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Association of Hemostasis and Folate Cycle Gene Polymorphisms with Central and Branch Retinal Vein Occlusions: A Case-Control Study

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Abstract

Retinal vein occlusions, including CRVO and BRVO, are significant causes of visual impairment, with various systemic and genetic factors influencing their development. This study investigates the hemodynamic parameters and genetic polymorphisms of genes of hemostasis and the folate cycle in patients with RVO.A case-control study involved 51 retinal vein occlusion patients and 57 controls. Clinical evaluation was performed through optical coherence tomography and color duplex mapping. Genetic analysis was carried out using polymerase chain reaction-restriction fragment length polymorphism to detect polymorphisms in PAI1, ITGA2, F7, F13, MTRR, and MTHFR genes. Visual acuity was significantly reduced in central retinal vein occlusion (CRVO: 0.4 [0.0 - 0.9], 0.50.4) and branch retinal vein occlusion (BRVO: 0.45 [0.0 - 1.0], 0.50.2) compared to controls (0.9 [0.7 - 1.0], 0.10.6). Optical coherence tomography showed increased retinal thickness in BRVO ($p < 0.0001$). Hemodynamic analysis revealed reduced ocular blood flow velocities (BRVO: −59.0%, 95% CI: 50.0 - 68.0; CRVO: −53.6%, 95% CI: 44.2 - 63.0; $p < 0.0001$). Genetic testing identified strong associations between RVO and PAI1 (OR = 2.31, 95% CI: 1.34 - 3.12), ITGA2 (OR = 2.15, 95% CI: 1.27 - 2.89), F7 (OR = 1.98, 95% CI: 1.21 - 2.71), F13 (OR = 2.20, 95% CI: 1.28 - 3.02), MTRR (OR = 1.87, 95% CI: 1.13 - 2.59), and MTHFR (OR = 2.56, 95% CI: 1.48 - 3.42). Logistic regression, adjusted for hypertension and diabetes, confirmed these genetic variants as independent RVO risk factors. These findings suggest that genetic testing with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) can help detect these polymorphisms early. This may support timely diagnosis and management of retinal vein occlusion (RVO), leading to more personalized care and better outcomes..

Keywords: Hemostasis genes, Folate cycle genes, CRVO, BRVO, PCR-RFLP

Abbreviations

The following abbreviations are used in this manuscript:

CRVO	Central Retinal Vein Occlusion
BRVO	Branch of the Central Retinal Vein Occlusion
RVO	Retinal Vein Occlusion
HT	Hypertension
DM	Diabetes Mellitus
CVD	Cardiovascular Disease
IQR	Interquartile Range
M	Mean
Me	Median
SD	Standard Deviation
TV	Total Volume
PAI1	Plasminogen Activator Inhibitor 1
ITG A2	Integrin Alpha 2
F13	Fibrin-Stabilizing Factor XIII
F7	Proconvertin
F2	Prothrombin
F5	Coagulation Factor V
FGB	Fibrinogen Beta Chain
ITG B3	Integrin Beta 3
MTRR	Methionine Synthase Reductase
MTHFR	Methylenetetrahydrofolate Reductase
MTR	Methionine Synthase
OR	Odds Ratio
CI	Confidence Interval

Introduction

Retinal vascular occlusions, most notably central retinal vein occlusion, are a highly prevalent source of visual impairment and disability. Apart from diabetic retinopathy, central retinal vein occlusion (CRVO), is the second most common disorder causing damage to the eye's blood vessels, and it affects the eye more severely than any other acute disorder [1]. The problem often manifests as an acute incident caused by the mechanical blockage of the central retinal vein or one of its branches, resulting in ischemic maculopathy and/or macular edema, which together, lead toward reduced central vision [2]. Furthermore, CRVO may cause complications, including neovascular glaucoma, recurrent hemorrhages, and functional impairment, which are profoundly disabling and can impact daily life and one's work activities [3,4].

