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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-AS1* (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-AS1*, *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.

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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 µl 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 (χ^2) test. The difference between the genotype frequencies in the patients and controls was analyzed by computing the χ^2 and the odds ratio (OR, 95% confidence interval [CI]) using the statistical software StatCalc EpiInfo 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 28.5%, 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 (χ^2)	p (χ^2)	OR (95% CI)
CDKN2B-AS1 rs4977756					
AA	281 (54.7)	135 (57.9)			Reference
AG	197 (38.5)	81 (34.8)	0.63 (0.91)	0.35 (0.85)	0.86 (0.61–1.21)
GG	35 (6.8)	17 (7.3)		0.97 (0.00)	0.99 (0.51–1.92)
CDKN2B rs1063192					
TT	270 (52.6)	127 (54.5)			Reference
CT	206 (40.2)	89 (38.2)	0.87 (0.26)	0.60 (0.26)	0.92 (0.65–1.29)
CC	37 (7.2)	17 (7.3)		0.94 (0.01)	0.98 (0.51–1.87)
ATOH7 rs1900004					
CC	230 (44.9)	110 (47.2)			Reference
CT	231 (45.0)	98 (42.1)	0.75 (0.57)	0.47 (0.51)	0.89 (0.63–1.25)
TT	52 (10.1)	25 (10.7)		0.98 (0.00)	0.99 (0.57–1.75)
CAV1 rs4236601					
GG	296 (57.7)	150 (64.4)			Reference
AG	183 (35.7)	76 (32.6)	0.06 (5.45)	0.23 (1.38)	0.82 (0.58–1.16)
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)			Reference
AG	91 (17.7)	69 (29.6)	<0.0001 (14.32)	<0.001 (13.68)	0.51 (0.35–0.74)
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)			Reference
CT	261 (50.9)	112 (48.1)	0.64 (0.87)	0.88 (0.02)	0.97 (0.63–1.50)
TT	148 (28.8)	75 (32.2)		0.54 (0.36)	0.87 (0.55–1.39)

Table 2. Genotype frequencies in POAG, PACG, PEXG patients and controls.

Genotypes	POAG n=268 (%)	Controls n=233 (%)	p (χ^2)	PACG n=125 (%)	p (χ^2)	PEXG n=120 (%)	p (χ^2)
CDKN2B-AS1 rs4977756							
AA	143 (53.3)	135 (57.9)		73 (58.4)		65 (54.2)	
AG	109 (40.7)	81 (34.8)	0.37 (1.95)	45 (36.0)	0.82 (0.39)	43 (35.8)	0.62 (0.93)
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)	89 (38.2)	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)	
TMCO1 rs4656461							
AA	219 (81.7)	162 (69.5)		103 (82.4)		98 (81.7)	
AG	47 (17.53)	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 (0.00)	
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 (%)	p (χ^2)	OR (95% CI)	PACG n=250 (%)	p (χ^2)	OR (95% CI)	PEXG n=240 (%)	p (χ^2)	OR (95% CI)
CDKN2B-AS1 rs4977756										
A	395 (73.7)	351 (75.3)	0.55 (0.35)	0.92 (0.68-1.23)	191 (76.4)	0.74 (0.10)	0.94 (0.65-1.37)	173 (72.1)	0.35 (0.87)	1.18 (0.82-1.71)
G	141 (26.3)	115 (24.7)			59 (23.6)			67 (27.9)		
CDKN2B rs1063192										
T	384 (71.6)	343 (73.6)	0.48 (0.48)	0.91 (0.68-1.21)	190 (76.0)	0.48 (0.49)	0.88 (0.61-1.28)	172 (71.7)	0.58 (0.30)	1.10 (0.77-1.58)
C	152 (28.4)	123 (26.4)			60 (24.0)			68 (28.3)		
ATOH7 rs1900004										
C	349 (65.1)	318 (68.2)	0.29 (1.10)	0.87 (0.66-1.14)	189 (75.6)	0.03 (4.26)	0.69 (0.48-1.00)	153 (63.75)	0.23 (1.44)	1.22 (0.87-1.72)
T	187 (34.9)	148 (31.8)			61 (24.4)			87 (35.25)		
CAV1 rs4236601										
G	395 (73.7)	376 (80.7)	0.008 (6.87)	1.49 (1.09-2.04)	193 (77.2)	0.27 (1.21)	0.81 (0.55-1.20)	187 (77.9)	0.38 (0.75)	1.18 (0.79-1.77)
A	141 (26.3)	90 (19.3)			57 (22.8)			53 (22.1)		
TMCO1 rs4656461										
A	485 (90.5)	393 (84.3)	0.003 (8.70)	0.57 (0.38-0.89)	228 (91.2)	0.009 (6.66)	0.52 (0.30-0.88)	218 (90.8)	0.01 (5.74)	0.54 (0.32-0.92)
G	51 (9.5)	73 (15.7)			22 (8.8)			22 (9.2)		
SIX1 rs10483727										
C	258 (48.1)	204 (43.8)	0.16 (1.90)	0.84 (0.65-1.09)	108 (40.0)	0.88 (0.02)	0.98 (0.71-1.35)	103 (42.9)	0.82 (0.05)	1.04 (0.75-1.44)
T	278 (51.9)	262 (56.2)			142 (60.0)			137 (57.1)		

