Investigation of Known Genetic Risk Factors for Primary Open Angle Glaucoma in Two Populations of African Ancestry

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See the appendix for the members of the International Consortium of African Ancestry REsearch in Glaucoma.

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Purpose. Multiple genes have been associated with primary open angle glaucoma (POAG) in Caucasian populations. We now examine the association of these loci in populations of African ancestry, populations at particularly high risk for POAG.

Methods. We genotyped DNA samples from two populations: African American (1150 cases and 999 controls) and those from Ghana, West Africa (483 cases and 593 controls). Our analysis included 57 single nucleotide polymorphisms (SNPs) in five loci previously associated with POAG at the genome-wide level, including *CDKN2B-AS1*, *TMCO1*, *CAV1/CAV2*, chromosome 8q22 intergenic region, and *SIX1/SIX6*. We evaluated association in the full datasets, as well as subgroups with normal pressure glaucoma (NPG, maximum IOP $\leq$21 mm Hg) and high pressure glaucoma (HPG, IOP >21 mm Hg).

Results. In African Americans, we identified an association of rs10120688 in the *CDNK2B-AS1* region with POAG ($P = 0.0020$). Several other SNPs were nominally associated, but did not survive correction for multiple testing. In the subgroup analyses, significant associations were identified for rs10965245 ($P = 0.0005$) in the *CDKN2B-AS1* region with HPG and rs11849906 in the *SIX1/SIX6* region with NPG ($P = 0.006$). No significant association was identified with any loci in the Ghanaian samples.

Conclusions. POAG genetic susceptibility alleles associated in Caucasians appear to play a greatly reduced role in populations of African ancestry. Thus, the major genetic components of POAG of African origin remain to be identified. This finding underscores the critical need to pursue large-scale genome-wide association studies in this understudied, yet disproportionately affected population.

Keywords: association, genetics, POAG, African, African American

Glaucoma is the second-leading cause of blindness in the world.[1] Primary open angle glaucoma (POAG) is the most common type and is inherited as a complex trait.[2–4] POAG is characterized by progressive retinal ganglion cell death, optic nerve head excavation, and visual field loss. POAG disproportionately affects individuals of African ancestry,[5,6] and is the most common cause of permanent blindness in African Americans.[7] The risk of POAG in persons older than 40 is 4- to 5-fold higher in African Americans (4%–5%) than in age-matched Caucasians (1%).[5,8–10] It is also more severe, with a 10-fold higher risk of blindness from glaucoma in African Americans.[1,11] POAG is even more common and severe in continental African populations.[12–16] In a major population-based study in Ghana, West Africa, POAG was diagnosed in 6% of those older than 40, which is one of the highest global prevalence rates ever reported.[14] In studies conducted in eye clinics serving populations of African ancestry, rates of blindness in one or both eyes have been observed in excess of 40%.[13,17–20]

Genetics has been shown to play an important role in the pathogenesis of POAG.[2–4,21,22] Previous linkage-based studies have identified several genes with varying contribution to glaucoma, including myocilin, *CYP1B1*, optineurin, and *WDR36*.[23–26] It has also been reported that DNA copy number changes in *TBK1* and *GALC* gene contribute to POAG pathogenesis.[27,28] Recently, genome-wide association studies (GWAS) of POAG in Iceland, Australia, Japan, and the United States have successfully identified and confirmed genetic associations that are significant at the genome-wide level with multiple genes involved in the pathogenesis of POAG, including *CAV1/CAV2*, *CDKN2B-AS1*, *TMCO1*, *SIX1/SIX6*, and an intergenic region on chromosome 8q22.[29–32] Multiple studies have confirmed or replicated these genetic associations in populations from Europe, the United States, Japan, and Barbados.[33–36] However, these loci have not been examined in populations of African ancestry, including African Americans and continental Africans. This study was designed to fill this significant gap.

We have assembled the International Consortium of African Ancestry REsearch in Glaucoma (ICAARE-Glaucoma) with glaucoma investigators from the United States and Africa (including Ghana, Nigeria, and South Africa). In this study, we used a haplotype-tagging approach to examine genetic associations in more than 3200 African American and Ghanaian (West African) glaucoma cases and controls from the ICAARE-Glaucoma.

