Primary open-angle glaucoma (POAG) is the predominant type of glaucoma worldwide and a leading cause of irreversible blindness. In African populations, the prevalence is approximately three times higher compared to European populations and it runs a more severe course with higher intraocular pressure (IOP). The IOP is a major risk factor for POAG and the only one that can be modified therapeutically to alter the progression rate of the disease. Therefore, it is essential that it is measured accurately. The reliability of IOP measurements, especially Goldmann applanation tonometry (GAT), is confounded by variations in central corneal thickness (CCT), and intraocular pressure (IOP) by estimating the genetic African ancestry (GAA) proportion in primary open-angle glaucoma (POAG) patients and controls from an admixed South African Colored (SAC) and a South African Black (SAB) population.

In this case-control study, 268 POAG patients and 137 controls were recruited from a university clinic in Cape Town, South Africa. All participants were genotyped on the Illumina HumanOmniExpress beadchip or HumanOmni2.5Exome beadchip. ADMIXTURE was used to infer participant’s GAA among 86,652 SNPs. Linear and logistic regression models were used to assess the relation between GAA, POAG, CCT, and IOP.

The median proportion of GAA was 60% in the study population. GAA was significantly associated with thinner CCT (P < 0.001) and IOP (P = 0.034) in POAG patients. The effect of GAA on CCT was marginally different among POAG patients versus controls (P = 0.066). In POAG patients, the CCT was significantly thinner compared to controls after adjusting for age and sex (P = 0.016). In a stratified analysis in participants with >60% GAA, CCT was not associated with POAG (P = 0.550).

This study demonstrated that a higher proportion of GAA was associated with a thinner CCT and a higher IOP in POAG patients. Remarkably, at higher proportions of GAA, the difference in CCT between POAG and controls was reduced. This suggests that thinner CCT is not associated with POAG in Africans.

Keywords: genetic African ancestry, glaucoma, central corneal thickness, intraocular pressure

Nevertheless, whether the effect of CCT on glaucoma is only due to its effects on IOP measurement error or whether an independent relationship between CCT and glaucoma truly exists remains controversial. Large population-based studies could not find any association of CCT with POAG. Other studies suggest that CCT is correlated with scleral and lamina cribrosa thickness, which affects the properties and vulnerability of the optic nerve and, therefore, increases the risk of glaucoma. However, histomorphometric studies in humans and monkeys could not confirm this correlation. Other biomechanical characteristics have been suggested to link CCT with POAG, such as the viscoelasticity of the cornea or corneal hysteresis. Lower corneal hysteresis has been associated with an increased risk of glaucoma and glaucoma progression.

CCT follows a diurnal rhythm and is affected by sex, age, and ethnicity. The ethnic variation of CCT has
Genetic African Ancestry in POAG Patients

Methods

Study Population

The Genetics in Glaucoma patients of African descent study (GIGA study) is a case-control study comprising open-angle glaucoma patients and healthy controls from South Africa. All participants (n = 405) provided a written informed consent in accordance with the ethical standards as stated in the Declaration of Helsinki. The institutional review boards of the Erasmus MC and the University of Cape Town granted ethical approval. Participants were ascertained at the ophthalmology outpatient department of the Groote Schuur Hospital in Cape Town, South Africa. In total, 268 POAG patients met the inclusion criteria of the study. Inclusion criteria were participants of South African Black (SAB) or admixed South African Colored (SAC) descent, over 55 years of age, and diagnosed with either POAG or normal tension glaucoma (NTG). All other types of glaucoma, including secondary causes or narrow/closed angle glaucoma, were excluded. Inclusion criteria for controls (n = 137) were persons aged over 55 years, of SAB or SAC descent, without a diagnosis of any form of glaucoma, and without a family history (first degree relatives) of glaucoma. All participants were examined by a local glaucoma specialist.

Ophthalmic Examination

The complete eye examination included visual acuity (VA) by using a Snellen or Tumbling E chart at 6 m with and without correction, refraction, IOP measurement with GAT, slit-lamp examination including peripheral anterior chamber depth assessment by the Van Herick method, indirect gonioscopy, funduscopy for optic nerve head examination, and digital fundus photography centered on the optic nerve by means of a Canon CF-60DSi fundus camera.