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-AS1*, 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-AS1* 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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REFERENCES

1. Quigley HA. Number of people with glaucoma worldwide. Br J Ophthalmol 1996; 80:389-93. [PMID: 8695555].
2. Schwartz M, Yoles E. Neuroprotection: a new treatment modality for glaucoma? Curr Opin Ophthalmol 2000; 11:107-11. [PMID: 10848215].
3. 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; 2014:S0161-6420. [PMID: 24974815].
4. Yip JL, Foster PJ. Ethnic differences in primary angle-closure glaucoma. Curr Opin Ophthalmol 2006; 17:175-80. [PMID: 16552253].
5. Shakya S, Gupta HR. Angle closure glaucoma: a cause for bilateral visual threat. Nepal Med Coll J 2006; 8:153-5. [PMID: 17203819].
6. Weinreb RN, Khaw PT. Primary open-angle glaucoma. Lancet 2004; 363:1711-20. [PMID: 15158634].
7. Quigley HA, Addicks EM, Green WR, Maumenee AE. Optic nerve damage in human glaucoma, II: the site of injury and susceptibility to damage. Arch Ophthalmol 1981; 99:635-49. [PMID: 6164357].
8. Fechtner RD, Weinreb RN. Mechanisms of optic nerve damage in primary open angle glaucoma. Surv Ophthalmol 1994; 39:23-42. [PMID: 7974188].
9. Burgoyne CF, Downs JC, Bellezza AJ, Suh JK, Hart RT. The optic nerve head as a biomechanical structure: a new paradigm for understanding the role of IOP-related stress and strain in the pathophysiology of glaucomatous optic nerve head damage. Prog Retin Eye Res 2005; 24:39-73. [PMID: 15555526].
10. Conway RM, Schlötzer-Schrehardt U, Küchle M, Naumann GO. Pseudoexfoliation syndrome: pathological manifestations of relevance to intraocular surgery. Clin Experiment Ophthalmol 2004; 32:199-210. [PMID: 15068440].
11. Izzotti A, Longobardi M, Cartiglia C, Saccà SC. Mitochondrial damage in the trabecular meshwork occurs only in primary open-angle glaucoma and in pseudoexfoliative glaucoma. PLoS ONE 2011; 6:e14567-[PMID: 21283745].
12. Gottanka J, Flugel-Koch C, Martus P, Jhonson DH, Lutjen-Drecoll E. Correlation of pseudoexfoliative material and optic nerve damage in pseuoexfoliative syndrome. Invest Ophthalmol Vis Sci 1997; 38:2435-46. [PMID: 9375560].
13. Schlötzer-Schrehardt U, Naumann GO. Trabecular meshwork in pseudoexfoliation syndrome with and without open-angle glaucoma. A morphometric, ultrastructural study. Invest Ophthalmol Vis Sci 1995; 36:1750-64. [PMID: 7635652].
14. Burdon KP, Macgregor S, Hewitt AW, Sharma S, Chidlow G, Mills RA, Danoy P, Casson R, Viswanathan AC, Liu JZ, Landers J, Henders AK, Wood J, Souzeau E, Crawford A, Leo P, Wang JJ, Rochtchina E, Nyholt DR, Martin NG, Montgomery GW, Mitchell P, Brown MA, Mackey DA, Craig JE. Genome-wide association study identifies susceptibility

loci for open angle glaucoma at TMCO1 and CDKN2B–AS1. Nat Genet 2011; 43:574-8. [PMID: 21532571].

