Replication of a Glaucoma Candidate Gene on 5q22.1 for Intraocular Pressure in Mongolian Populations: The GENDISCAN Project

Mi Kyeong Lee,[1,2] *Se Joon Woo,*[2,3] *Jong-il Kim,*[4] *Sung-il Cho,*[1] *Ho Kim,*[5] *Joohon Sung,*[*,1] *Jeong-Sun Seo,*[*,4] *and Dong Myung Kim*[*,6]

Purpose. Glaucoma is the second most frequent cause of visual impairment worldwide. Elevated intraocular pressure (IOP) causes glaucomatous optic nerve damage, especially in the primary open-angle glaucoma (POAG) subtype. As most previous studies on IOP genetics were analyses of glaucomatous families, a study of general pedigrees will provide additional information on genetic etiology.

Methods. This work was part of the GENDISCAN study (Gene Discovery for Complex Traits in Isolated Large Families of Asians of the Northeast), which recruited families from population isolates in Mongolia. IOP (obtained by a noncontact method), epidemiologic, and clinical information were collected from 1451 healthy individuals of 142 families. From these individuals, 390 genome-wide short tandem repeat markers were genotyped. Variance component-based linkage analysis was applied to pursue candidate loci explaining IOP variation.

Results. The mean IOP was 13.6 mm Hg in the men and 13.7 mm Hg in the women, inversely associated with aging ($\beta = -0.05$; $P \leq 0.0001$). The heritability of IOP was 0.48. Suggestive linkage evidence was found on the 5q22.1 region (LOD score, 2.4), which harbors *WDR36*, a candidate gene for POAG. In addition, possible linkage evidence was found on 2q37.1, 7p15.3, 17q25.3, and 20p13.

Conclusions. The findings support evidence that IOP regulation is associated with the 5q22.1 region, along with four other candidate regions. The present results further indicate that genetic factors regulating IOP in the general Mongolian population are linked to regions harboring POAG genes, suggesting that common genetic factors influence both normal IOP variation and POAG occurrence. In addition, the replication of previous findings concerning POAG regions from the white and African populations implies that the mutations regulating IOP levels did not occur recently. (*Invest Ophthalmol Vis Sci.* 2010;51:1335–1340) DOI:10.1167/iovs.09-3979

Glaucoma is the second most common cause of blindness worldwide, with a racially variable prevalence rate of 1% to 3%.[1,2] In 2010, glaucoma is predicted to affect 60.5 million people, with this number predicted to increase to 79.6 million by 2020.[3] The prevalence of primary glaucoma in the Mongolian population seems to be different from that in white populations. Primary angle-closure glaucoma (PACG) is more common (1.4%), and primary open-angle glaucoma (POAG) less common (0.5%) in the Mongolian population than in white populations.[4,5] For example, The Baltimore Eye Survey found a POAG prevalence of 1.4% in the examined white population.[5] Previous studies of subjects with elevated intraocular pressure (IOP) have shown a higher prevalence of glaucoma[6,7]; moreover, eyes with higher IOP showed more severe glaucomatous optic nerve damage and visual impairment. Although IOP is not a necessary condition for glaucoma, it is a major risk factor for the disease. Therefore, the investigation of genes that influence IOP may help to elucidate the genetic mechanisms of IOP regulation as well as the genetic background of glaucoma.

Three genes have been identified in families of patients with glaucoma: myocilin (1q24.3-q25.2, in a family affected with an autosomal dominant form of juvenile open-angle glaucoma[8]), optineurin (10p15-p14, in a large British family with a classic form of normal-tension open-angle glaucoma[9]), and WD40-repeat 36 (*WDR36*; 5q21.3-q22.1, in families with adult-onset POAG[10]).

The environmental risk factors of IOP are not well understood, although previous studies[11,12] support genetic contributions. Heritabilities of IOP were reported to be 0.35 in the Erasmus Rucphen Family study,[13] 0.36 in the Beaver Dam Eye Study,[11] and 0.29 in the Salisbury Eye Evaluation Study.[12] Several studies have reported quantitative trait loci (QTLs) that influence IOP levels. However, in general, QTLs for IOP have not been proven either by replication or identifying the causative variant. Duggal et al.[14] reported two regions on chromosomes 6 and 13 linked to IOP in a middle-aged normal population in the United States. Charlesworth et al.[15] demonstrated

From the [1]Complex Disease Genetic Epidemiology Branch, Department of Epidemiology, Graduate School of Public Health and Institute of Environment and Health, and the [5]Department of Epidemiology and Biostatistics, School of Public Health, Seoul National University, Seoul, Republic of Korea; the [3]Department of Ophthalmology, Seoul National University Hospital College of Medicine, Seoul National University Bundang Hospital, Seoul, Republic of Korea; the [4]Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul, Republic of Korea; and the [6]Department of Ophthalmology, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, Republic of Korea.