CCT was measured after topical instillation of lidocaine anaesthetics with an ultrasonic A-scan/pachymeter OcuScan RxP (Alcon Laboratories, Inc., Ft. Worth, TX, USA). Ten readings were automatically captured in both eyes.

Visual field testing was performed with the Humphrey Field Analyzer 24-2 Sita Fast (Carl Zeiss Meditec, Inc., Dublin, CA, USA) strategy. A definite visual field defect consistent with glaucoma was defined if the glaucoma hemifield test graded “outside normal limits” and if a cluster of 3 contiguous points was observed at the 5% level of the pattern deviation plot, including at least 1 of these points <5%. Field defects were not attributed to glaucoma in the presence of media opacities or nonglaucomatous optic nerve disease that could explain the visual field abnormality.

Inclusion Criteria

All patients were categorized as glaucomatous according to the ISGEO classification for open-angle glaucoma. After preliminary screening by local glaucoma specialists, and grading of fundus photographs by one senior ophthalmologist and one trained research grader, detailed grading was performed independently by one general ophthalmologist (AAT) and one glaucoma specialist (HGL). They interpreted fundus images and visual field results independently while being masked for other clinical information. In case of any discrepancy between the two graders, adjudication was solved by consensus. If no consensus was reached, participants were excluded. Category 1 or 2 ISGEO criteria had to be met to diagnose glaucoma. The highest level of evidence (category 1) requires a definite visual field defect, as mentioned above, and loss of the neuroretinal rim with a Vertical Cup Disc Ratio (VCDR) ≥ 0.7, or VCDR asymmetry ≥ 0.2 (both values represented the 97.5th percentile for the normal SAB population). Visual field testing results with less than 8% false-positive and false-negative responses, and less than 10% fixation losses were considered reliable. Category 2 requires a severely damaged optic disc, that is, a VCDR > 0.8 or VCDR asymmetry > 0.2 (both values determined by 99.5th percentile for the normal SAB population) in the absence of a satisfactory visual field test.

In addition, patients with POAG demonstrated an open angle on gonioscopy. Nonglaucomatous participants were those who met the following criteria in both eyes: IOP ≤ 21 mm Hg, a nonglaucomatous optic disc with VCDR < 0.5, and an intereye variation in VCDR < 0.2.

Estimation of Genetic Ancestry

Participants were genotyped on the Illumina HumanOmniExpressExome beadchip (n = 137) and the Illumina HumanOmni2.5Exome beadchip (n = 244). Before combining both genotype datasets, PLINK (v1.07) was used to perform extensive quality control checks. No within sample cryptic relatedness was observed during QC. To make inferences based on populations of known ancestry, we merged the combined dataset with 3 reference populations from Africa (Yoruba in Ibadan, Nigeria, and Luhya in Webuye, Kenya), East-Asia (Japanese in Tokyo, Japan, Southern Han Chinese, and Han Chinese in Beijing, China) and Europe (Utah Residents [CEPH] with Northern and Western Ancestry, Tuscany in Italy, Finnish in Finland, British in England and Scotland, and Iberian in Spain) appearing in the 1000 Genomes Project. PLINK then was used to perform linkage disequilibrium pruning on the merged genotype data to produce a reduced set of unlinked single nucleotide polymorphism (SNPs) (~indep-pairwise 50 10 0.1); 86,632 autosomal SNPs with an SNP call rate of 100% were selected from the merged datasets to estimate biogeographic ancestry (BGA). First we examined the genetic clustering by visualizing the principal components calculated in PLINK (Supplementary Fig. S1). The program ADMIXTURE v1.23 then was used to estimate the ancestral fractions of the three putative ancestral populations—African, Asian, and European—among the study samples. ADMIXTURE was run with default settings and K = 3 ancestral populations.

