Genomewide Scan and Fine Mapping of Quantitative Trait Loci for Intraocular Pressure on 5q and 14q in West Africans

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Purpose. High intraocular pressure (IOP) is a major risk factor for glaucoma, one of the leading causes of blindness worldwide. Because it has been demonstrated that African populations are at increased risk for glaucoma, the authors investigated the genetic basis of IOP in a sample of West Africans with type 2 diabetes (T2D) from Ghana and Nigeria.

Methods. Genomewide linkage analysis was conducted for loci linked to IOP (measured by applanation tonometry) in 244 affected sibling pairs with T2D using 372 autosomal short-tandem repeat markers at an average spacing of 9 cM.

Results. Multipoint variance components linkage analyses revealed suggestive linkage on chromosome 5 (5q22) with a logarithm of odds (LOD) score of 2.50 (nominal $P = 0.0003$; empiric $P = 0.0004$) and on chromosome 14 (14q22) with an LOD score of 2.95 (nominal $P = 0.0001$; empiric $P = 0.0003$). Fine mapping at a marker density of 2 cM in the 5q region confirmed the linkage signal, with an increase in peak LOD score to 4.91.

Conclusions. The strong signal on chromosome 5 lies in the region in which a novel gene, *WDR36*, in the GLC1G locus was recently identified as causative for adult-onset primary open-angle glaucoma and provides additional evidence that chromosome 5 contains susceptibility loci for glaucoma in multiple human populations. The evidence provided in this study is particularly important given the evolutionary history of these West African populations and the recent ancestral relationship to African Americans—a population with one of the highest rates of diabetes and associated complications (including glaucoma) in the world. (*Invest Ophthalmol Vis Sci.* 2006;47: 3262–3267) DOI:10.1167/iovs.05-1537

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Glaucoma, a leading cause of blindness, affects more than 70 million people worldwide.[1,2] With a prevalence between 1% and 3%, it is one of the most common causes of blindness in industrialized nations.[2] As a cause of blindness, it shows considerable ethnic disparity, accounting for approximately 3% of blindness in white Americans in contrast to 7.9% in African Americans.[3] Glaucoma is a heterogeneous group of optic neuropathies, characterized by an acquired loss of retinal ganglion cells and optic nerve atrophy. Primary open-angle glaucoma (POAG) is the commonest clinically defined subset of glaucoma.[2] It is usually asymptomatic in the early stages, and the diagnosis may not be made until the late stages of the disease, by which time major and irreversible optic nerve damage occurs.[4] Early detection, ideally before symptoms develop, is important to prevent irreversible loss of vision.

Elevated intraocular pressure (IOP) is the strongest known risk factor for glaucoma. Experimental elevation of IOP can induce glaucomatous neuropathy.[5] A recent meta-analysis of randomized, controlled trials showed that lowering IOP in patients with ocular hypertension or glaucoma helps to reduce the long-term risk of visual field loss.[6] Given that glaucoma can and does occur without high IOP (called normal tension glaucoma)[7] and that most persons with high IOP do not develop glaucoma,[8] it may be that there is considerable individual variation in disease susceptibility. Several studies also show variation in disease progression and response to treatment in patients with open-angle glaucoma.[9,10]

The genetic factors determining IOP level and susceptibility to pressure-induced damage are largely unknown, as are the specific processes that lead to retinal ganglion cell death. Several studies provide evidence for a significant genetic contribution.[5] In fact, positive family history has long been recognized as a major risk factor for glaucoma, with the risk for glaucoma in first-degree relatives estimated to be as high as 7 to 10 times that in the general population.[11–15] Several studies are directed at identifying the chromosomal regions and genes and the associated risk factors, including IOP, that contribute to glaucoma.[4,15–19] At least seven genetic loci—GLC1A,[20]

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GLC1B,[16] GLC1C,[18] GLC1D,[21] GLC1E,[22] GLC1F,[23] and another chromosome 2 locus[15]—have been reported for POAG. However, only three genes—myocilin (*MYOC*),[17] optineurin (*OPTN*),[24] and WD40-repeat 36 (*WDR36*)[4]—have been reported to be causally linked to glaucoma in these loci.

