Genetic prediction models for primary open-angle glaucoma: translational research

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KEY MESSAGES

1. This study investigated the association of 48 genetic markers in multiple genes with primary open-angle glaucoma (POAG).
2. This study confirmed the association of variant rs4236601 in the *CAV1/CAV2* gene locus with POAG in Chinese, and suggested a common variant in this locus, rs3801994, as a new genetic biomarker for POAG in Chinese.
3. This study revealed the association of the *CDNK2B* gene variant rs3217986 with normal-tension POAG, and rs2157719 with high-tension POAG in Chinese.
4. This study confirmed the association of variant rs33912345 in the *SIX6* gene with POAG, and identified a new variant rs12436579 for the disease in Chinese.
5. This study simulated a genetic prediction model that can incorporate multiple gene variants for the prediction of individual risk of POAG.

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Introduction

Glaucoma is a leading cause of irreversible blindness worldwide. Most glaucoma cases are primary open-angle glaucoma (POAG), estimated to affect ~2% of the world population. Development of POAG is resulted from the interaction of multiple environmental and genetic risk factors. Nonetheless, a single gene variant is of limited value for genetic counselling or identifying individuals at risk. A combination of multiple gene variants can significantly increase the predictive value for POAG. Therefore, we aimed to identify a relatively complete set of gene variants that are associated with POAG. These variants are useful in establishing genetic prediction model for POAG.

Methods

We recruited over 600 Hong Kong Chinese POAG patients with variable age of onset, highest intraocular pressure, and disease severity. We also recruited >1000 Hong Kong healthy controls. In addition, data were collected for over 300 POAG patients and 600 controls from Shantou, China, over 300 POAG patients and 400 controls from Beijing, China, and over 200 POAG and 200 controls from Osaka, Japan.

A total of 48 single-nucleotide polymorphisms (SNPs) were selected: (1) 13 SNPs from genes identified by genome-wide association studies: 2 SNPs in *CAV1/CAV2*, 2 SNPs in *TMCO1*, 4 SNPs in *CDKN2B-AS1*, 1 SNP each in *SRBD1* and *ELOVL5*, and 1 SNP each in chromosomal regions 8q22 and 14q23; (2) 5 SNPs from our previous studies, including *APOE* epsilon4, *TNF* rs1800629, TP53 rs1042522, *TLR4* rs7037117, and 2p16 rs1533428; (3) 20 SNPs from genes previously reported to be associated with POAG in other populations by candidate gene approaches; and (4) 10 SNPs in genes associated with endophenotypes of glaucoma, including *ZNF469*, *COL5A1*, *AKAP13*, and *AVGR8* for central corneal thickness, *ATOH7*, *RFTN1*, *TGFBR3*, *CARD10*, *CDC7*, and *SIX1*/SIX6 for optic disc parameters.

After identification of the associated SNPs in the *CAV1/CAV2*, *CDNK2B*, and *SIX6* genes, we expanded the number of SNPs to enable more comprehensive evaluation of the three genes. We conducted a related study to evaluate the association of the *TCF4* and *PTPRG* genes with Fuchs corneal dystrophy, which is associated with POAG risk. We found that *TCF4* rs613872 was strongly associated with Fuchs corneal dystrophy in Caucasians but not in Chinese, whereas SNPs in *PTPRG* were not associated with Fuchs corneal dystrophy in Caucasians or Chinese populations.[1]

The selected SNPs were genotyped in all Hong Kong samples using TaqMan genotyping assays (Applied Biosystems). SNPs that are significantly associated with POAG were genotyped in the replication cohorts using the same platform.

For each selected SNP, Hardy-Weinberg equilibrium was evaluated by Chi-squared test. The association of each SNP with POAG was assessed by Chi-squared tests or Fisher exact tests. Odds ratios (OR) of significant sequence alterations were

estimated. For SNP data generated in different cohorts, genotypes were combined using a Mantel-Haenszel model. An individual or pooled P value of <0.05 was considered statistically significant.

Fifty SNPs from our POAG genome-wide association study were adopted for the simulation of genetic model, involving 341 POAG patients and 1141 controls. The samples were randomly assigned into training and validation groups with a ratio of 7 to 3, respectively. Genotype was re-coded as the number of risk alleles for a SNP. The genetic risk score was the total number of risk alleles a subject had. Prediction equations were fitted using logistic regression with different combinations of age, sex, and re-coded genotypes/genetic risk scores. The discriminative accuracy of fitted equations was evaluated with the area under curve of the receiver operating characteristic analysis.