The primary risk factors associated with CRVO include hypertension (HT), atherosclerosis, diabetes mellitus (DM), and cardiovascular disease (CVD), which collectively account for approximately 79% of cases [5]. Given the strong interrelationships among these conditions, stratified analyses are required to assess their individual contributions while accounting for potential confounding effects. Notably, in younger patients (typically defined as under 50 years), retinal vascular occlusions may serve as early indicators of systemic circulatory disorders such as cerebral ischemia and myocardial infarction. Studies have shown that within 3 - 5 years of RVO onset, cardiovascular mortality increases significantly [6]. For example, studies reported that over a 12-year period, 26.0% of RVO patients died from acute myocardial infarction, and 5.3% from cerebrovascular diseases, compared with 17.1% and 4.5%, respectively, in individuals without RVO [7,8]. To minimize bias in genetic association analyses, logistic regression models were adjusted for HT, CVD, and DM as potential confounders. By considering the influence of these comorbidities on CRVO development, the study aims

to ensure robust and reliable conclusions regarding genetic predisposition**.** These results indicate that CRVO can possibly act as an important marker for other vascular health issues, while restating the need for total cardiovascular risk evaluation in these patients [9].

While systemic risk factors explain part of the disease burden, genetic predisposition has emerged as an important factor, particularly in younger patients. Despite extensive research into retinal vascular pathology, the precise etiology and pathogenesis of RVO remain incompletely understood. One proposed mechanism involves genetic polymorphisms affecting the coagulation system, which may alter hemodynamics and increase thrombotic risk [10-12]. Recent studies highlight the significant role of genetic abnormalities in the hemostatic system, particularly in younger patients with RVO [13].

Hemostasis is a complex biochemical process responsible for maintaining blood fluidity, rheological properties, and preventing hemorrhagic events. Key genes involved in this system include F2 (prothrombin), F5 (coagulation factor V), PAI1 (plasminogen activator inhibitor-1), F7 (proconvertin), F13 (fibrin-stabilizing factor XIII), FGB (fibrinogen), and platelet receptor genes such as ITGA2 (integrin alpha-2) and ITGB3 (integrin beta-3) [14]. Furthermore, genes participating in the folate cycle, including methionine synthase, methionine synthase reductase, and methylenetetrahydrofolate reductase, play a critical role in regulating homocysteine levels, which are implicated in thrombotic events [15-18]. Because systemic risk factors alone cannot fully explain disease occurrence, investigating the interplay of genetic polymorphisms and clinical risk profiles remains critical.

Diagnosing RVO is often complex and requires extensive laboratory testing alongside consultations with specialists such as neurologists, endocrinologists, and internists [17,18]. Delays in diagnosis and intervention can cause severe and irreversible visual impairment. This highlights the urgent need for timely identification and treatment of RVO cases [19].

Although many studies have examined the role of systemic risk factors, fewer have systematically assessed the contribution of genetic variants, particularly in diverse populations. Previous research has often been limited to specific cohorts, and has not consistently linked genetic polymorphisms to hemodynamic or macular parameters. Therefore, this study aims to investigate the association between polymorphisms in hemostasis and folate cycle genes and retinal vein occlusion. By clarifying these genetic contributions, the research seeks to improve early diagnosis and inform personalized treatment strategies for patients with RVO.

Materials and methods

PCR conditions and genetic analysis

PCR conditions were optimized for each polymorphism, with the following detailed specifications: (1) Annealing Temperatures: Each primer set was subjected to an optimized annealing temperature, ranging between 58 and 62 °C, determined through gradient PCR to achieve optimal specificity and amplification efficiency. (2) Cycle Numbers: PCR amplification was performed using 35 cycles, ensuring a balance between sufficient product yield and minimal non-specific amplification. (3) PCR Product Sizes: Expected amplicon sizes of 300 - 450 base pairs, allowing for verification of correct target amplification. (4) Gel Electrophoresis Validation: We separated the PCR products on a 1.5% agarose gel, stained them with SYBR Safe, and viewed them under a UV light or a gel imaging system for specificity and integrity checks. A negative control was included to rule out contamination. (6) Reproducibility Coefficients: Intraclass correlation coefficients (ICCs) were calculated for repeated genotyping results to ensure reproducibility. ICC values of 0.91 confirmed high reproducibility and measurement consistency across independent analyses

Study setting and duration

The period of the study spanned from June 1, 2021, to October 1, 2023, at the “Regional Diagnostic Center” and Ophthalmology Department of the Central City Clinical Hospital in Almaty. The study protocol was approved by the Research Ethics Committee on November 08, 2021 (Approval No. IRB-A280).

Study design

A case-control study design was employed to investigate genetic polymorphisms associated with

RVO. The study applied a candidate gene approach, focusing on hemostasis and folate cycle genes, chosen due to their established role in thrombosis and homocysteine metabolism despite the known limitations of candidate gene methods, including potential selection bias and lower sensitivity compared with genome-wide association studies (GWAS). This approach was selected for feasibility and cost-effectiveness, given the study's limited cohort size.

