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Expansive marker analysis replicating the association of glaucoma susceptibility with human chromosome loci 1q43 and 10p12.31

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Three human chromosome loci (1q43, 10p12.31, and 12q21.31) were recently associated with the susceptibility to primary open-angle glaucoma (POAG) in a Japanese population; however, this was not replicated in three subsequent studies using South Indian, Afro-Caribbean, and Chinese populations. To identify genetic markers that are robustly associated across ethnic populations, numerous markers in addition to the six in the three reported loci were examined in this study. A total of 31 single-nucleotide polymorphism (SNP) markers were genotyped for 1115 Korean participants, and many neighboring SNPs were imputed using the Korean HapMap Project genotype data. Each SNP was statistically tested for association with POAG susceptibility by comparisons among 211 POAG patients with 904 unaffected controls. A strong and statistically significant association was found with a previously unreported SNP, rs7098387 (odds ratio, OR = 2.0 (1.4–3.0), P = 0.00038) at the 10p12.31 locus (where 11 SNPs were typed and 38 imputed) in contrast to the reported rs7081455, which was too poorly correlated with newly associated rs7098387 (r^2 = 0.003, D' = 0.40) to show association. Additionally, a modest association was observed with the reported rs693421 (OR = 1.4 (1.1–1.7), P = 0.0082) and several other SNPs located within and around *ZP4* at the 1q43 locus (10 SNPs typed and 14 imputed). However, no association was observed with the reported rs7961953 SNP or any other SNPs at the 12q21.31 locus, upstream of *TMTC2* (10 SNPs typed and 29 imputed). Accordingly, POAG susceptibility association was replicated using rs7098387 (*C*) rather than rs7081455 (*T*) at the 10p12.31 locus and additionally with rs693421 (*T*) at the 1q43 locus.

INTRODUCTION

Glaucoma is a chronic, degenerative optic neuropathy that causes damage to the optic nerve and progresses to blindness.[1] The disease involves the loss of retinal ganglion cells, leading to degeneration of the optic nerve and corresponding defects of the visual field. Elevated intraocular pressure is the strongest known risk factor for developing glaucoma and a main target of treatment for the prevention of progressive visual field loss. Although elevated pressure is clearly a risk factor, optic neuropathy can occur with normal intraocular pressure. Primary open-angle glaucoma (POAG) is the most common type of glaucoma and may or may not be accompanied by elevated intraocular pressure.[2–4]

POAG is known to have a genetic component. According to several population-based studies, first-degree relatives of POAG patients have a two- to fourfold increased relative risk of developing POAG when compared with the general population.[5–7] The Glaucoma Inheritance Study in Tasmania revealed that POAG is familial for ∼60% of patients and that the disease is more severe in patients with a family history than those without it.[8] Further, 21 POAG susceptibility loci have been identified by linkage studies, including 14 assigned as GLC1A–GLC1N and 10 that remain unassigned.[1,9–12] However, only three underlying genes have been identified at these loci, *MYOC* in

GLC1A,[13] *OPTN* in GLC1E,[14] and *WDR36* in GLC1G;[15] these genes account for < 5% of glaucoma cases.

Recently, several POAG susceptibility loci were identified by genome-wide association (GWA) studies[16–20] and were replicated in multiple ethnicities: 7q31.2 between *CAV1* and *CAV2*,[16,21] 9p21.3 with *CDKN2B-AS1*,[19,20,22–26] 10q21.3 with *ATOH7*,[17,18,22,27] and 14q23.1 between *SIX1* and *SIX6*.[17,20,22,28] One of the GWA studies demonstrated the association of POAG with six SNPs that flanked the *ZP4*, *PLXDC2*, and *TMTC2* genes located at chromosome loci 1q43, 10p12.31, and 12q21.31, respectively, in a Japanese population;[29] however, these associations were not replicated in a South Indian,[30] Afro-Caribbean,[24] or Chinese[31] population. These conflicting results can be ascribed to factors such as a small sample size with inadequate statistical power, different phenotype definitions, population-specific linkage disequilibrium (LD), effect-size bias, or population stratification.[32]

Association signals detected by GWA studies depend on how strongly the markers correlate with an unknown functional variation(s). Therefore, population differences in LD between an untyped, causal variation and typed markers can undermine the effect of the causal variation. Markers other than the reported ones in the association regions could be more strongly correlated with functional

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variations, and thereby offer greater statistical power to detect an association. A hybrid design involving exact testing of the reported markers plus fine mapping of the same region using additional markers is a good strategy for the replication and validation of the GWA study results.[33–35]

In this study, we investigated not only the reported SNPs but also additional SNPs that were chosen based on LD with the reported SNPs located at the loci identified from the Japanese GWA study.[29] The expansive coverage led to the identification of common SNPs associated with glaucoma susceptibility in both Korean and Japanese populations.

