Genetic markers for primary open-angle glaucoma using next-generation sequencing: abridged secondary publication

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KEY MESSAGES

1. Single-nucleotide polymorphisms in multiple genes/loci—namely *AFAP1*, *CASC20*, *FNDC3B*, *FOXC1*, *LMX1B*, *GAS7*, *SPRED2/MIR4778*, and *TLCD5/ARHGEF12/TMEM136*—were associated with primary open-angle glaucoma (POAG), high-tension glaucoma, and/or normal-tension glaucoma in Hong Kong Chinese. However, no rare variants were associated with POAG.
2. Shared and distinct genes were identified between POAG and primary angle-closure glaucoma.
3. The *VAV3* gene was associated with progression of primary angle-closure glaucoma but not POAG.
4. Variants *SIX6* and *PAX6*, both POAG genes, were associated with retinal nerve fibre layer thickness and anisometropia development, respectively, in children but not in adults.

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Introduction

Glaucoma is a leading cause of irreversible blindness worldwide. In Hong Kong, approximately 20% of blindness cases are attributed to glaucoma. Most glaucoma cases are primary open-angle glaucoma (POAG), which affects 2% of the global population. Genome-wide association studies (GWAS) have identified common single-nucleotide polymorphisms (SNPs) in more than 30 genes associated with POAG; however, these genes have mainly been identified in Western populations. Primary angle-closure glaucoma (PACG) is another major subtype of glaucoma in Chinese individuals. We previously conducted a systematic review and meta-analysis of PACG and identified 15 SNPs in 13 genes/loci associated with PACG.[1] Refractive error is a known risk factor for glaucoma. High myopia is associated with increased risk of POAG, whereas hypermetropia is associated with increased risk of PACG. Therefore, investigation of genes related to refractive errors—including interocular differences (anisometropia)—may provide insights into the genetic architecture of glaucoma. This study aimed to investigate the associations of common and rare variants in multiple genes with POAG and its subtypes, 15 SNPs in 13 genes with PACG, and multiple gene variants with glaucoma-related phenotypes, such as retinal nerve fibre layer thickness, in Chinese individuals. We also evaluated the SNP effects on PACG progression. After identifying an association of *VAV3* with PACG progression, we evaluated its effect on POAG progression.

Methods

Patients with POAG or PACG at the Hong Kong Eye Hospital and Prince of Wales Hospital were recruited for ophthalmological examination and followed up for a minimum of 3 years. Additionally, individuals aged >30 years with normal visual acuity, normal intraocular pressure, and no major ocular disorders were recruited as controls. Schoolchildren were also recruited to investigate the effects of glaucoma genes on glaucoma-related phenotypes such as retinal nerve fibre layer (RNFL) thickness and anisometropia. Datasets of patients with POAG and controls were collected from the Joint Shantou International Eye Centre, Shantou, and the Department of Ophthalmology, Sichuan Provincial People's Hospital, Chengdu.

Candidate genes included: (1) 33 genes identified in previous GWAS of POAG—*ABCA1*, *AFAP1*, *ANKH*, *ANKRD55-MAP3K1*, *ATXN2*, *BICC1*, *CADM2*, *CASC20*, *CAV1/CAV2*, *CDKN2B-AS1*, *DGKG*, *EXOC2*, *FMNL2*, *FNDC3B*, *FOXC1*, *GAS7*, *GMDS*, *HMGA2*, *IKZF2*, *LHPP*, *LMX1B*, *LOXL1*, *MEIS2*, *PDE7B*, *PMM2*, *SIX1/SIX6*, *SPRED2/MIR4778*,

TFAP2B/PKHD1, *TGFBR3*, *TLCD5/ARHGEF12/TMEM136*, *TMCO1*, *TMTC2*, and *TXNRD2*; (2) four disease-causing genes—*MYOC*, *OPTN*, *WDR36*, and *NTF4*; (3) four genes associated with POAG in Hong Kong Chinese—*PAX6*, *TLR4*, *TNF*, and *TP53*; and (4) the 15 genes associated with PACG.[1]

Genomic DNA was extracted from whole blood. Targeted candidate genes and variants were analysed by sequencing or genotyping platforms in cases and controls from the Hong Kong cohort using in-house laboratory protocols.

A disease-causing mutation was defined as a coding variant present exclusively in cases, or with a frequency of <1/1000 in controls and showing a significant difference (P<0.05) between cases and controls after multiple corrections. For common (allele frequency >1%) and rare (<0.1%) variants, single-variant association analyses were performed using the Chi-squared test with adjustments for age and sex. Odds ratios (ORs) and 95% confidence intervals were estimated. Genotype-phenotype correlation analyses were conducted using logistic or linear regression.