Materials and Methods

Study Sample and Phenotype Description

This study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participating individuals. The research was reviewed and approved by the institutional review board from all participating institutions, including Duke University Medical Center (Durham, NC), the Massachusetts Eye and Ear Infirmary (Boston, MA), the University of Michigan (Ann Arbor, MI), New York Eye and Ear Infirmary (New York, NY), the University of Alabama at Birmingham (Birmingham, AL), and, for Ghanaian subjects, Noguchi Memorial Institute of Medical Research of the College of Health Sciences, University of Ghana in Accra.

Subjects with POAG were unrelated and met the following inclusion criteria[37]: glaucomatous optic neuropathy in at least one eye, and visual field loss consistent with optic nerve damage in at least one eye. Glaucomatous optic neuropathy was defined as a cup-to-disc ratio greater than 0.7 or focal loss of the nerve fiber layer resulting in a notch in the neuroretinal rim, associated with a glaucomatous visual field defect. Visual fields were performed using standard automated perimetry or frequency doubling test (FDT).[1] IOP was recorded but not used as an inclusion criterion. The exclusion criteria for POAG subjects included the diagnosis or history of any secondary glaucoma, history of ocular trauma, or significant use of systemic or ocular glucocorticoids. Medical records for all POAG cases and control subjects were reviewed by professionally trained glaucoma specialists. The examined control subjects were unrelated and met the following criteria: no known first-degree relative with glaucoma, IOP less than 21 mm Hg in both eyes without treatment, and no evidence of glaucomatous optic neuropathy in either eye. POAG cases were further stratified into high-pressure glaucoma (HPG) or normal-pressure glaucoma (NPG) based on maximum recorded IOP higher than 21 mm Hg or lower than 22 mm Hg, respectively.

DNA Genotyping and Association Analysis

Genomic DNA was extracted from peripheral blood by standard techniques (Gentra, Minneapolis, MN). HaploView version 4.2 (Broad Institute, Cambridge, MA) was used to design the tagging single nucleotide polymorphism (SNPs) of *CAV1/CAV2*, *CDKN2B-AS1*, *TMCO1*, and *SIX1/SIX6* in African (YRI) samples, using genotype data from the HapMap project (www.hapmap.org) with r^2 greater than 0.6 and minor allele frequency (MAF) greater than 0.05.[14] A total of 50 tagging SNPs were selected to cover the linkage disequilibrium (LD) blocks of the selected candidate regions (Supplementary Fig. S1). We also included seven additional SNPs (rs284489 on chromosome 8; rs1063192, rs4977756, rs10116277, and rs4977574 in the *CDKN2B-AS1* region; and rs33912345 and rs10483727 in the *SIX1/SIX6* region) that were strongly associated with POAG in Caucasians.[32,38] TaqMan allelic discrimination assays were employed for genotyping these 57 SNPs by use of Assays-On-Demand products with the ViiA7 Realtime PCR system with 384-well block according to the standard protocols from the manufacturer (Applied Biosystems, Foster City, CA). All 57 SNPs were genotyped in 1076 Ghanaians. Due to technical issues, 50 SNPs were genotyped on the whole set of 2149 African Americans. Seven SNPs (rs10800149 and rs7518099 in *TMCO1* region; rs2151280, rs1547705, rs10738607, and rs10811658 in *CDKN2B-AS1* region; and rs3759688 in *SIX1/SIX6* region) were genotyped in 1593 African Americans. For quality control (QC) purposes, two CEPH (the Centre d'Etude du Polymorphisme Humain, Foundation Jean Dausset, Paris, France) standards were included in each 96-well plate, and samples from two individuals were duplicated across all plates, with the laboratory technicians masked to their identities. Analysis of genotypes required matching QC genotypes within and across plates and at least 95% genotyping efficiency.

