• Basic Research •

Analysis of *SMOC2* gene variants in familial and non-familial primary open angle glaucoma Pakistani patients

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Abstract

● **AIM:** To find out the association of secreted protein acidic and rich in cysteine (SPARC)-related modular calcium binding 2 (*SMOC2)* gene variants rs2255680 and rs13208776 with genotypic and phenotypic characteristics in both familial and non-familial primary open angle glaucoma (POAG) patients.

● **METHODS:** A total of 212 POAG patients, comprising 124 familial and 88 non-familial, were enrolled. For genotyping the *SMOC2* variant rs2255680, amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) method and PCR-restriction fragment length polymorphism (PCR-RFLP) were utilized for analyzing rs13208776 variant.

● **RESULTS:** The mean age of familial POAG patients was 50.92±9.12y, with 78 males and 46 females. The mean age of non-familial POAG patients was 53.14±13.44y, with 52 males and 36 females. The *SMOC2* gene variant rs13208776 showed the significant association with POAG between familial and non-familial groups. The homozygous G/G variant was frequent among non-familial (60.2%) whereas the heterozygous G/A variant was more frequent in familial POAG patients (46%). There were significant differences in G/A variant between familial and non-familial glaucoma patients, and the risk was decreased to 0.53-fold in non-familial glaucoma patients [odds ratio (OR): 0.53; 95% confidence interval (CI): 0.29-0.94; *P*=0.033] in codominant model. The risk was further reduced to 0.49-fold (95%CI: 0.28-0.86; *P*=0.012) in dominant model for non-familial patients. No significant association of *SMOC2* gene variant rs2255680 between familial and non-familial glaucoma patients was found in our population. The haplotype analysis showed the decreased risk for TA [OR: 0.48 (95%CI: 0.29-0.79); *P*=0.004] and an increased risk for TG [OR=2.28 (95%CI: 1.22-4.25); *P*=0.01] haplotypes.

● **CONCLUSION:** Current findings show significant association of *SMOC2* gene variant rs13208776 with POAG between familial and non-familial Pakistani patients.

● **KEYWORDS:** glaucoma; primary open angle glaucoma; *SMOC2*; gene; variant; familial; non-familial
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INTRODUCTION

Glaucoma are group of optic neuropathies and stands as the leading cause of irreversible bilateral blindness globally[1]. The most common is the primary open angle glaucoma (POAG); further divided into familial or non-familial type. Its prevalence varies among different ethnicities and populations[2-3]. Glaucoma leads to progressive retinal ganglion cell death, leading to gradual peripheral vision loss and subsequent loss of central field of vision[4-5]. The underlying pathogenesis of POAG is not entirely clear; however, genetic factors, old age, increased intraocular pressure (IOP), corneal

thinning, inflammation, and oxidative stress contribute to its development[6-7].

Recent genomic advancements and study findings highlight the crucial role of genetic factors, and gene-environment interactions in POAG pathogenesis[8-9]. To date, numerous risk alleles have been identified, and 112 loci (68 novel) linked to increased IOP and the POAG development have been reported[10]. According to previous findings, genetic mutations have been identified as the direct causative factors for POAG[11]. Notably, *MYOC* gene mutations are linked to hereditary cases of both adult and juvenile POAG[12]. Furthermore, *CDKN2B-AS1* gene variants have been linked to increased predisposition to optic nerve damage, mainly inducing the apoptosis in retinal ganglion cells[13].