Results

We identified a SNP, rs2745572, in the *FOXC1* gene that was associated with high-tension glaucoma (HTG) [OR=0.73, P<0.001, Table 1]. The protective allele G of rs2745572 was also strongly correlated with lower intraocular pressure in patients with POAG (Beta= -1.43, P<0.001). In the Shantou cohort, SNP rs6596830 in the *FOXC1* locus, rather than rs2745572, showed significant associations with POAG (OR=0.75, P<0.001) and HTG (OR=0.75, P<0.001), suggesting population-specific effects.[2]

We also identified multi-gene SNPs associated with POAG, including SNP rs4414666 in the *SPRED2/MIR4778* locus (OR=1.18, P=0.023), rs62283813 in *FNDC3B* (OR=1.22, P=0.032), rs938604 in *AFAP1* (OR=0.74, P=0.004), rs3829849 in *LMX1B* (OR=0.73, P=0.013), and rs2326788 in *CASC20* (OR=1.23, P=0.022) [Table 2].

Relative to normal-tension glaucoma (NTG), HTG was more strongly associated with SNP rs4414666 in *SPRED2/MIR4778* (OR=1.28, P=0.004), rs62283813 in *FNDC3B* (OR=1.40, P=0.001), rs938604 in *AFAP1* (OR=0.65, P<0.001), rs3829849 in *LMX1B* (OR=0.68, P=0.011), rs2326788 in *CASC20* (OR=1.26, P=0.026), and rs9913911 in *GAS7* (OR=0.82, P=0.021) [Table 3]. In contrast, only two variants were associated with NTG: rs2326788 in *CASC20* (OR=1.24, P=0.041) and rs1893261 in the *TLCD5/ARHGEF12/TMEM136* locus (OR=0.81, P=0.025). No disease-causing mutations or rare variants were significantly associated with POAG in the Hong Kong cohort.

In a meta-analysis of PACG, we identified 15 SNPs in 13 genes/loci associated with the disease, then catalogued rare coding variants in 16 genes/loci associated with PACG. We demonstrated overlapping genes between PACG and POAG—namely *ABCA1*, *ATOH7*, *CALCRL*, *IL6*, and *VAV3*.[1] Notably, variants in *ABCA1*, *ATOH7*, *CALCRL*, *IL6*, and *VAV3* were not associated with POAG. Among the 15 SNPs, *LOXL1* rs3825942 (G153D, OR=0.65, P=0.0026) showed a significant association with PACG. When patients whose glaucoma remained

TABLE 1. Associations of single-nucleotide polymorphisms (SNPs) in *FOXC1* with primary open-angle glaucoma (POAG), high-tension glaucoma (HTG), and normal-tension glaucoma (NTG).

SNP	Gene/locus	Minor allele/ major allele	POAG		HTG		NTG	
			Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
rs7750978	*AL512329.2*	A/C	0.94 (0.81-1.09)	0.41	0.83 (0.69-0.99)	0.041	1.02 (0.86-1.21)	0.81
rs7774792	*AL512329.2*	A/T	0.90 (0.77-1.06)	0.22	0.92 (0.76-1.12)	0.41	0.87 (0.72-1.05)	0.15
rs6596830	*AL512329.2*	A/T	1.07 (0.91-1.25)	0.40	1.20 (1.00-1.44)	0.053	0.99 (0.83-1.19)	0.91
rs2745572	*50 kb upstream of FOXC1*	G/A	0.90 (0.77-1.05)	0.16	0.73 (0.61-0.88)	<0.001	1.06 (0.89-1.26)	0.55
rs56210598	*FOXC1*	G/C	1.08 (0.93-1.24)	0.31	1.04 (0.88-1.24)	0.64	1.09 (0.92-1.29)	0.30
rs2745596	*FOXC1*	T/G	0.83 (0.60-1.17)	0.30	0.77 (0.51-1.17)	0.23	0.87 (0.59-1.28)	0.48
rs2235716	*FOXC1*	T/C	0.95 (0.81-1.11)	0.51	0.97 (0.80-1.18)	0.76	0.90 (0.75-1.09)	0.27
rs2235717	*FOXC1*	G/C	0.95 (0.80-1.13)	0.57	0.96 (0.78-1.18)	0.68	0.90 (0.73-1.10)	0.31
rs4959583	*FOXC1*	A/C	1.16 (0.86-1.57)	0.32	1.01 (0.70-1.46)	0.95	1.31 (0.94-1.83)	0.11
rs984253	*FOXC1*	A/T	0.87 (0.65-1.16)	0.33	0.76 (0.53-1.09)	0.13	0.97 (0.70-1.33)	0.83
rs7763581	*FOXC1*	T/G	0.91 (0.75-1.11)	0.37	0.85 (0.67-1.09)	0.20	0.97 (0.77-1.21)	0.78
rs2569876	*FOXC1*	G/A	0.98 (0.84-1.14)	0.78	1.03 (0.86-1.24)	0.75	0.90 (0.75-1.08)	0.26

