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ORIGINAL RESEARCH

Untargeted Metabolomic Study of Patients with Macular Edema Secondary to Retinal Vein Occlusion in Aqueous Humor

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Purpose: The aim of this study was to identify metabolic biomarkers and investigate the metabolic changes associated with aqueous humor in retinal vein occlusion macular edema (RVO-ME).

Methods: Aqueous humor (AH) samples were collected from patients, including those diagnosed with central retinal vein occlusion macular edema (CRVO-ME), branch retinal vein occlusion macular edema (BRVO-ME), and a control group undergoing cataract surgery. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was utilized to analyze the metabolomic profiles in aqueous humor.

Results: A total of 28 metabolites were identified as potential biomarkers capable of distinguishing RVO-ME patients from the control group. Of these, 26 metabolites were specific for distinguishing CRVO-ME patients from controls, and 24 metabolites were specific for differentiating BRVO-ME patients from controls. Additionally, 9 metabolites were identified that could differentiate CRVO-ME patients from BRVO-ME patients.

Conclusion: This study successfully identified significant metabolic biomarkers that enhance our understanding of the pathogenesis of RVO-ME. These findings may offer new avenues for the treatment of RVO-ME and aid in differentiating between CRVO-ME and BRVO-ME patients.

Keywords: metabolomic, retinal vein occlusion, macular edema, aqueous humor

Introduction

Retinal vein occlusion (RVO) is a prevalent retinal condition that can result in irreversible vision loss. RVO can be classified based on the location of the blockage, specifically into central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO).[1,2] Known risk factors for RVO include hypertension, atherosclerosis, dyslipidemia, diabetes, thrombosis, and various inflammatory and myeloproliferative disorders.[3] Clinical manifestations of RVO encompass retinal hemorrhage, retinal venous tortuosity, optic disc swelling, and macular edema (ME). Among these, macular edema is the most common cause of visual impairment associated with RVO.[4,5]

Although the diagnosis of retinal vein occlusion macular edema (RVO-ME) is well-established, the pathogenesis and pathophysiology of RVO-ME remain controversial. Metabolomics, which involves identifying and quantifying metabolites in tissues or organisms, can provide insights into pathological and physiological changes.[6] Dynamic alterations in endogenous metabolites may indicate specific stages of disease progression. Metabolomics has been utilized to uncover metabolic changes in various diseases and to clarify their underlying mechanisms.[7] Aqueous humor (AH) serves to nourish the avascular cornea and lens while removing metabolic waste from the eye into the venous blood. Therefore, the biometabolic profile of AH can directly reflect the physiological state of the eye.[8] For example, metabolite analysis in the AH of myopia patients has revealed increased concentrations of 27 significant metabolites compared to controls.[9] Disruptions in metabolites related to osmoprotection, neuroprotection, and amino acid metabolism have been observed

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in the AH of glaucoma patients.[10] Additionally, differential metabolites have been detected in the AH of patients with age-related macular degeneration (AMD)[11] and diabetic retinopathy (DR).[12] However, metabolomics studies focusing on central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO) are limited. While there are a few studies examining metabolic changes in the AH of patients with RVO-ME and CRVO-ME,[13–15] there is a lack of specific metabolic analyses for BRVO-ME and no comparative studies between CRVO-ME and BRVO-ME.

Therefore, the objective of this study is to identify differential metabolites in RVO-ME, CRVO-ME, and BRVO-ME compared to a control group, and to screen for potential biomarkers among these metabolites. Additionally, we aim to determine the differential metabolites that can distinguish CRVO-ME from BRVO-ME.

Materials and Methods

Study Participants and Sample Collection

The study included 27 participants recruited between December 1, 2021, and March 1, 2023. All study procedures adhered to the principles of the Helsinki Declaration. The study was approved by the Research Ethics Committee of Tong Ren Hospital, Shanghai Jiao Tong University School of Medicine. Following the acquisition of written informed consent from the patients, samples were collected. Aqueous humor (AH) was collected following anesthesia with proparacaine hydrochloride eye drops. The anterior chamber was punctured using a 30-gauge needle to aspirate approximately 50–100 µL of AH, which was then transferred to an Eppendorf tube and stored at −80°C for future analysis.