[2]Contributed equally to the work and therefore should be considered equivalent authors.

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*Each of the following is a corresponding author: Joohon Sung, Department of Epidemiology, Graduate School of Public Health and Institute of Environment and Health, Seoul National University, 28 Yeongon-Dong, Chongro-gu, Seoul, Republic of Korea; jsung@snu.ac.kr.

Jeong-Sun Seo, Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, 28 Yeongon-Dong, Chongro-gu, Seoul, Republic of Korea; jeongsun@snu.ac.kr.

Dong Myung Kim, Department of Ophthalmology, Seoul National University College of Medicine, Seoul National University Hospital, 28 Yeongon-Dong, Chongro-gu, Seoul, Republic of Korea; dmkim@snu.ac.kr.

that the 10q22 region was linked to maximum IOP in one large POAG Australian pedigree. Rotimi et al.[16] reported 5q and 14q QTLs in families with type II diabetes in an African population. Finally, Duggal et al.[17] reported 19p as a novel candidate region controlling IOP in a white population.

The lack of replication among QTLs of IOP may be partly attributable to the differences in ascertainment strategy (glaucomatous families or general population) or in the age distribution of participants. Other explanations of the discrepancy include ethnic differences in the etiology represented by the difference in glaucoma epidemiology, as well as genetic heterogeneity across populations. The phenotypic variability, including intraindividual variation and measurement errors and the complex mechanisms of optic nerve damage, may be other reasons for the lack of replication.

Since most studies regarding the genetics of glaucoma or IOP have been performed in non-Asian populations, studies from Asians, with different glaucoma epidemiology, will augment existing evidence. There are several reasons for this. First, the ratio of POAG to angle-closure glaucoma (ACG) is substantially different between Asian and white populations.[5] Second, the age-related patterns of change in IOP differ between these populations, displaying negative age association in East Asian populations[18–21] and positive age associations in West Asian[22,23] and Caucasian[24,25] populations. In addition, mean IOP levels in Mongolian, Chinese, and Japanese populations are lower than those in white[26,27] populations. These ethnic differences in clinical and epidemiologic features of IOP suggest roles of both genetics and environment.

Studies of healthy participants from population isolates in Asia will not only reduce variations from environments and genetic heterogeneity, but will provide insight concerning epidemiologic differences. The GENDISCAN (GENe DIScovery for Complex traits in isolated large families of Asians of the Northeast) study is uniquely powerful in this regard. It is one of the rare studies of Northeast Asians tailored to gene discovery. Using homogeneous population isolates, using families not selected according to their health status, and recruiting large and extended families are several ways to increase the power of detecting genes that regulating IOP.

Materials and Methods

This study was performed as part of the GENDISCAN study designed to research the genetic backgrounds of several complex traits of the Asian population. In 2004, 142 large and complex pedigrees composed of 1451 family members were collected in 2004 in Orhongol, Selengae Province, Mongolia. The pedigree structure was complicated, with compound generations and numerous full and half-siblings, cousins, and spouses. Pedigree relationship information was determined by personnel interviews and confirmed by genotype data. The study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects, and each study protocol was approved by the institutional review board of Seoul National University (approval number H-0307-105-002).

One trained examiner measured IOP in both eyes by noncontact tonometry (NCT; model TM800; Kowa, Torrance, CA). The average of two successive measurements was regarded as the IOP for each eye. In cases in which the difference between the two consecutive IOP measurements was >3 mm Hg, one more measurement was performed, and the average of all three measurements was used. A total of 219 individuals <13 years-of-age without reliable IOP measurements and one individual with a self-reported history of diabetes were excluded. We collected additional epidemiologic data and clinical information, including basic demographics; anthropometries such as height, weight, waist circumference, and systolic/diastolic blood pressure; clinical tests such as fasting plasma glucose level and blood lipids; and personal and family histories of disease.