Secreted protein acidic and rich in cysteine (SPARC)-related modular calcium binding 2 (*SMOC2*) gene (ID: 64094), located on chromosome 6q27, encodes matricellular glycoproteins. It modulates the expression of matrix metalloproteinases and extracellular matrix proteins, thereby stimulating and stabilizing matrix assembly[14-15]. The *SMOC2* gene is predominantly expressed in the extracellular matrix of ocular tissues, including ciliary muscles, corneal keratocytes, and trabecular meshwork. In glaucomatous eyes, elevated levels of matrix metalloproteinases are observed compared to healthy ocular tissue. The *SMOC2* gene is also involved in collagen synthesis, establishing a linkage with the development of glaucoma[15-16]. *SMOC2* variant rs13208776 is strongly associated with primary glaucoma cases and increases susceptibility to POAG and primary angle-closure glaucoma (PACG). Given the significant correlation of *SMOC2* glycoprotein with primary glaucoma, *SMOC2* single nucleotide variants (SNVs) may have a notable association with POAG predisposition[15]. Therefore, this study was conducted to investigate the possible role of *SMOC2* gene variants rs13208776 and rs2255680 in the susceptibility to POAG development in both familial and non-familial patients within the Pakistani population.

PARTICIPANTS AND METHODS

Ethical Approval It was a cross-sectional study conducted at the Institute of Biotechnology and Genetics Engineering, University of Sindh, and Molecular Biology and Genetics Department, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan between years 2020 and 2023, after the approval from the Institutional Ethics Committee, (Approval No.1396). Written informed consent was obtained from all participants prior to their inclusion in the study. No stipend or financial compensation was provided to participants for their involvement in the study.

Inclusion and Exclusion criteria All familial and non-familial individuals affected by POAG were included. Glaucoma types other than POAG, systemic and autoimmune diseases, and signs of intracranial diseases causing optic nerve atrophy on imaging were excluded from study.

Table 1 Primers sequences for ARMS-PCR and PCR-RLFP of *SMOC2* gene

Primer	Primer sequence
For ARMS PCR	
FO	5′-TGAGGCCAATTAGACCGTGCTAAACT-3′
RO	5′-AACTAAAATTAAATTCTAAGCTGCAAGACG-3′
FI	5′-TCAGCCTTCTGCATTTTAGAAGTTAATTC-3′
RI	5′-TTAACAGCATTGACACACTCAAAACG-3′
For PCR RFLP	
R	5′-GTCTCCGGTTTAAGGGAGA-3′
F	5′-CTCAGAAATTGGCACCCTCT-3′

F: Forward; R: Reverse; O: Outer; I: Inner; ARMS: Amplification refractory mutation system; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism.

Clinical Evaluation of POAG Patients A total of 212 POAG patients were enrolled, comprising *n*=124 familial and *n*=88 non-familial patients. All participants were recruited from the Institute of Ophthalmology and clinics in Hyderabad and Jamshoro, Pakistan. Ophthalmologic tests, including anterior segment examination, IOP measurement, visual field test, visual acuity (VA), cup-to-disc ratio (C/D ratio), best-corrected visual acuity (BCVA), and anterior chamber angle assessment, were conducted by a glaucoma specialist. Air-puff tonometer and Goldman applanation tonometer were used for IOP measurement, slit lamp bio microscopy was employed for anterior segment disease examination, fundoscopy for C/D ratio assessment, and anterior chamber angle evaluation was performed using gonioscopy (Goldman single mirror Gonioscopy lens). Defects in the field of vision were examined through automated perimetry (Goldman perimeter). Optical coherence tomography was utilized for examination of peripapillary nerve fiber defect, optic nerve head, and depth of anterior chamber angle.

Analysis of *SMOC2* Gene Variants For genetic analysis, whole blood was collected from all study participants and stored at -80°C in ethylenediaminetetraacetic acid vacutainers for DNA extraction. The DNA extraction was performed using a standard non-organic method[17], and the quantification and quality assessment of the extracted DNA were conducted on a 0.8% agarose gel.

Amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) was utilized for the analysis of *SMOC2* gene variant rs2255680, while PCR-restriction fragment length polymorphism (RFLP) was conducted for the analysis of rs13208776 variant. The primers used in these PCR techniques were designed and developed using the online software Primer1 (Table 1).