Analysis of Hardy-Weinberg equilibrium (HWE) was performed separately for cases and controls from the two populations using GDA software (University of Connecticut, Storrs, CT).[15] Within each population, genotype frequencies of POAG cases and controls were compared by logistic regression with adjustment for age and sex using SAS software (SAS Institute, Inc., Cary, NC). SNP genotypes were coded according to a log-additive model, in which the relative risk for carriers of two variant (minor) alleles, compared to the reference group (homozygous wild type), was assumed to be the square of the relative risk for carriers of one variant. We also performed the association analysis based on classification of NPG or HPG. To correct for testing multiple SNPs in each genomic region, we calculated the effective number of independent marker loci (MeffLi) to control the experiment-wise level of significance and the false discovery rate based on the method reported by Li and Ji.[39] The values of MeffLi are 6.000 for *TMCO*, 9.106 for *CAV1/CAV2*, 19.146 for *CDKN2B-AS,* and 7.000 for *SIX1/SIX6*. The experiment-wide significance threshold required to limit type 1 error rate to 5% is 0.0085 for *TMCO*, 0.0056 for *CAV1/CAV2*, 0.0027 for *CDKN2B-AS1*, and 0.0073 for *SIX1/SIX6*. Because there is only one SNP in the chromosome 8 locus, the significance for *P* value cutoff is 0.05. Power calculations were performed with QUANTO software (University of Southern California, Los Angeles, CA) using previously described methods, assuming a population prevalence of 5% and a log-additive risk model.[37,40] To address the genetic heterogeneity between the African

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Table 1. Clinical Characteristics of Individuals in ICAARE-Glaucoma

Affection Status	*n*	% Female	Age, y	Maximal IOP, mm Hg
African American total	2149	51.9	58.1 ± 13.4	22.2 ± 9.0
African American cases	1150	49.5	57.0 ± 13.0	26.2 ± 8.8
African American HPG	870	48.7	56.0 ± 12.7	30.0 ± 7.4
African American NPG	280	49.8	58.9 ± 13.3	16.9 ± 3.6
African American Controls	999	54.6	59.4 ± 13.8	15.2 ± 3.1
Ghanaians total	1076	51.3	63.3 ± 11.8	31.2 ± 12.6
Ghanaian cases	483	43.9	63.4 ± 12.4	35.1 ± 11.6
Ghanaian controls	593	57.3	63.3 ± 9.6	18.4 ± 5.6

Age, age at diagnosis for cases and age of examination for controls.

American and Ghanaian populations, we conducted a formal meta-analysis of the SNPs with the African American and Ghanaian datasets with PLINK (Massachusetts General Hospital, Boston, MA).[41]

Results

The ICAARE-Glaucoma dataset at the time of this study consisted of 2149 African Americans and 1076 Ghanaians (West African). The African American cohort contained 1150 POAG cases and 999 fully ophthalmically examined controls. The Ghanaian cohort contained 483 POAG cases and 593 examined controls. Phenotypic information is provided in Table 1. Although elevated IOP was not required for the diagnosis of POAG, the Ghanaian POAG cases essentially all demonstrated elevated IOP at examination so NPG subset analysis was not performed.

All SNPs passed rigorous quality control and genotyping efficiency criteria (>95% with all the samples). All were in HWE in both cases and controls from both African Americans and Ghanaians ($P > 0.01$), except for two SNPs with minor deviations (rs1063192 in African American controls [HWE $P = 0.007$], rs4977574 in African American cases [HWE $P = 0.003$]). SNP rs1063192 in the *CDKN2B-AS1* region was monomorphic in Ghanaian controls and rare in Ghanaian POAG cases (a minor allele frequency of 0.002 for the C allele that is protective in the Caucasian POAG.)

In the full African American case/control dataset, we observed a significant association of SNP rs10120688 in the *CDKN2B-AS1* region with POAG risk ($P = 0.0020$; odds ratio [OR] 1.21, 95% confidence interval [CI] 1.07–1.37). Several other SNPs reached nominal significance ($P < 0.05$), including rs7518099 in the *TMCO1* region; rs1052990 and rs4236601 in the *CAV1/CAV2* region; and rs7049105, rs16905597, rs16905599, rs10811658, and rs10965245 in the *CDKN2B-AS1* region (Table 2). However, these associations did not survive correction for multiple testing. In the Ghanaian population, nominal associations were noted for several SNPs, including rs3807986, rs3815412, and rs8713 in the *CAV1/CAV2* region. However, none of these associations remained significant after correction for multiple testing in each genomic region. The meta-analysis with these two populations identified five nominally significant SNPs ($P < 0.05$), including rs7518099 and rs2814471 in *TMCO1*, rs4236601 in *CAV1/CAV2*, and rs10120688 and rs16905597 in *CDKN2B-AS1*, that did not survive correction for multiple testing.

