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Association of known common genetic variants with primary open angle, primary angle closure, and pseudoexfoliation glaucoma in Pakistani cohorts

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Purpose: Despite the different etiology of primary open angle glaucoma (POAG), primary angle closure glaucoma (PACG), and pseudoexfoliative glaucoma (PEXG), several studies have suggested that these forms of glaucoma have overlapping genetic risk factors. Therefore, the aim of this study was to evaluate the role of genetic variants recently associated with POAG in different types of glaucoma in Pakistani POAG, PACG, and PEXG patient cohorts.

Methods: Six variants in CDKN2B-ASI (rs4977756), CDKN2B (rs1063192), ATOH7 (rs1900004), CAV1 (rs4236601), TMCO1 (rs4656461), and SIX1 (rs10483727) were genotyped using TaqMan assays. A total of 513 unrelated patients with glaucoma (268 with POAG, 125 with PACG, and 120 with PEXG) and 233 healthy controls were included in the study. Genotypic and allelic associations were analyzed with a chi-square test.

Results: The frequency of the G allele of TMCO1 rs4656461 was significantly lower in the patients with POAG (p=0.003; OR [odds ratio]=0.57), PACG (p=0.009; OR=0.52), and PEXG (p=0.01; OR=0.54) compared to the control individuals. The T allele of ATOH7 rs1900004 was observed less frequently in the patients with PACG (p=0.03; OR=0.69) compared to the control individuals. The A allele of CAV1 rs4236601 was found more frequently in the patients with POAG (p=0.008; OR=1.49) compared to the control individuals. This study demonstrates that the TMCO1 rs4656461 variant is associated with POAG, PACG and PEXG in the Pakistani population. Our study was unable to confirm previous associations reported for variants in CDKN2B-ASI, CDKN2B, and SIX1 with any type of glaucoma.

Conclusions: In conclusion, we found consistent evidence of the significant association of three common variants in TMCO1, ATOH7, and CAV1.

Glaucoma encompasses a group of neurodegenerative disorders, which involves apoptotic death of retinal ganglion cells (RGCs). Retinal ganglion cell degeneration inevitably causes atrophy of the optic nerve and progressive visual field damage leading to irreversible vision loss [1,2]. A recent survey by Tham et al. estimated that worldwide 64.3 million people 40–80 years old have glaucoma, which will increase to 76.0 million in 2020 and 111.8 million in 2040. Primary open angle glaucoma (POAG) is the most common type of glaucoma with a higher prevalence in individuals of African ancestry. In contrast to European or African populations, primary angle closure (PACG) is the leading cause of bilateral blindness in East Asian populations [3-5].

In open angle and closed angle glaucoma, individuals often remain unaware they have the disorder until advanced visual loss has occurred due to the degeneration of the RGCs [6]. RGC death and damage to the optic nerve are related to an imbalance between the secretion and drainage of the aqueous humor produced in the posterior chamber of the eye by the ciliary body and drained into the anterior chamber of the eye. Due to this imbalance, the increased intraocular pressure (IOP) of the eye can cause mechanical stress to the posterior structures of the eye, remarkably the lamina cribrosa and adjacent tissues [7]. Stress induced by IOP might result in compression, deformation, and remodeling of the lamina cribrosa with consequent mechanical axonal damage and disruption of axonal transport [8,9].

Pseudoexfoliative glaucoma (PEXG) is a major type of secondary glaucoma, characterized by the production, aggregation, and accumulation of abnormal extracellular fibrillar material in the anterior segment of the eye [10]. PEXG is a secondary type of OAG, and in this case, pigment and abnormal basement membrane material from the anterior segment of the eye is deposited in the trabecular meshwork (TM), and because of malfunction of the TM, an increase in IOP occurs and causes damage to RGCs similar to POAG.
The deposition of pseudoexfoliative material also affects the cornea and the lens, and results in a significant loss in the number of axons. Oxidative stress caused by reactive oxygen species (ROS) is important in POAG and PEXG [11,12]. Izotti et al. observed that in POAG and PEXG oxidative damage arising from mitochondrial failure plays a role in the functional decay of the TM [13].