Genotyping was conducted with linkage mapping (Prism Linkage Mapping Set, ver. 2.5; Applied Bioscience, Inc. [ABI], Foster City, CA) containing 400 short tandem repeat markers. An additional 80 Marshfield microsatellite markers were adopted to make up intermarker gaps that resulted from genotype errors (markers with an error rate >1%) or insufficient information (a heterozygote index <0.4). Extensive quality checks were performed to verify consistency of marker genotyping and self-reported pedigree relationships. Genetic distances were based on the public Marshfield sex-averaged genetic map. Pedigree errors were found and corrected by using PREST.[28] Paternity errors were detected and removed with Simwalk2 software (http://www.genetics.ucla.edu/software/simwalk/ provided in the public domain by the Department of Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA).[29] Lists of original and additional markers are provided in

Table 1. Basic Characteristics of the Participants

Characteristic	Men	Women	Total
Study subjects, *n**	346 (40.2)	514 (59.8)	860
Age, y†	33.5 (16.5)	34.6 (16.5)	34.1 (16.5)
IOP, mm Hg by age†	13.6 (13.2–14.0)	13.7 (13.3–14.1)	13.7 (13.4–14.0)
13–19 y (*n* = 238)	14.6 (14.2–15.0)	15.8 (15.4–16.2)	15.3 (14.9–15.7)
20–29 y (*n* = 134)	14.6 (14.1–15.1)	14.3 (13.7–14.9)	14.4 (13.8–15.0)
30–39 y (*n* = 185)	14.0 (13.6–14.4)	13.7 (13.3–14.1)	13.8 (13.4–14.2)
40–49 y (*n* = 147)	14.0 (13.4–14.6)	13.2 (12.7–13.7)	13.6 (13.1–14.1)
50–59 y (*n* = 76)	13.7 (13.0–14.4)	13.8 (13.1–14.5)	13.7 (13.0–14.4)
>60 y (*n* = 80)	12.3 (11.7–12.9)	12.4 (11.7–13.1)	12.4 (11.7–13.1)
Range (median)	7–24 (13)	7–26 (13)	7–26 (13)
25%–75%	12–16	12–17	12–16
5%–95%	9–19	10–20	10–20
SBP, mm Hg†	121.5 (119.1–23.9)	120.3 (118.1–22.5)	120.8 (119.2–22.4)
DBP, mm Hg†	81.5 (80.2–82.8)	82.3 (81.2–83.8)	82.0 (81.0–83.0)
Fasting plasma glucose†	89.9 (88.4–91.4)	87.0 (85.7–88.3)	88.2 (87.2–89.2)
Total serum cholesterol†	149.2 (145.6–52.8)	153.5 (150.6–56.4)	151.8 (149.5–54.1)
Triglyceride†	72.6 (68.3–76.9)	68.1 (65.2–71.0)	69.9 (67.1–72.1)
Body mass index†	22.6 (22.2–23.0)	23.6 (23.2–24.0)	23.2 (22.9–23.5)

SBP, systolic blood pressure; DBP, diastolic blood pressure.

* Count (%).

† Mean (95% confidence interval).

TABLE 2. Age- and Sex-Adjusted Intraclass Correlations between Family Pairs of IOP Levels

Relationship Pair	Count	Correlation	95% CI
Parent–offspring	690	0.23	(0.19 to 0.27)
Sibling	374	0.37	(0.31 to 0.43)
Half-sibling	102	−0.01	(−0.13 to 0.11)
Grandparent	161	−0.02	(−0.11 to 0.07)
Avuncular	362	0.24	(0.17 to 0.31)
Half-avuncular	161	0.10	(−0.1 to 0.21)
Cousin	214	0.03	(−0.06 to 0.12)
Half-cousin	91	0.04	(−0.10 to −0.18)
Spouse	140	0.02	(−0.06 to 0.10)

Data are for genotyped individuals only.

Supplementary Table S1, http://www.iovs.org/cgi/content/full/51/3/1335/DC1.

The mean IOP measurement of the left eye was used in the analysis; the correlation of IOP between the left and right eyes was 0.86. The IOP levels were not normally distributed but instead were right skewed. *Z*-transformation was used for heritability and linkage analysis. Basic statistical analyses were performed (SAS ver. 9; SAS Institute, Cary, NC), and Familial Correlation[30] from SAGE (Statistical Analysis of Genetic Epidemiology, ver. 4.3; http://darwin.cwru.edu/sage/ provided in the public domain by the Department of Biostatistics and Epidemiology, Case Western Reserve University, Cleveland, OH) was used to estimate familial correlations and the asymptotic standard errors. In addition, heritability was estimated with the Variance Component algorithm in SOLAR (Sequential Oligogenic Linkage Analysis Routines), version 2.1.4[31] (http://www.vipbg.vcu.edu/software_docs/solar/doc/00.contents.html). Multipoint linkage analysis was performed with SOLAR, to localize the QTLs that influence IOP. Expected LOD scores and empiric locus-specific *P*-values were calculated by using a 10,000-permutation simulation.[32,33] From the results, LOD scores of 1.9 to 3.3 were taken as evidence suggestive of linkage.[34] All the analyses outlined were adjusted for age, age^2, sex, and interactions between each age term and sex.