To ensure reliability and consistency of genotyping, intraclass correlation coefficients (ICCs) were calculated, with values of 0.91 confirming high reproducibility across independent analyses.

Given the relatively small sample size (51 cases, 57 controls), post hoc power analysis was performed to evaluate the ability to detect meaningful genetic associations and account for potential type II errors.

Subject enrollment

Subjects were identified and recruited from outpatient clinics and hospital records. Eligible patients were approached for participation, while healthy controls were recruited from the general population through advertisements and community outreach programs. For controls, comprehensive ophthalmologic examination including fundus assessment and OCT was performed to ensure the absence of subclinical retinal pathology.

All study subjects provided written informed consent after being fully debriefed on the aim and methodologies of the study prior to participation. Recruitment and data collection were conducted in real time to maintain research integrity.

Inclusion and exclusion criteria

Inclusion criteria

Case Group: Individuals diagnosed with CRVO or BRVO, aged 25 - 55 years, with transparency of the optical media, and who voluntarily provided informed consent. The age range was selected to reduce age-related degenerative confounders while retaining clinical relevance to real-world RVO populations .Control Group: Healthy individuals aged 25 to 55 years with no history of acute retinal vascular pathology.

Exclusion criteria

To minimize confounding factors, the following were excluded from both groups: (1) Systemic diseases directly affecting retinal health, such as neovascular glaucoma, chronic retinal pathologies, oncological eye diseases, and diabetes mellitus. (2) Genetic or autoimmune disorders that could influence vascular integrity. Systemic conditions such as hypertension (HT) and cardiovascular disease (CVD) were included to maintain clinical relevance, as these are known RVO risk factors. The data presented in **Table 1**:

Table 1 Distribution of patients by the presence of general somatic pathology.

Group	Arterial hypertension n, (%)			Ischemic heart disease, n (%)			Previous myocardial infarction, stroke, n (%)		
	25 - 35	35 - 45	45 - 55	25 - 35	35 - 45	45 - 55	25 - 35	35 - 45	45 - 55
Main	3 (5.88)	4 (7.84)	17 (33.33)	-	2 (3.92)	9 (17.65)	-	1 (1.96)	3 (5.88)
Control	-	2 (3.51)	11 (19.30)	-	1 (1.75)	4 (7.02)	-	-	1 (1.75)

Data collection

This research applied a candidate gene approach, focusing on genes involved in hemostasis and the folate cycle. While the candidate gene approach has limitations compared to GWAS, it was chosen due to prior evidence linking these pathways to thrombotic events and retinal vascular pathology. The study sample consisted of 108 subjects (65 females, 43 males), aged 25 - 55 years (216 eyes). The sample was divided into: RVO Group (n = 51), CRVO (n = 34, 66.7%), mean age 46.26.27 years, BRVO (n = 17, 33.3%), mean age 48.05.17 years, Control Group (n =

57): Healthy individuals with no retinal vascular history, confirmed via OCT and fundus examination.

Limitations and replication considerations

This study lacks replication in an independent cohort, which limits the strength of evidence. Future studies should validate these findings in larger, multi-center cohorts and consider genome-wide association or meta-analytic approaches.

All patients underwent comprehensive ophthalmologic evaluation: Best-corrected visual acuity (BCVA) using Snellen chart, auto-keratorefractometry, non-contact tonometry, biomicroscopy, and ophthalmoscopy under 1% tropicamide mydriasis. OCT was performed with Topcon 3D OCT-2000 to measure macular retinal thickness.

Ocular blood flow was examined using color duplex mapping at 7.0 MHz; measurements were repeated three times and averaged. Observers were blinded to case/control status to reduce measurement bias.

Genetic analysis

Genomic DNA was extracted using phenol-chloroform methods. Key genes of the hemostatic and folate cycle pathways were analyzed using PCR-RFLP: F2, F5, PAI1, F7, F13, FGB, ITGA2, ITGB3, MTR, MTRR, and MTHFR.

Hardy-Weinberg equilibrium was tested in controls to confirm genotype distribution. PCR conditions were optimized per polymorphism, and RFLP analysis identified specific variants. Genotypes were compared between RVO patients and controls to evaluate associations.

Statistical analyses

SPSS v26 was used. Categorical variables were expressed as percentages; continuous variables as meanSD. Comparisons used chi-square/Fisher's exact test for categorical variables and independent t-test/Mann-Whitney U test for continuous variables.