Next, we stratified the African American cases by IOP history into NPG and HPG for association analysis using logistic regression. In the HPG subgroup, we identified significant association with SNP rs10965245 ($P = 0.0005$; OR 0.73, 95% CI 0.61–0.87) in the *CDKN2B-AS1* region. Nominal associations that did not survive correction for multiple testing were noted for several SNPs, including rs4236601 in the *CAV1/CAV2* region, and rs7049105, rs10120688, rs16905597, rs16905599, rs10965235, and rs17761446 in the *CDKN2B-AS1* region (Table 2).

In the NPG subgroup, we found significant association with SNP rs11849906 in the *SIX1/SIX6* regions ($P = 0.006$; OR 0.53, 95% CI 0.34–0.83). Nominal associations that did not survive correction for multiple testing were observed with several SNPs, including rs7518099 and rs2814471 in the *TMCO1* region, rs4236601 in the *CAV1/CAV2* region, and rs10120688 and rs4977756 in the *CDKN2B-AS1* region (Table 2). Additional details of the association with all 57 SNPs in these five genomic regions are presented in Supplementary Table S1. The Ghanaian POAG cases were almost all classified as HPG, so stratification by IOP was not performed.

To determine the statistical power of these datasets to detect association with these genetic loci, we selected an OR of 1.50 and 1.35 obtained from reports on the genetic associations in these regions. Reported ORs were 1.68 for *TMCO1*,[32] 1.4 to 1.5 for *CDKN2B-AS1*,[29,32] 1.32 for *SIX1/SIX6*,[29,35] and 1.36 for *CAV1/CAV2* variants.[33,42] Statistical power to detect association with these genomic loci was calculated using QUANTO software.[40] Assuming population risk $\alpha = 0.05$ and allele frequency of 0.10, our African American dataset has 98.9% power for OR of 1.50 and 87.2% power for OR of 1.35. The Ghanaian dataset has 85% power for OR of 1.50 and 59% power for OR of 1.35. In summary, our African American and Ghanaian datasets were well powered to detect associations in these regions.

Discussion

Our study represents the largest association study of glaucoma to date in populations of African ancestry. We replicated the association of POAG with the *CDKN2B-AS1* locus in the African Americans first reported in populations of European ancestry.[6,29,32,34,35,43,44] After stratification for IOP, this association remained significant in the HPG subgroup. We also found significant association of the *SIX1/SIX6* locus in African American cases in the NPG subgroup. Interestingly, we did not observe significant association with any of the previously reported genes and loci in POAG cases from the West African population of Ghana. This may be due to the relatively limited sample size of the Ghanaian dataset.

Several studies have reported the association of *CDKN2B-AS1* SNPs, and on stratification by IOP history, this association is restricted to the NPG but not the HPG subpopulation.[29,32,38,43–46] In African Americans, we see significant association with *CDKN2B-AS1* SNPs in the full dataset and in the HPG subgroup. This difference in pattern may be due to

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Table 2. The Genetic Association of 57 SNPs With POAG in the ICAARE-Glaucoma Samples of African Americans and Ghanaians (West Africans) Using Logistic Regression With Additive Model