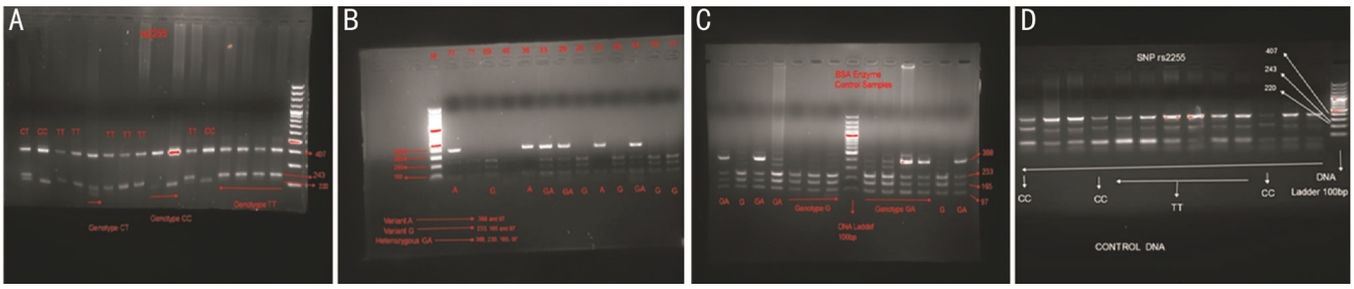

Figure 1 PCR conditions A: Genotyping of variant rs2255680, bands size: IC: 407, CC: 243, and TT: 220, by ARMS-PCR; B: Genotyping of variant rs13208776, bands size: GG: 233, 165, 97; GA: 388, 233, 165, 97 by PCR-RFLP; C: Genotyping of control variant rs13208776 by PCR; D: Genotyping of control variant rs2255680 by PCR. ARMS: Amplification refractory mutation system; PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; IC: Internal control.

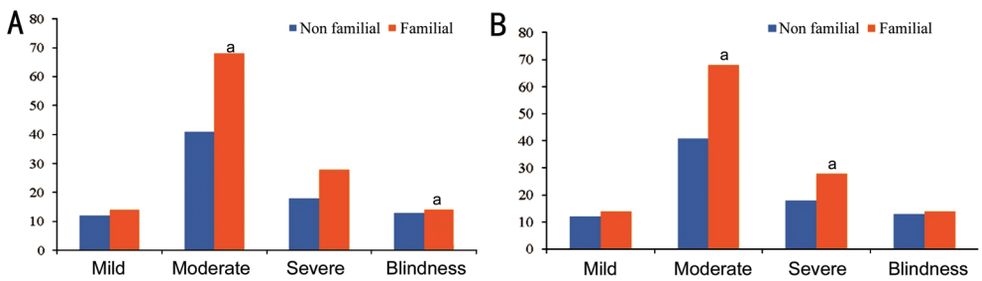

Figure 2 Comparison of visual acuity among familial and non-familial POAG patients A: Right eye; B: Left eye. POAG: Primary open angle glaucoma. [a]Statistically significant difference in visual acuity between the familial and non-familial POAG patients.

For ARMS assay (rs2255680), a total volume of 20 μL contained DNA (3 μL), dNTPs (2 μL), Taq polymerase (0.5 μL), inner primers (1 μL each), outer primers (2 μL each), and buffer (2 μL). To detect rs13208776 variant, PCR-RFLP involved a total volume of 25 μL, contained DNA (4 μL), dNTPs (2.5 μL), Taq polymerase (0.4 μL), forward and revers primers (1.2 μL each), and buffer (2.5 μL) followed by digestion with restriction enzyme BsaHI. PCR reaction mixture consisted of PCR product (10 μL), 10× buffer G (2 μL), nuclease free water (18 μL), and enzyme BsaHI (1 μL), mixed and incubated at 37°C for 16h.

PCR conditions were initial denaturation (95°C with 5 min), 45 cycles at 95°C with 30s, annealing with 45s, and extension at 72°C with 45s, and a final extension at 72°C with 10 min. Annealing temperature was 56°C for ARMS assay, and 54°C for PCR-RFLP. PCR products were resolved on a 2% agarose gel (Figure 1).

