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Analysis of interleukin-6 gene polymorphism and its serum levels in Indian age-related macular degeneration patients

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Background: Age-related macular degeneration (AMD) is a complex condition involving multiple factors. The condition is associated with numerous inflammatory indicators, including cytokines. Single-nucleotide polymorphisms in cytokine genes can also modify gene expression, perhaps contributing to the development of the disease. The objective of the present study was to examine the correlation among *IL-6* SNPs (rs1800795, rs1800796, and rs1800797) and the serum levels of IL-6 in AMD patients treated at the Regional Institute of Ophthalmology of Pt. B.D. Sharma PGIMS, Rohtak (Haryana), India.

Methods: This case-control study included 131 patients diagnosed with AMD using precise ophthalmic examinations, such as slit lamp examination, fundoscopy, and ocular coherence tomography. To provide a basis for comparison, we also enlisted 100 healthy individuals as controls. Serum IL-6 protein levels were measured in both patients and controls using an enzyme-linked immunosorbent assay kit (ELISA). Genotyping *IL-6* SNPs was performed using the PCR and DNA Sanger sequencing technique.

Results: IL-6 serum levels were considerably elevated in individuals with AMD compared to the control group ($p<0.05$). Statistically significant differences were seen in the genotype frequencies of rs1800795 ($p=0.027$) and rs1800797 ($p=0.0011$) among the AMD patients and the healthy controls. Furthermore, strong correlations were observed between rs1800795 and the likelihood of developing AMD based on the heterozygous (OR=2.04; $p=0.025$), dominant (OR=1.80; $p=0.035$), and over-dominant models (OR=2.10; $p=0.0094$). Additionally, there were notable associations between rs1800797 and vulnerability to AMD through heterozygous (OR=3.21; $p=0.009$), dominant (OR=2.74; $p=0.004$), and over-dominant (OR=3.11; $p=0.002$) models. The rs1800795, rs1800796, and rs1800797 haplotypes C-G-A and G-G-A were linked to an elevated risk of AMD ($p=0.005$, $p=0.024$. respectively).

Conclusions: Our findings indicated a significant elevation in IL-6 serum levels among the AMD patient group compared to the control group. The interleukin-6 gene polymorphisms rs1800795 and rs1800797 were linked to an elevated risk of AMD in our study population.

Age-related macular degeneration (AMD) is a multifactorial ailment defined by the gradual degradation of retinal cells. This condition results in irreversible visual loss in individuals aged 50 years and above [1,2]. Inflammation is a prevalent element in the development of many diseases, including AMD. It is often described as a complicated signaling cascade that is essential for recognizing and eliminating foreign chemicals as well as encouraging tissue recovery. However, continuous inflammation and excessive production of proinflammatory molecules can lead to chronic illnesses such as AMD, malignancies, type 2 diabetes mellitus, and neurologic disorders [3].

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The age-related eye disease study (AREDS) identifies three main stages of AMD—early, intermediate, and late. People with early AMD usually have no signs of the disease and are identified by the presence of drusen, along with retinal pigment defects. Drusen is the accumulation of lipids, proteins, and collagen in the retinal pigment epithelium (RPE) as well as Bruch's membrane [4]. In the intermediate stage, there is evidence of at least one large drusen, numerous small to medium-size drusen, or geographic atrophy (GA) that does not affect the macular region. There are two types of late-stage AMD: dry or atrophic AMD alongside GA affecting the RPE, and neovascular or exudative AMD that involves choroidal neovascularization (CNV) and detached units in the RPE. These conditions can lead to central vision impairment [5].

The exact causes of AMD remain unclear; hence, it is considered a multifactorial condition linked to genetic

predisposition, environmental factors, disrupted lipid metabolism, immune responses leading to chronic inflammation, oxidative stress, and extracellular matrix dysfunction [6]. Progressive damage to the photoreceptors is seen in late-stage AMD due to abnormal RPE function and an imbalance in oxygen and nutrient flow between the outer retina and blood vessels, leading to increased permeability of the choriocapillaris [7]. Drusen formation, inflammation, and subsequent neurodegeneration in the macular region are characteristic features of the disease [8].

It is widely recognized that inflammation plays an important role in the progression of AMD. The release of inflammatory mediators in the posterior segment of the eye can result in dysfunction of the RPE, thereby contributing to the progression of AMD. Research on AMD patients has explored single nucleotide polymorphisms (SNPs) in various inflammatory factors, such as toll-like receptor 3, complement factor H, CX3C chemokine receptor 1 (CX3CR1), interleukin 6 (IL-6), TNF-alpha, IL-17, 1L-18, interleukin-1 beta (IL-1β), and interleukin 8 (IL-8) [9–13]. Genetic polymorphisms within genes influence individual differences in cytokine production, making AMD a complex condition that is not easily classified as a single-gene disorder. As the gene responsible for AMD exhibits a high degree of variability and interference, it becomes rational to explore the genetic variants and evaluate the function of inflammatory mediators in AMD pathology [10].