15. Sharma S, Burdon KP, Chidlow G, Klebe S, Crawford A, Dimasi DP, Dave A, Martin S, Javadiyan S, Wood JP, Casson R, Danoy P, Griggs K, Hewitt AW, Landers J, Mitchell P, Mackey DA, Craig JE. Association of genetic variants in the TMCO1 gene with clinical parameters related to glaucoma and characterization of the protein in the eye. Invest Ophthalmol Vis Sci 2012; 53:4917-25. [PMID: 22714896].

16. Thorleifsson G, Walters GB, Hewitt AW, Masson G, Helgason A, DeWan A, Sigurdsson A, Jonasdottir A, Gudjonsson SA, Magnusson KP, Stefansson H, Lam DS, Tam PO, Gudmundsdottir GJ, Southgate L, Burdon KP, Gottfredsdottir MS, Aldred MA, Mitchell P, St Clair D, Collier DA, Tang N, Sveinsson O, Macgregor S, Martin NG, Cree AJ, Gibson J, Macleod A, Jacob A, Ennis S, Young TL, Chan JC, Karwatowski WS, Hammond CJ, Thordarson K, Zhang M, Wadelius C, Lotery AJ, Trembath RC, Pang CP, Hoh J, Craig JE, Kong A, Mackey DA, Jonasson F, Thorsteinsdottir U, Stefansson K. Common variants near CAV1 and CAV2 are associated with primary open angle glaucoma. Nat Genet 2010; 42:906-9. [PMID: 20835238].

17. Ramdas WD, van Koolwijk LM, Cree AJ, Janssens AC, Amin N, de Jong PT, Wolfs RC, Gibson J, Kirwan JF, Hofman A, Rivadeneira F, Oostra BA, Uitterlinden AG, Ennis S, Lotery AJ, Lemij HG, Klaver CC, Vingerling JR, Jansonius NM, van Duijn CM. Common genetic variants associated with open-angle glaucoma. Ophthalmology 2011; 118:2389-97. [PMID: 21872936].

18. Kawakami K, Sato S, Ozaki H, Ikeda K. Six family genes–structure and function as transcription factors and their roles in development. Bioessays 2000; 22:616-26. [PMID: 10878574].

19. Khor CC, Ramdas WD, Vithana EN, Cornes BK, Sim X, Tay WT, Saw SM, Zheng Y, Lavanya R, Wu R, Wang JJ, Mitchell P, Uitterlinden AG, Rivadeneira F, Teo YY, Chia KS, Seielstad M, Hibberd M, Vingerling JR, Klaver CC, Jansonius NM, Tai ES, Wong TY, van Duijn CM, Aung T. Genome-wide association studies in Asians confirm the involvement of ATOH7 and TGFBR3, and further identify CARD10 as a novel locus influencing optic disc area. Hum Mol Genet 2011; 20:1864-72. [PMID: 21307088].

20. Forsman E, Lemmela S, Varilo T, Kristo P, Forsius H, Sankila EM, Jarvela I. The role of TIGR and OPTN in Finnish glaucoma families: a clinical and molecular genetic study. Mol Vis 2003; 9:217-22. [PMID: 12789137].

21. Jansson M, Marknell T, Tomic L, Larsson LI, Wadelius C. Allelic variants in the MYOC/TIGR gene in patients with primary open-angle, exfoliative glaucoma and unaffected controls. Ophthalmic Genet 2003; 24:103-10. [PMID: 12789574].

22. Sambrook J, Russell DW, Sambrook J. The condensed protocols from Molecular cloning: a laboratory manual. Cold Spring Harbor (NY): 2006; Cold Spring Harbor Laboratory Press.