The study investigated aqueous humor samples from 7 patients with CRVO-ME, 10 patients with BRVO-ME, and 10 patients who underwent cataract surgery. All participants underwent a comprehensive eye examination. Cataract grading was assessed using the Lens Opacities Classification System III (LOCS III), with a grade of N2C2P2 assigned to both the RVO and control groups.[16] The diagnosis of RVO was based on the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM), with CRVO defined as ICD-9362.35 and BRVO defined as ICD-9362.36.[17] The inclusion criteria for RVO-ME were: 1) age ≥ 18 years, 2) diagnosis within the past year, and 3) central retinal thickness (CRT) ≥ 300 µm. Exclusion criteria included: 1) age-related macular degeneration, 2) diabetic retinopathy, 3) prior intravitreal injection of anti-vascular endothelial growth factor or steroids, 4) previous intraocular surgery, 5) prior retinal photocoagulation, 6) glaucoma (including neovascular glaucoma), 7) iritis and anterior chamber hemorrhage, 8) vitreous hemorrhage and other vitreoretinal diseases, 9) occurrence of cerebrovascular accidents or myocardial infarction in the past 3 months, and 10) use of any type of eye drops within the 3 months prior to sample collection. Control samples were collected from age- and sex-matched patients. None of the subjects or controls received steroid medications.

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis

The LC-MS/MS analysis was conducted by Biotree Biotech Co., Ltd. (Shanghai, China) using an ultrahigh-performance liquid chromatography (UPLC) system with a diode array detection system (Thermo Fisher Scientific). Separation was achieved with a UPLC BEH Amide column (2.1×100 mm, 1.7 µm) coupled to a Q Exactive HFX mass spectrometer (Thermo). The mobile phase comprised 25 mmol/L ammonium acetate, 25 mmol/L ammonia solution (pH 9.75), and acetonitrile. The autosampler operated at 4°C, with an injection volume of 3 µL. The Q Exactive HFX mass spectrometer was selected for its capability to collect tandem mass spectrometry (MS/MS) spectra in data-dependent acquisition mode, controlled by the acquisition software (Xcalibur, Thermo). In this mode, the software continuously evaluates full-scan MS spectra. The electrospray ionization source conditions for the Q Exactive HFX were as follows: sheath gas flow rate 30 Arb, auxiliary gas flow rate 25 Arb, capillary temperature 350°C, full MS resolution 60,000, MS/MS resolution 7,500, with collision settings.

Statistical Analysis

Principal Component Analysis (PCA) was utilized as an unsupervised model to assess the overall separation trend among the samples. Orthogonal Projection to Latent Structures Discriminant Analysis (OPLS-DA) was employed as a supervised model to identify significantly different metabolites between groups. To enhance the analysis, Variable Importance in Projection (VIP) values were calculated. Metabolites with VIP values greater than 1 were

initially selected as the most variable metabolites. These selected metabolites were further validated using a two-tailed test. Normality was assessed using the Shapiro–Wilk test. Statistical analyses included Student's *t*-test, ANOVA, and Fisher's exact test, with a p-value < 0.05 considered statistically significant.

Results

Characteristics of the Participants

To investigate the metabolic characteristics of AH in RVO-ME, we recruited 10 age- and sex-matched control subjects and 17 RVO-ME patients (7 CRVO-ME and 10 BRVO-ME) for untargeted metabolomics analysis. There were no significant differences in age, gender, hypertension, coronary heart disease, or diabetes among the groups (Table 1).

AH Metabolic Profiles

Untargeted metabolomics analysis was used to determine the metabolic profile of AH. PCA analysis revealed tight clustering of QC samples, indicating high repeatability and reliability of the data (Figure 1). OPLS-DA was then performed to assess the metabolic differences among the groups. The OPLS-DA score plot demonstrated clear separation between RVO-ME patients and the control group, as well as between CRVO and BRVO patients (Figure 2). The validation plot further confirmed the robustness of the model, with all R2 and Q2 values for permuted points on the left being lower than those for the original points on the right (Figure 3).