IL-6 is a potent pro-inflammatory cytokine with multiple functions. It is involved in regulating inflammatory processes and immune responses and plays a role in an array of physiologic functions, such as hematopoiesis, metabolism, and tissue repair. IL-6 is synthesized by various cell types, such as immune system cells (macrophages and T cells), endothelial cells, fibroblasts, and RPE cells. Changes in the *IL-6* gene can impact how the gene is transcribed and how the cytokine is released [14]. IL-6 plays an essential role in T-cell proliferation and differentiation, thereby influencing the inflammatory response. It also adds to the acute phase of inflammation by drawing neutrophils to the inflamed area, which in turn activates IL-6 receptors in nearby cells and the internal IL-6 signaling pathway. Transition from the acute phase to chronic inflammation involves the replacement of neutrophils with monocytes. Misregulation of IL-6 can lead to chronic inflammation and autoimmune disorders due to its pivotal role in the initiation and resolution of inflammation [15].

IL-6 induces the liver to produce acute-phase proteins, such as C-reactive proteins, serum amyloid A, and fibrinogen. It may also enhance endothelial permeability directly or indirectly through the stimulation of vascular endothelial growth factor (VEGF), resulting in increased vascular permeability and the onset of choroidal neovascularization (CNV) in AMD. Due to its high serum levels in AMD patients, IL-6 is considered a risk factor for CNV in AMD [16]. *IL-6* gene polymorphisms have been linked to several ocular disorders, including AMD [11,16–23].

Over 50 SNPs and five common haplotypes have been discovered in the *IL-6* gene; however, only three promoter region SNPs, −174G/C (rs1800795), −572G/C (rs1800796), and −597G/A (rs1800797), have been widely investigated in numerous immune-related disorders, including rheumatoid arthritis, multiple sclerosis, diabetes, allergic diseases, chronic periodontitis, systemic sclerosis, and cancer [24–28]. It has been reported that these promoter polymorphisms are important regulators of the *IL-6* gene and downstream protein levels, both in vitro and in vivo [17], affecting its expression in numerous diseases. The −174G/C polymorphism, in particular, plays a crucial role in regulating IL-6 transcription and plasma levels, affecting disease susceptibility and inflammatory responses. The activity of the IL-6 promoter is also modulated by the other two polymorphic sites, which interact to control the impact of the −174G/C polymorphism. This intricate regulation underscores the significant role of these polymorphisms in IL-6-mediated pathophysiology [29].

Recent studies, particularly those focusing on specific populations, including the Indian population, have highlighted the potential involvement of IL-6 in susceptibility to ocular disorders [11,16,17,19–22,30–32]. These findings indicate that IL-6 is a promising candidate for AMD research.

Given these insights, our study focused on *IL-6* gene SNPs rs1800795, rs1800796, and rs1800797 for evaluation as possible risk factors for the disease in our cohort of AMD patients. The current study aims to find and compare the serum levels of IL-6 in AMD patients and controls and to investigate the association of these levels with specific gene polymorphisms.

METHODS

Study subjects: We enrolled a group of 131 unrelated AMD patients from the Retina Clinic at the Regional Institute of Ophthalmology, PGIMS, Rohtak, Haryana, India, for this study. Before taking part in the study, the participants or their legal guardians graciously gave written informed consent. The research study followed the Declaration of Helsinki guidelines and received permission from the Institutional Human Ethical Committee (IHEC), Maharshi Dayanand University, Rohtak, Haryana, India.

Table 1. The primer sequences used for the study.

SNP	Genomic location	Minor allele	Primer (5′-3′)	Product size (bp)
1800795	7:22727026	C	F: TGACTTCAGCTTTACTCTTTGT	198
			R: CTGATTGGAAACCTTATTAAG	
1800796	7:22726627	C	F: GGAGACGCCTTGAAGTAACTGC	163
			R: GAGTTTCCTCTGACTCCATCGCAG	
1800797	7:22726602	A	F: CTCCTCTAAGTGGGCTGAAG	212
			R: CAAGCCTGGGATTATGAAGA	

The inclusion criteria for AMD include onset of the disease occurring after the age of 50, non-diabetic, normotensive, and having no ophthalmological ailment other than AMD. The patients recruited for this study were sporadic AMD cases.

One hundred unrelated healthy individuals over 50 years of age were enrolled from the general population during community outreach programs and health camps. All controls underwent detailed ophthalmic evaluation, including visual acuity, color vision, slit lamp biomicroscopy, photo stress testing, and the Amsler grid. Individuals with any ophthalmic condition (except refractive error), such as any stage of age-related maculopathy, macular hemorrhages, cataract, etc., were excluded from the control group. Furthermore, individuals with generalized acute or chronic inflammatory or infectious disorders were also excluded from the control group.

