BMC Ophthalmology

RESEARCH

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Suggestive evidence of male specific genetic association of *IL8* -251T>A promoter polymorphism with primary angle closure glaucoma in a north Indian Punjabi population

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Abstract

Background Overproduction of IL-8 in the retina and optic nerve may affect the survival of retinal ganglion cells (RGCs) and contribute to axonal damage in glaucoma. The -251T > A functional variant in the promoter region of the *IL8* gene is known to affect its transcriptional activity, as demonstrated in in vitro assays.

Methods The present study investigates the genetic association of this polymorphism with primary glaucoma in a North Indian Punjabi cohort. A total of 226 primary open angle glaucoma (POAG), 132 primary angle closure glaucoma (PACG) patients and 424 matched controls were recruited. Genotyping was performed using the restriction length polymorphism (RFLP) method.

Results Association analysis was done by PLINK software and appropriate corrections were applied for potential confounding variables. No significant differences in allele or genotype frequency were observed in pooled cases when compared to controls. However, after segregating the data into POAG and PACG and based on sex, significant difference was observed in the allele frequency among PACG males and control male subjects ($p = 0.014$, OR = 0.52, 95% CI = 0.31–0.88). The heterozygous 'AT' genotype provided 0.46 times protection for PACG among males ($p = 0.028$, OR = 0.46, 95% CI = 0.23–0.92). Genetic model analysis revealed that the combination of 'AT + AA' genotypes conferred protection against the development of PACG among male subjects under a dominant model ($p = 0.013$, OR = 0.44, 95% CI = 0.23–0.84; $\boldsymbol{p}_{corr} = 0.003$, OR = 0.30, 95% CI = 0.14–0.67).

Conclusions This study suggests a genetic association of the -251T > A variant with PACG in males in the targeted population and highlights the importance of sex- specific analysis in glaucoma. The biological mechanisms underlying these differences should be further explored to better understand the observed sex bias in PACG.

Keywords Primary open angle glaucoma, Primary angle closure glaucoma, Genetic association, IL8, Retinal ganglion cells

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Background

Glaucoma, a multifactorial optic neuropathy, is a major public health problem in the ageing population [1]. The vision loss is irreversible and occurs due to the gradual degeneration of retinal ganglion cells (RGCs) [1]. Primary open angle glaucoma (POAG)/angle closure glaucoma (PACG) are the most common clinical forms and account for bulk of the prevalence. Familial segregation and heritability studies consistently point towards contribution of host genetics to glaucoma development and progression [1, 2]. Genetic linkage and association studies have identified susceptibility loci for both POAG and PACG, yet more genes need to be identified to account for the missing heritability and to fully understand the genetic basis of the disease [2]. Further, the genetic spectrum of PACG is not as widely investigated as that of POAG. Among the different candidate genes implicated in the pathogenesis of glaucoma, those affecting the survival of RGCs are important [2]. Interleukin (IL)-8, a member of CXC family of chemokines, has been observed at higher levels in the aqueous humor (AH) of POAG patients [3, 4]. Kuchtey et al., in 2010, demonstrated severe visual field defects in POAG patients with higher IL-8 levels in the AH than the patients with mild visual field defects [4]. Also, an elevated levels of serum IL-8 levels have been observed in other ocular inflammatory diseases like proliferative diabetic retinopathy and intermediate uveitis [5]. Recent studies have highlighted the role of other cytokines such as IL-4, IL-15, VEGF, TNF-α, and TGF-β2 in POAG, which were found to be elevated in the AH and tear samples of glaucoma patients. These findings emphasize the importance of inflammatory markers in the pathogenesis of gaucoma. , However, the role of IL-8, especially in PACG, has not been widely explored [6, 7].

IL-8 is produced locally in the retina and optic nerve, and its responses are mediated by IL-8 receptors (CXCR1 and CXCR2), which are widely expressed by neurons and glial cells [8]. Overproduction of IL8 in the ocular microenvironment could contribute to RGC death in glaucoma because of its putative neurotoxic role [9, 10]. A functional variant at -251 base pairs upstream of transcriptional start site influences its production, as demonstrated by in vitro assays [11–13], and hence may act as a genetic risk factor for disease development and progression. A significant association of -251T > A (rs4073) promoter variant has been reported with bronchiolitis, atrophic gastritis, distal gastric cancer, breast cancer and neurodegenerative disorders like Alzheimer's disease (AD) and Parkinson's disease [14–16]. However, this variant has not been investigated as a genetic risk factor for glaucoma. Therefore, in the present study we investigate for the first time if -251T > A (rs4073) functional polymorphism modifies genetic susceptibility to POAG/PACG in a North Indian Punjabi cohort.