Identification of Potential Biomarkers

Compared to the control group, a total of 28 differential metabolites were identified in RVO-ME, with 26 differential metabolites in CRVO-ME and 24 in BRVO-ME. When comparing CRVO-ME to BRVO-ME, 9 differential metabolites were found (VIP > 1 and p < 0.05) (Tables 2–5). Volcano plots (Figure 4), heat maps, and hierarchical clustering analyses (Figure 5) were used to examine the trends of these differential metabolites. Compared to the control group, RVO-ME showed a significant increase in 20 metabolites and a significant decrease in 8 metabolites. CRVO-ME exhibited a significant increase in 18 metabolites and a significant decrease in 8 metabolites. BRVO-ME demonstrated a significant increase in 17 metabolites and a significant decrease in 7 metabolites. When comparing CRVO-ME and BRVO-ME, 3 metabolites were found to be increased, and 6 were found to be decreased. Three distinct differentially expressed metabolites, including PC(18:0_20:3), SQDG(33:2), and TG(16:0_10:4_16:0), were shared among the groups. Additionally, three differentially expressed metabolites were found to be specific to CRVO-ME and were also shared with RVO-ME. Two, four, and five differentially expressed metabolites were identified as being specific to RVO-ME, CRVO-ME, and RVO-ME, respectively (Figure 6).

Table 1 Clinical Characteristics of the Patients

	RVO-ME (27)		Control (10)	P Value
	CRVO-ME (7)	BRVO-ME (10)		
Gender (male/female)	3/4	5/5	4/6	0.899
Age (years),median	69.29±2.25	69.60±1.74	70.10±1.87	0.683
Hypertension (yes/no)	4/3	6/4	4/6	0.635
Diabetes (yes/no)	1/6	2/8	1/9	0.819
Coronary heart disease (yes/no)	1/6	1/9	1/9	0.953
Hyperlipidemia (yes/no)	4/3	5/4	3/7	0.425

Notes: The data are presented as the mean±SD.
Abbreviations: RVO-ME, retinal vein occlusion macular edema; CRVO-ME, central retinal vein occlusion macular edema; BRVO-ME, branch retinal vein occlusion macular edema.

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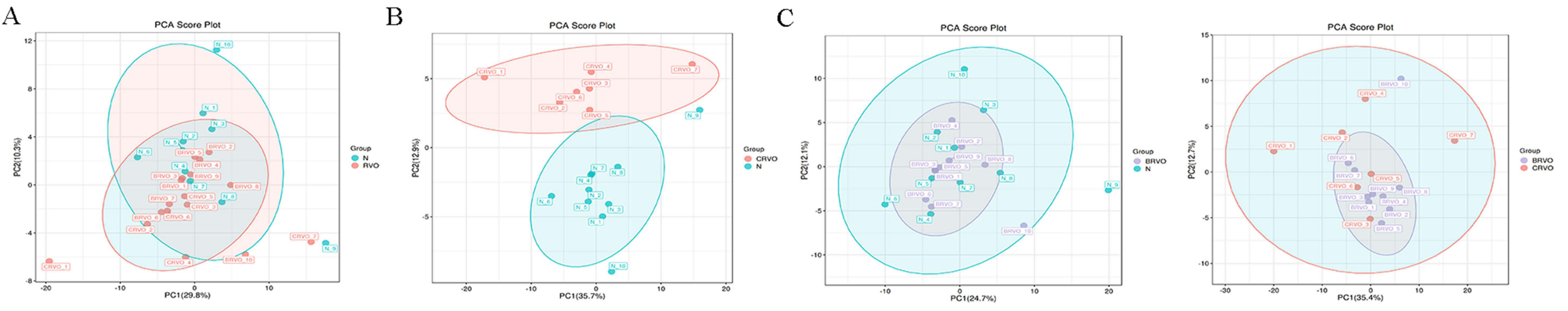

Figure 1 The score plot of PCA analysis. (**A**) Control vs RVO-ME, (**B**) Control vs CRVO-ME, (**C**) Control vs BRVO-ME, (**D**) CRVO-ME vs BRVO-ME.