Glaucoma is a complex disease influenced by a combination of genetic and environmental risk factors. Recently, several genome-wide association studies (GWASs) have been performed for POAG, which revealed several genetic variants associated with the disease. Among them are variants in or near the cyclin-dependent kinase inhibitor 2B antisense RNA (CDKN2B-AS1; OMIM: 613149) gene, the CDKN2B gene (OMIM: 613149), the transmembrane and coiled-coil domains 1 (TMCO1; OMIM: 614123) gene [14,15], the caveolin 1 (CAV1; OMIM: 601047) and caveolin 2 (CAV2; OMIM: 601048) genes [16], the SIX homeobox 1 (SIX1; OMIM:601205) gene [17,18], and the Atonal homolog 7 (ATOH7; OMIM: 609875) gene [19].

Despite the different etiology of POAG, PACG, and PEXG, several studies have suggested that these forms of glaucoma have overlapping genetic risk factors [20,21]. Therefore, the aim of the current study was to evaluate the role of the genetic variants recently associated with POAG, in different types of glaucoma in Pakistani patient cohorts with POAG, PACG, and PEXG.

**METHODS**

**Patients and clinical data:** The present case-control study included 513 patients with glaucoma (268 with POAG, 125 with PACG, 120 with PEXG) and 233 ethnically-matched healthy controls. Patients were recruited from the Al-Shifa Trust Eye Hospital in Rawalpindi, Pakistan. Complete ophthalmic examinations were performed for the patients and controls. Briefly, for the patients with POAG the inclusion criteria were IOP (>21 mmHg) measured using Goldmann applanation tonometry, a cup-to-disc ratio (CDR) >0.7 with thinning or notching of the disc rim, and nerve fiber layer defects. Visual field defects typical of glaucoma such as arcuate scotoma, nasal step, paracentral scotoma, and generalized depression were determined with a Humphrey Field Analyzer (Zeiss Humphrey Systems, Dublin, CA), and an open anterior chamber angle was confirmed with gonioscopy. Only individuals affected with advanced primary open angle glaucoma were included in the study while patients with normal tension glaucoma were excluded. The presence of symptoms such as eye pain, headache, blurred vision, and vomiting with a history of colored haloes suggested PACG.

On ocular examination, conjunctival congestion, a mid-dilated unreactive pupil, corneal edema and a gonioscopically closed anterior chamber angle (presenting eye), and occludable angles (fellow eye in unilateral cases) were imperative for diagnosis of PACG. PEXG was diagnosed in patients who presented with an accumulation of microfibrillar deposits or exfoliative material on the pupillary ruff, a clear annular zone, or flakes of exfoliative material with a grayish central disc on the anterior lens capsule, iris, or corneal endothelium in one or both eyes. Controls and cases were matched for age, gender, and ethnicity. The same detailed ophthalmological examination was performed for the controls as for the patients with glaucoma, and only control individuals without eye disease were selected.

**Genomic DNA and genotyping:** The current study was approved by the institutional review board of the Al-Shifa Eye Trust Hospital and adhered to the tenets of the Declaration of Helsinki. Whole-blood samples of patients and controls were drawn after written informed consent was obtained. Briefly white blood cells were lysed, followed by digestion of proteins, extraction of the DNA with phenol-chloroform, and precipitation of DNA with absolute ethanol. Genomic DNA was obtained from peripheral blood leukocytes using a standard phenol chloroform extraction method [22].

**Molecular genetic analysis:** Six single nucleotide polymorphism (SNPs: CDKN2B-AS1 rs4977756, CDKN2B rs1063192, ATOH7 rs1900004, CAV1 rs4236601, TMCO1 rs4656461, SIX1 rs10483727) were genotyped using TaqMan assays in an ABI PRISM 7900 real-time sequence detection system (Applied Biosystems, Foster City, CA), with 384-well microtiter plates. The 384-well plates contained 5 ul of final volume for each reaction in every well consisting of 2.5 μl TaqMan Universal PCR Master Mix (Applied Biosystems), 0.6 μl of each primer, and 10 ng of genomic DNA. The PCR cycling conditions were an initial denaturation step at 95 °C for 12 min and 40 cycles of 92 °C for 15 s and 60 °C for 1 min. Fluorescent signals were measured at 60 °C. The genotype of each sample was determined by measuring allele-specific fluorescence using SDS 2.3 software for allelic discrimination (Roche, Foster City, CA). The concordance for duplicate samples included in the 384-well plates was >97%.

