Genetics

Genetic Association of the *PARL-ABCC5-HTR3D-HTR3C* Locus With Primary Angle-Closure Glaucoma in Chinese

Fang Yao Tang,[1] Li Ma,[1] Pancy O. S. Tam,[1] Chi Pui Pang,[1] Clement C. Tham,[1,2] and Li Jia Chen[1,3]

[1]Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China
[2]Hong Kong Eye Hospital, Hong Kong, China
[3]Department of Ophthalmology and Visual Sciences, Prince of Wales Hospital, Hong Kong, China

Correspondence: Clement C. Tham, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Eye Hospital, 147K Argyle Street, Kowloon, Hong Kong; clemtham@cuhk.edu.hk.

Li Jia Chen, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Eye Hospital, 147K Argyle Street, Kowloon, Hong Kong; lijia_chen@cuhk.edu.hk.

FYT and LM contributed equally to the work presented here and should therefore be regarded as equivalent authors.

CCT and LJC are joint senior authors.

PURPOSE. This study evaluates the associations of haplotype-tagging single nucleotide polymorphisms (SNPs) in the *PARL-ABCC5-HTR3D-HTR3C* region with primary angle closure glaucoma (PACG), with a view to identify the responsible SNP in this region.

METHODS. Thirty SNPs from the *PARL-ABCC5-HTR3D-HTR3C* region were genotyped in a Hong Kong Chinese cohort of 422 PACG patients and 400 control subjects, using TaqMan SNP genotyping assays. Single marker and haplotype-based association analyses were performed.

RESULTS. Two synonymous *ABCC5* SNPs, namely rs939336 (p.Cys594=; $P = 0.013$; odds ratio [OR] = 1.46; 95% confidence interval [CI], 1.08 to 1.97) and rs1132776 (p.Ala395=; $P = 0.009$; OR = 1.47; 95% CI: 1.10 to 1.95), were associated with PACG. Mild associations were detected for *ABCC5* rs9838667 ($P = 0.024$) and *HTR3D* rs12493550 ($P = 0.035$). Conditional analysis revealed that no SNPs remained significant after adjusting for other SNPs, suggesting none of these tagging SNPs is fully responsible for the association in this region. In subgroup analysis, *ABCC5* SNPs rs939336, rs1132776, and rs983667 and *HTR3D* rs12493550 were associated only with the chronic form of PACG. However, these associations could not withstand the correction for multiple testing.

CONCLUSIONS. These findings enrich the allelic spectrum of *ABCC5* in PACG. We identified no tagging SNP responsible for the association of the whole region. Further deep sequencing analysis of this region should be warranted to uncover whether there is still disease associated variant in this region.

Keywords: *PARL-ABCC5-HTR3D-HTR3C*, genetic association, primary angle-closure glaucoma, glaucoma genetics

Glaucoma is a leading cause of irreversible visual impairment worldwide, characterized by typical optic disc cupping and visual field defect.[1] According to the status of the anterior chamber angle, primary glaucoma in adults can be classified into primary open angle glaucoma (POAG) and primary angle-closure glaucoma (PACG). Although POAG is more prevalent in the African and Caucasian populations, PACG is more prevalent in East Asians.[2]

Primary angle-closure glaucoma is characterized by closure of the anterior chamber drainage angle, thereby blocking the outflow of aqueous humor and resulting in elevated intraocular pressure (IOP) and progressive, irreversible optic neuropathy. PACG is a complex disease with multiple risk factors, such as older age, female, short axial length, East Asian origin, and genetic susceptibility.[3–5] To date, genome-wide association studies (GWAS) identified eight genetic loci that were significantly associated with PACG: *EPDR1*, *CHAT*, *GLIS3*, *FERMT2*, *DPM2-FAM102A*, *PLEKHA7*, *COL11A1*, and *PCMTD1-ST18*.[6,7] In another genome-wide association study, a single nucleotide polymorphism (SNP) rs1401999, located in the ATP-binding cassette, sub-family C (*CFTR/MRP*), member 5 (*ABCC5*) gene, was found to be significantly associated with

anterior chamber depth (ACD, a cardinal feature of PACG). This SNP was also found to be associated with PACG in Asians.[8]

The *ABCC5* gene is located on chromosome 3q27 with 3 adjacent genes: presenilin associated, rhomboid-like (*PARL*), 5-hydroxytryptamine (serotonin) receptor 3D, ionotropic (*HTR3D*), and 5-hydroxytryptamine (serotonin) receptor 3C (*HTR3C*). These four genes construct a strong linkage disequilibrium (LD) region spanning approximately 231 kb (Chr3:183,547,173-183,602,693) in the HapMap Chinese Han Beijing (CHB) population. The *ABCC5* SNP rs1401999 is located in the intronic region with unknown function. In view of the strong LD among the SNPs in this region, it remains unclear whether rs1401999 is associated with PACG by itself or it is a marker in LD with another exonic variant that is responsible for the association signal detected. Therefore, in this study, we performed a haplotype-tagging SNP analysis of the *PARL-ABCC5-HTR3D-HTR3C* locus, with a view to identify any coding associated variants and pinpoint the responsible SNP(s).