Ophthalmological evaluation: The corneal and lenticular transparency of all study subjects was assessed using slit-lamp biomicroscopy. The pupils were dilated using 1% tropicamide to prepare for a comprehensive examination of the retina using direct monocular ophthalmoscopy and slit-lamp biomicroscopy. All patients diagnosed with AMD underwent optical coherence tomography. AMD patients were divided into two categories: wet or dry AMD. Dry AMD was identified by the presence of drusen deposition beneath the RPE, with or without associated RPE atrophy underlying the central macula, while wet AMD was characterized by the presence of choroidal neovascularization (CNV), as well as other characteristics, including fluid accumulation.

Total protein estimation: IL-6 levels in serum were measured in 100 healthy controls and 131 patients with AMD. This analysis was conducted using the Interleukin-6 (human) ELISA Kit (Cayman Chemical, cat. No. 501,030), with a standard curve assay range of 7.8–250 pg/ml; sensitivity: 7.8 pg/ml, in accordance with the manufacturer's protocol. Absorbance was measured on the ELISA microplate reader (SPECTRA max 384plus, Molecular Devices, CA) at 450 nm.

Selection of single-nucleotide polymorphisms and Sanger sequencing: Genomic DNA was isolated from the venous blood samples using the phenol-chloroform-isoamylalcohol (PCI) technique. The SNPs selected in the present study were either previously examined for association with AMD or other ocular disorders in different populations. Previously published primers were used to perform polymerase chain reaction (PCR) for the selected SNPs: rs1800795 [11], rs1800796 [33], and rs1800797 [34]. Detailed information is outlined in Table 1.

PCR was performed for each SNP, and the purified PCR products were outsourced for DNA sequencing (Barcode Biosciences Pvt. Ltd., Bangalore, India). To ensure quality control, a random selection of 5% of the samples was chosen for the repetitive analysis of three SNPs.

Statistical analysis: The statistical analysis was performed using SPSS version 22.0 software. Subsequently, the Mann–Whitney U test was applied to examine age disparity and serum concentrations among the AMD patients and the controls. A chi-square test was conducted to ascertain gender-based disparities between the two groups. Hardy–Weinberg equilibrium (HWE) was also evaluated using χ^2 test. To compute the odds ratio (OR) and 95% confidence intervals (CI), we examined the allele and genotype distributions of the investigated SNPs using the χ^2 test and binary logistic regression. Statistical significance was determined at $p<0.05$. For multiple testing, the Bonferroni correction was used, setting the threshold for significance at $p<0.0167$ (0.05/3, corresponding to the analysis of three SNPs). A haplotype analysis was conducted using the online software SNPStats to examine the relationship between haplotypes and AMD. The haplotype block consisted of three IL-6 variants: rs1800795, rs1800796, and rs1800797. Linkage disequilibrium was evaluated using D' and r^2 measurements. Haplotype associations were reported in terms of ORs, 95% CI, and *p*-values. A p value below 0.05 was considered statistically significant.

Table 2. Demographic data of control group and AMD patient.

Characteristics		Control (n=100)	AMD patient (n=131)	P value
Sub-types	Wet AMD	–	92	–
	Dry AMD	–	39	–
Affected eye	Unilateral	–	34	–
	Bilateral	–	97	–
Smoking	Ever	29 (29%)	58(44.27%)	0.017
	Never	71 (71%)	73(55.73%)	
Sunlight exposure	Indoor workplace	69 (69%)	88 (67.17)	0.768
	Outdoor workplace	31 (31%)	43 (32.83%)	
Gender, n (%)	Male	64	87	0.7
	Female	36	44	
Age, median/min./max.		60/51/85	70/52/87	$8.3e^{-06}$

RESULTS

Symptoms used to diagnose AMD encompass the presence of drusen (small or large), subretinal CNV, hemorrhages, edema, and areas of geographic and RPE atrophy. Demographic features of the patients and controls are shown in Table 2. Sunlight exposure and gender differences were not statistically significant (p>0.05) between the AMD and control groups. However, age differences and smoking status between the two groups were found to be statistically significant (p<0.05).

Genotypic distribution of SNPs: An examination of the Hardy–Weinberg equilibrium (HWE) indicated that the genotypic distribution for the SNPs did not diverge from HWE (p<0.05) in either group except rs1800797 in the control group (Table 3).

Frequencies of SNP genotypes in AMD patients: We conducted a comparative analysis of the distribution of genotypes and alleles for three single nucleotide polymorphisms (SNPs; Appendix 1), rs18000795, rs1800796, and rs1800797—comparing patients with age-related macular degeneration (AMD) and a control group. The findings are presented in Table 4.

The statistical analysis revealed a notable disparity in the proportions of rs1800795 and rs1800797 genotypes between the two groups (p=0.027 and p=0.0011, respectively). Furthermore, the rs1800797 allele A exhibited a higher prevalence among the AMD patients compared to the control group (p=0.0036). However, the rs1800795 results did not survive the Bonferroni correction.