**Statistical analysis:** The detected genotypes of the six SNPs were assessed for Hardy–Weinberg equilibrium (HWE) using the Pearson chi-square (χ²) test. The difference between the genotype frequencies in the patients and controls was analyzed by computing the χ² and the odds ratio (OR, 95% confidence interval [CI]) using the statistical software StatCale EpilInfo package v.6 (Atlanta, GA).
RESULTS

The patients with POAG had a mean age of 54.6±1.4 years (52% male and 48% female), the patients with PACG had a mean age of 53.6±1.5 years (48.8% male, 51.2% female), the patients with PEXG had a mean age 52.4±2.6 years (49% male, 51% female), and the control subjects had a mean age of 54.1±1.3 years (49.5% male, 50.5% female). The p values for the age and gender differences were >0.05. Association of genotype and allele frequencies of six polymorphisms with different forms of glaucoma was determined. Genotype frequencies of all tested polymorphisms were in Hardy–Weinberg equilibrium.

Overall, a highly significant (p<0.001) protective effect of the AG genotype of rs4656461 in the TMCO1 gene was observed for glaucoma (Table 1). The association remained significant when the data were grouped based on the type of glaucoma (POAG, p=0.005; PACG, p=0.02; PEXG, p=0.03; Table 2). The frequency of the minor allele G was significantly lower in the patients with POAG (p=0.003; OR=0.57 [95% CI=0.38–0.89]), PACG (p=0.009; OR=0.52 [95% CI=0.30–0.88]), and PEXG (p=0.01; OR=0.54 [95% CI=0.32–0.98]) compared to the controls (Table 3). Therefore, the G allele may be protective for the disease.

The homozygous AA genotype of CAV1 rs4236601 was significantly associated with glaucoma (p=0.02, OR=2.46 [95% CI=1.01–6.24]). When grouped by glaucoma type, the AA genotype of the CAV1 SNP was significantly associated with POAG (p=0.02), but not with PACG and PEXG. A significant difference was found for the minor allele (A) frequency of rs4236601 between the controls and the patients with POAG only (p=0.008; OR=1.49 [95% CI=1.09–2.04]; Table 2).

A significant difference in genotype and allele frequencies was observed between the patients with PACG and the controls for the ATOH7 SNP rs1900004 (p=0.03; Table 2 and Table 3). The minor allele (T) was protective for the disease (OR=0.69 [95% CI=0.48–1.00]). No significant difference in genotype and allele frequencies was observed for the rs49777756 polymorphism in the CDKN2B-AS1 gene, rs1063192 in CDKN2B, and rs10483727 in SIX1, between the controls and the patients with glaucoma.

DISCUSSION

In the current study, we identified an association of the ATOH7 polymorphism rs1900004 with the PACG type of glaucoma. To the best of our knowledge, this is the first study in which an association of an SNP in ATOH7 was determined in patients with PACG. In a previous study, only patients with POAG were genotyped, and a marginal association was observed [16]. ATOH7 is involved in the development of the eye, and it has been reported that targeted deletion of ATOH7 causes optic nerve agenesis in mice [23]. The rs1900004 SNP in ATOH7 has also been associated with other eye diseases such as optic nerve hypoplasia [24,25].

The CAV1 SNP rs4236601 was associated with the POAG type of glaucoma in the current Pakistani patient cohort. The results of present study are consistent with a previous study by Thorleifsson et al. in which the A allele was associated with POAG susceptibility in Icelandic, Australian, and Chinese populations [16]. The minor allele frequencies identified in the Icelandic population (POAG 28.7%, controls 22.8%) are similar to those observed in this study in the Pakistani population (POAG 26.3%, controls 19.3%).

However, in a study of a U.S. population, no association was observed with the CAV1 variant (POAG 25.8%, controls 26.9%) [26]. No association was observed with PACG and PEXG glaucoma in the present study, which is in agreement with negative findings for PEXG in Icelandic and Swedish cohorts [16]. In a meta-analysis performed by Huang et al. [27], allele A in rs4236601 SNP was a significant risk factor in POAG p<0.001; [OR=1.23; 95% CI, 1.12–1.34]. Upon the stratification of data for ethnicity, the A allele conferred a higher disease risk among Caucasians (p<0.001 [OR=1.25; 95% CI, 1.18–1.33]) and Asians (p=0.003 [OR=3.33; 95% CI, 1.56–7.08]). The findings of the current study also support the results of meta-analysis since the A allele is significantly associated with POAG (p=0.008 [OR=1.49; 95% CI, 1.09–2.04]).