4384

Investigative Ophthalmology & Visual Science

METHODS

Study Subjects

A total of 422 PACG patients and 400 control subjects were recruited from the eye clinics at the Hong Kong Eye Hospital and the Prince of Wales Hospital, Hong Kong. All participants are Han Chinese and newly recruited. They received complete ocular examinations and investigations, including visual acuity assessment, IOP measurement by Goldmann applanation tonometry, anterior segment examination and gonioscopy with slit-lamp biomicroscope, fundal examination, visual field testing by standard automated perimetry, and retinal nerve fiber layer thickness measurement by optical coherence tomography (OCT). The recruited PACG patients fulfilled the diagnostic criteria from the International Society of Geographical and Epidemiological Ophthalmology (ISGEO).[9] A visual field defect was defined as glaucoma hemifield test outside normal limits, and pattern SD with $P < 0.05$, or a cluster of more than three points with $P < 0.05$ in the superior or inferior hemifield of the pattern deviation plot, one of which with $P < 0.01$.[10,11] The history of acute angle closure (AAC) attack of each PACG patient was collected according to the published criteria.[12] Patients who had AAC without any secondary causes were defined as acute primary angle closure (APAC). Otherwise, those without any record or history indicating an acute attack were subcategorized as non-APAC. Any patients with angle closure or ocular hypertension from the following causes were excluded: uveitis, neovascularization of iris/angle, iris/ciliary body cysts, posterior segment hemorrhage or tumor, Marfan syndrome, Axenfeld-Rieger syndrome, trauma, steroid, and/or iatrogenic.

Control subjects were recruited from patients attending the eye clinics for unrelated eye conditions. They were Han Chinese aged 60 years or above. Older control subjects were intentionally recruited, with the objective of excluding late-onset glaucoma. They underwent complete ocular assessment and had no history of any ocular diseases, except for mild cataract or refractive error. Also, all control subjects had open anterior chamber angle (Shaffer grade 3 or 4 open anterior chamber angle on gonioscopy) and IOP lower than 21 mm Hg without IOP-lowering medications. Any individuals with a family history of glaucoma were excluded.

The study protocol was approved by the Ethics Committee for Human Research, the Chinese University of Hong Kong. Informed consent was obtained from each participant. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

SNP Selection and Genotyping

Haplotype-tagging SNPs within the *PARL-ABCC5-HTR3D-HTR3C* region were selected from the CHB population, using the HapMap Genome Browser release #27 data set (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap27_B36/). A total of 29 haplotype-tagging SNPs were picked up based on the criteria of an r^2 cutoff of 0.8 between SNPs and a minor allele frequency (MAF) cutoff of 0.05. Moreover, a synonymous exonic SNP, rs939336, which was in complete LD with the reported SNP rs1401999,[8] was also selected. Thus, a total of 30 SNPs were included.

Genomic DNA was extracted from peripheral blood using the Qiagen QIAmap DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. All SNPs were genotyped in the PACG and control samples using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) with a Roche LightCycler 480 Real-Time PCR system (Roche Diagnostics; Basel, Switzerland) according to the manufacturer's instructions.

Statistical Analysis

The PLINK software package (version 1.07; http://pngu.mgh.harvard.edu/~purcell/plink/) was used for statistical analysis.[13] Hardy-Weinberg equilibrium (HWE) of each SNP in the control group was analyzed using the χ^2 test. Allelic and genotypic associations of each SNP with PACG were analyzed using the χ^2 or Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CIs) were estimated with the major allele as reference. Because the 30 selected SNPs are located in a continuous region with strong LD, the association was corrected for multiple testing using the SNPSpD program (http://gump.qimr.edu.au/general/daleN/SNPSpD/), which takes the correlations between SNPs into account.[14] After correction, an experiment-wide significance threshold of 0.0028 was required to keep the type I error rate at 0.05 level in these 30 SNPs. Logistic regression analyses were performed to adjust for age and sex. Furthermore, if multiple SNPs were found to be significant, logistic regression analyses were performed to adjust the association of individual SNP for the other SNPs to determine whether this SNP could explain the association of the other SNPs. If a SNP maintained the significance after adjustment, it is likely the responsible SNP in this region.