Statistical Analysis The association of SNVs between familial and non-familial groups in different genetic models, and the haplotype association test, were conducted using logistic regression through SNPStat software[18]. Odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the SNVs association between familial and non-familial groups. The *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Findings The mean age of familial POAG patients was 50.92±9.12y, with 78 males and 46 females. The mean age of non-familial POAG patients was 53.14±13.44y, with 52 males and 36 females. The IOP and C/D ratio for both the left and right eyes were higher in non-familial compared to familial POAG patients, and significant differences were noted in IOP (left eye only) and C/D ratio (both eyes; Table 2).

Table 2 Comparison of IOP and C/D ratio between familial and non-familial POAG patients

Variables	Non-familial (*n*=88)	Familial (*n*=124)	*P*
IOP (right), mm Hg	19.1±6.6	17.1±6.5	0.072
IOP (left), mm Hg	22.9±7.9	19.7±5.9	0.009
C/D ratio (right)	0.54±0.3	0.4±0.3	0.012
C/D ratio (left)	0.6±0.3	0.48±0.3	0.005

C/D: Cup-to-disc; IOP: Intraocular pressure; POAG: Primary open angle glaucoma.

Significantly higher frequency of complete blindness (P<0.01) was noted among POAG familial compared to non-familial patients in right eye. Similarly, there was a statistically higher moderate VA in familial POAG patients, while frequencies for mild and severe VA were equal in both groups (Figure 2). There were non-significant differences in VA (both eyes) between male and female POAG patients (P>0.05). Age-wise analysis showed a statistically insignificant difference among POAG patients.

For the BCVA, familial patients exhibited a significantly higher frequency for mild VA compared to non-familial POAG patients (P<0.001) in the right eye. However, no difference

Table 3 Allele and genotype frequencies of *SMOC2* gene variants in non-familial and familial POAG patients

Models	Genotypes/alleles	Non-familial (*n*=88)	Familial (*n*=124)	OR (95%CI)	*P*
rs2255680					
Alleles	C	101 (57)	130 (52)	1.00	0.31
	T	75 (43)	118 (48)	0.81 (0.55-1.21)	
Co-dominant	CC	41 (46.6)	48 (38.7)	1.00	
	CT	19 (21.6)	34 (27.4)	0.65 (0.33-1.32)	0.47
	TT	28 (31.8)	42 (33.9)	0.78 (0.41-1.47)	
Dominant	C/C	41 (46.6)	48 (38.7)	1.00	0.25
	C/T-T/T	47 (53.4)	76 (61.3)	0.72 (0.42-1.26)	
Recessive	C/C-C/T	60 (68.2)	82 (66.1)	1.00	0.75
	T/T	28 (31.8)	42 (33.9)	0.91 (0.51-1.63)	
Over-dominant	C/C-T/T	69 (78.4)	90 (72.6)	1.00	0.33
	C/T	19 (21.6)	34 (27.4)	0.73 (0.38 -1.39)	
Log-additive				0.87 (0.64-1.20)	0.41
rs13208776					
Alleles	G	136 (77)	163(66)	1.00	0.016
	A	40 (23)	85 (34)	0.56 (0.36-0.87)	
Co-dominant	G/G	53 (60.2)	53 (42.7)	1.00	
	G/A	30 (34.1)	57 (46)	0.53 (0.29-0.94)	0.033
	A/A	5 (5.7)	14 (11.3)	0.36 (0.12-1.06)	
Dominant	G/G	53 (60.2)	53 (42.7)	1.00	0.012
	G/A- A/A	35 (39.8)	71 (57.3)	0.49 (0.28-0.86)	
Recessive	G/G-G/A	83 (94.3)	110 (88.7)	1.00	0.15
	A/A	5 (5.7)	14 (11.3)	0.47 (0.16-1.37)	
Over-dominant	G/G-A/A	58 (65.9)	67 (54)	1.00	0.082
	G/A	30 (34.1)	57 (46)	0.61 (0.35-1.07)	
Log-additive				0.56 (0.36-0.88)	0.0097

OR: Odds ratio; CI: Confidence interval; POAG: Primary open angle glaucoma.