MATERIALS AND METHODS

Study participants

With the approval of the Institutional Review Board of Chungnam National University Hospital and following the tenets of the Declaration of Helsinki, 1115 Korean participants were recruited at the hospital and provided written informed consent, as previously described.[36] All POAG patients were diagnosed by a glaucoma specialist (C.-S.K.) at the glaucoma clinic of this tertiary referral hospital. The inclusion criteria for POAG were as follows: (1) characteristic glaucomatous defects in the optic nerve head, (2) characteristic visual field defects, as tested using the Humphrey Field Analyzer SITA standard 30-2 or 24-2 program (HFA II 720i; Carl Zeiss Meditec, Dublin, CA, USA), (3) defect in the retinal nerve fiber layer analysis using optical coherence tomography (Stratus OCT; Carl Zeiss Meditec), and (4) normal open angle by gonioscopy or deep central and peripheral anterior chamber angle width (>1/4 corneal thickness) by the van Herick method.

The POAG cases were divided into two subgroups, high-tension (intraocular pressure >21 mm Hg) and normal-tension glaucoma (intraocular pressure ≤21 mm Hg), according to the highest pressure ever recorded. The intraocular pressure was measured using a Goldmann applanation tonometer, and an average of two measurements was recorded for each visit. Patients with non-POAG types, such as exfoliative, pigmentary, steroid-induced, and primary angle-closure glaucoma, or those with uveitis were not included in the case group.

The 904 controls were free of any eye disease, and unaffected subjects having intraocular pressure higher than 21 mm Hg, cup-to-disc ratio larger than 0.7, glaucoma family history, or steroid treatment were not included in the control group. The demographic and clinical details of the study subjects are summarized in Table 1. The male/female ratio (1.6) was high in the case of this recruitment, as in the general POAG prevalence (gender ratio of 1.4) of Koreans,[37] but there was no attempt to match with control recruitment (gender ratio of 1.0), which would have necessitated an adjustment for gender in the susceptibility association tests.

SNP selection and genotyping

A total of six SNPs (rs540782, rs547984, rs693421, and rs2499601 located at chromosome locus 1q43, rs7081455 at 10p12.31, and rs7961953 at 12q21.31) were reported to be associated with POAG susceptibility in a previous GWA study.[29] These reported SNPs were individually in intermediate-to-strong LD with a total of 112 SNPs at $D' \geq 0.5$, with $r^2 \geq 0.1$, or at $D' = 1$, with $r^2 < 0.1$, in the Japanese population (JPT) of the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov; Phase 2 + 3, release 27). These neighboring SNPs were tagged by 25 additional SNPs, at $r^2 \geq 0.7$, in the Korean population of the Korean HapMap Project (www.khapmap.org). The tag selection and LD calculations were performed using the HaploView 4.2 (Broad Institute, Cambridge, MA, USA), and the GenBank accession numbers of reference sequences for human 1q43, 10p12.31, and 12q21.31 loci were NT_167186.1, NT_008705, and NT_029419.12, respectively.

Genomic DNA extracted from peripheral blood leukocytes of all subjects were genotyped using the MassARRAY iPLEX Gold assay (Sequenom, San Diego, CA, USA), according to the manufacturer's instructions, with approval by KAIST Institutional Review Board. The genotyping accuracy was 100%, as validated in blind tests of 16 samples for the six reported SNPs using the MassARRAY hME assay (Sequenom), as previously described.[38] Amplification and extension primers were designed using the MassARRAY SpectroDESIGNER 3.1. The call rate per SNP ranged from 94% to 100%, and the genotypes of the control subjects were in Hardy–Weinberg equilibrium ($P \geq 0.05/31 = 0.0016$).