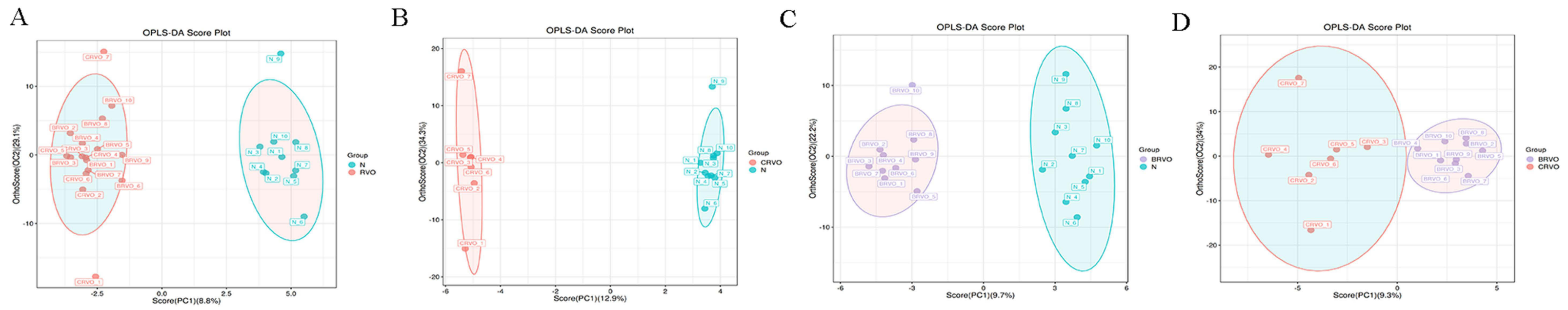

Figure 2 The score plot of the PLSDA model. (**A**) Control vs RVO-ME, (**B**) Control vs CRVO-ME, (**C**) Control vs BRVO-ME, (**D**) CRVO vs BRVO-ME.

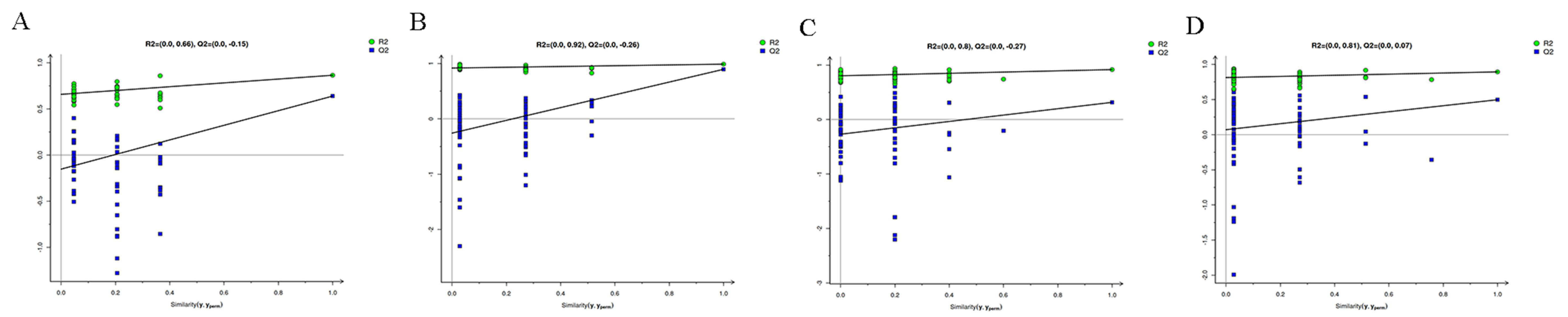

Figure 3 Validation plots for the OPLS-DA mode. (**A**) Control vs RVO-ME, (**B**) Control vs CRVO-ME, (**C**) Control vs BRVO-ME, (**D**) CRVO-ME vs BRVO-ME.