Statistical Analysis

Since the mean CCTs of right and left eyes were not statistically different (mean difference, 1.1 μm; P = 0.242; Pearson
correlation = 0.90), we only present the results of the right eye analyses. If measurements from the right eye were not available, then data from the left eye were used instead. In total, 383 right eyes and 22 left eyes were available for analysis. The average of the first 5 CCT readings was used in the analysis. The independent samples Student’s t-test was used to compare continuous variables among ethnic and diagnostic groups. We performed χ² tests on categorical variables. GAA fractions inferred from SNP data were used instead of self-reported ethnicity to determine any association with African ancestry. We studied four interrelationships among POAG, GAA, CCT, and IOP as depicted in Figure 1. Univariable and multivariable linear regression models, adjusting for age and sex, were applied to test the association between GAA and CCT, GAA and IOP, and CCT and IOP. Univariable and multivariable logistic regression models, adjusting for age and sex, were used to test the association of CCT with POAG. Also, effect modification of CCT by GAA was tested in the association of CCT with POAG by adding the multiplicative interaction term to the adjusted model. All statistical analyses were performed in SPSS (version 21.0; IBM Corp. Armonk, NY, USA) and R studio (R Core Team [2014]; R Foundation for Statistical Computing, Vienna, Austria).

Demographics and Clinical Characteristics

The demographic and clinical characteristics of the 268 POAG patients and 137 controls are given in Table 1. In the control group, there were significantly more women, and controls had undergone more ocular surgery (in particular cataract extraction) compared to the POAG patients. In the POAG patients, the untreated IOP was statistically significantly higher (P < 0.001) and the CCT was statistically significantly thinner than in control participants (P = 0.019). There was no statistically significant difference in mean CCT among participants with and without ocular surgery when adjusted for age, sex, and POAG status (surgery, 507.1 ± 40.5 μm, n = 194; no surgery, 504.5 ± 36.0, n = 211; P = 0.460). The CCT did not significantly vary with age (–0.23 μm per year; 95% confidence interval [CI], −0.57–0.11; P = 0.188). All CCT measurements had been performed during daytime (9 AM to 5 PM), and there was no association between CCT and the time of examination. The distribution of self-reported ethnicity/race was similar for POAG patients and controls. This was confirmed by an equal median percentage GAA (Mann-Whitney U Test, P = 0.750) for 381 participants that were genotyped successfully. Figure 2 and Supplementary Table S1 present the distribution of the percentage genetic Asian, African, and European ancestry across SAB and SAC. The median proportion of GAA was significantly different in SAC participants compared to SAB participants (Mann-Whitney U Test, P < 0.001). One individual

Table 1. Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th>Demographic and Clinical Characteristics</th>
<th>POAG, n = 268</th>
<th>Controls, n = 137</th>
<th>P Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (IQR)</td>
<td>68.0 (20)</td>
<td>66.0 (12)</td>
<td>0.828</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>144 (53.7)</td>
<td>91 (66.4)</td>
<td>0.019</td>
</tr>
<tr>
<td>Ocular surgery, n (%)</td>
<td>110 (41.0)</td>
<td>84 (61.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IOP (untreated), mm Hg ± SD</td>
<td>28.61 ± 9.73</td>
<td>14.20 ± 2.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCT, μm ± SD</td>
<td>502.53 ± 37.00</td>
<td>511.92 ± 39.82</td>
<td>0.019</td>
</tr>
<tr>
<td>Median VCDR (IQR)</td>
<td>0.90 (0.15)</td>
<td>0.30 (0.20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median proportion GAA, % (IQR)</td>
<td>61.24 (71.94)</td>
<td>55.37 (67.66)</td>
<td>0.750</td>
</tr>
</tbody>
</table>

IQR, interquartile range.