We conducted binomial logistic regression to assess the influence of these SNPs on AMD patients (Table 5). For rs1800795, our findings revealed that individuals with the heterozygous genotype (GC) were twice as likely to develop AMD (OR=2.04; 95% CI:1.15–3.63; p=0.025). Furthermore,

Table 3. Genotype distribution in the control group and AMD patients using Hardy–Weinberg equilibrium.

Group	SNP	Allele frequencies		Observed genotype distributions	Expected genotype distributions	Chi^2	P value
Control	rs1800795	G (0.825)	C (0.175)	70/25/5	68.06/ 28.87/ 3.07	1.8009	0.179
	rs1800796	G (0.64)	C (0.36)	39/50/11	40.96/46.08/12.96	0.72	0.396
	rs1800797	G (0.855)	A (0.145)	77/17/6	73.1/24.8/2.1	9.88	0.0016
Patient	rs1800795	G (0.771)	C (0.229)	74/54/3	77.87/46.25/6.87	3.6678	0.055
	rs1800796	G (0.7176)	C(0.2824)	65/58/8	67.45/53.09/10.45	1.1159	0.29
	rs1800797	G (0.7443)	A(0.2557)	72/51/8	72.56/49.86/8.56	0.0677	0.795
Total	rs1800795	G (0.7944)	C (0.2056)	144/79/08	145.77/75.46/9.76	0.5068	0.476
	rs1800796	G (0.6826)	C (0.3174)	104/108/19	107.17/99.67/23.17	1.6103	0.204
	rs1800797	G (0.7922)	A (0.2078)	149/68/14	144.97/76.05/9.97	2.5894	0.107

Table 4. *IL-6* (rs1800795, rs1800796, rs1800797) genotype and allele distributions in control group and AMD patients.

SNP	Genotype	Control (n=100)		AMD patient (n=131)		P value
		Count	Proportion	Count	Proportion	
rs1800795	GG	70	0.7	74	0.56	0.027
	GC	25	0.25	54	0.41	
	CC	5	0.05	3	0.02	
	G	165	0.82	202	0.77	0.155
	C	35	0.18	60	0.23	
rs1800796	GG	39	0.39	65	0.49	0.176
	GC	50	0.5	58	0.44	
	CC	11	0.11	8	0.06	
	G	128	0.64	188	0.72	0.076
	C	72	0.36	74	0.28	
rs1800797	GG	77	0.77	72	0.54	0.0011
	GA	17	0.17	51	0.38	
	AA	6	0.06	8	0.06	
	G	171	0.86	195	0.74	0.0036
	A	29	0.14	67	0.26	

AMD: Age-related macular degeneration; p: significance level; Bonferroni corrected p value: p<0.017 (0.05/3).

when comparing genotypes GC and CC to GG, there was a 1.8-fold increase in the odds of developing AMD (OR=1.80; 95% CI:1.04–3.11; p=0.035), but these results did not survive the Bonferroni correction. Compared to GG and CC, the GC genotype displayed a significant correlation with AMD (OR=2.10; 95% CI:1.19–3.72; p=0.0094), indicating a heightened risk for those carrying this genotype.

For polymorphism rs1800797, the patient group exhibited a notably higher frequency of allele A compared to the control group (25.6% versus 14.5%; OR=1.92; 95% CI:1.20–3.07; p=0.0048). The heterozygous genotype (GA) was linked to a 3.2-fold increase in the odds of developing AMD (OR=3.21; 95% CI:1.70–6.06; p=0.009). Additionally, the GA and AA genotypes, in comparison to GG genotypes, showed a 2.7-fold rise in the odds of developing AMD (OR=2.74; 95% CI:1.54–4.90; p=0.004). The GA genotype, compared to GG and AA, indicated a significant correlation with AMD (OR=3.11; 95% CI:1.66–5.84; p=0.002), implying a higher risk of developing AMD for individuals with the GA genotype. Binomial logistic regression analysis revealed no link between the rs1800796 polymorphism and AMD development.

Haplotype association: The polymorphisms studied demonstrated positive linkage disequilibrium (Table 6). Haplotype analysis revealed that those with the C-G-A haplotype were significantly more likely to develop AMD, with an odds ratio of 6.85 (95% CI:2.34–20.08; p=0.005), compared to the reference haplotype G-G-G. Similarly, haplotype G-G-A was present at a lower frequency (3.99%) but was associated with a notable OR of 11.05 (95% CI:1.38–88.43; p=0.024), indicating a considerable risk of developing AMD among individuals carrying this haplotype. Conversely, haplotypes with low frequencies showed no statistically significant association (Table 7).