Materials and methods

Study participants were recruited from Baba Deep Singh Eye hospital, Amritsar, India, after obtaining written informed consent. The study was approved by the Institutional Ethics Committee of Guru Nanak Dev University, Amritsar (No.702/HG/28/06/2019) and the study protocols were according to the tenets of the Declaration of Helsinki. A total 358 primary glaucoma cases (226 POAG and 132 PACG) and 424 controls were enrolled. The power of the study was calculated by using the CaTS power calculator (Skol et al.. 2006) and the sample size provided ≥80% power to detect allelic association for an effect size (odds ratio) of 1.5 at $p=0.05$ for a variant allele at frequency ≥ 0.2. POAG patients had intraocular pressure (IOP) ≥ 21 mm Hg in either eye, as tested using Goldmann Applanation Tonometry. Glaucomatous ocular nerve head (ONH) damage was defined as a vertical cup to disc ratio (VCDR) of ≥ 0.7, adjudged clinically on slit lamp biomicroscopy and confirmed using contrast-enhanced fundus photographs on optical coherence tomography. Individuals with known chronic systemic inflammatory, autoimmune or immunosuppressive diseases, as well as pre-existing ocular diseases (diabetic retinopathy, age-related macular degeneration), were excluded from the study. Individuals with a history of taking corticosteroids, non-steroidal anti-inflammatory drugs, ortopical steroids or prostaglandin analogues were also not recruited. Individuals suffering from any cardiovascular disease, and cancer were also excluded, as rs4073 has been shown to affect susceptibility to above mentioned diseases. Further details regarding the inclusion criteria are mentioned in detail elsewhere [17]. PACG cases were recruited based on the above-described criteria, along with the presence of a 180° closed angle in which the trabecular meshwork was not visible on gonioscopy. A total of 424 unrelated age- and sex- matched subjects without a family history of glaucoma were also recruited. The controls had IOP ≤ 21 mmHg, normal visual fields, and normal optic nerve heads with a VCDR of < 0.5.

Genomic DNA was extracted from venous blood (1 ml) using the standard organic method [18]. Quantification of extracted DNA was performed using ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The target promoter region of 348 bp flanking the −251T > A variant was amplified with primer sequences and reaction conditions mentioned previously [19]. The PCR products were digested with 3 units of Mfe-I (New England Biolabs) restriction endonuclease for 12 h at 37 °C. The digested products were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide (10 mg/ml) (GeNeiTM), and genotypes were scored based on the restriction fragment length polymorphism (RFLP) pattern (Fig. 1).