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Table 2 List of Significantly Different Metabolites in AH from RVO Compared with Controls

Metabolites	RT (s)	VIP	Fc	p Value	Up/Down Regulation
DG(16:0_16:0)	16.1645397	2.16002461	1.41	0.00063315	Up
DG(16:0_18:3)	12.783	1.407964	1.67	0.04949925	Up
DG(18:0_16:0)	17.3406595	2.20062501	1.43	0.0004502	Up
DG(18:0_18:0)	18.2535941	1.3130042	1.27	0.0463954	Up
DG(34:1e)	26.4698628	1.53153362	1.58	0.01693579	Up
DG(36:5e)	26.4770577	1.63627968	1.63	0.01525254	Up
DG(38:3)	5.3555635	2.19380712	1.15	0.00067891	Up
PC(18:0_20:3)	14.6279419	1.79561097	2.32	0.00180325	Up
SM(d42:2)	16.6088443	1.22717464	1.7	0.03209415	Up
TG(12:0e_6:0_16:0)	17.333	1.92559202	1.41	0.00288085	Up
TG(16:0_22:1_22:6)	16.7921441	1.68859729	1.24	0.00881451	Up
TG(16:2e_6:0_16:1)	5.359	2.19380712	1.15	0.00067891	Up
TG(17:1_17:1_17:1)	26.4782695	1.73785236	1.77	0.04310898	Up
TG(18:0_16:0_20:3)	21.5566462	1.73770022	1.31	0.00915491	Up
TG(18:0_18:0_20:3)	21.9726119	1.45063906	1.35	0.02713084	Up
TG(18:1_18:1_20:3)	26.4692811	1.65620429	1.63	0.01845193	Up
TG(18:1_18:1_22:5)	26.474965	1.59707249	1.59	0.00715751	Up
TG(18:1_18:2_20:5)	26.4779694	1.63357383	1.54	0.01663783	Up
TG(20:4e_10:1_10:1)	5.37005104	2.19380712	1.15	0.00067891	Up
TG(44:4)	7.55239105	2.0037523	1.57	0.00233267	Up
ChE(20:5)	26.4709388	1.5788257	0.71	0.01335454	Down
DG(34:5e)	10.696	1.14042173	0.77	0.04145359	Down
MGDG(16:1_18:3)	11.4565099	2.90385123	0.02	0.00004071	Down
PIP(26:0)	5.35907491	2.36533399	0.75	0.00001047	Down
PMe(16:0_20:3)	5.389	2.30808061	0.71	0.00001345	Down
SPH(t16:0)	1.52764084	1.40010509	0.83	0.04103888	Down
SQDG(33:2)	5.358	1.9595966	0.7	0.00048879	Down
TG(16:0_10:4_16:0)	5.30145632	2.29026443	0.39	0.00004727	Down

Table 3 List of Significantly Different Metabolites in AH from CRVO Compared with Controls

Metabolites	RT (s)	VIP	Fc	p Value	Up/Down Regulation
AcHexSiE(18:3)	17.456	1.34019207	2.61	0.04991518	Up
Cer(d47:7)	17.5528465	1.34034897	1.55	0.04848533	Up
DG(16:0_16:0)	16.1645397	1.88929624	1.51	0.0032522	Up
DG(18:0_16:0)	17.3406595	1.9412608	1.52	0.00208868	Up
DG(18:0_18:0)	18.2535941	1.48373019	1.43	0.02983032	Up
DG(36:5e)	26.4770577	1.73581379	1.85	0.00740201	Up
DG(38:3)	5.3555635	1.73706481	1.17	0.02887175	Up
PC(18:0_20:3)	14.6279419	2.05567913	3.33	0.0007053	Up
PC(38:5e)	13.5086393	1.60223537	2.08	0.01412463	Up
SM(d42:2)	16.6088443	1.62132541	2.38	0.01442581	Up
TG(12:0e_6:0_16:0)	17.333	1.7126885	1.55	0.01032575	Up
TG(16:2e_6:0_16:1)	5.359	1.73706481	1.17	0.02887175	Up
TG(18:0_16:0_18:3)	21.3642289	1.46694752	1.86	0.03646354	Up

(*Continued*)

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Table 3 (Continued).