Total protein estimation: Serum IL-6 levels were compared between AMD patients (n=131) and controls (n=100) using the Mann–Whitney U test. The analysis showed elevated serum levels in patients compared to controls [median (IQR): 22.71 (10.97) pg/ml versus 17.09 (3.41) pg/ml; p=0.0021]. The study participants were divided into dry and wet AMD groups to measure IL-6 serum levels. Both the dry and wet AMD groups showed elevated serum levels when compared with the control group. The individual values were dry AMD versus control [23.56 (10.44) pg/ml versus 17.09 (3.41) pg/ml; p=0.0263] and wet AMD versus control [21.85 (10.97) pg/ml versus 17.09 (3.41) pg/ml; p=0.00113]. However, no statistically significant differences were observed in IL-6

Table 5. Logistic regression analysis of *IL-6* (rs1800795, rs1800796, rs1800797) SNPs in control group and AMD patients.

Model	Genotype	Control	Patient	OR (95% CI)	P value	AIC	BIC
rs1800795							
Codominant	G/G	70 (70%)	74 (56.5%)	1	0.025	314.7	325
	G/C	25 (25%)	54 (41.2%)	2.04 (1.15–3.63)			
	C/C	5 (5%)	3 (2.3%)	0.57 (0.13–2.46)			
Dominant	G/G	70 (70%)	74 (56.5%)	1	0.035	315.6	322.5
	G/C-C/C	30 (30%)	57 (43.5%)	1.80 (1.04–3.11)			
Recessive	G/G-G/C	95 (95%)	128 (97.7%)	1	0.27	318.8	325.7
	C/C	5 (5%)	3 (2.3%)	0.45 (0.10–1.91)			
Overdominant	G/G-C/C	75 (75%)	77 (58.8%)	1	0.0094	313.3	320.2
	G/C	25 (25%)	54 (41.2%)	2.10 (1.19–3.72)			
Log-additive	C	—	—	1.43 (0.88–2.30)	0.14	317.9	324.8
rs1800796							
Codominant	G/G	39 (39%)	65 (49.6%)	1	0.18	318.6	328.9
	G/C	50 (50%)	58 (44.3%)	0.70 (0.40–1.20)			
	C/C	11 (11%)	8 (6.1%)	0.44 (0.16–1.18)			
Dominant	G/G	39 (39%)	65 (49.6%)	1	0.11	317.5	324.4
	G/C-C/C	61 (61%)	66 (50.4%)	0.65 (0.38–1.10)			
Recessive	G/G-G/C	89 (89%)	123 (93.9%)	1	0.18	318.3	325.2
	C/C	11 (11%)	8 (6.1%)	0.53 (0.20–1.36)			
Overdominant	G/G-C/C	50 (50%)	73 (55.7%)	1	0.39	319.3	326.2
	G/C	50 (50%)	58 (44.3%)	0.79 (0.47–1.34)			
Log-additive	C	—	—	0.68 (0.45–1.03)	0.064	316.6	323.5
rs1800797							
Codominant	G/G	77 (77%)	72 (55%)	1	0.009	308	318.3
	G/A	17 (17%)	51 (38.9%)	3.21 (1.70–6.06)			
	A/A	6 (6%)	8 (6.1%)	1.43 (0.47–4.31)			
Dominant	G/G	77 (77%)	72 (55%)	1	0.004	307.7	314.6
	G/A-A/A	23 (23%)	59 (45%)	2.74 (1.54–4.90)			
Recessive	G/G-G/A	94 (94%)	123 (93.9%)	1	0.97	320.1	326.9
	A/A	6 (6%)	8 (6.1%)	1.02 (0.34–3.04)			
Overdominant	G/G-A/A	83 (83%)	80 (61.1%)	1	0.002	306.4	313.3
	G/A	17 (17%)	51 (38.9%)	3.11 (1.66–5.84)			
Log-additive	A	—	—	1.92 (1.20–3.07)	0.0048	312.1	319

AMD: Age-related Macular degeneration; OR: odds ratio; CI: confidence interval; AIC: Akaike information criterion; BIC: Bayesian Information Criterion; Bonferroni corrected p value: $p<0.017$ (0.05/3).

Table 6. Linkage disequilibrium between the *IL-6* polymorphisms. Haplotype analysis.

SNPs	rs1800795 (D'; r^2)	rs1800796 (D'; r^2)	rs1800797 (D'; r^2)
rs1800795 (D'; r^2)	–	0.1836; 0.0040	0.5235; 0.2705
rs1800796 (D'; r^2)	–	–	0.3812; 0.0176
rs1800797 (D'; r^2)	–	–	–

D′ is the deviation between the expected haplotype frequency and the observed frequency [D′ scale: 0,1]. r^2 is squared correlation coefficient of the haplotype frequencies [r^2 scale: 0,1]

Table 7. Haplotype association with AMD occurrence.