CAV1 is involved in regulating endothelial nitric oxide synthase (eNOS), an enzyme that produces nitric oxide, and transforming growth factor beta (TGF-β). eNOS is involved in regulating blood flow to ocular tissues and nitric oxide levels in plasma [28]. Excessive production of nitric oxide causes cytotoxicity, neurodegeneration, and apoptotic cell death [29,30]. Therefore, a functional change in CAV1 might cause an imbalance in eNOS levels that could potentially result in RGC degeneration, an important process in glaucoma.

We observed a significant association of the TMCO1 gene polymorphism rs4656461 in Pakistani patients with POAG, PACG, and PEXG. In previous studies, a higher frequency of the G allele was found in patients compared to controls of Australian and European descent, whereas in the present study the A allele was found more frequently in the patients compared to the controls [14,15]. The contradictory association observed for this variant in the TMCO1 gene suggests that either the G allele has a different effect in different populations, possibly due to the involvement of other
### Table 1. Genotype frequencies in patients and controls.

| Genotypes          | Patients n=513 (%) | Controls n=233 (%) | \(p (\chi^2)\) | \(p (\chi^2)\) | OR (95% CI) |
|--------------------|--------------------|--------------------|----------------|----------------|-------------|
| **CDKN2B-AS1 rs4977756** |                    |                    |                |                |             |
| AA                 | 281 (54.7)         | 135 (57.9)         | 0.63 (0.91)    | 0.35 (0.85)    | 0.86 (0.61–1.21) |
| AG                 | 197 (38.5)         | 81 (34.8)          |                | 0.97 (0.00)    | 0.99 (0.51–1.92) |
| GG                 | 35 (6.8)           | 17 (7.3)           |                |                |             |
| **CDKN2B rs1063192** |                    |                    |                |                |             |
| TT                 | 270 (52.6)         | 127 (54.5)         | 0.87 (0.26)    | 0.92 (0.65–1.29) |
| CT                 | 206 (40.2)         | 89 (38.2)          |                | 0.98 (0.51–1.87) |
| CC                 | 37 (7.2)           | 17 (7.3)           | 0.94 (0.01)    |                |             |
| **ATOH7 rs1900004** |                    |                    |                |                |             |
| CC                 | 230 (44.9)         | 110 (47.2)         | 0.75 (0.57)    | 0.89 (0.63–1.25) |
| CT                 | 231 (45.0)         | 98 (42.1)          |                | 0.99 (0.57–1.75) |
| TT                 | 52 (10.1)          | 25 (10.7)          | 0.98 (0.00)    |                |             |
| **CAV1 rs4236601** |                    |                    |                |                |             |
| GG                 | 296 (57.7)         | 150 (64.4)         | 0.06 (5.45)    | 0.82 (0.58–1.16) |
| AG                 | 183 (35.7)         | 76 (32.6)          | 0.23 (1.38)    |                |             |
| AA                 | 34 (6.6)           | 7 (3.0)            | 0.02 (4.71)    | 2.46 (1.01–6.24) |
| **TMCO1 rs4656461** |                    |                    |                |                |             |
| AA                 | 420 (81.9)         | 162 (69.5)         | 0.0001 (14.32) | 0.51 (0.35–0.74) |
| AG                 | 91 (17.7)          | 69 (29.6)          | <0.0001 (13.68)|                |             |
| GG                 | 2 (0.4)            | 2 (0.9)            | 0.32 (0.97)    | 0.39 (0.04–3.86) |
| **SIX1 rs10483727** |                    |                    |                |                |             |
| CC                 | 104 (20.3)         | 46 (19.7)          | 0.64 (0.87)    | 0.97 (0.63–1.50) |
| CT                 | 261 (50.9)         | 112 (48.1)         | 0.88 (0.02)    |                |             |
| TT                 | 148 (28.8)         | 75 (32.2)          | 0.54 (0.36)    |                |             |

- \(\chi^2\) indicates the chi-squared test statistic.
- OR (95% CI) indicates the odds ratio with 95% confidence interval.
## Table 2. Genotype frequencies in POAG, PACG, PEXG patients and controls.