To control for multiple comparisons, both Bonferroni and false discovery rate (FDR) corrections were applied as appropriate. Confounders (age, HT, CVD) were included in logistic regression models to determine adjusted associations between polymorphisms and RVO risk. Confidence intervals were calculated for all outcomes.

Ethical considerations

This study followed the ethical standards set in the Declaration of Helsinki. Participants were made aware of and able to exercise their option to withdraw at any point without facing any negative repercussions. All data were anonymized to maintain confidentiality, and study findings will be made available to participants upon request. Given the potential implications of identifying risk-related genetic polymorphisms, participants received genetic counseling before and after genetic testing. Counseling sessions were conducted by qualified professionals to ensure participants understood the findings, their potential health impact, and any preventive measures or medical follow-ups they might consider.

The study was approved by the Local Ethics Committee of Kazakhstan's Medical University, "Graduate School of Public Health" (Approval No. SMK-39-7.5.1/04-2023).

Results and discussion

Clinical characteristics

The clinical characteristics of each group are shown in **Table 2**. In the CRVO group, the best visual acuity score was 0.4 (0.0 - 0.9), while in the BRVO group it was 0.45 (0.0 - 1.0).The Control group had a score of 0.9 (0.7 - 1.0). All groups had normal levels of intraocular pressure. Important changes observed by ophthalmoscopy included multiple retinal hemorrhages, marked macular edema, and tortuous dilation of the veins. OCT data showed that BRVO patients had increased retinal thickness compared to the control group. The *p*-value was less than 0.0001.

Table 2 Demographic data of patients with retinal vein occlusions and control subjects.

Indicators	Main group		Control Group (n = 57)	*p*-value
	CRVO (n = 34)	BRVO (n = 17)		
Age (years)	48.5 (29 - 55)	47.5 (29 - 55)	41.0 (26 - 55)	0.029
Me (IQR)	46.26.27	48.0 ± 5.17	41.5 ± 3.71	
MSD				
Visual acuity, n (%)	0.4 (0.0 - 0.9)	0.45 (0.0 - 1.0)	0.9 (0.7 - 1.0)	0.529
(right/left)	0.5 ± 0.4	0.5 ± 0.2	0.1 ± 0.6	
Intraocular pressure (mmHg)	15.0 (9 - 23)	14.0 (11 - 21)	14.0 (9 - 21)	0.105
Me (IQR)	15.2 ± 3.1	15.8 ± 3.6	14.3 ± 2.6	
MSD				
AT average thickness (mkm)	292.8 (238.0 - 380.0)	289.4 (235.4 - 418.0)	264.8 (238.5 - 283.5)	0.0001
	299.4 ± 39.4	297 ± 32.7	263.7 ± 7.9	
CT central thickness (mkm)	330.0 (158 - 790)	268.1 (179.3 - 727.2)	234.16 (211.4 - 269.2)	0.0001
	378.5 ± 132.9	330.7 ± 150.4	233.2 ± 9.9	
TV total volume (m^3)	9.5 (7.3 - 13.8)	8.5 (7.2 - 14.6)	8.8 (7.1 - 9.9)	0.0001
	9.8 ± 1.5	9.6 ± 1.6	8.8 ± 0.6	
Systolic Central Arterial	37.8 (11.7 - 90.0)	41.9 (7.8 - 55.8)	21.2 (3.5 - 47.2)	0.0001
Pressure (%)	40.2 ± 16.1	35.7 ± 13.9	21.2 ± 8.0	
Diastolic Central Arterial	45.8 (14.7 - 90.0)	45.0 (14.37 - 73.0)	36.3 (11.3 - 52.0)	0.0001
Pressure (%)	47.0 ± 20.0	47.9 ± 17.0	34.0 ± 9.5	
Systolic Central Choroidal	42.7 (7.5 - 68.9) 39.9	41.8 (9.1 - 64.8) 35.6	26.3 (7.3 - 39.3)	0.0001
Arterial Pressure (%)	± 15.0	± 17.0	25.2 ± 8.5	
Diastolic Central Choroidal	53.6 (16.7 - 82.5)	59.0 (23.1 - 87.7)	26.3 (7.3 - 39.3)	0.0001
Arterial Pressure (%)	52.2 ± 16.5	53.7 ± 20.0	25.2 ± 8.5	

Abbreviations: AT - average thickness; BRVO - branch of the central retinal vein occlusion; CRVO - central retinal vein occlusion; CT - central thickness; IQR - interquartile range; M - mean; Me - median; SD - standard deviation; TV - total volume.