23. Brown NL, Dagenais SL, Chen CM, Glaser T. Molecular characterization and mapping of ATOH7, a human atonal homolog with a predicted role in retinal ganglion cell development. Mamm Genome 2002; 13:95-101. [PMID: 11889557].

24. Moshiri A, Gonzalez E, Tagawa K, Maeda H, Wang M, Frishman LJ, Wang SW. Near complete loss of retinal ganglion cells in the math5/brn3b double knockout elicits severe reductions of other cell types during retinal development. Dev Biol 2008; 316:214-27. [PMID: 18321480].

25. Macgregor S, Hewitt AW, Hysi PG, Ruddle JB, Medland SE, Henders AK, Gordon SD, Andrew T, McEvoy B, Sanfilippo PG, Carbonaro F, Tah V, Li YJ, Bennett SL, Craig JE, Montgomery GW, Tran-Viet KN, Brown NL, Spector TD, Martin NG, Young TL, Hammond CJ, Mackey DA. Genome-wide association identifies ATOH7 as a major gene determining human optic disc size. Hum Mol Genet 2010; 19:2716-24. [PMID: 20395239].

26. Kuehn MH, Wang K, Roos B, Stone EM, Kwon YH, Alward WL, Mullins RF, Fingert JH. Chromosome 7q31 POAG locus: ocular expression of caveolins and lack of association with POAG in a US cohort. Mol Vis 2011; 17:430-5. [PMID: 21321670].

27. Huang W, Wang W, Zhou M, Zhang X. Association of SNP rs4236601 near CAV1 and CAV2 with primary open-angle glaucoma: a meta-analysis. Clin Experiment Ophthalmol 2013; epub:.

28. Liu B, Neufeld AH. Activation of epidermal growth factor receptor signals induction of nitric oxide synthase-2 in human optic nerve head astrocytes in glaucomatous optic neuropathy. Neurobiol Dis 2003; 13:109-23. [PMID: 12828935].

29. Fuchshofer R, Tamm ER. Modulation of extracellular matrix turnover in the trabecular meshwork. Exp Eye Res 2009; 88:683-8. [PMID: 19385040].

30. Toda N, Nakanishi-Toda M. Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. Prog Retin Eye Res 2007; 26:205-38. [PMID: 17337232].

31. van Koolwijk LM, Ramdas WD, Ikram MK, Jansonius NM, Pasutto F, Hysi PG, Macgregor S, Janssen SF, Hewitt AW, Viswanathan AC, ten Brink JB, Hosseini SM, Amin N, Despriet DD, Willemse-Assink JJ, Kramer R, Rivadeneira F, Struchalin M, Aulchenko YS, Weisschuh N, Zenkel M, Mardin CY, Gramer E, Welge-Lüssen U, Montgomery GW, Carbonaro F, Young TL. DCCT/EDIC Research Group. Bellenguez C, McGuffin P, Foster PJ, Topouzis F, Mitchell P, Wang JJ, Wong TY, Czudowska MA, Hofman A, Uitterlinden AG, Wolfs RC, de Jong PT, Oostra BA, Paterson AD; Wellcome Trust Case Control Consortium 2, Mackey DA, Bergen AA, Reis A, Hammond CJ, Vingerling JR, Lemij HG, Klaver CC, van Duijn CM. Common genetic determinants of intraocular pressure and primary open-angle glaucoma. PLoS Genet 2012; 8:e1002611-[PMID: 22570627].

32. Llobet A, Gasull X, Gual A. Understanding trabecular meshwork physiology: a key to the control of intraocular pressure? News Physiol Sci 2003; 18:205-9. [PMID: 14500801].

33. Gibson J, Griffiths H, De Salvo G, Cole M, Jacob A, Macleod A, Yang Y, Menon G, Cree A, Ennis S, Lotery A. Genome-wide association study of primary open angle glaucoma risk and quantitative traits. Mol Vis 2012; 18:1083-92. [PMID: 22605921].

34. Fan BJ, Wang DY, Pasquale LR, Haines JL, Wiggs JL. Genetic variants associated with optic nerve vertical cup-to-disc ratio are risk factors for primary open angle glaucoma in a US Caucasian population. Invest Ophthalmol Vis Sci 2011; 52:1788-92. [PMID: 21398277].

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