Haplotype	rs1800795	rs1800796	rs1800797	Frequency	OR (95% CI)	P value
1	G	G	G	0.4675	1	–
2	G	C	G	0.2473	1.42 (0.84–2.38)	0.19
3	C	G	A	0.1162	6.85 (2.34–20.08)	0.005
4	C	G	G	0.0605	3.02 (0.99–9.19)	0.053
5	G	G	A	0.0399	11.05 (1.38–88.43)	0.024
6	C	C	G	0.0375	0	1
7	G	C	A	0.0191	0.46 (0.04 - 5.44)	0.54
rare	C	C	A	0.0121	0	1

Rare: pooled rare haplotypes; OR: odds ratio; CI: confidence interval; p: significance level when p<0:05

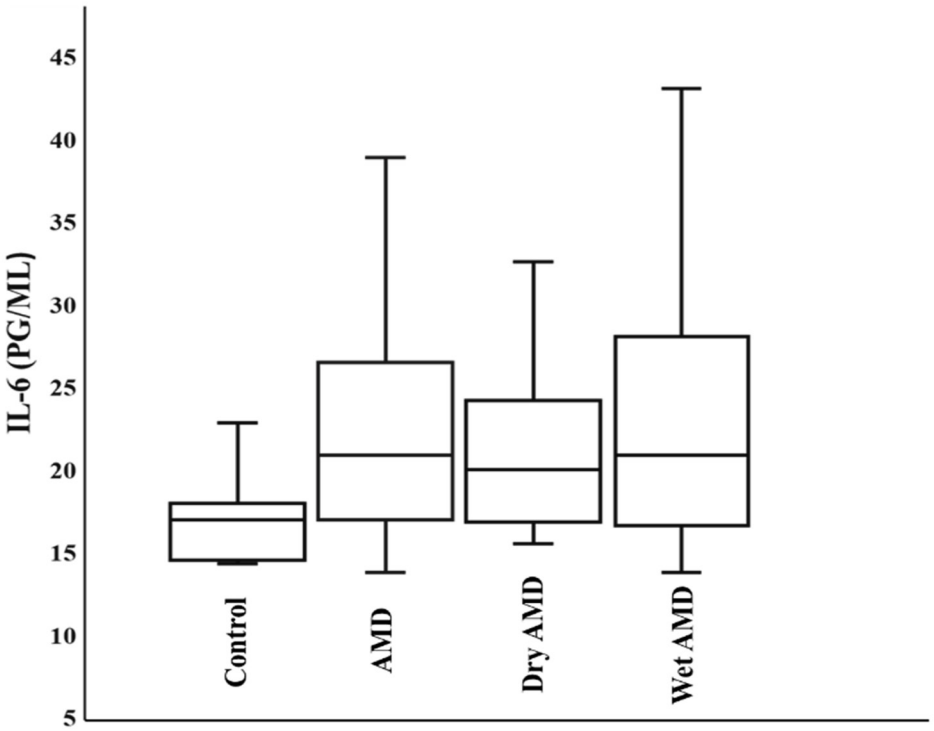

Figure 1. The box-and-whisker plot represents the serum levels of IL-6 in the controls and the patients. AMD: age-related macular degeneration; IL-6: interleukin-6.

Table 8. Serum IL-6 serum levels in relation to the genotypes of *IL-6* polymorphisms.

Genotypes	Control	P value	AMD patients	P value
	(pg/ml), Median (IQR)		(pg/ml), Median (IQR)	
rs1800795				
G/G	17.097 (3.59)	0.758	21.36 (10.06)	0.44
versus	versus		versus	
G/C + C/C	16.121 (3.536)		22.7 (10.97)	
rs1800796				
G/G	17.463(3.048)	0.234	21 (10.73)	0.688
versus	versus		versus	
G/C + C/C	16.122(3.17)		22.95 (10.42)	
rs1800797				
G/G	16.61 (3.475)	0.319	21.85 (10.85)	0.815
versus	versus		versus	
G/A + A/A	17.22 (6.463)		22.95 (10)	

concentrations within the dry and wet subtypes [23.56 (10.44) pg/ml versus 21.85 (10.97) pg/ml; p=0.335, respectively] (Figure 1). IL-6 serum levels were also examined with different genotypes of the rs1800795, rs1800796, and rs1800797 polymorphisms, but no associations between genotypes and IL-6 serum levels were observed (Table 8).

Analyzing the association between serum IL-6 levels, smoking status, and IL-6 polymorphisms in patients with AMD and controls: The analysis of serum levels in AMD patients to compare smokers and non-smokers showed significantly higher IL-6 levels in smokers [median (IQR): 23.37 (10.01) pg/ml versus 20.27 (10.24) pg/ml; p=0.033]. In the control group, there was no significant difference in serum IL-6 levels between smokers and non-smokers [median (IQR): 16.73 (2.19) pg/ml versus 17.09 (3.41) pg/ml; p=0.96].