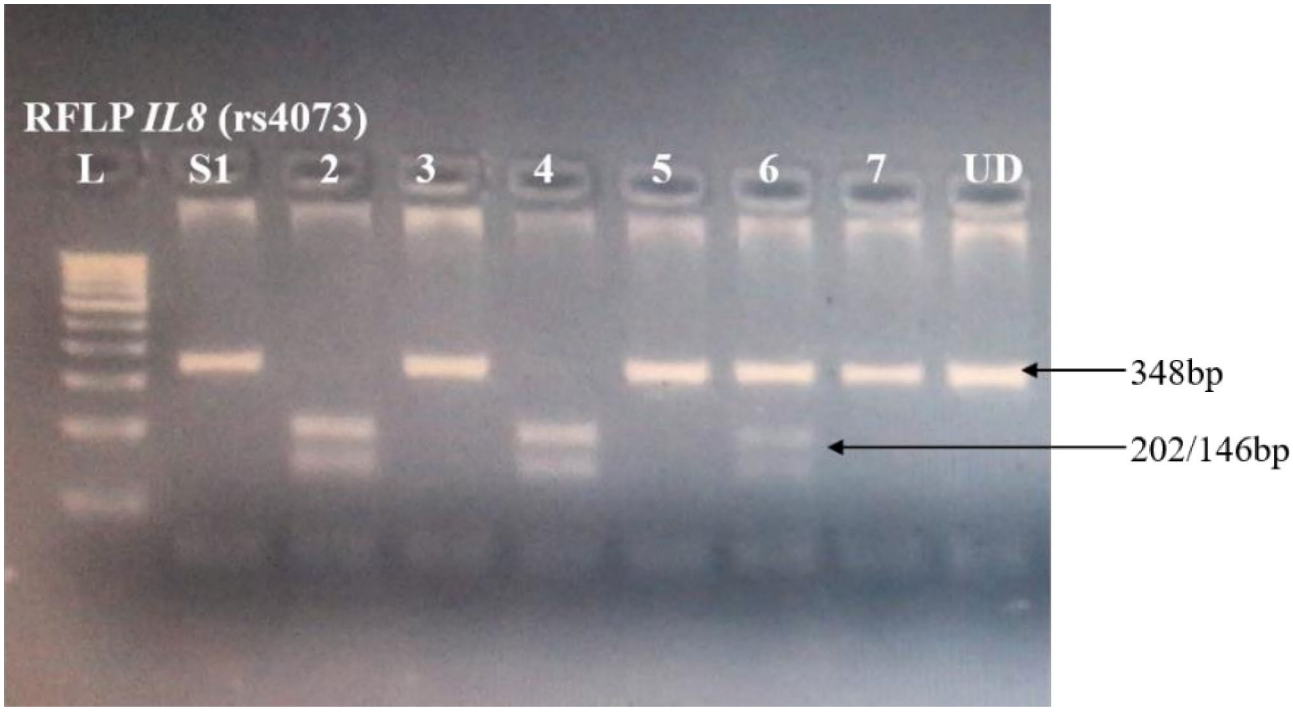

Fig. 1 Agarose gel (3.0%) depicting the digested products of the *IL8* -251T > A (rs4073) polymorphism. Lane 1 includes the 100 bp molecular ladder; S1, 3, 5 and S7 = TT homozygous (348 bp); S2 and S4 = AA homozygous (202/146 bp) and S6 = AT heterozygous (348/202/146 bp) genotype. Last well: undigested (UD) PCR product

Table 1 Baseline demographic and clinical parameters among cases and controls

Factors	Cases (Mean ± SD)	Controls (Mean ± SD)	*p*-value
Age	59.92 ± 12.81	59.46 ± 11.56	0.573
VCDR Right eye	0.72 ± 0.39	0.23 ± 0.08	**0.000***
VCDR Left eye	0.73 ± 0.39	0.25 ± 0.09	**0.000***
IOP Right eye	22.41 ± 8.89	14.09 ± 3.51	**0.000***
IOP Left eye	22.90 ± 9.89	14.24 ± 3.43	**0.000***

p value < 0.05* was considered to be statistically significant

Descriptive statistical analysis for the demographic and clinical characteristics for study participants was performed using GraphPad Prism 5.0. Association analyses were performed using PLINK software (v1.07). *p*-values of less than 0.05 were considered to be statistically significant. To investigate the association of the variant with IOP and VCDR, the values for the right eye was chosen arbitrarily (as the mean for both eyes were the same, Table 1) for ANOVA. Binary logistic regression was applied to account for possible confounders (age and family history) using SPSS software.

Results

Demographic and clinical characteristics of the study participants

Males had a higher prevalence of POAG (62.39%) while females showed a higher prevalence of PACG (61.36%) (Table 1; Fig. 2) in accordance with several epidemiological surveys where higher prevalence of POAG has been reported in males while females are at a higher risk for PACG [11]. The mean VCDR for the right and left eye in cases was 0.72 and 0.73, respectively, which was higher with respect to control subjects (0.23 and 0.25) for both right and left eyes, respectively. The mean IOP in both eyes was higher in cases as compared to controls (Table 1) consistent with the inclusion criteria for recruitment of patients.