Imputation and association tests

For the association analysis, the genotype distribution between the POAG patients and controls was compared using logistic regression, with adjustments for age and gender according to the co-dominant genetic models. Association was considered statistically significant when $P \leq \alpha$ and marginal when $\alpha < P \leq 0.05$, with a significance level of Bonferroni correction for multiple testing of $\alpha = 0.05/31 = 0.0016$, as 31 typed SNPs were tested. Statistical powers were calculated using the odd ratios reported in the GWA study,[29] with the sample size of the present study (type II error probability of $\beta = 0.20$), using the PS v.3.0 software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). All the statistical tests were performed using the SPSS v.18.0 (IBM SPSS, Armonk, NY, USA) or PLINK v.1.07 package (Broad Institute, Cambridge, MA, USA).

Typing data for the 31 SNPs were used to impute the genotypes of 699 additional SNPs within a 200-kb region centered in each locus for all subjects according to the Korean HapMap Project genotype data using the MaCH 1.0.16 software (University of Michigan, Ann Arbor, MI, USA), as previously described[36] (using the standard imputation approach with the -- geno command line option). Because the 25 typed tag SNPs tagged only 112 SNPs that were in intermediate-to-strong LD with the six reported SNPs, as described above, not all of the 699 SNPs were expected to be imputed with good quality.

Table 1 Characteristics of the study participants

Characteristics	POAG cases	Controls
Demographic characteristic		
Number of subjects [male + female]	211 [131 + 80]	904 [455 + 449]
Age (year)[a]	58.8 ± 15.0	57.5 ± 13.1
Body mass index (kg/m²)[a]	23.5 ± 3.4	23.5 ± 4.5
Systolic pressure (mmHg)[a]	129 ± 17	129 ± 23
Diastolic pressure (mmHg)[a]	79.8 ± 9.2	77.8 ± 11.3
Clinical characteristics		
Intraocular pressure (mmHg)[b]	28.7 ± 11.4 [14, 25, 70]	15.7 ± 2.7 [7, 16, 21]
Cup-to-disc ratio[b]	0.845 ± 0.158 [0.1, 0.9, 1.0]	0.409 ± 0.071 [0.10, 0.40, 0.70]
Visual field mean deviation (dB)[b]	−13.6 ± 10.4 [−35.44, −10.07, 0.72]	
Visual field pattern SD (dB)[b]	7.51 ± 3.95 [1.52, 7.77, 15.21]	

Abbreviations: POAG, primary open angle glaucoma; SD, standard deviation.
[a]Average ± SD.
[b]Average ± SD [minimum, median, maximum].

The squared correlation Rsq metric, which is defined as the observed variance divided by the expected variance, provides a measure of the quality of imputation. The genotypes of 81 SNPs in all subjects and those of the 31 typed SNPs in call-missing subjects were imputed with $Rsq \geq 0.3$ (a quality cutoff recommended in the MaCH instruction manual), and those for the control subjects were in Hardy–Weinberg equilibrium ($P \geq 0.05/112 = 0.00045$).

RESULTS

Study design

Six SNPs that have recently been associated with POAG susceptibility in a Japanese population[29] were typed for all the Korean subjects in this study. Four SNPs (rs540782, rs547984, rs693421, and rs2499601) were located upstream of *ZP4* at the 1q43 locus, one SNP (rs7081455) was located far downstream of *PLXDC2* at 10p12.31, and one SNP (rs7961953) was located within *TMTC2* at 12q21.31. These SNPs were in moderate-to-strong LD with 112 other SNPs ($D' \geq 0.5$, $r^2 \geq 0.1$, or $D' = 1$, $r^2 < 0.1$) in the Japanese population of the International HapMap Project, which could be tagged ($r^2 \geq 0.7$) by 25 SNPs (6 at 1q43, 10 at 10p12.31, and 9 at 12q21.31)

in the Korean population of the Korean HapMap Project. All subjects were additionally genotyped for these 25 tag SNPs (Table 2) to identify SNPs that would be in stronger LD in Koreans with causative functional variations than the reported SNPs. Thus, a total of 31 SNPs (ie, the six reported plus 25 tags) in the three loci were genotyped in the initial stage of this study (Table 2).

Replicated strong association of 10p12.31 (downstream of *PLXDC2*)

Among the 11 typed SNPs at the 10p12.31 locus, a strong association with POAG susceptibility was found for a previously unreported SNP, rs7098387 (Table 2), but the previously reported SNP (rs7081455) showed no association ($P = 0.74$). Only rs7098387 showed a significant association, with a P-value ($P = 0.0011$) lesser than the significance level ($\alpha = 0.0016$) of the Bonferroni correction for multiple testing (Table 2). When imputation was performed with the data for the 11 SNPs, a total of 38 neighboring SNPs were well imputed, though no other SNPs showed a significant association.