LD and haplotype-based association were assessed using the Haploview software.[15] The global haplotype associations of SNPs of interest (e.g., those in the same LD block) were assessed by omnibus test, followed by haplotype-specific association analysis. Correction for multiple testing in haplotype association analysis was performed by using the permutation test (number of iterations = 10,000).

Statistical Power Analysis

For the 30 SNPs for fine mapping, their MAFs ranged from 0.089 to 0.488. Therefore, assuming an allelic OR of 1.64 (derived from the PACG GWAS study),[8] our sample size provides 70.3% to 94.2% of statistical power to detect a significant association at α level of 0.05.

RESULTS

Supplementary Table S1 showed the characteristics of the PACG patients and controls. There was no significant difference in sex between the PACG and control groups. Among the PACG patients, 93 had a history of AAC attack and 329 participants developed PACG without an acute attack history.

All of the SNPs conformed to HWE ($P > 0.05$) in the control group and were included for further analysis. The call rate of each SNP was >98%. In single SNP association analysis, one SNP (rs3749446) in *PARL*, two in *ABCC5* (rs939336 and rs1132776), and one in *HTR3D* (rs12493550) were associated with PACG (Supplementary Table S2). Logistic regression analysis revealed that three SNPs in *ABCC5* (rs939336: $P = 0.013$, OR = 1.46, 95% CI: 1.08 to 1.97; rs1132776: $P = 0.009$, OR = 1.47, 95% CI: 1.10 to 1.95; rs9838667: $P = 0.024$, OR = 1.27, 95% CI: 1.03 to 1.57; Table 1) and rs12493550 in *HTR3D* ($P = 0.035$, OR = 1.71, 95% CI: 1.04 to 2.82; Table 1) were nominally associated with PACG after adjusted for age and sex. However, conditional analysis revealed that no SNPs remained significant when adjusting each other (Supplementary Table S3), suggesting the associations of the four SNPs were not independent. Notably, none of the associations could withstand the correction for multiple testing ($P > 0.0028$).

TABLE 1. Allelic Association of SNPs in *PARL-ABCC5-HTR3D-HTR3C* Region With PACG After Adjusted for Age and Sex