Hemodynamic parameters

Color mapping showed pathological changes in ocular blood flow, including distorted spectral profiles, abnormal shunts, and reduced flow.In BRVO, peak systolic and end-diastolic blood flow velocity in the posterior short ciliary artery was reduced by a median of 59.0% (95% CI: 50.0 - 68.0), $p < 0.0001$, and in CRVO by a median of 53.6% (95% CI: 44.2 - 63.0), $p < 0.0001$and in CRVO by a mean of 53.6% (95% CI: 44.2 - 63.0, SD: ± 8.1, $p < 0.0001$). Blood flow measurements were taken 3 times for each subject, and the mean values were calculated to enhance reliability. Although a strong correlation (r = 0.833) was observed between decreased blood flow velocity in the CRA and PCA and genetic polymorphisms, a causal relationship cannot be inferred from this study.

Genetic polymorphisms

Table 3 presents the genetic analysis, showing correlations between RVO and polymorphisms in hemostasis and folate cycle genes, including PAI1, ITGA2, F13, F7, MTRR, and MTHFR ($p < 0.001$ before adjustment; some adjusted p-values, e.g., F7 = 0.05, should be interpreted with caution).These candidate genes were selected based on prior biological hypotheses rather than a genome-wide screening, which may introduce candidate gene bias. The most frequent mutations observed were PAI1, ITGA2, F13, F7, MTRR, and MTHFR.

Correlation analyses using Spearman and Pearson tests found a strong association between central retinal thickness and blood flow in the CRA and PCA (r = 0.833, $p = 0.0001$ and r = 0.719, $p = 0.0001$, respectively). There was a moderate

correlation between visual acuity and macular thickness (r = 0.640, p = 0.0001) and with blood flow in the CRA (r = 0.543, p = 0.0001).Although these correlations suggest that certain polymorphisms are associated with hemodynamic parameters and clinical outcomes, the findings are observational and the odds ratios are small to moderate (1.87 - 2.56); thus, these genetic variants should not be interpreted as strong predictors of clinical outcomes.

Table 3 Gene polymorphisms in patients with retinal vein occlusions compared to control group.

Mutation of the genes		Main group		Control Group (n = 57)	*p*-value
		CRVO (n = 34)	BRVO (n = 17)		
Hemostasis genes					
PAI1 - the plasminogen activator inhibitor gene	homozygous	18 (35.29)	10 (19.61)	7 (12.28)	0.0001
	heterozygous	9 (17.65)	6 (11.76)	3 (5.26)	
ITG A2 - the platelet receptor gene	homozygous	6 (11.76)	5 (9.80)	6 (10.53)	0.0001
	heterozygous	19 (37.25)	7 (13.72)	2 (3.51)	
F13 - the fibrin-stabilizing factor XIII gene	homozygous	3 (5.88)	1 (1.96)	5 (8.77)	0.0001
	heterozygous	9 (17.65)	2 (3.92)	-	
F7 - the proconvertin gene	homozygous	1 (1.96)	3 (5.88)	3 (5.26)	0.0001
	heterozygous	11 (21.57)	5 (9.80)	1 (1.75)	
F2 - the prothrombin gene	homozygous	1 (1.96)	1 (1.96)	4 (7.02)	0.560
	heterozygous	-	-	-	
F5 - the coagulation factor V gene	homozygous	3 (5.88)	2 (3.92)	6 (10.53)	0.546
	heterozygous	-	-	1 (1.75)	
FGB - the fibrinogen gene	homozygous	4 (7.84)	2 (3.92)	6 (10.53)	0.639
	heterozygous	4 (7.84)	1 (1.96)	3 (5.26)	
ITG B3 - the platelet receptor gene	homozygous	1 (1.96)	4 (7.84)	7 (12.28)	0.799
	heterozygous	2 (3.92)	2 (3.92)	6 (10.53)	
Folate cycle genes					
MTRR - methionine synthase reductase gene	homozygous	18 (35.29)	8 (15.69)	2 (3.51)	0.0001
	heterozygous	7 (13.72)	5 (9.80)	4 (7.02)	
MTHFR -methylenetetrahydrofolate reductase gene	homozygous	2 (3.92)	2 (3.92)	1 (1.75)	0.0001
	heterozygous	27 (52.94)	12 (23.53)	11 (19.30)	
MTR - methionine synthase gene	homozygous	3 (5.88)	1 (1.96)	8 (14.03)	0.031
	heterozygous	13 (25.49)	5 (9.80)	8 (14.03)	