Genotyping of -251T > A variant

The presence of the 'A' allele creates a site for Mfe-I. Two bands of 202 and 146 bp were observed after digesting the PCR amplicon if 'A' allele was present at this promoter site. The bands were visualized on a 3.0% agarose gel (Fig. 1.). The frequency of TT, AT and AA genotypes did not vary among combined cases, POAG and PACG subjects with respect to controls (Table 2). No association of the variant was observed either with POAG or PACG (Table 2). However, stratification of the data based on sex revelaed that the minor allele 'A' conferred a 0.52-fold protection ($p = 0.014$, OR = 0.52) and the 'AT' genotype provided a 0.46-fold protection ($p = 0.028$, OR = 0.46) in males with PACG (Table 3). No significant difference in allele or genotype frequency was observed among males with POAG or females with POAG or PACG. A dominant genetic model revealed a statistically significant association of rs4073 with PACG in males ($p = 0.013$,

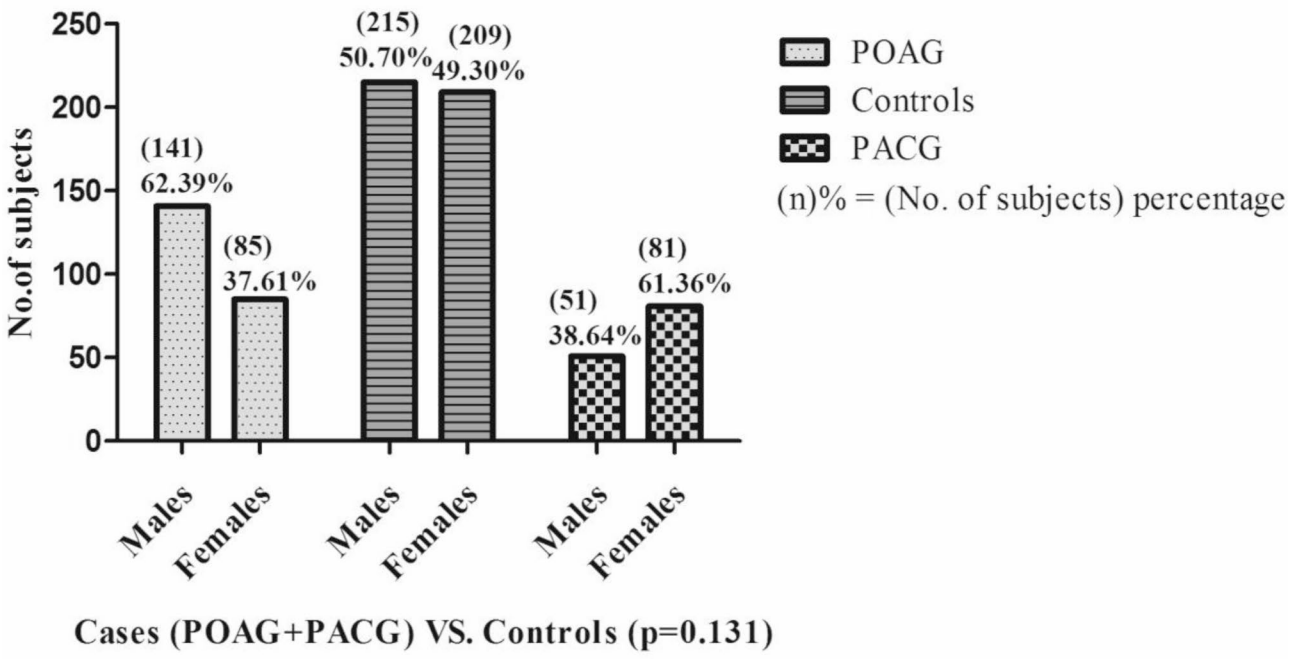

Fig. 2 Frequency distribution of males and females among POAG and PACG cases with respect to control subjects

OR=0.44; p_{corr}=0.003, OR=0.30), which remained significant after correcting for confounding variables. The genotypes at the promoter polymorphism of *IL8* were also compared for their distribution pattern in relation to VCDR and IOP. No significant difference in the mean values of VCDR and IOP was observed among patients with AA, AT and TT genotypes at -251T>A locus (Table S1).