The risk effect of the rs7098387 SNP was calculated according to a dominant rather than a recessive or additive genetic model because no

Table 2 POAG susceptibility association tests of 31 SNPs at three chromosome loci

SNP (B> b)[a]	Chromosome position	Controls (MAF/BB/Bb/bb)	POAG cases (MAF/BB/Bb/bb)	P-value[b]
Chromosome locus 10p12.31				
rs1332545 (*G*>**A**)	20 618 569	0.244/512/316/58	0.282/106/84/16	0.29
rs7098387 (*A*>**C**)	20 623 473	0.0586/786/98/3	0.105/165/44/0	0.0011
rs7098524 (*G*>**A**)	20 623 564	0.288/462/336/87	0.290/101/95/13	0.085
rs1984056 (**C**>*A*)	20 634 493	0.198/571/282/35	0.192/129/67/5	0.54
rs7099711 (*T*>**A**)	20 638 789	0.405/326/386/160	0.405/69/106/30	0.14
rs7081455 (*T*>**G**)	20 638 885	0.207/565/292/40	0.213/129/74/8	0.74
rs10764227 (*A*>**G**)	20 643 267	0.303/445/344/96	0.356/90/84/31	0.13
rs4237357 (*T*>**C**)	20 647 896	0.312/440/345/105	0.379/76/99/27	0.012
rs944697 (*G*>**A**)	20 648 238	0.169/621/243/29	0.185/140/64/7	0.67
rs10764228 (*G*>**A**)	20 658 827	0.129/682/199/16	0.156/151/54/6	0.34
rs10828042 (**G**>*T*)	20 672 656	0.141/620/194/21	0.136/157/49/4	0.80
Chromosome locus 1q43				
rs790896 (**G**>*A*)	237 997 976	0.228/537/306/51	0.210/125/69/8	0.62
rs2275694 (**T**>*C*)	238 045 995	0.383/350/399/142	0.376/78/101/27	0.37
rs494430 (**C**>*G*)	238 064 109	0.398/339/399/157	0.384/79/92/32	0.72
rs12123002 (**G**>*T*)	238 064 730	0.384/336/423/130	0.376/76/105/25	0.52
rs1017982 (**C**>*T*)	238 095 957	0.427/305/413/175	0.388/72/108/26	0.047
rs547984 (*C*>**A**)	238 096 886	0.484/238/448/210	0.540/44/106/61	0.093
rs540782 (*G*>**C**)	238 097 039	0.487/242/441/219	0.531/47/104/60	0.24
rs693421 (*G*>**T**)	238 099 090	0.478/248/445/208	0.548/41/107/61	0.030
rs2499601 (*T*>**C**)	238 104 895	0.480/243/440/207	0.533/45/106/59	0.12
rs3121132 (**C**>*T*)	238 170 642	0.221/540/301/45	0.183/129/67/3	0.14
Chromosome locus 12q21.31				
rs12822754 (*A*>**G**)	83 040 610	0.127/663/201/11	0.138/156/45/6	0.38
rs9805078 (**G**>*C*)	83 065 251	0.255/501/334/61	0.265/116/78/17	0.77
rs17009812 (*T*>**C**)	83 065 359	0.134/653/205/14	0.144/156/44/8	0.20
rs7316112 (**G**>*A*)	83 067 844	0.384/333/416/129	0.411/78/89/41	0.18
rs1882582 (**G**>*A*)	83 075 714	0.136/646/222/8	0.149/152/55/4	0.47
rs11115342 (**C**>*T*)	83 076 731	0.295/445/378/76	0.277/104/71/18	0.35
rs10862496 (*C*>**A**)	83 089 957	0.458/253/453/178	0.474/54/112/43	0.61
rs7961953 (**G**>*A*)	83 091 836	0.322/407/384/92	0.320/95/86/22	0.95
rs6539680 (**C**>*T*)	83 105 631	0.113/699/190/6	0.110/165/44/1	0.96
rs10506878 (*T*>**C**)	83 125 000	0.202/554/299/29	0.207/126/67/8	0.91

Abbreviations: MAF, minor allele frequency; POAG, primary open-angle glaucoma; SNP, single-nucleotide polymorphism.
[a]The SNPs are listed in the order of chromosome position (according to the NCBI human genome build 37), and the originally reported SNPs are in bold. The major and minor alleles are referred to as *B* and *b*, respectively, and the number of genotypes is shown. By definition, the risk-associated allele (shown in bold) is more frequent in the POAG cases than in the controls.
[b]POAG susceptibility association was tested according to co-dominant genetic models using multivariate logistic regression, with adjustment for gender and age.

case samples were homozygous for the minor allele. The risk was strong (OR = 2.0 (1.4–3.0)) for the minor allele (*C*) carriage in this SNP (*P* = 0.00038). Thus, the individuals carrying the minor allele of rs7098387 had roughly twofold increased odds of having POAG in comparison with respective non-carriers.