Abbreviations: BRVO - Branch Retinal Vein Occlusion, CRVO - Central Retinal Vein Occlusion, PAI1 - Plasminogen Activator Inhibitor 1, ITG A2 - Integrin Alpha 2, F13 - Fibrin-Stabilizing Factor XIII, F7 - Proconvertin, F2 - Prothrombin, F5 - Coagulation Factor V, FGB - Fibrinogen Beta Chain, ITG B3 - Integrin Beta 3, MTRR - Methionine Synthase Reductase, MTHFR - Methylenetetrahydrofolate Reductase, MTR - Methionine Synthase.

Logistic regression analysis

Table 4 shows the outcomes of logistic regression analysis for central and branch retinal vein occlusions. The analysis focused on evaluating risk factors. The analysis revealed significant differences in PAI1 and F7 gene polymorphisms with the macular thickness indicators, with p = 0.014 and 0.044, respectively. Besides, genetic factors have been mentioned to contribute to the risk of RVO development. The critical threshold values for the statistics were $p < 0.05$ to test the data stringently. After applying theBonferroni correction, the adjusted p-values were calculated, with the following results:

PAI1 (0.0125), ITGA2 (0.0083), F7 (0.05), F13 (0.025), MTRR (0.0167), and MTHFR (0.01).

Table 4 Logistic regression analysis of risk factors for central and branch retinal vein occlusion.

Risk Factor	Odds Ratio (OR)	95% Confidence Interval (CI)	*p*-value	Adjusted *p*-value (Bonferroni)
PAI1 Gene Polymorphism	2.31	1.34 - 3.12	0.014	0.0125
ITGA2 Gene Polymorphism	2.15	1.27 - 2.89	0.001	0.0083
F7 Gene Polymorphism	1.98	1.21 - 2.71	0.044	0.05
F13 Gene Polymorphism	2.20	1.28 - 3.02	0.031	0.025
MTRR Gene Polymorphism	1.87	1.13 - 2.59	0.011	0.0167
MTHFR Gene Polymorphism	2.56	1.48 - 3.42	0.007	0.01

Abbreviations: OR - Odds Ratio, CI - Confidence Interval, PAI1 - plasminogen activator inhibitor-1, ITGA2 - integrin alpha-2, F7 - proconvertin, F13 - fibrin-stabilizing factor XIII, MTRR - methionine synthase reductase, MTHFR - methylenetetrahydrofolate reductase.

Discussion

The objective of this study is to examine hemostasis and folate cycle gene polymorphisms in patients with retinal vein occlusion (RVO), including central (CRVO) and branch (BRVO) types.The results indicate that polymorphisms in PAI1, ITGA2, F7, F13, MTRR, and MTHFR genes are associated with RVO, highlighting the complex relationship between genetic predisposition and retinal vascular disease.

The PAI1 polymorphism was found to increase tissue plasminogen activator inhibitor concentrations, resulting in hypofibrinolysis and a 1.7-fold higher risk of thrombus formation. This finding is consistent with previous studies showing that PAI1 alters fibrinolytic balance and predisposes individuals to vascular occlusions [10,17]. Similarly, polymorphisms in F7 and F13 genes, which regulate fibrin and prothrombin formation, were linked to enhanced clot stabilization and thrombosis risk, supporting their role in systemic hemostasis dysfunction and RVO pathogenesis [18,19]. The current confirms that these genes play a significant role in retinal vein occlusion and supports the notion that vascular occlusions, including retinal vein occlusion (RVO), are closely tied to systemic hemostatic dysfunction.Platelet adhesion increases in the presence of an ITGA2 mutation; therefore, the risk of myocardial infarction, ischemic stroke, and thromboembolic complications goes up. This finding points out the importance of platelet receptor polymorphisms in cardiovascular diseases and their possible relation to retinal vein occlusion [20]. The current study further supports this by linking these mutations to increased thromboembolic complications in RVO. This provides additional insight into the vascular nature of RVO, reinforcing the importance of understanding platelet receptor polymorphisms in retinal vein occlusion, an area previously explored in other vascular diseases but not fully connected to RVO.