Results

Among the 48 SNPs, only the SNPs in the *CAV1/CAV2*, *CDNK2B*, and *SIX6* genes were significantly associated with POAG. The other SNPs were not significantly associated with POAG.

Association of CAV1/CAV2 with POAG

In the Hong Kong cohort, the SNP rs4236601 conferred an increased risk of POAG[2] (A allele, P=0.0072, OR=4.72, Table 1), with a population attributable risk of 1.47%. A common SNP, rs3801994, showed a borderline association with POAG (A allele, P=0.036, OR=1.31, Table 1). This SNP presented in 18% of patients and 15% of controls, conferring a population attributable risk of 4.33%.

SNP rs4236601 was associated with POAG in the Shantou cohort (P=0.0079, OR=4.23). It had a minor allele frequency of 0.49% in controls and 2.0% in patients. Also, rs4236601 showed a significant association with POAG in the Beijing cohort (P=0.030, OR=3.92). The minor allele frequency was higher in the Beijing cohort: 1.3% in controls and 3.6% in POAG patients. In contrast, rs3801994 was not significantly associated with POAG in the Shantou or Beijing cohort, but their ORs were toward the same direction with that in the Hong Kong cohort (OR=1.14 in the Shantou cohort and 1.09 in the Beijing cohort, Table 1). By pooling the data of rs4236601 and rs3801994 from the three Chinese cohorts, the SNP rs4236601 was strongly associated with POAG (P=1.1 $\times 10^{-4}$, OR=3.80), whereas SNP rs3801994 showed a borderline association with POAG (P=0.022, OR=1.23, I^2=0).[3]

Association of the CDNK2B gene SNPs with POAG

We genotyped 11 SNPs of the *CDNK2B* gene. Among the nine candidate SNPs, rs3217986 was associated with normal-tension glaucoma, and

TABLE 1. Allelic association of the CAV1/CAV2 single nucleotide polymorphisms (SNPs) with primary open-angle glaucoma (POAG) in Chinese and Japanese populations

SNP	Predicted function	MA	MAF		POAG vs control		POAG vs control (adjusted for age and sex)	
			Control	POAG	P value	OR (95% CI)	P value	OR (95% CI)
Chinese - Hong Kong	n=436	n=454						
rs6466579	Intergenic	T	0.18	0.19	0.52	1.08 (0.85-1.37)	0.46	1.10 (0.85-1.42)
rs7801950	Intergenic	T	0.12	0.14	0.22	1.19 (0.90-1.58)	0.21	1.21 (0.90-1.62)
rs4236601	Intergenic	A	0.0043	0.020	0.0072	4.72 (1.36-16.36)	0.0086	6.25 (1.60-24.50)
rs3779512	c.102+4320T>G	T	0.061	0.056	0.68	0.92 (0.62-1.37)	0.93	0.98 (0.65-1.49)
rs3807989	c.103-12759A>G	A	0.22	0.25	0.083	1.21 (0.97-1.51)	0.11	1.21 (0.96-1.53)
rs3801994	c.103-8531G>A	A	0.15	0.18	0.036	1.31 (1.02-1.69)	0.042	1.32 (1.01-1.74)
rs1049337	c.*1246C>T	C	0.46	0.49	0.21	1.13 (0.93-1.36)	0.091	1.19 (0.97-1.46)
Chinese – Shantou	n=731	n=123						
rs4236601	Intergenic	A	0.0049	0.020	0.0079	4.23 (1.33-13.43)	0.0037	6.09 (1.80-20.63)
rs3801994	c.103-8531G>A	A	0.19	0.21	0.44	1.14 (0.82-1.59)	0.54	1.11 (0.80-1.54)
Chinese – Beijing	n=192	n=170						
rs4236601	Intergenic	A	0.013	0.036	0.057	2.86 (0.92-8.85)	0.030	3.92 (0.71-21.49)
rs3801994	c.103-8531G>A	A	0.19	0.20	0.67	1.09 (0.72-1.66)	0.85	1.04 (0.49-2.20)
Japanese – Osaka	n=207	n=101						
rs4236601	Intergenic	-	-	-	-	-	-	-
rs3801994	c.103-8531G>A	A	0.33	0.28	0.22	0.79 (0.55-1.15)	0.033	0.58 (0.35-0.96)

rs2157719 with high-tension glaucoma in the Chinese cohort (Table 2). SNP rs3217986 (OR=0.67, 95% CI=0.46-0.99, P=0.045) was for the first time identified for normal-tension glaucoma. It did not alter the risk of high-tension glaucoma (P=0.857) or POAG (P=0.386). SNP rs2157719 reduced the risk of high-tension glaucoma (OR=0.69, 95% CI=0.48-0.98, P=0.040), but not POAG (P=0.084) or normal-tension glaucoma (P=0.542).