				MAF		Allelic Association	
SNP	Gene	Nucleotide Change	Minor Allele	PACG	Control	Adjusted P	Adjusted OR (95% CI)
rs6443905	*PARL*	c.1028+681A>T	T	0.070	0.059	0.548	1.13 (0.75–1.70)
rs10937152	*PARL*	c.829-1385G>A	A	0.462	0.480	0.341	0.90 (0.73–1.11)
rs3811725	*PARL*	c.512-162T>G	G	0.396	0.353	0.052	1.23 (1.00–1.51)
rs12107202	*PARL*	c.511+7751G>A	A	0.290	0.310	0.280	0.88 (0.71–1.05)
rs12631031	*PARL*	c.511+4771C>T	T	0.261	0.250	0.673	1.05 (0.84–1.31)
rs953419	*PARL*	c.126-35T>C	C	0.463	0.469	0.694	0.96 (0.79–1.73)
rs3749446	*PARL*	c.126-157G>A	G	0.139	0.175	0.051	0.76 (0.58–1.00)
rs4148594	*ABCC5*	c.4213-1195A>G	G	0.149	0.168	0.480	0.91 (0.69–1.19)
rs2139559	*ABCC5*	c.3854+1752C>A	A	0.225	0.236	0.929	0.98 (0.78–1.26)
rs3749442	*ABCC5*	p.Leu1208=	T	0.454	0.485	0.169	0.87 (0.71–1.06)
rs9838667	*ABCC5*	c.3414+434A>C	A	0.393	0.350	0.024	1.27 (1.03–1.57)
rs1016752	*ABCC5*	c.3414+50C>G	C	0.090	0.066	0.061	1.42 (0.98–2.05)
rs3792584	*ABCC5*	c.1834-621A>G	G	0.059	0.069	0.400	0.85 (0.57–1.25)
rs939336	*ABCC5*	p.Cys594=	T	0.152	0.109	0.013	1.46 (1.08–1.97)
rs1132776	*ABCC5*	p.Ala395=	T	0.162	0.116	0.009	1.47 (1.10–1.95)
rs7636910	*ABCC5*	p.Gln382=	G	0.383	0.402	0.535	0.94 (0.76–1.15)
rs4148569	*ABCC5*	c.129+9273C>T	C	0.152	0.167	0.250	0.85 (0.65–1.12)
rs4148568	*ABCC5*	c.129+9031G>A	A	0.249	0.256	0.717	0.96 (0.76–1.20)
rs2292997	*ABCC5*	c.129+7980C>T	T	0.371	0.385	0.529	0.94 (0.76–1.15)
rs4148562	*ABCC5*	c.129+5834G>A	A	0.080	0.086	0.558	0.90 (0.62–1.29)
rs939334	*HTR3D*	c.-198+372T>C	C	0.263	0.261	0.639	1.06 (0.84–1.33)
rs4912518	*HTR3D*	c.228-927T>C	T	0.076	0.059	0.144	1.34 (0.90–1.98)
rs12493550	*HTR3D*	c.228-181G>A	A	0.057	0.035	0.035	1.71 (1.04–2.82)
rs7622660	*HTR3D*	c.257+354C>T	C	0.085	0.071	0.271	1.23 (0.85–1.78)
rs6792482	*HTR3D*	c.381-134T>C	C	0.477	0.459	0.428	1.08 (0.89–1.32)
rs1467257	*HTR3D*	c.517-278A>C	A	0.235	0.236	0.770	0.97 (0.77–1.22)
rs7648564	*HTR3C*	c.68-523C>T	C	0.344	0.335	0.765	1.03 (0.84–1.27)
rs6808122	*HTR3C*	c.234+146G>A	G	0.251	0.229	0.351	1.11 (0.89–1.40)
rs6766410	*HTR3C*	p.Asn163Lys	C	0.357	0.348	0.895	1.01 (0.82–1.25)
rs6807670	*HTR3C*	c.*158G>A	G	0.261	0.253	0.822	1.03 (0.82–1.28)

In LD analysis, six LD blocks were defined across the whole region (Figure). There were three blocks in *PARL*, one block in *ABCC5*, one in *HTR3D*, and one in *HTR3C*. However, none of them showed a significant association with PACG in the omnibus test. We then performed a sliding-window test in PLINK to identify the association of specific haplotypes with PACG. Using a window size of two SNPs, a total of 29 blocks were included into the omnibus haplotype analysis, and 7 of them gave an association with PACG ($P < 0.05$; Table 2). The most significant omnibus association was identified from a two-SNP window defined by *ABCC5* SNPs rs939336 and rs1132776 ($P_{omnibus} = 0.003$ at one degree of freedom).

In subgroup analysis, we divided the PACG patients into those with ($n = 93$, APAC) and without ($n = 329$, non-APAC) history of AAC attack and analyzed their association profiles. One SNP (rs10937152, $P = 0.032$, OR = 1.49, 95% CI: 1.03 to 2.15) in *PARL* was associated with APAC (Supplementary Table S4). In contrast, one SNP (rs3811725, $P = 0.025$, OR = 1.31, 95%, CI: 1.03 to 1.66) in *PARL*, four (rs9838667, $P = 0.013$, OR = 1.25, 95% CI: 1.01 to 1.54; rs1016752, $P = 0.031$, OR = 1.56, 95% CI: 1.04 to 2.35; rs939336, $P = 0.010$, OR = 1.56, 95% CI: 1.13 to 2.19; and rs1132776, $P = 0.004$, OR = 1.61, 95% CI: 1.17 to 2.22) in *ABCC5*, and one (rs12493550, $P = 0.036$, OR = 1.80, 95% CI: 1.04 to 3.13) in *HTR3D* showed nominal associations with non-APAC (Supplementary Table S4). In the haplotype analysis, there was no significant omnibus association. A sliding-window test was also performed, and haplotypes in five blocks were associated with non-APAC (Supplementary Table S5). Again, the most significant omnibus association was identified from a two-SNP window defined by

ABCC5 SNPs rs939336 and rs1132776 ($P_{omnibus} = 0.002$ at one degree of freedom).