Discussion

The present study reports an association of the *IL8* -251T>A variant with PACG among male subjects in a North Indian Punjabi cohort. While this polymorphism has been investigated in other retinal and neurodegenerative disorders, it has not been explored extensively in glaucoma [20]. Evidence suggests the upregulation of pro-inflammatory cytokine genes, including *IL8*, in neurodegenerative diseases. Immunohistochemistry assays conducted on human eyes indicates that IL8 is endogeneously produced by the retina and optic nerve [4]. Another local source of this chemotactic cytokine in the eyes are the trabecular meshwork endothelial (TME) cells, which also produce significant amounts of CXCL6 and monocyte chemoattractant protein-1 (MCP-1) [21]. By producing these cytokines, TME cells can regulate the permeability of Schlemm's canal endothelial (SCE) cells, thereby affecting the homeostatic mechanisms involved in the AH outflow [22].

Recent studies demonstrated that IL-8 plays a critical role in inducing TM cell contractibility, fibrogenic activity, and plasticity [23]. Clinical findings are in agreement with these in vitro studies, where elevated concentrations of IL-8 have been observed in the AH of glaucoma patients, including those with the PACG phenotype [4, 24]. Interestingly, a positive correlation between levels of IL-8 and TGF-β has also been reported, suggesting that these cytokines may reflect alterations in extracellular matrix (ECM) at the trabecular meshwork (TM) in both open angle and pseudoexfoliative glaucoma (PEX) eyes [25]. TGF-β is a well- known regulator of ECM remodeling and fibrosis. IL-8 and TGF-β may therefore synergistically contribute to ECM alterations at the TM, and their combined action could create a more fibrotic and less permeable outflow pathway, which is a hallmark of glaucoma pathogenesis. IL-8 has also been implicated in the induction of senescence in TM-SC conventional outflow pathway cells [26, 27]. Thus, overproduction of IL-8 could possibly contribute to RGC death and axonal damage indirectly by affecting TM-SC cells and thereby altering the IOP dynamics. In a study by Chono et al., high IL-8 levels in AH were significantly associated with preoperative IOP and visual field defects in PEX eyes [28]. Additionally, IL-8 levels may show spatio-temporal variations depending on the stage of the disease, with elevated mRNA expression in eyes during the early stages of PEX glaucoma but not in the later stages [29].

Promoter region polymorphisms can significantly affect cytokine expression levels by altering transcription factor binding sites, thereby influencing the susceptibility to various diseases. The *IL8* -251T>A polymorphism, located in the promoter region of the *IL8* gene, has been studied in the context of several retinal

Table 2 Distribution of allele/genotype frequencies and genetic model analysis for *IL8* (-251T > A) rs4073 SNP among all cases, POAG/PACG subtypes with respect to controls

SNP – 251T > A (rs4073)				
Genotype/Allele	**All Cases *n* = 358(%)**	**Control *n* = 424(%)**	***p*-value**	**OR (95% CI)**
T	503 (70.25)	579 (68.28)	**Ref**	
A	213 (29.75)	269 (31.72)	0.399	0.92 (0.72–1.16)
TT	180 (50.28)	202 (47.64)	**Ref**	
AT	143 (39.94)	175 (41.27)	0.569	0.91 (0.68–1.23)
AA	35 (9.78)	47 (11.09)	0.465	0.83 (0.51–1.35)
AT + AA > TT	178 > 180	222 > 202	0.462	0.89 (0.67–1.19)
Dominant model			0.470	0.89 (0.65–1.21)
AA > AT + TT	35 > 323	47 > 377	0.552	0.86 (0.54–1.37)
Recessive model			0.765	0.92 (0.55–1.54)
AT > AA + TT	143 > 215	175 > 249	0.706	0.94 (0.71–1.26)
Co-dominant			0.583	0.91 (0.66–1.26)
	POAG *n* = 226(%)	**Control *n* = 424(%)**		
T	307 (67.92)	579 (68.28)	**Ref**	
A	145 (32.08)	269 (31.72)	0.895	1.01 (0.79–1.29)
TT	107 (47.35)	202 (47.64)	**Ref**	
AT	93 (41.15)	175 (41.27)	0.985	1.00 (0.71–1.41)
AA	26 (11.50)	47 (11.09)	0.873	1.04 (0.61–1.78)
AT + AA > TT	119 > 107	222 > 202	0.942	1.01 (0.73–1.39)
Dominant model			#0.859	1.03 (0.71–1.50)
AA > AT + TT	26 > 200	47 > 377	0.871	1.04 (0.62–1.73)
Recessive model			#0.760	1.09 (0.61–1.94)
AT > AA + TT	93 > 133	175 > 249	0.975	0.99 (0.71–1.38)
Co-dominant			#0.986	0.99 (0.68–1.45)
	PACG *n* = 132(%)	**Control *n* = 424(%)**		
T	196 (74.24)	579 (68.28)	**Ref**	
A	68 (25.76)	269 (31.72)	0.065	0.74(0.54–1.01)
TT	73 (55.30)	202 (47.64)	**Ref**	
AT	50 (37.88)	175 (41.27)	0.264	0.79 (0.52–1.19)
AA	9 (6.82)	47 (11.09)	0.102	0.52 (0.24–1.13)
AT + AA > TT	59 > 73	222 > 202	0.124	0.73 (0.49–1.08)
Dominant model			#0.122	0.70 (0.44–1.09)
AA > AT + TT	9 > 123	47 > 377	0.159	0.58 (0.27–1.23)
Recessive model			#0.355	0.67 (0.29–1.55)
AT > AA + TT	50 > 82	175 > 249	0.487	0.86 (0.58–1.29)
Co-dominant			#0.309	0.78 (0.49–1.24)