Association of the SIX6 gene with POAG

We identified a new SNP, rs12436579, for normal-tension glaucoma (P=0.021), high-tension glaucoma (P=0.017), and POAG (P=0.0064) in Hong Kong Chinese (Table 3). The SNP rs33912345 showed significant association with normal-tension glaucoma (P=0.044). However, rs10483727 and the other eight SNPs (rs7152532, rs1010053, rs4400969, rs2179970, rs10148202, rs10133871, rs761557, and rs7156317) did not show significant association with POAG (P≥0.11, Table 3).

We tested the two significant SNPs (rs33912345 and rs12436579) and one significant SNP (rs10483727) in the Shantou Chinese and Japanese populations. All three SNPs were associated with POAG in Shantou Chinese (P≤0.015, Table 3). The OR of these three SNPs in Japanese pointed to the same direction as that in the Chinese populations. In the pool-analysis, we confirmed the associations: rs33912345 (P=0.0023, OR=0.78, I^2=0), rs10483727 (P=0.0020, OR=0.78, I^2=0), and rs12436579 (P=0.00081, OR=0.79, I^2=10%).

TABLE 2. Allelic association of genotyped single nucleotide polymorphisms (SNP) with primary open-angle glaucoma (POAG) in Chinese and Japanese populations

SNP	Associated allele	Allele frequency				High-tension glaucoma vs control		Normal-tension glaucoma vs control		POAG vs control	
		Control	High-tension glaucoma	Normal-tension glaucoma	POAG	OR (95% CI)	P_{emp}	OR (95% CI)	P_{emp}	OR (95% CI)	P_{emp}
Association analysis in Chinese cohort		n=733	n=437	n=285	n=722						
rs2518723	T	0.337	0.326	0.333	0.329	0.95 (0.77-1.19)	0.727	0.99 (0.77-1.25)	1.000	0.97 (0.79-1.18)	1.000
rs3217992	C	0.465	0.458	0.430	0.447	0.97 (0.79-1.19)	1.000	0.87 (0.69-1.09)	0.236	0.93 (0.77-1.12)	0.778
rs3217986	G	0.115	0.112	0.081	0.100	0.97 (0.70-1.34)	0.857	0.67 (0.46-0.99)	0.045	0.85 (0.63-1.15)	0.386
rs573687	A	0.103	0.069	0.089	0.085	0.79 (0.55-0.99)	0.035	0.86 (0.58-1.27)	0.483	0.81 (0.59-1.12)	0.244
rs545226	A	0.415	0.395	0.354	0.379	0.92 (0.75-1.13)	0.386	0.77 (0.61-0.98)	0.028	0.86 (0.71-1.04)	0.171
rs10965215	G	0.309	0.282	0.277	0.280	0.87 (0.70-1.10)	0.317	0.86 (0.67-1.10)	0.217	0.87 (0.71-1.07)	0.353
rs2157719	C	0.107	0.077	0.093	0.083	0.69 (0.48-0.98)	0.040	0.85 (0.58-1.25)	0.542	0.75 (0.55-1.03)	0.084
rs17694493	C	0.015	0.011	0.023	0.016	0.79 (0.32-1.95)	0.750	1.61 (0.68-3.80)	0.294	1.11 (0.51-2.41)	0.750
rs4977756	G	0.207	0.211	0.190	0.202	1.02 (0.79-1.32)	1.000	0.90 (0.67-1.19)	0.469	0.97 (0.77-1.23)	1.000
rs10757278	A	0.489	0.489	0.493	0.490	1.00 (0.81-1.23)	1.000	1.02 (0.81-1.28)	1.000	1.01 (0.83-1.21)	1.000
rs1333049	G	0.490	0.490	0.493	0.491	1.00 (0.81-1.23)	1.000	1.01 (0.81-1.27)	1.000	1.00 (0.83-1.21)	1.000
Association analysis in Japanese cohort		n=207	n=102	n=154	n=256						
rs2518723	T	0.377	0.352	0.333	0.341	0.90 (0.63-1.27)	0.539	0.83 (0.61-1.13)	0.221	0.85 (0.65-1.12)	0.231
rs3217992	C	0.466	0.460	0.422	0.437	0.98 (0.70-1.37)	0.727	0.83 (0.62-1.12)	0.455	0.89 (0.68-1.15)	0.321
rs3217986	G	0.043	0.069	0.062	0.065	1.64 (0.80-3.37)	0.233	1.46 (0.75-2.82)	0.386	1.53 (0.85-2.76)	0.196
rs573687	A	0.118	0.139	0.092	0.110	1.20 (0.73-1.97)	0.441	0.75 (0.46-1.22)	0.278	0.92 (0.61-1.39)	0.857
rs545226	A	0.391	0.411	0.353	0.376	1.09 (0.77-1.53)	0.611	0.85 (0.62-1.15)	0.378	0.94 (0.72-1.22)	0.778
rs10965215	G	0.358	0.332	0.284	0.303	0.89 (0.63-1.27)	0.469	0.71 (0.52-0.98)	0.038	0.78 (0.59-1.03)	0.108
rs2157719	C	0.116	0.134	0.095	0.110	1.18 (0.71-1.95)	0.571	0.80 (0.49-1.30)	0.360	0.94 (0.63-1.42)	0.857
rs17694493	C	0.029	0.020	0.023	0.022	0.68 (0.22-2.13)	0.600	0.78 (0.31-2.02)	0.857	0.74 (0.32-1.70)	0.370
rs4977756	G	0.225	0.238	0.222	0.228	1.08 (0.72-1.60)	1.000	0.99 (0.69-1.41)	1.000	1.02 (0.75-1.39)	0.857
rs10757278	A	0.544	0.465	0.431	0.445	0.73 (0.52-1.02)	0.065	0.64 (0.47-0.86)	0.003	0.67 (0.52-0.87)	0.003
rs1333049	G	0.515	0.460	0.431	0.443	0.81 (0.57-1.13)	0.221	0.72 (0.53-0.96)	0.022	0.75 (0.58-0.97)	0.021