To our knowledge, no genomewide linkage study for POAG has been conducted in sub-Saharan African populations, and only one[25] has been conducted in any population of African descent living on other continents.[19] In this report, we provide evidence of quantitative trait loci (QTL) for IOP in West Africa (a major source population for present-day African Americans) and show strong evidence of replication of a recently reported POAG locus.[4]

Materials and Methods

Families with affected sibling pairs with T2DM were enrolled from four major ethnic groups in two countries in West Africa—Igbo and Yoruba in Nigeria and Gaa and Akan in Ghana. The research was conducted according to the tenets of the Declaration of Helsinki. The study protocol was approved by the institutional review boards (IRBs) of all participating institutions, and all participants gave written, informed consent of their participation in the study after explanation of the study background, procedures, and possible consequences. A detailed description of the study is available in earlier publications.[26,27]

All subjects were affected sibling pairs with T2D and were enrolled from five centers in two countries in West Africa: Enugu, Ibadan, and Lagos in Nigeria and Accra and Kumasi in Ghana. Diabetes diagnosis was based on the criteria established by the America Diabetes Association Expert Committee, as follows: fasting plasma glucose (FPG) concentration greater than 126 mg/dL (7.0 mM) or a 2-hour postload value in oral glucose tolerance test (OGTT) greater than 200 mg/dL (11.1 mM) on more than one occasion. The detection of autoantibodies to glutamic acid decarboxylase (GAD) antibody or a fasting C-peptide level less than 0.03 nM was used to exclude subjects with type 1 diabetes. Eye examination was part of a comprehensive physical examination of each participant in the study, as previously described.[26] Measurement of IOP was performed with a Goldmann applanation tonometer after instillation of a drop of fluorescein in each eye. Before measuring IOP in the right eye, the tonometer was set to 10 mm Hg, and pressure was recorded only after the tonometer was moved back from the cornea. The tonometer was then reset to 10 mm Hg for measurement of the left eye. Measurements were taken in duplicate on each eye, and the average was recorded. None of the subjects was taking glaucoma medication at the time of the measurement. Those found to have elevated IOP were referred for management.

Genotyping was performed at the Center for Inherited Disease Research (CIDR). The CIDR microsatellite marker set is composed primarily of trinucleotide and tetranucleotide repeats, with an average spacing of 8.9 cM and no gaps in the map larger than 18 cM. Average marker heterozygosity was 0.76. Approximately 10% of the marker loci are different between the current CIDR marker set and the Marshfield Genetics screening set version 8. For this study, 372 autosomal short tandem repeat markers were genotyped for an average sex-equal distance of 9 cM, with no gaps greater than 18 cM. The error rate was 0.1% per genotype, and the inconsistency rate was 0.11%. Extensive quality checks were carried out to verify consistency of marker genotyping and stated pedigree relationships, as previously described.[27]

Descriptive statistics and regressive statistical values were calculated using the Statistical Analysis System (SAS Institute, Cary, NC). The potential confounding influences of age and sex on the distribution of IOP were removed using a multiple regression model. Because IOP displayed a non-Gaussian distribution with a right skew, we performed a logarithmic transformation to obtain a normal (Gaussian) distribution. Transformed data were used in all subsequent analyses. QTL linkage analysis was performed with software (SOLAR, Southwest Foundation for Biomedical Research, San Antonio, TX) through the multipoint variance components approach.[28] In variance components linkage analysis, the variance of a trait is decomposed into locus-specific effects determined by the identity-by-descent (IBD) relationships (additive QTL variance), the residual additive genetic effects (additive polygenic variance), and individual specific random environmental effects (random environmental variance).[28,29] The null hypothesis is that the additive QTL variance equals zero (no linkage), and this was tested by comparing the likelihood of the restricted model with that of a model in which the additive QTL variance is estimated. The difference between the two log likelihoods produces a logarithm of odds (LOD) score. Twice the difference between the two log likelihoods of these models yields a test statistic that is asymptotically distributed as a 50:50 mixture of a χ^2 variable and a point mass at zero. One-LOD unit support intervals were obtained by identifying the peaks of the maximum LOD score on the plot of the multipoint results, dropping down 1 LOD unit and finding the chromosomal region defined by the shoulders of the curve.[30] An LOD score ≥3.3 was taken as evidence of significant linkage, and an LOD score ≥1.9 but <3.3 was taken as evidence of suggestive linkage.[31] Marshfield age- and sex-averaged maps were used in the linkage analyses.