DISCUSSION

In this study, we identified two synonymous *ABCC5* SNPs, namely rs939336 (p.Cys594=) and rs1132776 (p.Ala395=), and one intronic *ABCC5* SNP, rs9838667, to be associated with PACG in the Chinese population. Also, LD analysis revealed that the LD block defined by rs939336 and rs1132776 had the strongest association with PACG. Apart from the three *ABCC5* SNPs, mild associations were also detected for the SNP in *HTR3D* (rs12493550), whereas no SNP in *PARL* and *HTR3C* was associated with PACG. Conditional analysis revealed that none of the four SNPs in *ABCC5* and *HTR3D* achieved significance in PACG after adjusting for each other, suggesting that none of these four SNPs can fully explain the association detected in this region, and thus the responsible SNP remains to be identified. In subgroup analysis, only SNP rs10937152 in *PARL* was found to be associated with APAC, whereas rs3811725 in *PARL*, rs9838667, rs1016752, rs939336, and rs1132776 in *ABCC5* and rs12493550 in *HTR3D* were associated with non-APAC.

In a previous study, an intronic SNP rs1401999 in *ABCC5* was found to be associated with ACD and PACG (OR = 1.3).[8] In this study, we identified two synonymous coding variants rs939336 (p.Cys594=; OR = 1.46) and rs1132776 (p.Ala395=; OR = 1.47) to be associated with PACG. Of note, the SNP rs939336 is in complete LD with rs1401999 in Chinese, suggesting the associations of the two coding SNPs with PACG
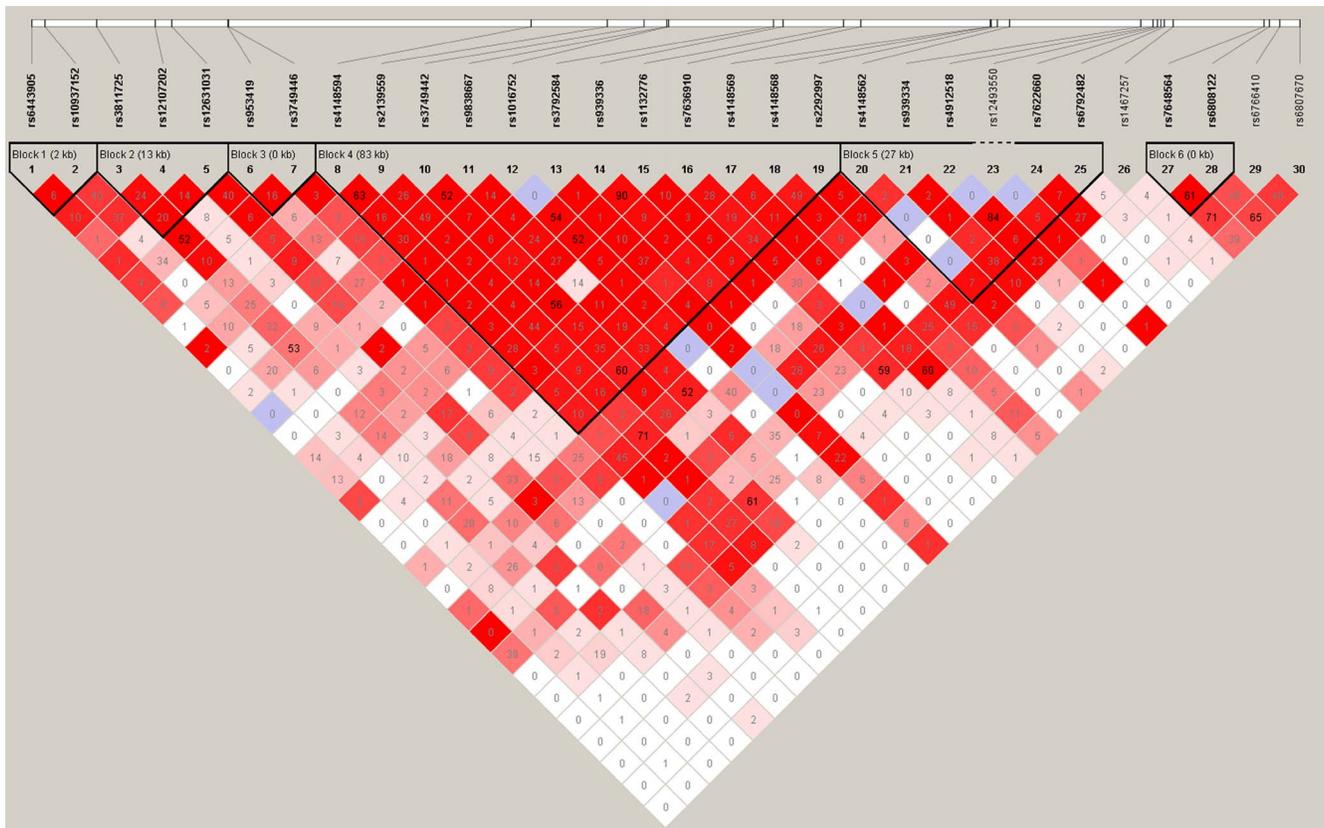
FIGURE. Linkage disequilibrium structure of the *PARL-ABCC5-HTR3D-HTR3C* region.

are genuine. Although these two SNPs do not result in an alteration of amino acid, they can probably influence the processes of translation and expression, although their exact biological mechanism awaits further investigation.