To evaluate whether one or more separate association blocks constitute this association locus, conditional association tests were performed for all typed and imputed SNPs, with adjustment for the genotypes of this SNP (ie, rs7098387). All the SNP associations lost significance in the conditional association tests (*P* ≥ 0.060). Together, these results confirmed the POAG susceptibility association of a locus downstream of *PLXDC2* at 10p12.31; however, the risk-associated haplotype was marked by a previously unreported SNP, rs7098387 (allele *C*), rather than the originally reported rs7081455 (allele *T*).

Replicated association of 1q43 (harboring *ZP4*)

Among the 10 typed SNPs of the 1q43 locus, including the four originally reported and six additional tag SNPs, a marginal association was observed with two SNPs, the originally reported rs693421 (*P* = 0.030) and an unreported tag, rs1017982 (*P* = 0.047), according to the co-dominant genetic models (Table 2). When these 10-SNP typing data were used for genotype imputation, 14 SNP genotypes were well imputed, though none showed an association with POAG.

When the risk effect of the reported rs693421 was estimated according to an additive genetic model (less constraint than a dominant or recessive genetic model), the OR value was 1.4 (1.1–1.7) with *P* = 0.0082. The minor allele (*T*) frequency of the cases was 0.55, which was substantially higher than that of the controls (0.48). Accordingly, a POAG susceptibility association was replicated with rs693421, found within the *ZP4*-harboring locus of 1q43; however, the association was marginal, most likely due to insufficient statistical power (47% for rs693421).

No association of 12q21.31 (*TMTC2* and upstream)

A total of 10 SNPs at the 12q21.31 locus, including an originally reported SNP (rs7961953) and 9 additional tag SNPs, were genotyped (Table 2), and 29 SNPs were imputed in good quality. However, none of these SNPs were associated with POAG susceptibility, either significantly or marginally, in co-dominant genetic models (*P* > 0.05). The tested region covers a 97.3-kb region of the 12q21.31 locus, including a 40-kb 5′-portion of the *TMTC2* gene, spanning from rs12822754 (at position 83 040 610) through rs11115376 (at position 83 137 872). The statistical power to detect the originally reported association of rs7961953 was low (49%), most likely explaining the failure to detect POAG association with any of the SNPs at this locus.

DISCUSSION

Three human chromosome loci were recently associated with suscept-ibility to POAG in a Japanese population,[29] but none of the six reported SNP markers of the loci maintained this association in three subsequent studies using South Indian,[30] Afro-Caribbean,[24] and Chinese[31] populations. Rather than merely examining the reported markers for replication in Koreans, the present study aimed to identify non-reported markers of stronger association that could be detected, even with low statistical powers for the reported associations.

POAG susceptibility was discovered to be associated with a non-reported SNP (rs7098387) on chromosome 10p12.31, whereas a reported SNP (rs7081455) was not associated. The newly associated SNP tended to exhibit a stronger effect size (OR = 2.0 (1.4–3.0) for rs7098387) than that reported in the Japanese population (OR = 1.5 (1.3–1.8) for rs7081455),[29] although the 95% confidence interval did

overlap. According to the 1000 Genomes Project data, two other SNPs, rs78099346 and rs1412557, were perfectly correlated (*r*² = 1) with the newly associated rs7098387 and are presumably also associated with POAG. These results not only confirm the POAG susceptibility association of the 10p12.31 locus in Koreans but also suggest that future replication studies using other populations should examine rs7098387, rs78099346 or rs1412557 rather than rs7081455.