TABLE 3. Allelic association of the SIX6/SIX1 locus with normal-tension glaucoma, high-tension glaucoma, and primary open-angle glaucoma (POAG) in Chinese and Japanese populations

No.	SNP	MA	MA frequency				Normal-tension glaucoma vs control		High-tension glaucoma vs control		POAG vs control	
			Control	Normal-tension glaucoma	High-tension glaucoma	POAG	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Chinese - Hong Kong			n=375	n=349	n=436	n=785						
1	rs33912345	A	0.209	0.168	0.185	0.177	0.76 (0.58-0.99)	0.044	0.86 (0.67-1.10)	0.22	0.82 (0.65-1.02)	0.067
2	rs12436579	A	0.313	0.259	0.260	0.259	0.76 (0.61-0.96)	0.021	0.77 (0.62-0.95)	0.017	0.77 (0.63-0.93)	0.0064
3	rs7152532	C	0.445	0.423	0.428	0.426	0.91 (0.74-1.12)	0.39	0.93 (0.77-1.13)	0.49	0.92 (0.78-1.10)	0.38
4	rs1010053	A	0.449	0.430	0.456	0.445	0.92 (0.75-1.14)	0.46	1.03 (0.85-1.25)	0.79	0.98 (0.82-1.17)	0.83
5	rs4400969	A	0.136	0.150	0.148	0.149	1.12 (0.83-1.50)	0.45	1.10 (0.83-1.45)	0.51	1.11 (0.86-1.42)	0.42
6	rs2179970	C	0.110	0.100	0.090	0.094	0.90 (0.63-1.27)	0.54	0.80 (0.58-1.12)	0.19	0.84 (0.63-1.13)	0.25
7	rs10148202	A	0.241	0.244	0.240	0.242	1.01 (0.80-1.29)	0.92	0.99 (0.79-1.25)	0.95	1.00 (0.82-1.23)	0.99
8	rs10133871	A	0.253	0.264	0.257	0.260	1.06 (0.84-1.34)	0.64	1.02 (0.82-1.28)	0.86	1.04 (0.85-1.27)	0.72
9	rs10483727	C	0.207	0.174	0.183	0.179	0.81 (0.62-1.05)	0.117	0.86 (0.67-1.10)	0.22	0.84 (0.67-1.04)	0.11
10	rs761557	T	0.138	0.144	0.146	0.145	1.05 (0.78-1.42)	0.73	1.07 (0.81-1.41)	0.63	1.06 (0.83-1.37)	0.63
11	rs7156317	G	0.153	0.166	0.163	0.164	1.10 (0.83-1.45)	0.52	1.08 (0.83-1.41)	0.58	1.09 (0.86-1.38)	0.50
12	rs3783820	T	0.429	0.463	0.482	0.474	1.15 (0.93-1.41)	0.20	1.24 (1.02-1.51)	0.031	1.20 (1.01-1.43)	0.043
Chinese – Shantou			n=731	n=34	n=123	n=157						
1	rs33912345	A	0.208	0.152	0.126	0.131	0.68 (0.34-1.35)	0.27	0.55 (0.37-0.82)	0.0029	0.58 (0.41-0.82)	0.0020
2	rs12436579	A	0.294	0.235	0.213	0.218	0.74 (0.42-1.31)	0.29	0.65 (0.47-0.90)	0.0091	0.67 (0.50-0.89)	0.0064
3	rs10483727	C	0.209	0.162	0.126	0.134	0.73 (0.38-1.41)	0.35	0.55 (0.37-0.81)	0.0025	0.58 (0.41-0.83)	0.0024
Japanese – Osaka			n=207	n=154	n=102	n=256						
1	rs33912345	A	0.198	0.171	0.172	0.171	0.84 (0.57-1.23)	0.37	0.84 (0.54-1.31)	0.45	0.84 (0.60-1.18)	0.31
2	rs12436579	A	0.316	0.311	0.297	0.305	0.97 (0.71-1.34)	0.86	0.91 (0.63-1.32)	0.63	0.95 (0.72-1.26)	0.71
3	rs10483727	C	0.203	0.170	0.168	0.169	0.80 (0.55-1.18)	0.26	0.80 (0.51-1.23)	0.31	0.80 (0.57-1.12)	0.19