ABCC5, also known as multidrug resistance protein 5 (MRP5), participates in transmembrane transportation of various molecules and mediation of cellular export of cyclic nucleotides including a second messenger cyclic guanosine monophosphate (cGMP), which plays a role in cell prolifera-

TABLE 2. Two-SNP Sliding Window Analysis Across the *PARL-ABCC5-HTR3D-HTR3C* Region in PACG

Locus ($P_{omnibus}$)	SNPs	Haplotype	Frequency		Association		
			PACG	Control	P	OR (95% CI)	$P_{permutation}$
Window 6 (0.041)	rs953419-rs3749446	TG	0.138	0.179	0.026	0.73 (0.50–1.07)	0.105
		CA	0.464	0.469	0.85	0.98 (0.75–1.29)	0.158
		TA	0.398	0.353	0.062	1.21 (0.91–1.61)	0.968
Window 7 (0.043)	rs3749446-rs4148594	AG	0.149	0.168	0.301	0.87 (0.60–1.26)	0.554
		GA	0.139	0.175	0.04	0.76 (0.52–1.11)	0.11
		AA	0.712	0.657	0.016	1.29 (0.96–1.73)	0.041
Window 13 (0.014)	rs3792584-rs939336	AT	0.151	0.11	0.014	1.44 (0.95–2.17)	0.03
		GC	0.059	0.069	0.434	0.85 (0.48–1.48)	0.718
		AC	0.79	0.821	0.108	0.82 (0.58–1.16)	0.205
Window 14 (0.003)	rs939336-rs1132776	TT	0.151	0.11	0.014	1.44 (0.95–2.17)	0.026
		TC	0	0	NA	NA	NA
		CC	0.849	0.89	0.014	0.70 (0.46–1.05)	0.005
Window 15 (0.037)	rs1132776-rs7636910	GC	0.383	0.415	0.209	0.88 (0.66–1.18)	0.702
		TA	0.162	0.116	0.011	1.47 (0.99–2.20)	0.022
		CA	0.455	0.469	0.589	0.95 (0.72–1.24)	0.529
Window 22 (0.038)	rs4912518-rs12493550	CA	0.057	0.035	0.037	1.67 (0.85–3.27)	0.089
		TG	0.076	0.059	0.176	1.31 (0.78–2.27)	0.381
		CG	0.867	0.906	0.014	0.68 (0.44–1.05)	0.035
Window 23 (0.050)	rs12493550-rs7622660	GC	0.085	0.071	0.303	1.22 (0.73–2.03)	0.526
		AT	0.058	0.035	0.031	1.70 (0.87–3.32)	0.089
		GT	0.857	0.894	0.027	0.71 (0.47–1.08)	0.068

tion.[16] ABCC5 also provides resistance of anticancer drugs.[17,18] The expression of *ABCC5* was detected in multiple human tissues, including the structures of the anterior segment of the eye, such as iris, ciliary body, lens, and cornea.[8,19] Interestingly, the association of *ABCC5* with PACG argues favorably for a role in eye development through the regulation of cGMP, which potentially influences anterior chamber depth. A significant reduction of body length and ocular size occurred after activity of endogenous ABCC5 in zebrafish was inhibited.[20] The *ABCC5* gene was first identified to influence ACD,[8] which is a key anatomical risk factor of PACG.[21,22] A shallow anterior chamber remarkably increased the risk of angle closure.[3] Eyes with an ACD less than 2.80 mm conferred a 42.5-fold of increased risk of angle closure compared with eyes with an ACD greater than 3.00 mm.[3] Also, people with a shallow anterior chamber have greater susceptibility of optic neuropathy.[23]

HTR3D encodes the subunit D of the type 3 receptor for 5-hydroxytryptamine (serotonin), which is a biogenic hormone that functions as a neurotransmitter. It initiates intestinal peristalsis and secretory reflexes and transmits information to the central nervous system.[24] *HTR3D* was found to be associated with vomiting within 24 hours after chemotherapy.[25] However, due to the very limited studies on this gene, the expression of *HTR3D* is to be detected in human tissues. In this study, we found an SNP rs12493550 in *HTR3D* that was associated PACG, suggesting that the abnormality in signal transmission may have a role in the pathogenesis of PACG.