To estimate the probability of obtaining false-positive evidence of linkage, we conducted gene-dropping simulations using MERLIN.[32] Marker data were simulated under the null hypothesis of no linkage or of association to observed phenotypes while retaining the same pedigree structures, maps, marker allele frequencies, and missing data patterns. We simulated 10,000 replicates and conducted the same linkage analyses described earlier. The probability of obtaining a false-positive result was defined as the proportion of replicates for which we obtained a specified LOD or higher score.

Table 1. Demographic Characteristics of Study Participants

	Men	Women
No. participants (%)	188 (38.5)	301 (61.5)
Age (y)	53.4 ± 10.6	52.7 ± 11.1
Body mass index (kg/m²)*	24.6 ± 4.1	27.2 ± 5.4
Fasting glucose (mg/dL)	208.1.7 ± 89.8	207.7 ± 90.4
C-peptide (ng/mL)*	1.2 ± 0.7	1.5 ± 0.9
Insulin (uU/mL)	20.9 ± 23.6	26.2 ± 36.7
SBP (mm Hg)	135.2 ± 24.4	138.4 ± 24.8
DBP (mm Hg)	82.5 ± 12.4	83.2 ± 13.1
Cholesterol (mg/dL)*	178.3 ± 44.4	199.1 ± 54.1
Triglyceride (mg/dL)	94.8 ± 57.9	97.5 ± 56.3
Intraocular pressure (mm Hg)	17.0 ± 4.3	16.8 ± 4.4

Data are mean ± SD unless otherwise indicated.
* Significant difference comparing men and women ($P < 0.01$).

Results

A total of 244 sibships (210 full-sibling and 36 half-sibling pairs for a total number of 489 persons, 188 men and 301 women) was included in the analysis (Table 1). Average duration of diabetes was 7.0 years, and mean age at diagnosis of diabetes was 46.5 years. Mean body mass index was 24.6 (±4.1) kg/m² for men and 27.2 (±5.4) kg/m² for women. Mean fasting blood glucose level was 208 mg/dL (±11.6 mM), indicating the poor control of blood glucose in these subjects with T2D. The mean value of IOP was 17.0 (±4.3) mm Hg for men and 16.8 (±4.4) mm Hg for women. In the general population, the acceptable normal range for IOP is 10 to 21 mm Hg, with a mean of 16 mm Hg.[1]

A plot of the LOD scores on all 22 autosomes obtained from the initial multipoint variance component linkage analyses is