TABLE 2. Associations of single-nucleotide polymorphisms (SNPs) with candidate genes for primary open-angle glaucoma (POAG).

Chromosome	Nearest gene	SNP	Minor allele	Major allele	Minor allele frequency		P value	Odds ratio (95% confidence interval)
					POAG	Controls		
2	*SPRED2/MIR4778*	rs4414666	G	T	0.47	0.43	0.023	1.18 (1.02-1.37)
3	*FNDC3B*	rs62283813	G	A	0.22	0.19	0.032	1.22 (1.02-1.46)
4	*AFAP1*	rs938604	A	G	0.13	0.16	0.0039	0.74 (0.61-0.91)
9	*LMX1B*	rs3829849	T	C	0.08	0.11	0.013	0.73 (0.58-1.94)
20	*CASC20*	rs2326788	G	A	0.25	0.21	0.022	1.23 (1.03-1.47)

TABLE 3. Associations of single-nucleotide polymorphisms (SNPs) with candidate genes for high-tension glaucoma (HTG) and normal-tension glaucoma (NTG).

Chromosome	Nearest gene	SNP	Minor allele	Major allele	Minor allele frequency			NTG vs controls		HTG vs controls	
					HTG	NTG	Controls	P value	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)
2	*SPRED2/MIR4778*	rs4414666	G	T	0.49	0.45	0.43	0.36	1.08 (0.91-1.28)	0.0039	1.28 (1.08-1.51)
3	*FNDC3B*	rs62283813	G	A	0.25	0.20	0.19	0.61	1.06 (0.85-1.31)	0.0011	1.40 (1.14-1.72)
4	*AFAP1*	rs938604	A	G	0.13	0.14	0.16	0.16	0.85 (0.67-1.07)	<0.001	0.65 (0.51-0.83)
9	*LMX1B*	rs3829849	T	C	0.08	0.09	0.11	0.099	0.79 (0.59-1.05)	0.011	0.68 (0.51-0.92)
11	*TLCD5/ARHGEF12/ TMEM136*	rs1893261	A	G	0.32	0.27	0.31	0.025	0.81 (0.67- 0.97)	0.77	1.03 (0.86-1.23)
17	*GAS7*	rs9913911	G	A	0.46	0.49	0.51	0.29	1.10 (0.93-1.30)	0.021	0.82 (0.69-0.97)
20	*CASC20*	rs2326788	G	A	0.25	0.25	0.21	0.041	1.24 (1.01-1.51)	0.026	1.26 (1.03-1.54)

stable for 10 years were regarded as controls, *VAV3* rs6689476 was associated with PACG progression at the 3rd year (OR=2.86, P=0.045), 5th year (OR=2.84, P=0.037), and 10th year (OR=2.74, P=0.030).[3] In contrast, these variants were not associated with POAG or its progression, suggesting genetic diversity between POAG and PACG.

We investigated the effects of multiple SNPs in glaucoma-associated genes on glaucoma-related ocular phenotypes, including RNFL thickness. In Hong Kong children, temporal-inferior p-RNFL thickness was associated with SNPs rs33912345 ($P=7.7 \times 10^{-4}$) and rs10483727 (P=0.0013) after adjustments for age, sex, and axial length.[4] In contrast, SIX6 variants were not associated with RNFL thickness in adult patients with POAG.

We also investigated multiple gene variants in refractive error, including anisometropia, in children to determine the roles of certain glaucoma genes (eg, *PAX6*) in refractive error, given that high myopia is a known risk factor for POAG. At the 3-year follow-up, *PAX6* rs644242 was associated with anisometropia in terms of axial length (P=0.0003; OR=1.61) and spherical equivalent (P=0.03) among children.[5] *PAX6* was associated with glaucoma, especially early-onset glaucoma. However, *PAX6* variants were not associated with POAG in adults.