PARL encodes a mitochondrial integral membrane protein and has a key role in the regulation of mitochondrial function.[26] Expression of an ortholog of human PARL, Rhomboid-7 (rho-7), was shown to cause neurodegeneration in *Drosophilia*.[27] Recently, mitochondrial abnormalities have been linked with psychiatric neurodegenerative disorders, such as schizophrenia and Parkinson's disease.[28-31] In addition, *PARL* was also associated with ocular diseases, such as Leber's hereditary optic neuropathy (LHON).[32] SNP rs3749446 in *PARL* was associated with LHON in previous studies.[33,34] In this study, nominal associations were detected between *PARL* rs10937152 and APAC and between rs3811725 and non-APAC, suggesting the potential involvement of mitochondrial function in the mechanisms of glaucoma. Further studies are needed to link the *PARL* variants to mitochondrial dysfunction in the context of PACG.

In a subgroup analysis, one SNP had an association with APAC. In contrast, six SNPs showed a significant association with non-APAC. This discrepancy could be explained by the fact that *ABCC5* could be a gene for chronic primary angle closure, whereas the difference between APAC and non-APAC could indicate that APAC requires other or additional triggering factors for acute onset. Near work, pupil dilation, and emotional factors, such as intense concentration, emotional stress, and excitement, are precipitating factors.[35] Another explanation could be the relatively small sample size of the APAC group, which might provide insufficient statistical power to reveal the association of the SNPs with APAC. With 93 APAC cases, the statistical power was less than 40% to detect an association of the strongest SNPs (i.e., rs939336 and rs1132776) with PACG.

There are some other limitations in this study. First, the statistical significance of the nominally significant SNPs did not withstand correction for multiple testing, likely due to limited sample size. However, the ORs of the associated SNPs were consistent with that of the SNP rs1401999 in a previous study.[8] Second, the classification of PACG in this study was based on whether the patients had a history of acute angle closure attack. Because all patients had glaucomatous changes, they were all PACG patients. Whether the SNPs detected in this study are also associated with primary angle closure suspect

(PACS) and/or primary angle closure (PAC), which are earlier stages in the primary angle closure disease (PACD) spectrum, remains unknown. Elucidation of this may help to explain whether the genes at the *PARL-ABCC5-HTR3D* locus have a role in early pathogenesis of PACG.

In summary, this study has enriched the spectrum of associated SNPs at the *PARL-ABCC5-HTR3D* locus in PACG. Two coding variants in *ABCC5* were associated with PACG. Although the responsible SNP had not been pinpointed, our data suggested it is more likely to locate in the region of *ABCC5* and *HTR3D*. Furthermore, we identified discrepancies in the association profiles between APAC and non-APAC. These findings provide new clues for further genetic and biological investigations on PACG.

Acknowledgements

References

1. Resnikoff S, Pascolini D, Mariotti SP, Pokharel GP. Global magnitude of visual impairment caused by uncorrected refractive errors in 2004. *Bull World Health Organ*. 2008; 86:63–70.
2. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014;121:2081–2090.
3. Lavanya R, Wong TY, Friedman DS, et al. Determinants of angle closure in older Singaporeans. *Arch Ophthalmol*. 2008; 126:686–691.
4. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311:1901–1911.
5. Rong SS, Tang FY, Chu WK, et al. Genetic associations of primary angle-closure disease: a systematic review and meta-analysis. *Ophthalmology*. 2016;123:1211–1221.
6. Vithana EN, Khor CC, Qiao C, et al. Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. *Nat Genet*. 2012;44:1142–1146.
7. Khor CC, Do T, Jia H, et al. Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. *Nat Genet*. 2016;48:556–562.
8. Nongpiur ME, Khor CC, Jia H, et al. ABCC5, a gene that influences the anterior chamber depth, is associated with primary angle closure glaucoma. *PLoS Genet*. 2014;10: e1004089.
9. Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. *Br J Ophthalmol*. 2002;86:238–242.
10. Anderson DR, Chauhan B, Johnson C, Katz J, Patella VM, Drance SM. Criteria for progression of glaucoma in clinical management and in outcome studies. *Am J Ophthalmol*. 2000;130:827–829.
11. Tham CC, Kwong YY, Leung DY, et al. Phacoemulsification versus combined phacotrabeculectomy in medically uncontrolled chronic angle closure glaucoma with cataracts. *Ophthalmology*. 2009;116:725–731.
12. Aung T, Friedman DS, Chew PT, et al. Long-term outcomes in Asians after acute primary angle closure. *Ophthalmology*. 2004;111:1464–1469.

13. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.

14. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet*. 2004;74:765–769.

15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21:263–265.

16. Oshita AK, Rothstein G, Lonngi G. cGMP stimulation of stem cell proliferation. *Blood*. 1977;49:585–591.

17. Jedlitschky G, Burchell B, Keppler D. The multidrug resistance protein 5 functions as an ATP-dependent export pump for cyclic nucleotides. *J Biol Chem*. 2000;275:30069–30074.

18. Pratt S, Shepard RL, Kandasamy RA, Johnston PA, Perry W III, Dantzig AH. The multidrug resistance protein 5 (ABCC5) confers resistance to 5-fluorouracil and transports its mono-phosphorylated metabolites. *Mol Cancer Ther*. 2005;4:855–863.

19. Karla PK, Quinn TL, Herndon BL, Thomas P, Pal D, Mitra A. Expression of multidrug resistance associated protein 5 (MRP5) on cornea and its role in drug efflux. *J Ocul Pharmacol Ther*. 2009;25:121–132.

20. Long Y, Li Q, Li J, Cui Z. Molecular analysis, developmental function and heavy metal-induced expression of ABCC5 in zebrafish. *Comp Biochem Physiol B Biochem Mol Biol*. 2011;158:46–55.

21. Foster PJ, Devereux JG, Alsbirk PH, et al. Detection of gonioscopically occludable angles and primary angle closure glaucoma by estimation of limbal chamber depth in Asians: modified grading scheme. *Br J Ophthalmol* 2000;84:186–192.

22. Devereux JG, Foster PJ, Baasanhu J, et al. Anterior chamber depth measurement as a screening tool for primary angle-closure glaucoma in an East Asian population. *Arch Ophthalmol*. 2000;118:257–263.

23. Aung T, Nolan WP, Machin D, et al. Anterior chamber depth and the risk of primary angle closure in 2 East Asian populations. *Arch Ophthalmol*. 2005;123:527–532.

24. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology*. 2007;132:397–414.

25. Hammer C, Fasching PA, Loehberg CR, et al. Polymorphism in HTR3D shows different risks for acute chemotherapy-induced vomiting after anthracycline chemotherapy. *Pharmacogenomics*. 2010;11:943–950.

26. Cipolat S, Rudka T, Hartmann D, et al. Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* 2006;126:163–175.

27. Lessing D, Bonini NM. Maintaining the brain: insight into human neurodegeneration from *Drosophila melanogaster* mutants. *Nat Rev Genet*. 2009;10:359–370.

28. Gaki GS, Papavassiliou AG. Oxidative stress-induced signaling pathways implicated in the pathogenesis of Parkinson's disease. *Neuromolecular Med*. 2014;16:217–230.

29. Shao L, Martin MV, Watson SJ, et al. Mitochondrial involvement in psychiatric disorders. *Ann Med*. 2008;40:281–295.

30. Uranova N, Orlovskaya D, Vikhreva O, et al. Electron microscopy of oligodendroglia in severe mental illness. *Brain Res Bull*. 2001;55:597–610.

31. Shi G, Lee JR, Grimes DA, et al. Functional alteration of PARL contributes to mitochondrial dysregulation in Parkinson's disease. *Hum Mol Genet*. 2011;20:1966–1974.

32. Nikoskelainen EK, Savontaus ML, Wanne OP, Katila MJ, Nummelin KU. Leber's hereditary optic neuroretinopathy, a maternally inherited disease. A genealogic study in four pedigrees. *Arch Ophthalmol*. 1987;105:665–671.

33. Phasukkijwatana N, Kunhapan B, Stankovich J, et al. Genome-wide linkage scan and association study of PARL to the expression of LHON families in Thailand. *Hum Genet*. 2010;128:39–49.

34. Istikharah R, Tun AW, Kaewsutthi S, et al. Identification of the variants in PARL, the nuclear modifier gene, responsible for the expression of LHON patients in Thailand. *Exp Eye Res*. 2013;116:55–57.

35. Lowe RF. Primary angle-closure glaucoma. Inheritance and environment. *Br J Ophthalmol*. 1972;56:13–20.

Investigative Ophthalmology & Visual Science