* Indicates significant *p*-value ≤ 0.05; OR = Odds Ratio, CI = Confidence Interval, Ref = Reference

# indicates *p*-value and OR at 95% CI, after applying correction for age, sex and family history

and neurodegenerative disorders, although its role in glaucoma has not been extensively investigated. This polymorphism can potentially modulate the expression of IL-8, contributing to the inflammatory environment observed in glaucomatous eyes.

In the present study, no significant association of *IL8* -251 A > T variant was observed with either POAG or PACG overall. However, sex-based stratification of the data revealed a significant difference in allele and genotype frequencies between PACG males and control male subjects.

The differential results between PACG and POAG may be attributed to the distinct pathophysiology of these two forms of glaucoma [3, 30]. PACG is primarily driven by anatomical factors such as a narrow anterior chamber angle leading to angle closure, which results in elevated IOP [30]. In this scenario, the role of inflammation and cytokines like IL-8 could exacerbate the fibrotic changes and TM contractility, influencing the AH outflow, but the primary pathological mechanism is mechanical obstruction. In contrast, POAG is typically associated with chronic ECM remodeling and TM dysfunction. The lack of association between IL-8 and POAG may be due to the involvement of other genetic or inflammatory pathways that are more central to its pathogenesis, overshadowing the potential role of IL-8. Thus, while IL-8 may play a contributory role in both types of glaucoma, its association might be more pronounced in the context

Table 3 Sex wise distribution of allele/genotype frequencies and genetic model analysis for *IL8* -251T > A (rs4073) among POAG and PACG cases with respect to controls