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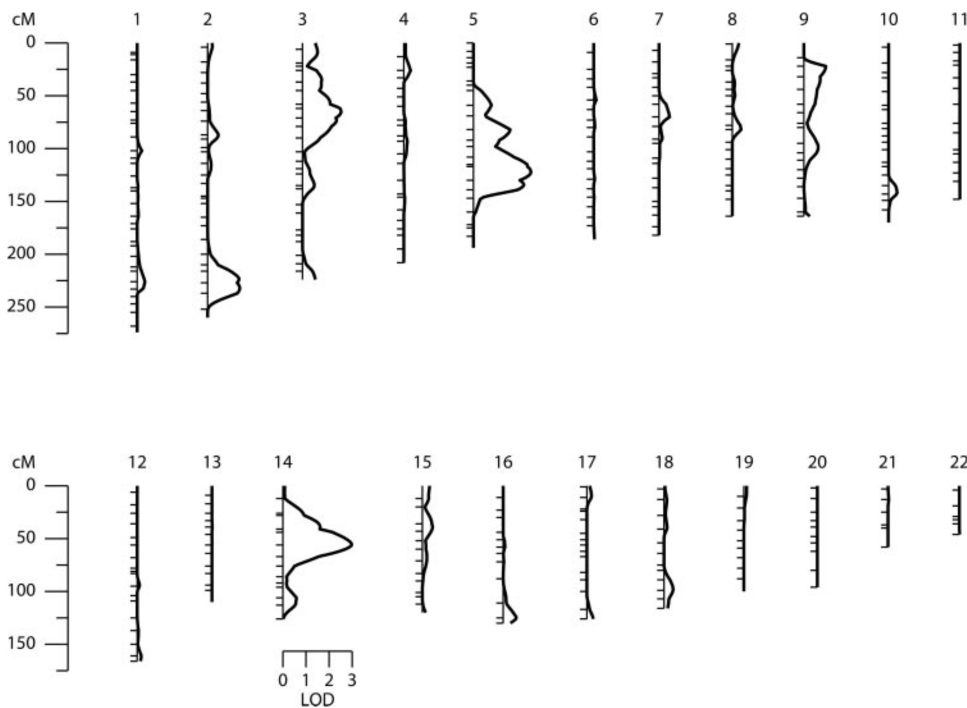

FIGURE 1. Multipoint variance component linkage results for intraocular pressure.

presented in Figure 1. Peak LOD scores on this first-pass genome scan are shown in Table 2. The strongest evidence for linkage to IOP was observed on chromosome 5, with a maximum LOD score of 2.50 (nominal $P = 0.0003$; empiric $P = 0.0004$) near marker D5S2501 and on chromosome 14, with a maximum LOD score of 2.95 (nominal $P = 0.0001$; empiric $P = 0.0003$) near marker D14S587. Two other regions had LOD scores above 1.5, on chromosome 3 near marker D3S1768 (LOD score, 1.67; nominal $P = 0.0026$; empiric $P = 0.0039$) and on chromosome 5 near marker D5S424 (LOD score, 1.60; nominal $P = 0.0003$; empiric, $P = 0.0048$). Based on the 10,000 simulations conducted, the probability of obtaining an LOD score of 3.0 or higher was 0.0002, and the probability of obtaining an LOD score of 1.5 or higher was 0.0064, suggesting that the reported linkage regions were unlikely to have resulted from chance.

After the first-pass genome scan, these two linkage peaks (5q22, 14q22) met the Lander-Kruglyak criterion[31] for *suggestive linkage* and deserved to be followed up with genotyping with markers at a higher density to refine the linkage peaks. Because of resource constraints, we were able to follow up only one of these two regions. We chose to follow up the 5q linkage region given that other investigators have reported a POAG locus in this genomic region and that confirmation of this QTL in this population will support the existence in multiple populations of a "true" QTL in this genomic region. A 2-cM microsatellite fine-mapping scan of the chromosome 5q region was performed on the same sample. Linkage analysis of this fine map confirmed the existence of a linkage peak with an increase in LOD scores (Fig. 2). An examination of the two LOD score plots (first-pass genomewide scan and fine-mapping scan) on chromosome 5 showed that the peak at approximately 82 cM from *p-ter* had a 0.6-Unit increase in LOD score, whereas the maximum LOD score at approximately the 135-cM (from *p-ter*) signal rose to 4.91 (1-LOD unit support interval, 130–139 cM). This QTL meets the Lander-Kruglyak criterion[31] for *significant linkage*. However, it should be noted that this linkage signal comprises three sharp peaks, each with a peak LOD score greater than 4 on the fine-mapping scan (Fig. 2;