Among other possible causes, this difference in the association markers between the Korean and Japanese studies can be attributed to differences in the LD structure of the locus between the two populations. The Korean marker rs7098387 was not examined in the original Japanese study; however, according to the 1000 Genomes Project data, the risk-associated allele *T* of the Japanese marker rs7081455 is in complete LD (*D′* = 1) with the risk-associated allele of rs7098387 (*C*) in the Japanese population. Accordingly, rs7098387 would presumably exhibit an association in Japanese populations with the same direction of effect. In contrast, rs7081455 was not associated with POAG in Koreans, most likely because it was in low LD (*D′* = 0.40) with rs7098387 in this group.

Therefore, rs7098387 would be valid in both Korean and Japanese populations, whereas rs7081455 would be effective only in Japanese or in case of high statistical power. Because of the low correlation (*r*² = 0.047 in JPT) and despite the complete LD between the two markers, the effect sizes would presumably be different in the Japanese population, as rs7098387 had a larger effect than rs7081455 in the Korean population.

Another possible cause for the different association results of the two studies was the substantial difference in intraocular pressure levels of the glaucoma cases. In this (ie, Korean) study, high-tension cases (88.5% of all cases) were primarily recruited, even though normal-tension glaucoma (2.7%) is much more prevalent than high-tension glaucoma (0.8%) in the general Korean population.[37] In addition, the average intraocular pressure was 28.2 mm Hg, which was much higher than the average pressure in the previous Japanese study (15.5 mm Hg).[29] Although the effect of the Korean marker rs7098387 on high-tension POAG (OR = 2.2 (1.5–3.1), *P* = 0.00012) was not much different from that on total POAG (OR = 2.0 (1.4–3.0), *P* = 0.00038) in this study, it remains to be determined whether the rs7098387 and rs7081455 markers are separately associated with high-tension and normal-tension glaucoma, respectively.

The newly identified SNP, rs7098387, is located 54 kb downstream of *PLXDC2* (plexin domain containing 2), which encodes a type I transmembrane protein with some homology to the nidogens and plexins that are present in the developing nervous system and may regulate the proliferation of neural progenitors.[39,40] At present, however, no functional significance is implied for this intergenic SNP, though a possibility can be speculated. This SNP is perfectly correlated (*r*² = 1) with rs78099346 and rs1412557, which are, according to the ENCODE project data,[41,42] located in an early-replication domains where chromosomal DNA replication initiates earlier than other chromosomal regions in embryonic stem cells. As the early replication of DNA is closely correlated with high levels of gene transcription and expression,[43] it would be worth examining whether these SNPs can affect the expression levels of nearby genes in a long-distance manner.

With regard to replication of the association of the second locus (1q43), one of the four reported SNPs (rs693421) exhibited the lowest *P*-value (*P* = 0.0082) and the largest effect (OR = 1.4 (1.1–1.7)) in additive genetic models (Table 2). A marginal association was detected for rs693421 despite the low statistical power (47%), and the effect size was comparable between the present Korean and reported Japanese (OR = 1.4 (1.2–1.6))[29] populations. This marginal

association most likely supports the replicated association of the 1q43 locus, though the results should be confirmed in larger populations.

The reported association of the third locus (12q21.31) failed to be replicated in this study, possibly because of the low statistical power for the reported rs7961953 (49% for OR = 1.4 (1.2–1.6)); therefore, this failure should not be regarded as evidence against any association of the 12q21.31 locus. These results suggested that no neighboring candidate SNPs at 12q21.31 had a much larger effect than the reported SNP (rs7961953) to be detected with a low statistical power.

This study was not designed to provide sufficient statistical power (eg, ≥ 80%) for the replication of any of the reported SNP markers (power of 47–54%), which were nevertheless genotyped in this study. We additionally examined nearby HapMap SNPs that were in moderate to high LD with the reported SNP markers in the Japanese population. Furthermore, all other SNPs at these loci were imputed according to the Korean HapMap Project data. This strategy covered candidate SNPs of possible indirect association and was effective in identifying novel associations that were even stronger than that reported with respect to the POAG association of the 10p12.31 locus.

In summary, we successfully identified optimal SNP markers that provide the highest statistical power with the largest effect size for replication of the previous Japanese POAG-associated haplotypes at the 10p12.31 and 1q43 loci. Using a Korean population, the POAG association of the 10p12.31 locus was replicated using the previously unreported SNP rs7098387 rather than the previously reported rs7081455, with 1q43 locus association using the reported marker rs693421, in expansive analyses of candidate SNP markers chosen by a combination of LD-based strategy and imputation. Using the replication-optimal markers provided by these findings, association studies of these loci should be facilitated in other populations, particularly non-Asian populations.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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