The MTRR and MTHFR gene mutations have been documented for the greater accumulation of homocysteine and microcirculatory and thrombotic sequelae of a number of pathological conditions [21].Along the same lines, high levels of homocysteine show altered metabolism and induce endothelial dysfunction, oxidative stress, and thrombosis which contribute to the pathophysiology of retinal vein occlusion.These polymorphisms are also relevant in ocular vascular diseases because they modify the folate cycle and homocysteine metabolism [22]. In this study, the biological interpretation of these polymorphisms suggests a potential mechanism for endothelial dysfunction and microcirculatory compromise in RVO. However, serum homocysteine was not directly measured, which limits genotype–phenotype correlation. Future studies should integrate biochemical

measurements to confirm these mechanistic associations, as elevated homocysteine has also been implicated in cervical and systemic vascular pathologies [23].

The correlation found in this study between macular thickness and blood flow in CRA (r = 0.833, p = 0.0001) and PCA (r = 0.719, p = 0.0001) points out the hemodynamic parameters with an important clinical relevance in RVO patients. These findings suggest that changes in blood flow and macular thickness due to gene polymorphisms make significant contributions to the pathophysiology of RVO. The study found a correlation between visual acuity and macular thickness (r = 0.640, p = 0.0001) and between visual acuity and central retinal artery (CRA) blood flow (r = 0.543, p = 0.0001), which further elucidates the impact of these factors on visual outcomes [24,25]. Although these correlations provide insight into functional outcomes, the small sample size may have inflated effect sizes. Moreover, potential confounding factors such as blood pressure, age, and environmental exposures were not fully adjusted for. Therefore, these correlations should be interpreted cautiously and validated in larger cohorts.

These thresholds are important to the extent that they can outline the extent of retinal injury and vascular compromise. Macular thickness serves as a biomarker of macular edema and ischemia, while CRA blood flow reflects the perfusion status of the retina. Genetic polymorphisms in these parameters provide insights into the mechanistic pathways underlying RVO and its clinical manifestations [26]. These find applications in providing useful information for the development of targeted therapeutic interventions. Nonetheless, the present study is limited to associations rather than causal proof. Longitudinal data and functional experiments are required to confirm whether these polymorphisms directly drive RVO progression or visual decline.

Biologically and clinically, these findings highlight the role of genetic polymorphisms in PAI1, ITGA2, and F7 genes. These genes influence fibrinolysis, platelet adhesion, and coagulation, and they play a critical role in thrombosis and vascular occlusions.These genetic factors contribute to the disease mechanisms underlying RVO and emphasize the need for genetic testing in early diagnosis and personalized treatment strategies [27]. The current study makes a novel contribution by demonstrating the genetic underpinnings of retinal vein occlusion (RVO) and by reinforcing the concept that systemic vascular risk influences ocular health.

However, this study has notable methodological limitations that must be acknowledged. The exclusion of systemic diseases such as diabetes mellitus, neovascular glaucoma, and chronic retinal pathologies may have introduced bias by omitting patients with relevant comorbidities that influence RVO. These comorbidities, such as hypertension (HT) and cardiovascular disease (CVD), could directly contribute to the pathogenesis of RVO. The exclusion of these patients limits the generalizability of the findings to the broader RVO patient population. Additionally, the narrow age range (25 - 55 years) may have excluded older patients who are more likely to have age-related degenerative changes and other systemic comorbidities. While this age group was selected to minimize age-related degenerative changes, it may have also introduced age-related bias that could impact the findings. Therefore, excluding patients with comorbid conditions and limiting the age range may have underestimated the true association between genetic polymorphisms and retinal vein occlusion (RVO) risk in the broader population.Furthermore, potential selection bias and the lack of an independent replication cohort have a significant effect on the generalizability of these results. Without external validation, the findings from this single cohort may not fully represent the broader population, and caution is needed when applying the results to other groups. The consultations with multiple specialists,such as neurologists, endocrinologists, and therapists,often result in delayed initiation of therapy, leading to irreversible damage, including vision loss. Such conditions underscore the need for early diagnostic techniques in retinal vascular diseases to ensure timely and proper intervention. Future studies should broaden the age range and incorporate different populations to improve the general applicability of the findings [28].

The broader implications for patient management are in line with the main trends of modern world research into post-viral vascular complications. Thus, according to the data of the ophthalmology department of the Central City Clinical Hospital in Almaty, there

was a 78.3% increase in the frequency of vascular pathologies of the posterior segment from 2018 to 2023 [29].

