR was confirmed by direct ophthalmoscope, and polygenic multifactorial inheritance was suggested in this study. Based on stereoscopic optic disc photography of extended families from the population-based Beaver Dam Eye Study, Klein et al.[16] reported that the correlation coefficient of vertical CDR in siblings was 0.25; in parents and children, 0.24; in avuncular relatives, 0.14; and in spouses, 0.01. Heritability of the ratio was estimated as 0.48 according to the parent–child correlation.[16] Chang et al.[17] reported a similar heritability (0.58) based on the siblings of the population-based Salisbury Eye Evaluation study. The CDR measurement was performed mainly with a +90-D lens during slit lamp examination, and heritability was estimated as twice the residual between-sibling correlation after the adjustment of possible confounding factors. However, the estimation of heritability based on familial aggregation in extended families is often cumbersome, as the

TABLE 3. Genetic and Environmental Effects Estimated by Age- and Sex-Adjusted Maximum-Likelihood Model

Variables/Models	A (95% CI)	D (95% CI)	E (95% CI)	−2LL	*df*	$\Delta\chi^2$	Δ *df*	*P**
Disc area								
ADE	0.459 (0–0.800)	0.315 (0–0.791)	0.226 (0.191–0.268)	955.411	1108	—	—	—
AE	0.773 (0.730–0.808)	—	0.227 (0.192–0.270)	957.125	1109	1.714	1	0.190
E	—	—	—	1301.54	1110	341.44	2	<0.001
Cup area								
ADE	0.526 (0.024–0.848)	0.301 (0–0.803)	0.173 (0.146–0.205)	127.351	1108	—	—	—
AE	0.827 (0.794–0.855)	—	0.173 (0.145–0.206)	129.078	1109	1.727	1	0.189
E	—	—	—	558.964	1110	431.61	2	<0.001
Cup disc area ratio								
ADE	0.521 (0.009–0.814)	0.266 (0.002–0.779)	0.213 (0.180–0.253)	−2019.268	1108	—	—	—
AE	0.786 (0.746–0.820)	—	0.214 (0.180–0.254)	−2018.00	1109	1.268	1	0.260
E	—	—	—	−1653.92	1110	365.35	2	<0.001

A, additive genetic; D, dominant genetic; E, unique environment; *df,* degree of freedom; −2LL, twice the negative log-likelihood; $\Delta\chi^2$, difference in χ^2 values; Δ*df,* difference in degrees of freedom; *P*, χ^2 test in model fitting.

* Statistics when the model is reduced.

environmental factors, particularly those that are not measurable and therefore are not adjusted for, are often diversified to an unknown extent across various generation cohorts (parent–child aggregation) and even among siblings when they are not at the same age.

The twin study offers a unique opportunity for dissecting the genetic and environmental component in the phenotypic variance. As mentioned earlier, an MZ twin pair shares 100% of genes, whereas the DZ pair shares approximately half of them. Thus, greater similarities of phenotypes in MZ, compared with DZ, pairs suggest additional gene sharing if we assume the intrapair similarities of environmental exposure between MZ and DZ pairs is equal (the so-called equal-environment assumption). A few small-sample twin studies have given a higher but rough estimation of CDR heritability. Armaly[27] suggested that cup-disc ratio was genetically determined by using 33 twin pairs; however, the twins in the study were not differentiated as MZ and DZ, and comparison of similarities between MZ and DZ twins was not attempted. Teikari and Airaksinen [28] gave a general picture of greater similarities of CDR in MZ (10 pairs) than DZ (7 pairs) twins. Schwartz et al.[29] confirmed a greater ICC on horizontal CDR in MZ (0.846, $n = 37$) than in DZ (0.490, $n = 26$) pairs, with an estimated heritability of 0.60 to 0.80.[29] However, horizontal CDR is often less relevant to glaucoma, and the slit lamp observation may introduce systematic observer bias, as described before. These problems may affect the accuracy of the estimation of heritability in these studies. Although the methodology is considerably different, our study confirmed a heritability of approximately 80% for CDAR as well as disc and cup size.

In our study, the optic disc area, cup area, and CDAR were treated as intermediate phenotypes for glaucoma, given they were considered to be important clinical signs and even diagnostic criteria of glaucoma. The continuous distribution of these optic disc parameters was used to estimate the importance of genetic and environmental determinants. Studying these intermediate phenotypes may provide evidence of genetic and environmental effects that drive the pathways and link them to glaucoma. The high heritability of these intermediate phenotypes of glaucoma may support further linkage study on these phenotypes and may be promising in the efforts of identify the susceptible genes for glaucoma. Because of the age effects on optic disc parameters, the findings of heritability may have to be further confirmed in the elderly twin population.

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