Table 4 Haplotype and linkage disequilibrium analysis of *SMOC2* gene variants in familial and non-familial POAG patients

Haplotype	rs2255680	rs13208776	Non-familial	Familial	Total frequency	OR (95%CI)	*P*
1	C	G	0.5739	0.5242	0.5448	1.00	-
2	T	A	0.2273	0.3427	0.2948	0.48 (0.29-0.79)	0.004
3	T	G	0.1989	0.1331	0.1604	2.28 (1.22-4.25)	0.01

Global haplotype association *P*-value: 0.0011. Linkage disequilibrium analysis: D'=0.9997, r^2=0.5001; *P*<0.001; OR: Odds ratio; CI: Confidence interval; POAG: Primary open angle glaucoma.

was observed for moderate VA between both groups. Severe and blind cases were not corrected for VA. For the left eye, low frequency of BCVA was noted when comparing familial and non-familial POAG patients. A significant difference was observed for moderate and severe glaucoma cases (*P*=0.001). Gender-wise, there was an insignificant difference in BCVA (both eyes). Left eye BCVA exhibited a significant correlation with age groups (below and above 40y) when compared with BCVA in the right eye.

Genotype and Haplotype Analysis The allele and genotype frequency distribution of *SMOC2* gene variants between familial and non-familial glaucoma patients are illustrated in Table 3. No significant association of *SMOC2* gene variant rs2255680 between familial and non-familial glaucoma patients was found in our population. For *SMOC2* gene variant rs13208776 the significant association was noted between familial and non-familial glaucoma groups. The homozygous G/G variant was frequent among non-familial (60.2%) whereas the heterozygous G/A variant was more frequent in familial POAG patients (46%). There were significant differences in G/A variant between familial and non-familial glaucoma patients, and the risk was decreased to 0.53-fold in non-familial glaucoma patients (OR: 0.53; 95%CI: 0.29-0.94; *P*=0.033) in codominant model and further reduced to 0.49-fold (95%CI: 0.28-0.86; *P*=0.012) in dominant model. The haplotype analysis showed the decreased risk for TA (OR: 0.48; 95%CI: 0.29-0.79; *P*=0.004) and an increased risk for TG (OR=2.28; 95%CI: 1.22-4.25; *P*=0.01) haplotypes (Table 4).

DISCUSSION

POAG, a common optic nerve neuropathy with multifactorial origins, affects a significant portion of the global population. More than 79.6 million people worldwide have glaucoma, with an expected rise to 111.8 million by 2040[19-20]. Genetic investigations have offered promising indications for genetic contribution to the glaucoma pathogenesis. Genes linked to increased IOP and POAG risk include *CAV1*, *ABCA1*, *SIX1/SIX6*, *ATXN2*, *CDKN2B-AS1*, *TMCO1*, *AFAP1*, *ARHGEF12*, *GMDS*, *FOXC1*, *GAS7*, and *TXNRD2*. However, differences in both risk and genetic factors exist due to ethnic and geographical variations[21-24]. Therefore, this study investigated the role of *SMOC2* gene variants (rs2255680 and rs13208776) in both familial and non-familial POAG Pakistani patients.

In our study, the *SMOC2* gene variant rs13208776 showed a significant association between familial and non-familial glaucoma patients. The homozygous G/G variant was more common among non-familial (60.2%), while the heterozygous G/A variant was more prevalent in familial POAG patients (46%). The risk decreased by 0.53-fold in non-familial glaucoma patients in the codominant model and further reduced to 0.49-fold in the dominant model. In contrast to our findings, Al-Dabbagh *et al*[15] reported a strong correlation between the G/A genotype of the *SMOC2* gene variant rs13208776 and the risk of primary glaucoma development in the non-familial cases of Saudi Arabian population, although their study showed increased risk with PACG rather than POAG. In contrast, another study found no significant relationship between the polymorphism of the *SMOC2* gene and an increased risk of macular degeneration among the Jordanian Arab population[25].