* P value obtained from a Student’s t test for continuous variables and with χ² test for categorical variables. For median age and median VCDR and median proportion GAA, P values were obtained with the Mann-Whitney U test.
Table 2. Association of GAA, CCT, IOP, and POAG as Graphically Depicted in the Directed Acyclic Graph (Fig. 1)

<table>
<thead>
<tr>
<th></th>
<th>Univariable Regression Model</th>
<th>Multivariable Regression Model†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td>1. CCT ~ GAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All, n = 581</td>
<td>−3.58</td>
<td>−4.65 to −2.51</td>
</tr>
<tr>
<td>Control, n = 130</td>
<td>−5.23</td>
<td>−7.10 to −3.36</td>
</tr>
<tr>
<td>POAG, n = 251</td>
<td>−2.80</td>
<td>−4.09 to −1.51</td>
</tr>
<tr>
<td>2. IOP ~ GAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n = 129</td>
<td>−0.14</td>
<td>−0.29 to 0.01</td>
</tr>
<tr>
<td>POAG, n = 200</td>
<td>0.63</td>
<td>0.24 to 1.02</td>
</tr>
<tr>
<td>3. IOP ~ CCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, n = 136</td>
<td>0.09</td>
<td>−0.04 to 0.21</td>
</tr>
<tr>
<td>POAG, n = 214</td>
<td>0.00</td>
<td>−0.35 to 0.36</td>
</tr>
<tr>
<td>4. POAG ~ CCT, n = 405</td>
<td>1.067†</td>
<td>1.01 to 1.13</td>
</tr>
</tbody>
</table>

*β = effect per 10% increase in GAA or 10 μm decrease in CCT.
† Multivariable regression model adjusted for age and sex.
* Odds ratio. Corresponds to the effect of 10 μm decrease in CCT.

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South African population was considerably thinner; it was even the thinnest in any African study performed by means of ultrasound pachymetry to date (i.e., 512 ± 39.8; Fig. 4). Associations between GAA and CCT have been studied previously in African Americans and Europeans in the ADAGES study. This study found a similar correlation for CCT and GAA in the entire group. However, a significant association between GAA and CCT in the African American subgroup could not be detected due to a limited degree of admixture in this group.

The association between IOP and GAA has been studied in Latinos in the LALES study. This population-based study found that IOP increases by 0.38 mm Hg for every 10% increase in GAA. Although West Coast Latinos have a modest contribution of GAA, our study found similar results, that is, a 0.46 mm Hg increase per 10% increase in GAA, for POAG patients. For controls, we did not find any association between GAA and IOP. This could be explained by selection bias that was induced by selecting only controls with an IOP < 21 mm Hg. We did not observe a significant linear correlation between IOP and CCT in either POAG patients or controls. Although most population-based studies find a correlation between IOP and CCT, case control studies could not always detect this association in POAG patients due to selection of severe cases with critical elevated IOP.

Most of the studies investigating the relationship between glaucoma and CCT were based predominantly on European ancestral populations and focused on ocular hypertension and NTG patients. There have been conflicting reports about the CCT of POAG patients versus controls. Several studies did not find any statistically significant differences in CCT between these groups. Yet, various other studies have, indeed, reported such differences. A few studies have investigated this relationship in African populations. Only one of these detected a statistically significantly thinner CCT in POAG patients, but this difference was only present in the left eye. Also, the Barbados eye study found a thinner CCT in POAG patients, but this difference was not statistically significant. Recently, the Tema eye survey, the largest population-based study of CCT on the African continent, could not find an association between CCT and POAG as well.

A novel finding of our study was that the difference in CCT between POAG patients and controls attenuated by increasing GAA (Fig. 3). This highlights that in individuals with a high percentage of African ancestry, differences in CCT are little, if at all, associated with POAG. This stresses the importance of anatomic variation between ethnic/racial groups and the

### Table 3. Association of CCT With POAG Stratified by Median Proportion GAA

<table>
<thead>
<tr>
<th>Strata</th>
<th>n&lt;sub&gt;POAG&lt;/sub&gt;</th>
<th>n&lt;sub&gt;control&lt;/sub&gt;</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; median GAA (&lt;59.6%)</td>
<td>122; 69</td>
<td>1.09</td>
<td>1.00–1.18</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>≥ median GAA (≥59.6%)</td>
<td>129; 61</td>
<td>1.04</td>
<td>0.94–1.14</td>
<td>0.477</td>
<td></td>
</tr>
</tbody>
</table>

OR, odds ratio per 10 µm decrease in CCT.