Males with POAG and PACG vs. Control male subjects								
Allele/Genotype	**POAG** ***n*** **= 141 (%)**	**Control** ***n*** **= 215 (%)**	***p*-value**	**OR(CI)**	**PACG** ***n*** **= 51 (%)**	**Control** ***n*** **= 215 (%)**	***p*-value**	**OR(CI)**
T	195 (69.15)	283 (65.81)	**Ref**		80 (78.43)	283 (65.81)	**Ref**	
A	87 (30.85)	147 (34.19)	0.354	0.85 (0.62–1.18)	22 (21.57)	147 (34.19)	**0.014***	0.52 (0.31–0.88)
TT	72 (51.06)	97 (45.11)	**Ref**		33 (64.71)	97 (45.11)	**Ref**	
AT	51 (36.17)	89 (41.40)	0.270	0.77 (0.48–1.22)	14 (27.45)	89(41.40)	**0.028***	0.46 (0.23–0.92)
AA	18 (12.77)	29 (13.49)	0.596	0.83 (0.43–1.62)	4 (7.84)	29 (13.49)	0.113	0.40 (0.13–1.23)
Genetic Models								
AT + AA > TT	69 > 72	118 > 97	0.272	0.78 (0.51–1.20)	18 > 33	118 > 97	**0.013***	0.44 (0.23–0.84)
Dominant model			#0.318	0.78 (0.48–1.26)			**#0.003***	0.30 (0.14–0.67)
AA > AT + TT	18 > 123	29 > 186	0.843	0.93 (0.49–1.76)	4 > 47	29 > 186	0.277	0.54 (0.18–1.62)
Recessive model			#0.980	0.99 (0.48–2.01)			#0.687	0.79 (0.26–2.43)
Females with POAG and PACG vs. Control female subjects								
Allele/Genotype	**POAG** ***n*** **= 85 (%)**	**Control** ***n*** **= 209 (%)**	***p*-value**	**OR(CI)**	**PACG** ***n*** **= 81 (%)**	**Control** ***n*** **= 209 (%)**	***p*-value**	**OR (CI)**
T	112 (65.88)	297 (71.05)	**Ref**		116 (71.60)	297 (71.05)	**Ref**	
A	58 (34.12)	121 (28.95)	0.217	1.27 (0.86–1.86)	46 (28.40)	121 (28.95)	0.895	0.97 (0.65–1.45)
TT	35 (41.18)	106 (50.72)	**Ref**		40 (49.39)	106 (50.72)	**Ref**	
AT	42 (49.41)	85 (40.67)	0.137	1.49 (0.87–2.54)	36 (44.44)	85 (40.67)	0.671	1.12 (0.65–1.91)
AA	8 (9.41)	18 (8.61)	0.525	1.34 (0.53–3.36)	5 (6.17)	18 (8.61)	0.569	0.73 (0.25–2.11)
Genetic Models								
AT + AA > TT	50 > 35	103 > 106	0.138	1.47 (0.88–2.44)	41 > 40	103 > 106	0.838	1.05 (0.63–1.76)
Dominant model			#0.147	0.15 (0.85–2.82)			#0.656	1.13 (0.64–2.01)
AA > AT + TT	8 > 77	18 > 191	0.826	1.10 (0.46–2.64)	5 > 76	18 > 191	0.492	0.69 (0.25–1.94)
Recessive model			#0.598	1.30 (0.48–3.47)			#0.359	0.55 (0.15–1.95)

* Indicates significant *p*-value; OR = Odds Ratio, CI = Confidence Interval, Ref = Reference

p-value and OR at 95% CI, after applying correction for age and family history

of the acute IOP changes seen in PACG. The interaction between inflammation and mechanical blockage likely provides an additive effect, which may explain the observed association of the *IL8* -251T>A polymorphism in PACG.

The A allele and the heterozygous AT genotype provided 0.52- and 0.46-times protection against PACG among males ($p=0.014$; 0.028), respectively. Genetic model analysis after segregation revealed that the combination of 'AT+AA' genotypes conferred protection against the development of PACG among male subjects under a dominant model, indicating that the presence of at least one A allele is associated with a reduced risk of PACG in males.

The lack of significant variation in the AA genotype could be attributed to its relatively low frequency in the studied population. This aligns with the known allele frequency distribution of the *IL8* -251T>A polymorphism. The observed protective effects were likely driven by the more common heterozygous AT genotype, which provided higher statistical power for detecting associations. In contrast, the low occurrence of the AA genotype may have limited our ability to detect significant differences between PACG subjects and controls, particularly given the small sample size of PACG cases. The dominant model analysis (AT+AA vs. TT) further supports that the presence of at least one A allele is sufficient to confer a protective effect, explaining why the AT genotype showed significant association while the AA genotype did not.

The molecular reasons for this male-specific association in PACG may involve several factors. Higher levels of testosterone in males may interact with the *IL8* promoter polymorphism, influencing gene expression and modifying the inflammatory response and ECM remodeling in the TM. Sex hormones like testosterone and estrogen have immunomodulatory effects, with testosterone generally suppressing certain immune responses [30–32]. In monocytes, testosterone has been shown to inhibit the release of IL-8 [33]. IL-8 is a pro-inflammatory cytokine, and reduced expression of *IL8* in males carrying the A allele could lead to lower levels of inflammation in the TM, reducing the risk of developing PACG.