Discussion

We demonstrated an association between rs2745572 in *FOXC1* and HTG in Hong Kong Chinese. The OR of the risk allele was comparable to the GWAS results (OR=1.23) in POAG. However, in the Shantou Chinese cohort, SNP rs6596830 in the *FOXC1* locus, rather than rs2745572, showed significant associations with POAG and HTG, suggesting population-specific effects.[2]

We identified SNPs in multiple genes/loci associated with POAG and its subtypes. Notably, rs2326788 in *CASC20* was the only variant associated with both HTG and NTG, suggesting that genetic profiles differ between these two subtypes. No significant associations were identified between other genes and POAG (HTG/NTG), suggesting possible ethnic differences in the POAG genetic profiles of Chinese and Western populations. Moreover, we identified no rare variants associated with POAG in the Hong Kong cohort, likely due to the low minor allele frequencies of these variants

(<0.1%), which resulted in insufficient statistical power to detect significant differences.

It has been suggested that the genetic components of PACG and POAG are distinct. However, the identification of overlapping genes suggests that PACG and POAG share certain genetic markers or even biological pathways. Functional characterisation of these overlapping genes may shed new light on the pathogenesis of various glaucoma subtypes.

The association between the missense variant *LOXL1* rs3825942 and PACG indicated that it may serve as a reliable genetic marker for PACG. Ten-year follow-up data suggested that *VAV3* rs6689476 represents a genetic marker for PACG progression.[3] In contrast, none of these gene variants was associated with POAG in the Hong Kong cohort, suggesting genetic diversity between PACG and POAG.

SIX6 was associated with p-RNFL thickness in children, suggesting a role for *SIX6* in RNFL variation during neural retinal development in childhood. The effect of *SIX6* on glaucoma susceptibility might begin during childhood. Timely follow-up of children carrying the risk alleles may help identify those at risk of POAG onset.

We found that *PAX6* rs644242 was associated with anisometropia onset in Hong Kong Chinese children, implying roles in imbalanced refractive change and axial elongation between the eyes. Eyeball elongation in early life may increase the risk of future glaucoma. Accordingly, timely follow-up of children carrying the risk alleles may help identify those at risk of developing POAG later in life.

Conclusion

Common SNPs in multiple genes/loci showed associations with POAG, HTG, and/or NTG in Hong Kong Chinese. In contrast, no rare variants were associated with POAG. Variants in multiple genes may serve as useful genetic biomarkers for glaucoma, endophenotypes, and glaucoma progression in Chinese individuals. Gene variants could represent cost-effective biomarkers for disease risk assessment.

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Disclosure

The results of this research have been previously published in:

1. Liang YJ, Ling A, Chan PP, et al. Genetic association of primary angle-closure glaucoma and disease progression. Clin Exp Ophthalmol 2025;53:660-7.
2. Liang YJ, Wang YY, Rong SS, et al. Genetic associations of primary angle-closure disease: a systematic review and meta-analysis. JAMA Ophthalmol 2024;142:437-44.
3. Wang YY, Zhang XJ, Kam KW, et al. Association of polymorphisms in ZFHX1B and PAX6 with anisometropia in Chinese children: The Hong Kong Children Eye Genetics Study. Invest Ophthalmol Vis Sci 2023;64:6.
4. Lu SY, Zhang XJ, Wang YM, et al. Association of *SIX1-SIX6* polymorphisms with peripapillary retinal nerve fibre layer thickness in children. Br J Ophthalmol 2023;107:1216-22.

References

1. Liang YJ, Wang YY, Rong SS, et al. Genetic associations of primary angle-closure disease: a systematic review and meta-analysis. JAMA Ophthalmol 2024;142:437-44.
2. Wu Z, Huang C, Zheng Y, et al. Primary open-angle glaucoma risk prediction with ABCA1 and LOC102723944 variants and their genotype-phenotype correlations in southern Chinese population. Mol Genet Genomics 2023;298:1343-52.
3. Liang YJ, Ling A, Chan PP, et al. Genetic association of primary angle-closure glaucoma and disease progression. Clin Exp Ophthalmol 2025;53:660-7.
4. Lu SY, Zhang XJ, Wang YM, et al. Association of *SIX1-SIX6* polymorphisms with peripapillary retinal nerve fibre layer thickness in children. Br J Ophthalmol 2023;107:1216-22.
5. Wang YY, Zhang XJ, Kam KW, et al. Association of polymorphisms in ZFHX1B and PAX6 with anisometropia in Chinese children: The Hong Kong Children Eye Genetics Study. Invest Ophthalmol Vis Sci 2023;64:6.