Genomic Region	SNP	Allele	AA, $n = 1150$ Case/999 Control		AA HPG, $n = 870$ Case/999 Control		AA NPG, $n = 280$ Case/999 Control		Ghanaian, $n = 483$ Case/593 Control	
			P	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
TMCO1	rs10800149	A	0.66	1.04 (0.89–1.21)	0.74	0.97 (0.82–1.15)	0.09	1.21 (0.97–1.51)	0.10	1.19 (0.97–1.46)
TMCO1	rs10800150	C	0.49	0.96 (0.85–1.08)	0.74	0.98 (0.86–1.11)	0.29	0.90 (0.75–1.09)	0.24	0.90 (0.76–1.07)
TMCO1	rs4656461	C	0.74	1.02 (0.89–1.18)	0.82	1.02 (0.87–1.19)	0.62	1.06 (0.85–1.32)	0.19	1.14 (0.94–1.40)
TMCO1	rs1913845	C	0.74	1.03 (0.88–1.20)	0.40	1.08 (0.91–1.27)	0.31	0.89 (0.71–1.12)	0.76	0.97 (0.78–1.20)
TMCO1	rs12059327	C	0.71	0.97 (0.80–1.16)	0.93	1.01 (0.83–1.23)	0.28	0.85 (0.63–1.15)	0.22	1.17 (0.91–1.51)
TMCO1	rs6426940	C	0.94	1.00 (0.89–1.13)	0.98	1.00 (0.88–1.14)	0.92	1.01 (0.84–1.22)	0.31	0.91 (0.77–1.09)
TMCO1	rs7518099	C	0.048	1.27 (1.00–1.62)	0.12	1.23 (0.95–1.59)	0.05	1.39 (0.99–1.93)	0.11	1.29 (0.94–1.75)
TMCO1	rs2814471	C	0.27	1.11 (0.93–1.32)	0.80	1.03 (0.85–1.24)	0.019	1.37 (1.05–1.77)	0.76	1.04 (0.81–1.34)
CAV1/CAV2	rs8940	C	0.051	0.86 (0.74–1.00)	0.08	0.86 (0.73–1.02)	0.15	0.84 (0.66–1.06)	0.10	0.83 (0.67–1.04)
CAV1/CAV2	rs1052990	A	0.029	0.87 (0.77–0.99)	0.06	0.88 (0.77–1.00)	0.08	0.85 (0.70–1.02)	0.11	0.87 (0.73–1.03)
CAV1/CAV2	rs6466578	C	0.23	0.91 (0.78–1.06)	0.52	0.95 (0.80–1.12)	0.08	0.80 (0.62–1.03)	0.19	0.86 (0.70–1.07)
CAV1/CAV2	rs3919515	C	0.41	0.95 (0.84–1.08)	0.44	0.95 (0.83–1.09)	0.59	0.95 (0.78–1.15)	0.56	0.95 (0.79–1.13)
CAV1/CAV2	rs10227696	A	0.13	1.13 (0.97–1.31)	0.20	1.11 (0.94–1.32)	0.18	1.17 (0.93–1.49)	0.09	1.21 (0.97–1.51)
CAV1/CAV2	rs4236601	A	0.020	1.16 (1.02–1.31)	0.050	1.14 (1.00–1.31)	0.05	1.21 (1.00–1.47)	0.06	1.19 (0.99–1.42)
CAV1/CAV2	rs917664	A	0.72	1.02 (0.91–1.16)	0.75	1.02 (0.90–1.16)	0.79	1.03 (0.85–1.25)	0.98	1.00 (0.84–1.19)
CAV1/CAV2	rs3779512	A	0.43	1.05 (0.93–1.20)	0.28	1.08 (0.94–1.24)	0.90	0.99 (0.81–1.21)	0.74	1.03 (0.86–1.24)
CAV1/CAV2	rs3807986	C	0.97	1.00 (0.88–1.13)	0.93	1.01 (0.88–1.15)	0.84	0.98 (0.81–1.19)	0.015	0.80 (0.66–0.96)
CAV1/CAV2	rs3807989	C	0.97	1.00 (0.88–1.13)	0.56	0.96 (0.84–1.10)	0.28	1.12 (0.92–1.36)	0.35	0.91 (0.76–1.10)