RESULTS

The pedigree data used for this linkage study were obtained from 1451 individuals from 142 families with 1720 parent–offsprings, 660 siblings, 946 grandparents, 795 avunculars, 548 cousins, and 452 spousal pairs. With the largest pedigree having a bit rate of 207, the average pedigree size was 10.2. Table 1 shows a detailed description of the study population and the distribution of the trait IOP. Approximately 40% of the subjects were male. The mean age of the men was 33.5 years and that of the women, 34.5 years. The mean (95% confidence interval) IOP was 13.7 (13.4–14.0) mm Hg ranging from 7–26 mm Hg for all individuals, and 13.6 (13.2–14.0) mm Hg for the men and 13.7 (13.3–14.1) mm Hg for the women. As shown in Table 1, a decreasing tendency of IOP with age was evident in both sexes. The mean IOP in the 13- to 19-year and >60-year age groups was 15.3 mm Hg and 12.4 mm Hg, respectively. The decreasing trend of IOP with age was significant (−0.25 by 5-year with $P < 0.0001$.)

Table 2 shows the age- and sex-adjusted familial correlations of IOP for each relationship. The intraclass correlation between sibling pairs was the largest. Notably, spousal pairs showed a correlation of 0.02, indicating that environment influences, if any, would only marginally contribute to IOP variation. The higher correlations evident in closer familial relationship pairs were strongly suggestive of a role of genetic factors in controlling IOP levels.

The heritability (SE) for IOP was 0.48 (0.06; $P < 0.0001$), which was compatible with the findings in familial correlation analyses that indicated the importance of genetic factors. The multivariate normality assumption was met for models under analysis (residual kurtosis, 0.21) when we adjusted for age, age^2, sex, and interactions between each age term and sex.

On genome-wide linkage scanning, we found several linkage regions with empiric $P < 0.001$. The highest multipoint LOD score of IOP was 2.4, with an empiric $P = 0.0002$, on 5q22.1 with the nearest marker *D5S2027*; the strongest signal met the Lander-Kruglyak criterion[34] for suggestive linkage results. The one drop from maximum LOD score region spanned roughly 26 cM, from 126 to 152 cM. Other candidate regions were located on chromosome 2 with an LOD score of 1.6 (empiric $P = 0.0032$), chromosome 7 with an LOD score of 1.4 ($P = 0.0051$), chromosome 17 with an LOD score of 1.5 ($P = 0.0043$), and chromosome 20 with an LOD score of 1.5 ($P = 0.0043$), having the nearest marker (cytogenetic region) of *D2S260* (2q37.1), *D7S493* (7p15.3), *D17S784* (17q25.3), and *D20S117* (20p13), respectively. Table 3 shows detailed chromosomal information for these regions, including LOD score and the empiric *P* from simulation analyses. Figure 1A is a graphic display of the multipoint linkage analysis across 22 chromosomes. Figure 1B is a more detailed description of chromosome 5.

DISCUSSION

To our knowledge, this is the first report on QTLs that influence IOP levels in an Asian population. In our study, suggestive linkage evidence was observed on 5q22.1, with an LOD score of 2.4. Previously, 5q22 was reported as a suggestive linkage region for IOP in a study of a West African population.[16] The 5q22.1 region has also been linked to POAG in several other populations. *GLC1G* and *WDR36*, containing glaucoma-causing mutations, were identified in the 5q22.1 region.[10] Several variants of *WDR36* have been associated with POAG.[35,36] In a recent study, alterations in *WDR36* in Japanese patients with POAG were not associated with normotensive POAG, whereas one variant of *WDR36* was significantly associated with high-tension POAG.[37] Our findings not only replicate those of previous studies but further suggest the possibility that normal variation in IOP, elevated IOP, and POAG may be regulated by common genetic factors. The LOD scores in this study did not