The analysis of genotypic and allelic distribution between smokers and non-smokers revealed that, among non-smokers, *IL-6* rs1800795, the C allele was more frequent in patients than in the control group (55.48% versus 43.66%; p=0.049; Table 9). However, this result did not survive the Bonferroni correction.

DISCUSSION

Common ocular diseases, including uveitis, glaucoma, AMD, diabetic retinopathy, and cataract, can result in visual impairment and eventually lead to irreversible blindness [35], significantly diminishing quality of life. The development and progression of these conditions are heavily influenced by inflammation. While earlier research on inflammation in eye diseases has focused mainly on uveitis and dry eye disease, inflammation is now recognized as a factor in various other ophthalmic disorders. Identifying links between inflammatory biomarkers and ocular diseases could enable early diagnosis and timely intervention [22]. The objective of this study was to examine the potential relationship between *IL-6* gene polymorphisms, serum levels, and AMD, a multifactorial condition with a complex cause.

Our study revealed that serum levels of IL-6 were significantly increased in AMD patients compared to controls, indicating an association between elevated IL-6 levels and AMD. Additionally, when analyzing the subtypes of AMD (dry and wet) separately against the controls, significant associations were found between IL-6 levels and AMD status for both types. These findings align with previous studies linking elevated IL-6 levels to AMD pathogenesis. For instance, Ambreen et al. (2015) reported significantly elevated serum IL-6 levels in patients compared to controls ($p<0.0001$; Wilk's $\lambda=0.064$; $F=278.7$), with a stronger association observed in individuals with dry AMD [11]. Čolak et al. (2017) observed that higher serum IL-6 levels were present exclusively in patients with neovascular AMD, while those with early AMD did not show increased levels ($r=-0.999$) [36]. Haas et al. (2015) observed significantly elevated serum IL-6 levels in patients with neovascular AMD (p=0.037). The authors proposed that this could be partly linked to changes in visceral fat distribution in patients with AMD [37]. Klein and co-authors, on the other hand, published different results on the population of Beaver Dam, Wisconsin, which did not show an association between IL-6 serum levels and AMD (OR=0.97; 95% CI=0.80–1.17; p=0.74) [38].

Our results demonstrated a significant difference in the genotype frequencies of *IL-6*rs1800795 and rs1800797

Table 9. *IL-6* (rs1800795, rs1800796, rs1800797) genotype and allele distributions in smokers and non-smokers in AMD patients and control.

SNP	Genotype	Smokers		P value	Non-smokers		P value
		Control (29)	Patients (58)		Control (71)	Patients (73)	
rs1800795	GG	12(41.38%)	12(20.69%)	0.11	22(30.99%)	13(17.81%)	0.117
	GC	11(37.93%)	33(56.89%)		36(50.70%)	39(53.42%)	
	CC	06(20.69%)	13(22.42%)		13(18.31%)	21(28.77%)	
	G	35(60.34%)	57(49.14%)	0.163	80(56.34%)	65(44.52%)	0.049
	C	23(39.66%)	59(50.86%)		62(43.66%)	81(55.48%)	
rs1800796	GG	24(82.76%)	55(94.83%)	0.0739	59(83.1%)	55(75.34%)	0.446
	GC	04(13.79%)	1(01.72%)		09(12.68%)	15(20.55%)	
	CC	01(03.45%)	2(03.45%)		03(04.22%)	03(4.11%)	
	G	52(89.66%)	111(95.69%)	0.123	127(89.44%)	125(85.62%)	0.327
	C	6(10.34%)	5(04.31%)		15(10.56)	21(14.38%)	
rs1800797	GG	26(89.66%)	48(82.76%)	0.525	58(81.69%)	59(80.82%)	0.314
	GA	03(10.34%)	08(13.79%)		12(16.90%)	12(16.44%)	
	AA	00(00%)	02(03.45%)		01(1.41%)	02(2.74%)	
	G	55(94.83%)	104(89.66%)	0.252	128(90.14%)	130(89.04%)	0.76
	A	3(05.17%)	12(10.34%)		14(09.86%)	16(10.96%)	

P value: significance level; Bonferroni corrected p value: $p<0.017$ (0.05/3).

between AMD patients and controls, along with a significant difference in the allele frequency of *IL-6*rs1800797. For *IL-6*rs1800796, no statistically significant differences were observed in the distribution of allele or genotype frequencies between the two groups. Multiple clinical and observational studies have demonstrated a substantial association between *IL-6* polymorphisms and an elevated risk of AMD [11,31]. However, findings from other studies, including genome-wide association studies (GWAS), have not consistently replicated these associations [22,39].