* Multivariable regression model adjusted for age and sex.
possible susceptibility for development of POAG. Investigation into the racial and ethnic differences in the anatomy of the optic nerve head showed, for instance, a thicker retinal nerve fiber layer, and larger optic discs with deeper cups in African individuals.\textsuperscript{57–61} In particular, a correlation between thinner CCT and larger optic discs seems to be present in POAG patients.\textsuperscript{62,63} Larger optic disc diameters may be associated with increased vulnerability to pressure-induced deformation. Therefore, eyes with thinner corneas are more susceptible to glaucomatous damage in comparison with those having thicker corneas. This hypothesis may explain why African persons are more vulnerable to glaucomatous optic nerve head damage. Although conflicting evidence exists that CCT is associated with other disc topographic parameters (i.e., rim area, cup area and VCDR).\textsuperscript{64–66} A new property of the cornea, corneal hysteresis, has shown to be a better predictor of glaucomatous damage.\textsuperscript{15,21}

Our study has strengths and weaknesses. A strength of our study is that by applying BGA estimation, we were able to objectively measure variation in CCT and IOP related to ethnic/racial differences. Self-reported ethnicity/race frequently is used in epidemiologic studies to assess an individual’s background origin. Often participants are asked to specify a single ethnic/racial group based on categories. This method can be unreliable, since these definitions can be imprecise and inconsistent over time.\textsuperscript{67,68} Also, self-reported ethnicity/race can be based on subjective physical characteristics and intrinsic beliefs. Skin color, for example, often is used as surrogate of race, although visual classification of skin color can be interpreted differently.\textsuperscript{69–71} Especially in complex admixed populations, such as SAC, self-reported ethnicity does not reveal the extent of admixture, which is because admixed individuals can have multiple ancestries, and these ancestry proportions can vary greatly per individual. Recent advances in genome-wide genotyping that allow the inference of BGA can set aside the use of proxy methods, such as self-reported ethnicity/race.\textsuperscript{71}

The high degree of admixture in our study population also is a valuable asset of the study, since it enabled detailed evaluation of the differences in CCT and IOP in relation to African ancestry. A limitation of this study is its relatively small sample size. As a result, this study had limited statistical power to find significant interaction between GAA and CCT when studying the association between CCT and POAG. Also, we performed several numbers of tests. Therefore, chance finding should be considered when interpreting the data. Post hoc power analysis showed that this study was sufficiently powered (power > 80%; $P < 0.013$; considering four tests), for the multivariable associations in the POAG patients. We currently are extending our genotyping efforts in a Tanzanian population. As genotyping progresses, we will have more statistical power to detect any significant differences. Preliminary data from Tanzania strengthens our current findings and confirm that in this African black population CCT is not different among POAG patients and controls. Another limitation of this study includes potential selection bias. For selecting POAG patients based on functional damage (ISGEO category 1), we applied rather strict criteria for assessing the reliability of the visual fields. This might have led to a selection of super-test takers, and, therefore, an undercalling of POAG patients. It turned out, however, that 96% of the probable glaucoma cases

![Figure 4. CCT in African POAG patients and non-glaucomatous African individuals. Error bars: Standard deviation. *Significant difference between POAG patients and controls; Sng et al., no standard deviation available.](image-url)
that failed our strict reliability criteria were later identified as glaucomatous, based on advanced structural optic nerve head damage (ISGEO category 2). Therefore, the effects of our strict visual field reliability criteria on our results probably were insignificant. In controls, the IOP cutoff for enrolment could have biased the associations in this group. Although high IOP is the main risk factor for POAG, we might have overlooked potentially healthy participants with elevated IOP.

In conclusion, this study shows that in African admixed individuals GAA measurement is an unprejudiced tool to distinguish associations with POAG and their endophenotypes. We found that a higher proportion of GAA is associated with a thinner CCT, and that an increase in GAA in POAG patients is associated with a higher IOP. Interestingly, our current study shows that the difference in CCT between POAG patients and controls is reduced at higher proportions of GAA. This confirms previous studies that did not find significant differences in CCT between POAG patients and controls in Africans. Therefore, some biomechanical properties of the African eye may be different compared to those in other ethnic groups. However, it is not yet clear to what extent they relate to the increased glaucoma susceptibility of Africans.

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