Simulation of genetic modelling using multiple SNPs

Equations using more SNPs performed better. Equations using re-coded genotypes and genetic risk score of 10 SNPs were comparable. Age and sex contributed minimally to the discriminative power. The best equation used the genetic risk score of 50 SNPs and the area under the curve of 75.1% was attained. The test using a cut-off probability of 0.3 had a sensitivity of 62% and specificity of 83% for detection of subjects at risk of glaucoma.

Discussion

We performed genetic association analysis for POAG in the Hong Kong Chinese and other Asian cohorts. A total of 48 SNPs from multiple genes were screened. After identifying the SNPs in the *CAV1/CAV2*, *CDNK2B*, and *SIX6* genes that were significantly associated with POAG, we expanded the number of SNPs in these three genes and identified new associated SNPs for POAG. These three genes are important genetic biomarkers for the molecular pathogenesis of POAG in Chinese. For the remaining genes that were not significantly associated with POAG, further studies are warranted to involve more SNP markers to confirm their role in the disease. We have successfully identified disease-associated genes and SNPs by using haplotype-tagging SNP analysis in both glaucoma[3] and age-related macular degeneration.[4,5] This method can be used in future studies of glaucoma gene identification.

The current number of significant SNPs is not sufficient for building up an applicable genetic model. We therefore simulated the genetic model using data from our POAG genome-wide association studies. We demonstrated a method in assessing the risk of POAG using expandable prediction equations based on SNPs. We found that equations using more SNPs performed better. Equations using re-coded genotypes and genetic risk score of 10 SNPs were comparable. The best equation used the genetic risk score of 50 SNPs and the area under the curve of 75.1% was attained. The test using a

cut-off probability of 0.3 had a sensitivity of 62% and specificity of 83% for detection of subjects at risk of POAG. This model sets up a foundation for future establishment of genetic prediction model for POAG. Further studies are warranted to identify more associated gene SNPs for POAG for such models to be applicable.

Understanding glaucoma genetics provides useful information for its management. Discovery of all genetic factors for glaucoma is of great importance. Obtaining a complete and universal picture of glaucoma genetic components will help guide the biological investigations and translational studies. In addition, uncovering the pathogenic mechanisms behind the association between genetic variations and glaucoma should be achieved through studies of multiple disciplines. Furthermore, expanding the options for translation of genetic discoveries into clinical practices should increase the possibilities of creation of a useful product. Gene therapy based on virus vector, genome editing, and prediction model of risk of POAG using genetic information are promising research area.

Conclusion

SNPs at the *CAV1/CAV2* locus, the *CDNK2B* gene, and the *SIX6* gene are associated with POAG in the Hong Kong Chinese population. These SNPs (rs4236601 and rs3801994 in *CAV1/CAV2*, rs3217986 and rs2157719 in *CDNK2b*, and rs33912345 and rs12436579 in *SIX6*) are potentially useful genetic biomarker for POAG. With more SNPs are being identified, they can be incorporated into the genetic model that we simulated and be applied to patient care in future.

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Results from this study have been published in:

(1) Rong SS, Chen LJ, Leung CK, et al. Ethnic specific association of the CAV1/CAV2 locus with primary open-angle glaucoma. Sci Rep 2016;6:27837.

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