Metabolites	RT (s)	VIP	Fc	p Value	Up/Down Regulation
TG(18:0_16:0_20:3)	21.5566462	1.50091866	1.35	0.02584976	Up
TG(18:1_18:1_20:3)	26.4692811	1.647668	1.83	0.0128773	Up
TG(18:1_18:2_20:5)	26.4779694	1.69218697	1.74	0.00987267	Up
TG(20:4e_10:1_10:1)	5.37005104	1.73706481	1.17	0.02887175	Up
TG(44:4)	7.55239105	1.83420006	1.86	0.00362061	Up
ChE(20:5)	26.4709388	1.76885317	0.63	0.00704227	Down
MGDG(16:1_18:3)	11.4565099	2.46838356	0.01	0.00002874	Down
PIP(26:0)	5.35907491	2.18325341	0.7	0.00376116	Down
PMe(16:0_20:3)	5.389	2.19190895	0.63	0.00488725	Down
SPH(t16:0)	1.52764084	1.58914253	0.73	0.01522368	Down
SPH(t18:0)	2.13396923	1.42007825	0.74	0.03334845	Down
SQDG(33:2)	5.358	2.06362033	0.57	0.00806381	Down
TG(16:0_10:4_16:0)	5.30145632	2.27116371	0.28	0.00006563	Down

Table 4 List of Significantly Different Metabolites in AH from BRVO Compared with Controls

Metabolites	RT (s)	VIP	Fc	p Value	Up/Down Regulation
DG(16:0_16:0)	16.1645397	2.21175581	1.35	0.0014485	Up
DG(18:0_16:0)	17.3406595	2.15266979	1.36	0.00246052	Up
DG(34:1e)	26.4698628	1.71291644	1.61	0.01541432	Up
DG(38:3)	5.3555635	2.3105047	1.14	0.00042249	Up
PC(18:0_20:3)	14.6279419	1.49665683	1.6	0.03460422	Up
TG(12:0e_6:0_16:0)	17.333	1.88640823	1.31	0.01037196	Up
TG(16:0_12:3_14:4)	5.36873279	1.3281529	1.1	0.0498961	Up
TG(16:0_16:1_17:1)	20.4318785	1.58145942	1.6	0.02928055	Up
TG(16:0_22:1_22:6)	16.7921441	1.54162766	1.23	0.02820913	Up
TG(16:2e_6:0_16:1)	5.359	2.3105047	1.14	0.00042249	Up
TG(17:1_17:1_17:1)	26.4782695	1.57168978	1.79	0.04421369	Up
TG(18:0_16:0_20:3)	21.5566462	1.54097047	1.27	0.03088355	Up
TG(18:0_18:0_20:3)	21.9726119	1.89685163	1.36	0.00479349	Up
TG(18:1_18:1_22:0)	21.955	1.41517667	1.71	0.0429274	Up
TG(18:1_18:1_22:5)	26.474965	1.90232042	1.53	0.00919275	Up
TG(20:4e_10:1_10:1)	5.37005104	2.3105047	1.14	0.00042249	Up
TG(44:4)	7.55239105	1.66384319	1.37	0.02700662	Up
MGDG(16:1_18:3)	11.4565099	2.72322573	0.02	1.7763E-06	Down
PG(37:0)	7.54685356	1.7823985	0.87	0.01381605	Down
PIP(26:0)	5.35907491	2.41804932	0.78	0.00038289	Down
PMe(16:0_20:3)	5.389	2.76773411	0.77	1.7994E-05	Down
SQDG(33:2)	5.358	2.03074015	0.78	0.00809567	Down
TG(16:0_10:4_16:0)	5.30145632	2.23377742	0.47	0.0008195	Down
WE(30:1)	9.671	1.48066066	0.68	0.03646081	Down

Discussion

RVO is the second most common cause of vision loss in elderly patients with retinal vascular diseases, following diabetic retinopathy.[18–20] The formation of retinal vein thrombosis in RVO leads to multifactorial pathophysiological changes, including increased capillary hydrostatic pressure, endothelial dysfunction, disruption of the blood-retinal barrier,

Table 5 List of Significantly Different Metabolites in AH from CRVO Compared with BRVO