TABLE 2. Results from Genomewide Linkage Analysis of Intraocular Pressure Showing LOD Score Peaks >1.5

Chromosome	Nearest Marker	Flanking Markers	Linkage Peak (cM)*	1-LOD Unit Support Interval (cM)	Maximum LOD Score
Suggestive linkage					
5	D5S2501	D5S1503 D5S816	122	98–143	2.50
14	D14S587	D14S599 D14S592	55	31–70	2.95
Other linkage regions with LOD >1.5					
5	D5S424	D5S424 D5S641	82	75–95	1.60
3	D3S1768	D3S1768 D3S2409	65	51–83	1.67

* Distance from *p-ter* on Marshfield map.

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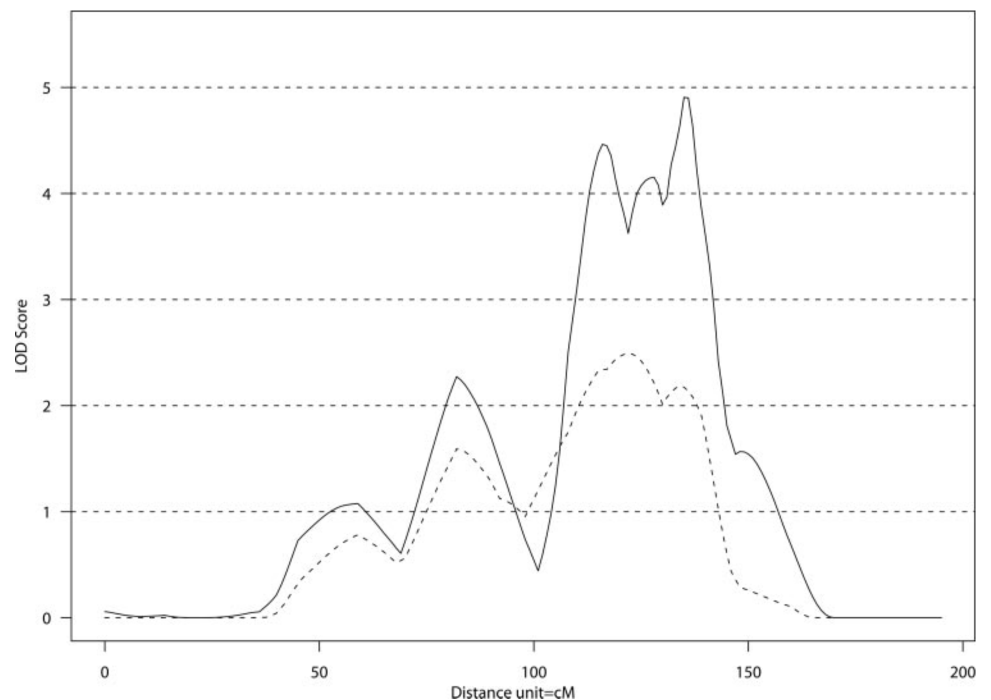

FIGURE 2. Linkage evidence on chromosome 5 for intraocular pressure (9-cM scan, *dotted lines*) and (2-cM fine-mapping scan, *solid line*). Results were obtained from variance components linkage analyses as implemented in SOLAR. Peak LOD on fine mapping was 4.91 at 135 cM from *p-ter*.

Table 3) and each peak separated from the next by a map distance of at least 7 cM.

The average information content of this region was 0.484 for the 10-cM map and was increased to 0.662 for the 2-cM fine-mapping scan. The maximum linkage disequilibrium (LD), as estimated by the D' statistic, in this region was 0.2 for both the 10-cM and the 2-cM maps. Given these results, the increase in LOD is likely to be attributed to the increased information content provided by the 2-cM fine-mapping scan rather than to marker LD in this linkage region. The breadth of the region and the multiple peaks within it suggest several susceptibility genes may be present in this region. On the other hand, this may just be a reflection of the low resolution of the genome scan at this density with this collection of pedigrees.