| Genotypes       | POAG n=268 (%) | Controls n=233 (%) | p (χ²) | PACG n=125 (%) | p (χ²) | PEXG n=120 (%) | p (χ²) |
|-----------------|----------------|--------------------|--------|----------------|--------|----------------|--------|
| **CDKN2B-AS1 rs4977756** |                |                    |        |                |        |                |        |
| AA              | 143 (53.3)     | 135 (57.9)         | 0.37 (1.95) | 73 (58.4)     | 0.82 (0.39) | 65 (54.2)     | 0.62 (0.93) |
| AG              | 109 (40.7)     | 81 (34.8)          |        | 45 (36.0)      |        | 43 (35.8)      |        |
| GG              | 16 (6.0)       | 17 (7.3)           |        | 7 (5.6)        |        | 12 (10.0)      |        |
| **CDKN2B rs1063192** |                |                    |        |                |        |                |        |
| TT              | 134 (50.0)     | 127 (54.5)         |        | 72 (57.6)      |        | 64 (53.3)      |        |
| CT              | 116 (43.3)     | 99 (42.1)          | 0.51 (1.33) | 46 (36.8)     | 0.76 (0.53) | 44 (36.7)     | 0.67 (0.77) |
| CC              | 18 (6.7)       | 17 (7.3)           |        | 7 (5.6)        |        | 12 (10.0)      |        |
| **ATOH7 rs1900004** |                |                    |        |                |        |                |        |
| CC              | 111 (41.4)     | 110 (47.2)         |        | 68 (54.4)      |        | 51 (42.5)      |        |
| CT              | 127 (47.4)     | 98 (42.1)          | 0.41 (1.76) | 53 (42.4)     | 0.03 (6.54) | 51 (42.5)     | 0.45 (1.57) |
| TT              | 30 (11.2)      | 25 (10.7)          |        | 4 (3.2)        |        | 18 (15.0)      |        |
| **CAV1 rs4236601** |                |                    |        |                |        |                |        |
| GG              | 145 (54.1)     | 150 (64.4)         |        | 76 (60.8)      |        | 75 (62.5)      |        |
| AG              | 105 (39.2)     | 76 (32.6)          | 0.02 (7.16) | 41 (32.8)     | 0.30 (2.40) | 37 (30.8)     | 0.26 (2.62) |
| AA              | 18 (6.7)       | 7 (3.0)            |        | 8 (6.4)        |        | 8 (6.7)        |        |
| **TMCOI rs4656461** |                |                    |        |                |        |                |        |
| AA              | 219 (81.7)     | 162 (69.5)         |        | 103 (82.4)     |        | 98 (81.7)      |        |
| AG              | 47 (17.5)      | 69 (29.6)          | 0.005 (10.31) | 22 (17.6)     | 0.02 (7.51) | 22 (18.3)     | 0.03 (6.52) |
| GG              | 2 (0.75)       | 2 (0.9)            |        | 0 (0.0)        |        | 0 (0.0)        |        |
| **SIX1 rs10483727** |                |                    |        |                |        |                |        |
| CC              | 65 (24.2)      | 46 (19.7)          |        | 19 (15.2)      |        | 20 (16.7)      |        |
| CT              | 128 (47.8)     | 112 (48.1)         | 0.39 (1.88) | 70 (56.0)     | 0.32 (2.23) | 63 (52.5)     | 0.68 (0.76) |
| TT              | 75 (28.0)      | 75 (32.2)          |        | 36 (28.8)      |        | 37 (30.8)      |        |
### Table 3. Allele frequencies in POAG, PACG, PEXG patients and controls.