In our study, although differences in serum IL-6 levels were noted across genotypes, these differences did not reach statistical significance. Similar findings have been reported in other studies, where no significant association between *IL-6* polymorphisms and serum IL-6 levels has been observed in various inflammatory disorders [40–43]. However, conflicting results have also been reported [11,17,22]. Our results suggest that although these SNPs are recognized for their regulatory effects on *IL-6* gene expression in vitro and in vivo, their impact on serum IL-6 levels may vary depending on population characteristics and study design. The absence of a significant association may reflect the intricate interplay between genetic and environmental factors affecting IL-6 levels, rather than indicating that these SNPs lack biologic relevance [22,44]. Additionally, the deviation of rs1800797 from the Hardy–Weinberg equilibrium in the control group highlights the need to consider potential population stratification and other factors that may influence the genetic associations observed in our study.

External factors, such as smoking, may also contribute to the variability in IL-6 expression. Smoking is a well established risk factor for AMD [31]. The significant elevation in serum IL-6 levels observed in smokers compared to non-smokers indicates that smoking may contribute to increased systemic inflammation in AMD patients [32].

Additionally, our haplotype analysis found that the C-G-A and G-G-A haplotypes were significantly associated with an increased risk of AMD. This finding is notable, as it adds a new dimension to the understanding of genetic risk factors for AMD, suggesting that specific haplotype combinations may be more informative than single SNP analyses.

Overall, our findings revealed that *IL-6* polymorphisms, particularly rs1800795 and rs1800797, were linked with a higher possibility of developing AMD. Elevated serum IL-6 levels further underscore the potential role of inflammation in AMD pathogenesis. However, controversial results in the literature indicate that these associations may be influenced by population-specific factors and interactions with other genetic or environmental elements. Additional research is required to clarify these complex relationships and to better understand the mechanisms through which IL-6 and its polymorphisms contribute to AMD.

Role of interleukin-6 in drusen formation: IL-6 can trigger and perpetuate inflammatory responses in the retina [45]. Chronic inflammation can result in structural and functional disruptions of the RPE and adjacent tissues, possibly contributing to drusen formation. It may activate retinal immune cells, such as microglia and macrophages. These cells may produce additional cytokines and proteases, contributing to inflammation and potentially promoting drusen formation.

IL-6 may impact lipid metabolism in the retina by affecting the balance between lipid production and clearance [46,47]. Disruptions in lipid homeostasis could lead to the accumulation of lipid-rich material [46], a component of drusen. IL-6 may interact with apolipoproteins, such as APOE and APOB, which play roles in lipid transport and metabolism [48,49]. Any dysregulation could contribute to drusen formation in the eye. IL-6 can regulate the expression and function of components, such as collagens and proteoglycans, as well as enzymes, such as matrix metalloproteinases (MMPs) [50]. Changes in these components may impact the deposition and clearance of extracellular material, including drusen. IL-6 may enhance the vascular permeability of retinal pigment epithelial cells in the retina [51], leading to plasma proteins and other substances leaking into the subretinal space. This leakage could cause the build-up of extracellular deposits, such as drusen. IL-6 may enhance the expression of vascular endothelial growth factor [52], which plays a crucial role in angiogenesis. This can disrupt the blood–retinal barrier and contribute to drusen accumulation.

Potential therapeutic strategies for AMD: • Targeting IL-6 or its downstream pathways could offer therapeutic advantages in managing drusen formation and slowing the progression of AMD.

• **IL-6 inhibitors.** Monoclonal antibodies [48] or small molecule inhibitors that target IL-6 [49] or its receptor may help mitigate drusen formation and slow AMD progression.

• **Combined treatments.** Pairing IL-6 inhibitors [49] with other therapies, such as anti-VEGF treatment [53], may offer improved efficacy in addressing AMD.

Limitations of the study and future prospects: Although our candidate gene study offers initial insights, it is hindered by a small sample size and lacks validation in independent cohorts. Future research should prioritize larger, more diverse cohorts to enhance reliability, validate identified SNPs, conduct functional studies for mechanistic understanding, and explore broader genetic factors implicated in AMD.

These steps are vital for advancing our understanding and identifying potential therapeutic targets.

Conclusion: This study revealed a substantial elevation in serum IL-6 levels among AMD patients compared to controls and also implicates IL-6 variants rs1800795 and rs1800797 in AMD development. IL-6 plays multiple roles in the formation of drusen by affecting inflammation, lipid metabolism, extracellular matrix regulation, and vascular permeability. IL-6 contributes to the accumulation of extracellular deposits in the retina. Targeting IL-6 may present a promising therapeutic approach for AMD. Further research is essential for comprehending the intricate correlation between IL-6 and the development of drusen, and it is also important in developing effective approaches for treatment.

APPENDIX 1. SUPPLEMENTARY FIGURE 1:

To access the data, click or select the words "Appendix 1." Chromatogram for different genotypes of *IL-6* genetic polymorphisms. A. rs1800795 (I-G/G, II-G/C, III-C/C); B. rs1800796 (I-C/C, II-G/C, III-G/G); C. rs1800797(I-A/A, II-G/A, III-G/G).

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