DISCUSSION

General understanding of the genetic basis of the group of disorders collectively known as glaucoma is improving, and several linkage and association studies based on genomewide and candidate loci approaches are making significant contributions to that understanding.[5,15,19,25] A recent review[33] identified at least 20 genetic loci linked to POAG and at least 16 POAG-associated genes from association studies, noting that most results of candidate genes are inconsistent and that only three POAG genes (*MYOC*, *OPTN*, and *WDR36*) are recognized. Despite these, novel loci are still being found. For example, investigators from the Beaver Dam Eye Study[34] recently reported two novel genetic loci for IOP on chromosomes 6 and 13 that have not been identified in any previous genomewide scans for POAG. These and other observations suggest that POAG is genetically heterogeneous, but more work is needed to elucidate the various forms of the condition.

The 5q QTL in this study, the first genome scan for IOP in West Africans, overlaps with a recently described adult-onset POAG locus on 5q22.1,[4] designated GLC1G by the HUGO Gene Nomenclature Committee (HGNC). For several reasons, we believe the 5q QTL identified in the present study is a replication of the GLC1G locus. First, the locus identified in the first-pass genome scan in the present study overlaps with the GLC1G locus.[4] Second, fine mapping of the locus confirmed and increased the LOD score to greater than 4. Third, the 2-Mb critical interval for GLC1G corresponded to one of our fine-mapping linkage peaks at 116 cM. The present study, in repli-

TABLE 3. Comparison of Peak LOD Scores for IOP on First-pass Genome Scan and Fine-Mapping Scan of Chromosome 5

	Original 9-cM Genome Scan		2-cM Fine-Mapping Scan	
	Peak LOD	Location (1 LOD support interval*), cM Nearest marker†	Peak LOD	Location (1 LOD support interval*), cM Nearest marker†
First chr5 linkage region	1.60	82 (75–96) D5S424	2.27	82 (74–93) D5S424
Second chr5 linkage region	2.50	122 (99–130) D5S2501	4.46	116 (111–122) D5S654
	—	—	4.15	128 (122–130) D5S471
	2.20	134 (130–143) D5S1505	4.91	135 (130–139) D5S2120

* 1 LOD unit support interval, except where a trough occurs before 1 LOD unit drop on plot.
† Nearest marker on the map of the STR markers genotyped for this study. All distances are from *p-ter* on the Marshfield map.

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cating linkage evidence for IOP at a 5q locus for POAG, suggests that this QTL may be important in several geographic populations. On the other hand, a recent study[33] mapped a juvenile-onset POAG (JPAG) locus to 5q22.1-q32; this locus did not overlap with the GLC1G minimal interval, and the authors ruled out WDR36 coding sequence mutations in the pedigree studied.

The story of how WDR36 was identified from within the GLC1G locus is instructive. A mutation screen of seven candidate genes from the GLC1G critical region, which spans approximately 2 Mb (D5S1466-D5S2051), identified a significant alteration (D658G) in the *WDR36* (WD40-repeat 36) gene.[4] Additional screening of *WDR36* in 130 POAG families revealed 24 DNA variations with four mutations (N355S, A449T, R529Q, D658G) identified in 17 unrelated POAG subjects but absent in more than 200 healthy control chromosomes.[4] These mutations were conserved between WDR36 orthologs in mouse, rat, dog, chimp, and human. *WDR36*, a novel gene with 23 exons encoding 951 amino acids, is expressed in lens, iris, sclera, ciliary muscles, ciliary body, trabecular meshwork, retina, and optic nerve, as established by RT-PCR.[4] However, several lines of evidence suggest that other POAG genes may exist in this region. For example, the region is large and often has several linkage peaks (as in the present study); moreover, previous studies have identified POAG families that map to this region but show no *WDR36* mutations. An examination of the annotated human genome sequence (build 35.1) of this region shows other potential candidate genes for POAG apart from the *WDR36* gene. These genes include *SEMA6A* and *TGFBI* (transforming growth factor β-induced), which share the "visual perception" gene ontology (GO) annotation with MYOC; *DMXL1* and *WDR55* (formerly *NP_060176*), which share the G-protein β WD-40 repeat Interpro domain with *WDR36*; and *ACSL6*, which shares the adenosine monophosphate (AMP)–dependent synthetase and ligase Interpro domain with *WDR36*. However, it should be noted that these are just a few of the potential candidate genes in the region. We are in the process of screening some of these genes in our West African patients for the potential identification of novel variants for subsequent functional analysis.

