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 Chen1,3
1Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China
2Hong Kong Eye Hospital, Hong Kong, China
3Department 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.

ABSTRACT

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; OR=1.46; 95% confidence interval [CI], 1.08 to 1.97) and rs1132776 (p.Ala395=p; P=0.009; OR=1.47; 95% CI: 1.10 to 1.95), were associated with PACG. Mild associations were detected for ABC5 (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, ABC5 SNPs rs939336, rs1132776, and rs9838667 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

G laucoma is a leading cause of irreversible visual impairment worldwide, characterized by typical optic disc cupping and visual field defect. 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.7

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.7-5 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-ST18.6,5 In another genome-wide association study, a single nucleotide polymorphism (SNP) rs14019999, 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 (Chr3:183,547,173-183,545,693) in the HapMap Chinese Han Beijing (CHB) population. The ABCC5 SNP rs14019999 is located in the intrinsic region with unknown function. In view of the strong LD among the SNPs in this region, it remains unclear whether rs14019999 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).
**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 (ISGE). 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 \( P < 0.01 \). The history of acute angle closure (AAC) attack of each PACG patient was collected according to the published criteria. 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**

Haploview software was used for multiple testing correction, and the SNPSpD program (http://gump.qimr.edu.au/general/daleN/SNPSpD/) which corrects for multiple testing using the SNPSpD program was used for statistical analysis. The global haplotype associations of SNPs of interest (e.g., those in the same LD block) were assessed using 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 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), our sample size provides 70.3% to 94.2% of statistical power to detect a significant association at \( \alpha = 0.05 \).

**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), our sample size provides 70.3% to 94.2% of statistical power to detect a significant association at \( \alpha = 0.05 \).

**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: 1.05 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 (\( P > 0.0028 \)).
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 = 0.002 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 = 0.051, 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). 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...
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 prolifer-

**Figure.** Linkage disequilibrium structure of the PARL-ABCC5-HTR3D-HTR3C region.

<table>
<thead>
<tr>
<th>Locus (P_{emul})</th>
<th>SNPs</th>
<th>Haplotype</th>
<th>Frequency</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Window 6 (0.041)</td>
<td>rs953419-rs3749446</td>
<td>TG</td>
<td>0.138</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA</td>
<td>0.464</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA</td>
<td>0.398</td>
<td>0.062</td>
</tr>
<tr>
<td>Window 7 (0.043)</td>
<td>rs3749446-rs4148594</td>
<td>AG</td>
<td>0.149</td>
<td>0.301</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA</td>
<td>0.139</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>0.712</td>
<td>0.016</td>
</tr>
<tr>
<td>Window 13 (0.014)</td>
<td>rs3792584-rs939336</td>
<td>AT</td>
<td>0.151</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GC</td>
<td>0.059</td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AC</td>
<td>0.79</td>
<td>0.108</td>
</tr>
<tr>
<td>Window 14 (0.003)</td>
<td>rs939336-rs1132776</td>
<td>TT</td>
<td>0.151</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Window 15 (0.037)</td>
<td>rs1132776-rs7636910</td>
<td>GC</td>
<td>0.383</td>
<td>0.209</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA</td>
<td>0.162</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA</td>
<td>0.455</td>
<td>0.589</td>
</tr>
<tr>
<td>Window 22 (0.038)</td>
<td>rs4912518-rs12493550</td>
<td>CA</td>
<td>0.057</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>0.076</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CG</td>
<td>0.867</td>
<td>0.014</td>
</tr>
<tr>
<td>Window 23 (0.050)</td>
<td>rs12493550-rs7622660</td>
<td>GC</td>
<td>0.085</td>
<td>0.503</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT</td>
<td>0.058</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT</td>
<td>0.857</td>
<td>0.027</td>
</tr>
</tbody>
</table>
tion.\textsuperscript{16} ABC5 also provides resistance of anticancer drugs.\textsuperscript{17,18} The expression of \textit{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.\textsuperscript{8,19} Interestingly, the association of \textit{ABCC5} with PACG argues favorably for a role in eye development through the regulation of \textit{ABCC5} which potentially influences anterior chamber depth. A significant reduction of body length and ocular size occurred after activity of endogenous ABC5 in zebrafish was inhibited.\textsuperscript{20} The \textit{ABCC5} gene was first identified to influence ACD, which is a key anatomical risk factor of PACG.\textsuperscript{21,22} A shallow anterior chamber of endogenous \textit{ABCC5} in zebrafish was inhibited.\textsuperscript{20} The \textit{PARL-ABCC5-HTR3D-HTR3C} association of the strongest SNPs (i.e., rs939336 and rs1132776) with PACG.

\textit{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.\textsuperscript{24} \textit{HTR3D} was found to be associated with vomiting within 24 hours after chemotherapy. However, due to the very limited studies on this gene, the expression of \textit{HTR3D} is to be detected in human tissues. In this study, we found an SNP rs12493550 in \textit{HTR3D} that was associated PACG, suggesting that the abnormality in signal transmission may have a role in the pathogenesis of PACG. \textit{PARL} encodes a mitochondrial integral membrane protein and has a key role in the regulation of mitochondrial function.\textsuperscript{26} Expression of an ortholog of human \textit{PARL}, Rhomboid-7 (rho-7), was shown to cause neurodegeneration in \textit{Drosophila}.\textsuperscript{27} Recently, mitochondrial abnormalities have been linked with psychiatric neurodegenerative disorders, such as schizophrenia and Parkinson's disease.\textsuperscript{28–31} In addition, \textit{PARL} was also associated with ocular diseases, such as Leber's hereditary optic neuropathy (LHON).\textsuperscript{32} SNP rs5749446 in \textit{PARL} was associated with LHON in previous studies.\textsuperscript{33,34} In this study, nominal associations were detected between \textit{PARL} and PACG, suggesting the potential involvement of mitochondrial dysfunction in the mechanisms of glaucoma. Further studies are needed to link the \textit{PARL} variants to mitochondrial dysfunction in the context of PACG.

In a subgroup analysis, one SNP had an association with PACAP. In contrast, six SNPs showed a significant association with non-PACAP. This discrepancy could be explained by the fact that \textit{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.\textsuperscript{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 rs1152776) 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.\textsuperscript{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 \textit{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 \textit{PARL-ABCC5-HTR3D} locus in PACG. Two coding variants in \textit{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 \textit{ABCC5} and \textit{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

Supported in part by Research Grants 14112514 and 14105916 (CPP) and 474111 (CCYT) from the General Research Fund, Hong Kong, and Direct Grants 4054198 (CCYT) and 4054559 (IJC) from the Chinese University of Hong Kong, Hong Kong.

Disclosure: F.Y. Tang, None; L. Ma, None; P.O.S. Tam, None; C.P. Pang, None; C.C. Tham, None; L.J. Chen, None

References