| Alleles         | POAG n=536 (%) | Controls n=466 (%) | PACG n=250 (%) | PEXG n=240 (%) |
|-----------------|----------------|-------------------|----------------|----------------|
|                 | p (χ²)         | OR (95% CI)       | p (χ²)         | OR (95% CI)    |
| CDKN2B-AS1 rs4977756 |                |                   |                |                |
| A               | 395 (73.7)     | 0.55              | 0.92 (0.68-1.23)| 0.74           |
| G               | 141 (26.3)     | (0.35)            | 59 (23.6)      | (0.10)         |
|                 |                | 0.94 (0.65-1.37)  | 67 (27.9)      | (0.87)         |
|                 |                | 1.18 (0.82-1.71)  |                |                |
| CDKN2B rs1063192 |                |                   |                |                |
| T               | 384 (71.6)     | 0.48              | 0.91 (0.68-1.21)| 0.48           |
| C               | 152 (28.4)     | (0.48)            | 60 (24.0)      | (0.49)         |
|                 |                | 0.88 (0.61-1.28)  | 68 (28.5)      | (0.30)         |
|                 |                | 1.10 (0.77-1.58)  |                |                |
| ATOH7 rs1900004 |                |                   |                |                |
| C               | 349 (65.1)     | 0.29              | 0.87 (0.66-1.14)| 0.03           |
| T               | 187 (34.9)     | (1.10)            | 61 (24.4)      | (4.26)         |
|                 |                | 0.69 (0.48-1.00)  | 87 (35.25)     | (1.44)         |
|                 |                | 1.22 (0.87-1.72)  |                |                |
| CAV1 rs4236601  |                |                   |                |                |
| G               | 395 (73.7)     | 0.008             | 1.49 (1.09-2.04)| 0.27           |
| A               | 141 (26.3)     | (0.87)            | 57 (22.8)      | (1.21)         |
|                 |                | 0.81 (0.55-1.20)  | 53 (22.1)      | (0.75)         |
|                 |                | 1.18 (0.79-1.77)  |                |                |
| TMCO1 rs4656461 |                |                   |                |                |
| A               | 485 (90.5)     | 0.003             | 0.57 (0.38-0.89)| 0.009          |
| G               | 51 (9.5)       | (8.70)            | 22 (8.8)       | (6.66)         |
|                 |                | 0.52 (0.30-0.88)  | 22 (9.2)       | (5.74)         |
|                 |                | 0.54 (0.32-0.92)  |                |                |
| SIX1 rs10483727 |                |                   |                |                |
| C               | 258 (48.1)     | 0.16              | 0.84 (0.65-1.09)| 0.88           |
| T               | 278 (51.9)     | (1.90)            | 142 (60.0)     | (0.02)         |
|                 |                | 0.98 (0.71-1.35)  | 137 (57.1)     | (0.05)         |
|                 |                | 1.04 (0.75-1.44)  |                |                |
contributing factors, or that it might be in linkage disequilibrium with a yet unidentified causative variant in or near the TMCO1 gene. The rs4656461 polymorphism is located downstream of the TMCO1 gene [14,15] in the same linkage disequilibrium block as a second polymorphism, rs7518099, located within intron 2 of TMCO1, which has been associated with increased IOP in POAG [31]. In an expression study on ocular tissues and human retina, predominant expression of TMCO1 was observed in the ciliary body, which is responsible for producing the aqueous humor, and in the trabecular meshwork (TM), a crucial determinant of the IOP due to its resistance during the drainage of aqueous humor from the eye [32,33]. Therefore, TMCO1 might also be involved in regulating IOP levels together with ciliary body and TM since the rs4656461 polymorphism is also associated with increased IOP in various studies [14,15,31].

No association of rs4977756 in CDKN2B-ASI, rs1063192 in CDKN2B, and rs10483727 in SIX1 was observed. The results of the present study are surprisingly different from previous studies, in which a strong association for rs4977756 was observed for Australian patients of European descent with POAG, and for rs1063192 in patients in the Afro-Caribbean population of Barbados with POAG. In a meta-analysis by Ramdas et al., which included data from six independent studies, a significant association of rs1063192 in CDKN2B and rs10483727 in SIX1 was observed with POAG [16]. Similarly, in a GWAS by Burdon et al. rs4977756 in CDKN2B-ASI was highly associated with POAG [6]. Similar to our study, no association of rs10483727 in the SIX1 gene was observed in patients in the Afro-Caribbean population of Barbados with POAG, while strong association was observed in patients in a U.S. Caucasian population with POAG [34].

In conclusion, the results of the present study revealed that the TMCO1 rs4656461 SNP is significantly associated with glaucoma, irrespective of the type (POAG, PACG, PEXG). The ATOH7 rs1900004 polymorphism was protective for PACG in Pakistani patients, while the CAV1 rs4236601 SNP conferred increased risk of POAG. Further replication studies of these polymorphisms in different types of glaucoma in various populations are required, along with functional studies to better understand the disease mechanisms.

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