The 14q22 linkage signal (LOD = 2.5; $P = 0.000038$) is also noteworthy in that other groups have identified evidence for POAG susceptibility loci in this region.[15,25] For example, Wiggs et al.[15] reported a maximum LOD score greater than 2.0 for five regions, including chromosome 14. This finding was subsequently supported, though weakly, by the genome scan for POAG in the Barbados Family Study of Open-Angle Glaucoma.[25] Interestingly, the 14q22 region is the location for the methylenetetrahydrofolate dehydrogenase gene, *MTHFD1*, a trifunctional enzyme involved in de novo purine and pyrimidine biosynthesis and in homocysteine metabolism.[35] It has been reported that mild hyperhomocysteinemia was associated with POAG and secondary open-angle glaucoma.[36–38] Furthermore, a polymorphism of another enzyme directly involved in homocysteine metabolism, methylenetetrahydrofolate reductase (*MTHFR*), showed significant association with hypercysteinemia and POAG.[39]

Other important glaucoma loci, not observed in the present study, have been reported.[5] These loci include 1q23 to 25 (*MYOC*); 2cen-q13 (*GLC1B*); 3q21 to 3q24 (*GLC1C*); 8q23 (*GLC1D*); 10p15 to 10p14 (*OPTN*); 7q35 (*GLC1F*); 9q22 (*GLC1*); and 20q12 (GLC1).[4,16–19,21–23] In the only other study with participants from a population exclusively of recent African descent, Nemesure et al.[25] found no support for the myocilin (*MYOC*) or optineurin (*OPTN*) genes as causative genes for glaucoma in an Afro-Caribbean population in Barbados.

The present study has some limitations. First, study participants were affected sibling pairs with T2D, but it is uncertain whether the linkage regions tracked IOP, the underlying T2D, or both. However, our genome scan for T2D in this sample[27] found no chromosome 5 linkage regions but obtained suggestive linkage to T2D on chromosomes 12 and 20. Therefore, we think it unlikely that the chromosome 5 QTL tracked T2D. Second, we focused on IOP as the phenotype rather than POAG or glaucoma, reasoning that the continuous variable provides more power to detect linkage than a binary variable such as the presence or absence of glaucoma. However, because IOP is an intermediate physiologic phenotype whose elevation does not always correlate with disease (American Academy of Ophthalmology, 2001), it is uncertain whether a study of IOP can be directly compared with one of POAG. Third, we did not measure central corneal thickness, which is a major determinant of IOP. Central corneal thickness itself is highly heritable, and our linkage regions may track (at least in part) this phenotype rather than IOP. Despite these limitations, however, we believe that the data presented here provide an additional important step in the understanding of the genetic susceptibility to glaucoma in various world populations.

Based on our findings and the support provided by other studies, we plan to conduct fine mapping of our linkage signals on chromosomes 5 and 14 with densely placed single nucleotide polymorphisms (SNPs). We are of the opinion that this data set of West Africans with T2DM and associated complications, including IOP, is likely to be highly useful for fine mapping and gene localization given that the region of linkage disequilibrium around a susceptibility allele is likely to be narrower in Africans than in Europeans or Asians.[40] Because glaucoma is the leading cause of blindness in African Americans who share an ancestral relationship with West African populations, we anticipate that findings from this project will be directly relevant to the ongoing effort to help reduce the substantial ethnic disparity in glaucoma rates in the United States.

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