A sex specific association of this promoter region polymorphism in *IL8* has also been reported in coronary artery diseases (CAD wherein the allele 'T' confers a 1.85-fold higher risk towards CAD among male subjects ($p<0.001$; OR=1.85) [34]. This suggests that the *IL8* promoter polymorphism may have broader implications in male-specific disease susceptibility. In the present study, although we did not get significant association of this functional variant with either POAG or PACG in the combined analysis, we obtained evidence of an association with PACG in males after stratified analysis. However, the study has some limitations. Firstly, the relatively small sample size of the PACG cohort ($n=51$) limits the statistical power of the study. Although Bonferroni correction was applied to minimize false positives, the small sample size limits the generalizability of our findings. Secondly, while we observed a significant association in PACG males, we did not have sufficient data to account for potential confounding factors such as smoking, lifestyle, or other environmental influences that could modulate IL-8 levels. Additionally, we were unable to directly measure IL-8 levels in AH due to the unavailability of samples, which would have provided more insight into the functional impact of the *IL8* polymorphism. Finally, our study focused on a single population, and the findings may not be universally applicable to other ethnic or geographical groups. Further studies with more comprehensive ocular measurements, including axial length, anterior chamber depth and lens thickness, are needed to fully explore the potential relationship between *IL8* variants and these anatomical parameters in glaucoma patients. Nevertheless, the pilot result of a male specific association of -251 A>T variant with PACG provides a strong impetus for further validation of these results in larger multiethnic cohorts and to establish a more definitive understanding of the underlying genetic causes of a strong sex bias observed in glaucoma. It will be interesting to investigate the correlation between the systemic IL-8 levels and the corresponding genotypes in PACG, which could not be done in the present study. The sex- specific association of -251 A>T variant in *IL8* with PACG observed in this study further corroborates the consistent epidemiological finding that sex predilection is higher for PACG and inconclusive for POAG [35, 36].

Conclusion

This study, for the first time, suggests a potential genetic association of -251T>A variant in *IL8* with PACG among males in a North Indian Punjabi cohort. If replicated in larger PACG cohorts in other glaucomatous populations, these results may provide important insights into the contribution of sex-specific factors in modifying genetic susceptibility to PACG.

Abbreviations

AD	Alzheimer's disease
AH	Aqueous humor
CAD	Coronary artery disease
CI	Confidence Interval
ECM	Extracellular matrix
IL8	Interleukin 8
IOP	Intra-ocular pressure
MCI	Mild cognitive impairment
MCP	Monocyte chemoattractant protein
ONH	Optic nerve head
OR	Odds ratio
PACG	Primary angle closure glaucoma
PEX	Pseudoexfoliation syndrome
POAG	Primary open angle glaucoma

RFLP	Restriction fragment length polymorphism
RGCs	Retinal ganglion cells
SCE	Schlemm's canal endothelial
SNP	Single Nucleotide Polymorphism
TGFB	Transforming growth factor-B
TME	Trabecular Meshwork Endothelial
TM	SC-Trabecular meshwork-Schlemm's canal
VCDR	Vertical cup to disc ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12886-024-03786-y.

Supplementary Material 1

Acknowledgements
We are sincerely thankful to all study subjects, who participated in the study by donating their precious blood samples.

Author contributions
NT: was involved in sample and data collection, experiments and methodology, analysis and writing of original draft. VKV: were clinical collaborators and were involved in clinical investigations RKP and SM: Conceptualization, Supervision, funding acquisition and data analysis.

Funding
The work was funded by Department of Science and Technology (DST), Science& Engineering Research Board (SERB), Grant No. SB/YS/LS-140/2014 and DST-PURSE Program [(No.SR/PURSE Phase 2/18 (G)]. Indian Council of Medical Research (ICMR, New Delhi, India) is duly acknowledged for providing senior research fellowship to author NT (45/07/2019-HUM/BMS). The funding agencies had no role in the design of study and collection, analysis, and interpretation of data and in writing the manuscript. Also, no funding was provided by any funding agency for publication of research articles.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethical approval from Ethics committee of Guru Nanak Dev University, Amritsar (India) (No.702/HG/28/06/2019) and proper informed consent was taken from all the study participants. The study protocols were according to the tenets of the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 17 July 2024 / Accepted: 25 November 2024
Published online: 02 December 2024

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