SMOC2 is an extracellular glycoprotein comprising two domains of EF-hand calcium-binding and thyroglobulin type-I, a follistatin-like domain, and a signal peptide. The *SMOC2*, with its multiple domains, may act as a protease inhibitor as the thyroglobulin domains have been observed to bind and inhibit different proteases, including cysteine and serine proteases. Similarly, the follistatin-like domain typically functions as serine protease inhibitors. Dysregulation of protease activity has been implicated in abnormal matrix accumulation, potentially contributing to the glaucoma pathogenesis. It's hypothesized that *SMOC2* gene variants could lead to reduced levels of protease inhibitors, resulting in elevated levels of matrix metalloproteinases. These play a role in remodeling the trabecular meshwork in the eye, affecting aqueous humor outflow and increasing IOP[15,26-28].

SMOC2 plays a role in various cellular processes such as cell cycle regulation, cell attachment and movement, angiogenesis, fibrosis, and tissue calcification[26]. Gene variants of *SMOC2* have been associated with generalized vitiligo[29] autoimmune thyroid disease, papillary thyroid carcinoma[30], as well as dental disorders such as oligodontia and microdontia[31-32]. Additionally, *SMOC2* expression has been studied in cancers like colon[33], liver[34], lung[35], and endometrial cancers[36]. While one study has indicated its role in PACG in non-familial cases[15], the impact of haplotypes and familial genetic components remains unexplored. To the best of our knowledge, this study is the first to investigate *SMOC2* gene variants in Pakistani glaucoma patients and compare genotypes in both familial and non-familial patients. Furthermore, it reports an increased risk with the TG haplotype and a decreased risk with the TA haplotype in POAG development. These findings not only enhance our understanding of genetic factors associated with glaucoma but also offer new insights into potential differences in genetic predisposition between familial and non-familial cases of the disease. Identifying genetic variants associated with susceptibility to glaucoma can provide valuable insights into the diverse risk factors contributing to the development of this condition. Recent research highlighting the importance of matricellular proteins in glaucoma suggests that further studies on genes encoding these proteins could offer promising avenues for therapeutic intervention[15]. The Pakistani population is diverse, with multiple ethnic groups, each possessing distinct genetic lineages, contributing to genetic diversity[37]. Additionally, the high rate of consanguinity in Pakistan[22] provides a unique opportunity to investigate the genetic aspects of primary glaucoma. Further research on various genes through association studies could elucidate the complex mechanism of POAG.

The present study also investigates and compares the demographics and clinical variables among patients with familial and non-familial POAG. According to the previous studies, old age is a significant risk factor for POAG progression[38-39]. Consistent to that we found that 85% of familial and 90% of non-familial patients were above 40y of age. Previous study conducted on 107 new diagnosed glaucoma patients reported an association between old age and visual field defects. Moreover, patients with a family history of glaucoma showed 10-fold higher risk for visual field defects and loss of visual field at the time of presentation[40]. We also observed that 63% of familial and 59% of non-familial POAG patients were male. Previous studies have also reported a male dominance pattern among familial and non-familial glaucoma patients[41]. In our study, IOP and C/D ratio were markedly higher in non-familial glaucoma than in familial POAG patients. No significant difference was observed for C/D ratio and IOP between male and female patients in either study groups. Elevated IOP and C/D ratio were noted in glaucoma

patients older than 40y compared to younger patients. Further studies are necessary to distinguish clinical findings between familial and non-familial POAG patients.

In conclusion, the present study suggests an association of *SMOC2* gene variant rs13208776 with POAG between familial and non-familial patients. The heterozygous G/A variant was more frequent in familial POAG patients, suggesting a distinction in risk compared to non-familial cases among Pakistani population. Expanding research to include diverse ethnicities and populations will enhance the validity of our study's findings. Moreover, utilizing next-generation sequencing (NGS) in future studies may uncover a broader spectrum of potential pathogenic variants, necessitating functional analysis for comprehensive characterization.

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