CAV1/CAV2	rs3779514	A	0.69	1.03 (0.89–1.19)	0.41	1.07 (0.92–1.24)	0.47	0.92 (0.73–1.16)	0.84	0.98 (0.80–1.20)
CAV1/CAV2	rs3815412	A	0.51	1.04 (0.92–1.18)	0.63	1.03 (0.90–1.18)	0.54	1.06 (0.88–1.29)	0.03	0.82 (0.69–0.98)
CAV1/CAV2	rs8713	A	0.85	1.01 (0.89–1.16)	0.89	1.01 (0.87–1.17)	0.79	1.03 (0.83–1.27)	0.04	0.82 (0.68–0.99)
Cbr 8q22	rs284489	C	0.69	0.98 (0.86–1.10)	0.92	0.99 (0.87–1.13)	0.44	0.93 (0.77–1.12)	0.66	1.04 (0.86–1.27)
CDKN2B-AS1	rs2069422	G	0.96	1.01 (0.82–1.23)	0.86	0.98 (0.79–1.22)	0.57	1.09 (0.80–1.49)	0.75	0.95 (0.71–1.28)
CDKN2B-AS1	rs7049105	A	0.009	0.84 (0.73–0.96)	0.013	0.83 (0.72–0.96)	0.12	0.85 (0.69–1.05)	0.75	1.03 (0.85–1.25)
CDKN2B-AS1	rs2151280	A	0.19	1.11 (0.95–1.29)	0.38	1.08 (0.91–1.27)	0.11	1.19 (0.96–1.48)	0.25	0.89 (0.73–1.08)
CDKN2B-AS1	rs7851706	C	0.26	1.11 (0.92–1.34)	0.24	1.13 (0.92–1.38)	0.66	1.07 (0.80–1.43)	0.06	0.80 (0.64–1.01)
CDKN2B-AS1	**rs10120688**	A	**0.0020**	**1.21 (1.07–1.37)**	0.012	1.18 (1.04–1.35)	0.004	1.32 (1.09–1.60)	0.10	0.86 (0.72–1.03)
CDKN2B-AS1	rs16905597	A	0.010	0.78 (0.64–0.94)	0.006	0.74 (0.60–0.92)	0.38	0.88 (0.66–1.18)	0.57	1.07 (0.85–1.36)
CDKN2B-AS1	rs16905599	C	0.04	0.86 (0.75–1.00)	0.04	0.85 (0.73–0.99)	0.33	0.90 (0.72–1.12)	0.28	1.12 (0.92–1.36)
CDKN2B-AS1	rs16923583	A	0.47	0.94 (0.80–1.11)	0.44	0.93 (0.78–1.11)	0.71	0.95 (0.73–1.24)	0.94	1.01 (0.81–1.25)
CDKN2B-AS1	rs1547705	A	0.43	1.07 (0.91–1.27)	0.33	1.10 (0.91–1.31)	0.97	1.01 (0.79–1.28)	0.89	1.01 (0.84–1.23)
CDKN2B-AS1	rs1537370	C	0.06	0.88 (0.77–1.00)	0.11	0.89 (0.77–1.03)	0.09	0.84 (0.68–1.03)	0.24	1.12 (0.93–1.36)
CDKN2B-AS1	rs10738607	C	0.32	0.92 (0.78–1.09)	0.16	0.88 (0.73–1.05)	0.82	1.03 (0.81–1.30)	0.95	0.99 (0.80–1.23)
CDKN2B-AS1	rs10965235	A	0.06	1.13 (1.00–1.27)	0.03	1.16 (1.01–1.32)	0.70	1.04 (0.86–1.25)	0.43	0.93 (0.79–1.11)
CDKN2B-AS1	rs4990722	G	0.55	1.05 (0.89–1.25)	0.65	1.04 (0.87–1.25)	0.41	1.12 (0.85–1.47)	0.79	0.97 (0.79–1.20)
CDKN2B-AS1	rs17761446	G	0.08	0.79 (0.60–1.03)	0.05	0.74 (0.55–1.00)	0.76	0.94 (0.63–1.41)	0.86	1.04 (0.70–1.53)
CDKN2B-AS1	rs1333049	C	0.10	0.89 (0.78–1.02)	0.07	0.87 (0.75–1.01)	0.78	0.97 (0.78–1.20)	0.68	1.05 (0.84–1.31)