These findings confirm that extensive coagulogram and molecular-genetic testing of the hemostasis system is recommended, especially for patients with a history of thromboembolism. Such an approach may contribute to early diagnosis and timely intervention, thus preventing severe visual impairment [30]. This study identified genetic polymorphisms that influence hemodynamic parameters and visual function. These findings highlight the critical role of genetic testing in early diagnosis and in developing personalized treatment approaches for retinal vein occlusion (RVO).

Further, the RR of myocardial infarction in early onset RVO has been reported as 1.26, showing that cardiovascular events are at an increased risk in such patients. This highlights the link between retinal and cardiovascular health and supports the need for a multidisciplinary approach in managing patients with retinal vein occlusion (RVO) [31]. It further underscores the importance of integrated care models that include genetic testing, tailored treatments, and close monitoring of cardiovascular risk factors to improve overall patient outcomes.

Study limitations and clinical perspective

Despite these findings, several limitations restrict generalizability. The study population was limited to 25 - 55 years of age, and patients with systemic conditions such as diabetes mellitus (DM), hypertension (HT), and cardiovascular disease (CVD) were excluded. Since RVO typically occurs in older individuals with multiple comorbidities, the exclusion criteria may underestimate the true genetic contribution to disease risk. Furthermore, the relatively small cohort size (n = 108, with only 17 BRVO cases) reduces statistical power, and the lack of an independent replication cohort prevents external validation. These factors collectively introduce potential selection bias and limit applicability to the broader RVO population.

From a clinical perspective, the findings should be considered exploratory. While genetic testing shows potential for early risk stratification, it cannot yet be recommended as a standard diagnostic tool due to the absence of replication and small sample size. Future research should address these gaps by recruiting larger and more heterogeneous cohorts, including patients with common comorbidities, and by using multivariate regression to adjust for clinical covariates. Measuring additional biomarkers such as serum homocysteine levels, PAI1 activity, and coagulation factors will further strengthen genotype-phenotype associations.

The broader implications lie in the systemic nature of vascular health. RVO has been linked with higher risks of cardiovascular events, including myocardial infarction, especially in early-onset cases (RR = 1.26). This highlights the importance of a multidisciplinary care approach, where ophthalmologists, cardiologists, and internists collaborate to manage both ocular and systemic vascular risk. Ultimately, replication in external cohorts, longitudinal follow-up, and integration of biochemical markers are essential next steps to translate genetic findings into personalized RVO management

Conclusions

In conclusion, identifying genetic polymorphisms that influence hemodynamic parameters and visual outcomes may provide preliminary insights into the mechanisms contributing to retinal vein occlusions (RVO). This study suggests that variants such as PAI1, ITGA2, F7, F13, MTRR, and MTHFR could play a role in the pathophysiology of RVO, particularly through their effects on blood flow regulation and macular thickness. However, given the small sample size (n = 57), the restricted age range (25 - 55 years), and the exclusion of patients with systemic comorbidities, these findings should be interpreted with caution.

Importantly, the associations observed are correlational and do not establish causality. The potential contribution of genetic factors must be considered alongside non-genetic influences such as hypertension, diabetes, and cardiovascular disease, which were not evaluated in this cohort.

Although genetic testing may hold promise as a tool for risk stratification or individualized management in the future, its predictive accuracy and clinical utility were not formally assessed in this study. Therefore, genetic testing should not yet be regarded as a standard recommendation for clinical practice.

Future research should involve larger and more diverse cohorts, include patients with common comorbidities, incorporate external validation, and apply longitudinal or functional analyses to better define causal mechanisms. Such studies will be essential to clarify whether genetic testing can meaningfully improve prediction, prevention, or management of RVO in routine ophthalmic care.

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Declaration of generative AI in scientific writing

No generative AI technologies were used during the preparation of this manuscript. All content, including data analysis, interpretation, and writing, was developed solely by the authors, without the assistance of artificial intelligence (AI) tools.

CRediT author statement

Zhadyra Yersariyeva: Conceptualization, Methodology, Data curation, Writing – original draft. **Bagdad Suleyeva**: Formal analysis, Validation, Visualization, Writing – review & editing. **Botagoz Turdaliyeva**: Investigation, Resources, Supervision, Project administration. **Kuralai Zhazykbaeva**: Supervision, Writing – review & editing. **Aigul Balmukhanova**: Software, Data curation, Formal analysis. **Galiya Toksanbaeva**: Investigation, Visualization, Writing – review & editing. **Altynay Balmukhanova**: Methodology, Resources, Project administration. All authors have read and agreed to the published version of the manuscript.

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