TABLE 3. Suggestive Regions from Genome-Wide Linkage Scan

Chromosome (Location)	Empirical *P*	Maximum LOD Score	1-LOD Unit Support Interval	Locus-Specific Heritability	Nearest Marker	Cytogenetic Region
5 (133)	0.0004	2.4	125–145	0.39	*D5S2027*	5q22.1
2 (254)	0.0032	1.6	240–266	0.39	*D2S206*	2q37.1
7 (35)	0.0051	1.4	17–52	0.36	*D7S493*	7p15.3
17 (130)	0.0043	1.5	118–139	0.35	*D17S784*	17q25.3
20 (4)	0.0043	1.5	2–16	0.32	*D20S117*	20p13

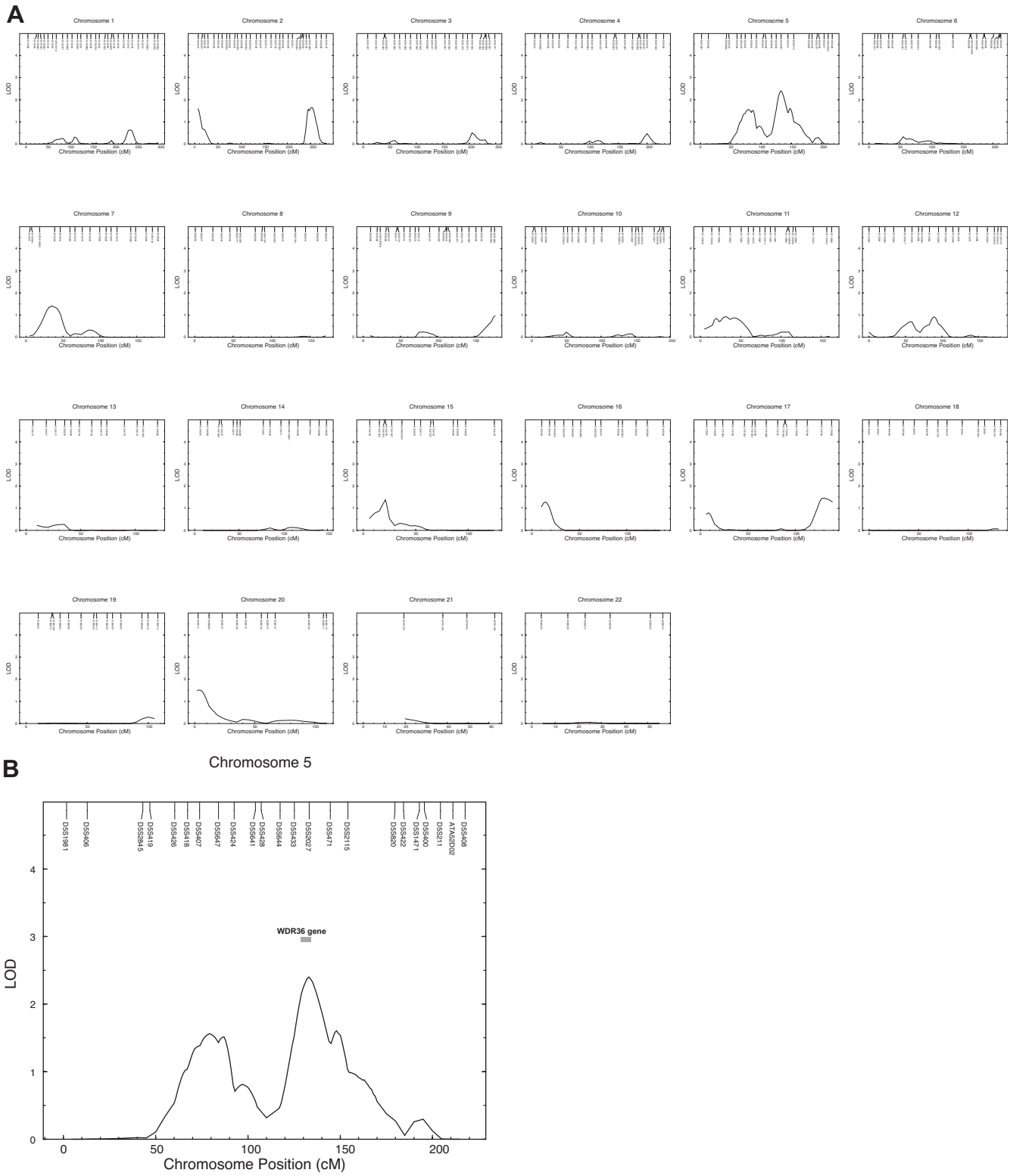

FIGURE 1. (**A**) Genome-wide multipoint linkage analysis results for IOP. The regions in chromosomes 2, 7, 5, 17, and 20 showed LOD scores greater than or ~1.5. (**B**) Multipoint linkage results for chromosome 5. The highest LOD score was 2.4, with an empiric $P = 0.0002$. The location of the *WDR36* gene and the highest peak from our analysis were very close.