CDKN2B-AS1	rs1333050	C	0.37	1.07 (0.92–1.24)	0.43	1.07 (0.91–1.26)	0.53	1.08 (0.85–1.36)	0.10	1.28 (0.95–1.73)
CDKN2B-AS1	rs10811658	A	0.04	1.17 (1.01–1.35)	0.07	1.16 (0.99–1.35)	0.08	1.21 (0.98–1.49)	0.48	1.07 (0.90–1.27)
CDKN2B-AS1	rs12347779	C	0.97	1.00 (0.78–1.27)	0.89	1.02 (0.79–1.32)	0.73	0.94 (0.65–1.35)	0.28	0.82 (0.57–1.18)
CDKN2B-AS1	**rs10965245**	A	0.006	0.80 (0.68–0.94)	**0.0005**	**0.73 (0.61–0.87)**	0.97	1.00 (0.78–1.27)	0.38	1.10 (0.89–1.35)
CDKN2B-AS1	rs2383208	A	0.24	0.91 (0.78–1.06)	0.42	0.94 (0.79–1.10)	0.18	0.85 (0.67–1.08)	0.47	1.09 (0.87–1.35)
CDKN2B-AS1	rs1063192	C	0.15	0.85 (0.67–1.06)	0.39	0.90 (0.70–1.15)	0.06	0.68 (0.46–1.01)	0.98	N/A
CDKN2B-AS1	rs4977756	C	0.16	0.91 (0.81–1.04)	0.41	0.95 (0.83–1.08)	0.04	0.82 (0.67–1.00)	0.44	1.07 (0.90–1.29)
CDKN2B-AS1	rs10116277	G	0.88	0.99 (0.81–1.20)	0.89	1.02 (0.82–1.26)	0.43	0.88 (0.64–1.21)	0.23	1.82 (0.68–4.85)
CDKN2B-AS1	rs4977574	A	0.48	1.05 (0.91–1.22)	0.41	1.07 (0.91–1.26)	0.99	1.00 (0.80–1.26)	0.48	1.10 (0.85–1.41)
SIX1/SIX6	rs2350890	C	0.31	1.07 (0.94–1.20)	0.42	1.06 (0.93–1.21)	0.36	1.09 (0.90–1.32)	0.65	1.04 (0.87–1.24)
SIX1/SIX6	rs4901977	A	0.99	1.00 (0.88–1.13)	0.79	0.98 (0.86–1.12)	0.67	1.04 (0.86–1.26)	0.28	1.10 (0.92–1.32)
SIX1/SIX6	rs8012339	A	0.56	0.95 (0.82–1.12)	0.31	0.92 (0.77–1.09)	0.64	1.06 (0.83–1.36)	0.69	0.96 (0.78–1.17)
SIX1/SIX6	rs1266416	C	0.50	0.96 (0.85–1.08)	0.74	0.98 (0.86–1.12)	0.24	0.90 (0.74–1.08)	0.14	1.14 (0.96–1.35)
SIX1/SIX6	rs3759688	A	0.88	1.01 (0.86–1.20)	0.97	1.00 (0.83–1.20)	0.63	1.06 (0.83–1.35)	0.86	0.98 (0.82–1.18)
SIX1/SIX6	**rs11849906**	G	0.43	0.91 (0.72–1.15)	0.77	1.04 (0.81–1.32)	**0.006**	**0.53 (0.34–0.83)**	0.18	1.23 (0.91–1.67)
SIX1/SIX6	rs10148202	A	0.13	0.88 (0.74–1.04)	0.18	0.88 (0.74–1.06)	0.24	0.85 (0.65–1.12)	0.72	1.04 (0.84–1.30)
SIX1/SIX6	rs7156317	A	0.73	1.02 (0.90–1.16)	0.61	1.04 (0.90–1.19)	0.87	0.98 (0.81–1.20)	0.52	1.06 (0.89–1.26)
SIX1/SIX6	rs7146104	A	0.17	1.24 (0.91–1.68)	0.23	1.22 (0.88–1.69)	0.25	1.32 (0.82–2.11)	0.70	0.93 (0.66–1.32)
SIX1/SIX6	rs10483727	C	0.67	0.96 (0.78–1.18)	0.97	0.99 (0.77–1.28)	0.66	0.92 (0.64–1.32)	0.76	1.13 (0.52–2.44)
SIX1/SIX6	rs33912345	A	0.25	0.88 (0.71–1.10)	0.91	0.99 (0.77–1.27)	0.57	0.90 (0.62–1.30)	0.93	0.96 (0.42–2.23)