reach the level of significance proposed by Lander and Kruglyak.[34] However, recent studies on age-related macular degeneration and the association of *TCF7L2* and type 2 diabetes[38,39] demonstrated that replication with suggestive or possible evidence is more convincing than unreplicated findings with strong LOD scores.

In addition, we found potential linkage evidence on chromosomes 2, 7, 17, and 20, with LOD scores higher than and or

equal to 1.5. The locus on 2q37.1, *MYP12*, is related to high myopia[40] and is also linked to common myopia.[41] This region should be investigated further, as myopia is one of the risk factors of elevated IOP[42] and glaucoma.[43] In 2006, a region on chromosome 7 (7p15.3) was reported to be related to congenital cataract.[44] As 17q25 is one of the loci previously reported to be responsible for POAG,[45] it would be worthwhile to focus on this region for candidate genes as well, although what we found in the present work is only less than suggestive linkage evidence. Moreover, 17q25.3 has been linked to systolic blood pressure,[46] which focuses attention on the relationship between systolic blood pressure and IOP.[12,24,47] We used systolic blood pressure as a covariate in linkage analysis; however, adjusting for it did not materially alter the LOD score for this region. Chromosome 20 also showed a potential linkage in this study; however, 20p13 has not been linked to IOP or glaucoma to date.

The heritability of IOP was 0.48, which is higher than that in other studies.[11,12,48] The higher heritability is most likely due, at least in part, to the lesser variation in other environments and more genetic heterogeneity underlying IOP levels.

Given the higher frequencies of PACG in Mongolia,[4] if the same population were analyzed for genes influencing glaucoma risk, the results would have explained the risk of PACG rather than POAG. Our findings alone cannot exclude or include the possibility that those who have elevated IOP will develop higher risk of POAG in the Mongolian population. However, considering our findings on IOP genes and previous reports on POAG genes, together with the pathogenesis underlying POAG, it is logical to suggest that there is a common genetic variation in the 5q region that influences both IOP and POAG risk. Furthermore, the possibility of a common genetic variant among the Caucasian, African, and Asian populations suggests that the genetic variation regulating IOP levels is ancient and is not selected by the evolutionary process.

In this study, we obtained IOP data by using NCT. Although NCT readings tend to show slightly higher values than Goldmann applanation tonometry,[20] good correlations between NCT and Goldmann readings have been reported previously.[49–51] Thus, NCT is a reasonable substitute for the gold standard method, especially in large-scale epidemiologic studies.

Our study was a large family-based examination of an isolated Asian population. Population admixture is a critical limitation in many genetic studies and may lead to biased results.[52] Collecting related individuals from a genetically homogeneous population not only decreases subpopulation effects but has greater statistical power.[53]

There are several limitations to our study. First, we did not check central corneal thickness in our subjects. As NCT is more sensitive to the level of central corneal thickness than Goldmann tonometry,[54] we would have obtained more accurate IOP data if we had adjusted IOP according to central corneal thickness. Second, the subjects did not undergo thorough ophthalmic examinations. More detailed examinations, such as slit lamp examination, gonioscopic angle assessment, optic disc examination, and visual field testing, which were all unavailable in our survey setting, would have yielded additional information regarding related types of glaucoma. The IOP levels used in this study were of cross-sectional measurements that include intraindividual variation as well as measurement errors. However, it is unlikely that the variation in IOP measurement is associated with genetic predisposition and the resultant biasing of the results. The intraindividual variation and errors in measurements partly account for the weaker linkage evidence in this study.

In conclusion, by primary genome-wide linkage analysis in a general population in Mongolia, we replicated the genomic region 5q22.1 containing the *WDR36* gene. Region 5q22.1 was previously reported to be linked to IOP in a West African glaucomatous pedigree, and *WDR36* is a causative gene in POAG. In addition, we discovered four new candidate loci of IOP regulation, each of which has been reported to be associated with IOP or IOP-related traits.

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