P value from the logistic regression using additive model with the justification of age and sex. AA, African American. Bold indicates significance.

the small sample size of our NPG dataset (280 cases and 999 controls), which reflects the smaller proportion of African Americans with NPG. Cao et al.[47] reported the association of rs1063192 in this region in the Afro-Caribbean populations of Barbados, West Indies. Consistent with our other findings, we do not observe this association in either African Americans or Ghanaians. The protective C allele of this SNP is rare in the Ghanaian population (MAF = 0.002 in cases versus 0 in controls). In addition, the mean IOP of POAG cases in the Barbados dataset was 22.5 mm Hg, significantly lower than the 26.2 mm Hg observed in our African American cases. These facts may contribute to the lack of association with the *CDKN2B-AS1* locus in Ghanaians and African Americans.

We have also identified a significant association of rs11849906 in the *SIX1/SIX6* region in the African American NPG subgroup ($P = 0.006$; OR = 0.53 ± 0.34–0.83). This SNP is monomorphic in the Caucasian and Asian populations from the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov) and is polymorphic only in populations of African ancestry, including African Americans and Africans in Nigeria, Kenya. Additional studies in populations of African ancestry are required to replicate this finding.

The lack of association with *TMCO1*, *CAV1/CAV2*, or the chromosome 8q22 intergenic region may be secondary to the limited sample size or perhaps due to smaller effect sizes in populations of African ancestry compared with Caucasians. The lack of associations with specific variants previously identified in Caucasian populations may be explained by the rarity of those alleles in African populations. Several associated SNPs common in Caucasians were rare in the Ghanaians, including rs10483727 and rs33912345 in the *SIX1/SIX6* region, and rs10116277 and rs1063192 in the *CDKN2B-AS1* region, potentially limiting the effect that these alleles could have on POAG risk.

Admixture in the African Americans and genetic heterogeneity between the African American and Ghanaian populations may also influence our association findings. Our meta-analysis of these loci with the African American and Ghanaian data sets indicates that five SNPs were nominally significant ($P < 0.05$) in the meta-analysis (rs7518099 and rs28144471 in *TMCO1*, rs4236601 in *CAV1/CAV2*, and rs10120688 and rs16905597 in *CDKN2B-AS1*). Interestingly, for the SNPs in *TMCO1* and *CAV1/CAV2*, the ORs and risk alleles were consistent between populations, while, for *CDKN2B-AS1*, the odds ratios and risk alleles were opposite in the two populations (Table 2, Supplementary Table S1). This suggests that some of the risk for POAG in the African American population may be explained by Caucasian admixture (*CDKN2B-AS1*), while other POAG risk factors may be driven by African-derived risk alleles.

Mutations in myocilin and optineurin have been documented in POAG cases. Previous studies[14,48–52] found that, despite the high prevalence of POAG in the populations of African ancestry, the mutations in these two genes play a relatively limited role. The increased prevalence of glaucoma in the African populations is not due to a higher prevalence of myocilin mutations. In this study, approximately 573 African American and 100 Ghanaian POAG cases have been screened for myocilin mutations.[49,50] Patients carrying any known mutations were excluded from our analysis. Given the relatively rare frequency of myocilin mutations in these African populations, we expect that the association results will not be affected by the lack of myocilin screening in the remaining POAG cases. In addition, several studies have confirmed the lack of significant association of common variants in myocilin with POAG in Caucasian individuals.[38,53] Collectively, these results suggest that other currently unknown genetic risk factors contribute to POAG in these African populations.

Our findings suggest that genetic associations for POAG found in Caucasian populations appear to play a much smaller role in populations of African ancestry, leaving a large portion of genetic architecture of African POAG to be determined. This is not surprising, as Africa in general, and Sub-Saharan Africa in particular, contains the anthropologically oldest and most heterogeneous populations on Earth who have experienced environmental conditions that have historically contributed unique selection pressures. In the long term, multiple GWASs of POAG in populations of African descent will be required to not only validate the known glaucoma loci, but also to identify novel variants and loci in specific populations of African ancestry that contribute to POAG pathogenesis and related blindness.

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Appendix

The International Consortium of African Ancestry REsearch in Glaucoma (ICAARE–Glaucoma) is led by Michael A. Hauser and R. Rand Allingham, and includes the following investigators: Stephen Akafo, Adeyinka Ashaye, Allison E. Ashley-Koch, Rupert Bourne, Donald L. Budenz, Pratap Challa, John H. Fingert, Douglas E. Gaasterland, Christopher A. Girkin, Fielding Hejtmancik, Leon Herndon, Rachel Kuchtey, Paul R. Lichter, Yutao Liu, Sayoko E. Moroi, Barbara Nemesure, Louis R. Pasquale, Julia E. Richards, Robert Ritch, Janey L. Wiggs, and Susan Williams.

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