Metabolites	RT (s)	VIP	Fc	p Value	Up/Down Regulation
Cer(d47:7)	17.5528465	1.55479746	1.57	0.03946196	Up
PC(18:0_20:3)	14.6279419	1.81255774	2.08	0.01086749	Up
SM(d42:2)	16.6088443	1.63294143	1.94	0.03074999	Up
Cer(d30:0)	9.18163223	2.05497395	0.84	0.02581543	Down
Cer(t34:0)	10.454	2.09150814	0.8	0.04211113	Down
SPH(t16:0)	1.52764084	2.12477607	0.81	0.01277615	Down
SQDG(33:2)	5.358	1.47324036	0.73	0.03960702	Down
TG(16:0_10:4_16:0)	5.30145632	2.40700536	0.59	0.0073171	Down
TG(27:1)	7.59265063	2.12624993	0.45	0.04729948	Down

inflammation, and ischemia-induced neovascularization, resulting in macular edema (ME) and/or complications from neovascularization.[21–23] In these complex processes, vascular and pro-inflammatory cytokines play a crucial role, as they are released by endothelial and inflammatory cells, facilitating communication between them.[21] Excessive release of cytokines during the disease can lead to oxidative stress, cell apoptosis, and disruption of the blood-retinal barrier. Indeed, the severity of ME has been shown to be associated with cytokine imbalance.[22,23] Our research group has previously reported elevated levels of VEGF, MCP-1, IP-10, IL-6, and IL-8 in AH of patients with BRVO and their correlation with morphological parameters observed through spectral-domain optical coherence tomography (SD-OCT).[14]

In this study, we identified elevated levels of PC in the aqueous humor (AH) of patients with RVO, CRVO, and BRVO through LC-MS analysis. PC, a type of phospholipid and a major component of biological membranes, plays a crucial role in membrane structure and function. The composition of fatty acyl chains in phospholipids determines the biophysical properties of membranes, which in turn influence various biological processes. PC is a specific type of phospholipid.[24,25] The oxidation of esterified unsaturated fatty acids in phospholipids leads to the formation of mediators with various biological activities, including pro-inflammatory, pro-thrombotic, endotoxin-neutralizing, and immune-modulatory effects.[26–28] These activities may contribute to the onset and progression of RVO.

Recent studies have demonstrated that the etiology of RVO is multifactorial. Risk factors include various systemic conditions such as hypertension (HT), dyslipidemia, high blood viscosity, coagulation abnormalities, obesity, and atherosclerosis.[29,30] Dyslipidemia can lead to a pro-inflammatory state, endothelial dysfunction, increased blood viscosity, heightened blood cell aggregation, and reduced antioxidant defense.[31,32] Triglycerides (TG) are fat molecules commonly found in the blood, and some studies have suggested a correlation between elevated triglyceride levels and retinal artery sclerosis as well as retinal arteriolar occlusion.[33,34] This correlation may serve as a risk factor for RVO. Our study has also identified high levels of TG in the aqueous humor (AH) of RVO patients, indicating that managing lipid metabolism through diet and medication could influence RVO-related macular edema (RVO-ME), potentially offering new strategies for intervention.

SQDG is a lipid compound found in photosynthetic organisms, primarily in plants, algae, and certain bacteria.[35,36] It consists of a sugar moiety (sulfoquinovose) linked to a diacylglycerol backbone. SQDG plays a crucial role in photosynthesis, particularly in the thylakoid membranes of chloroplasts.[37] It contributes to the organization and stability of photosynthetic complexes and regulates the dynamics of cell membranes.[38] Additionally, SQDG helps protect cells from oxidative damage by scavenging and neutralizing reactive oxygen species (ROS).[39] Its role in cellular defense against oxidative stress is vital for maintaining cellular health and preventing potential damage to cellular components.[40] Research suggests that SQDG may exhibit various biological activities and potential applications, including anti-inflammatory, antimicrobial, antioxidant, anti-tumor, and anti-metabolic disease effects.[41–44] Our study found reduced levels of the anti-inflammatory and antioxidant molecule SQDG in the aqueous humor (AH) of patients with RVO, including CRVO and BRVO. This finding suggests that SQDG may be involved in the pathogenesis of RVO through inflammatory and oxidative stress pathways.

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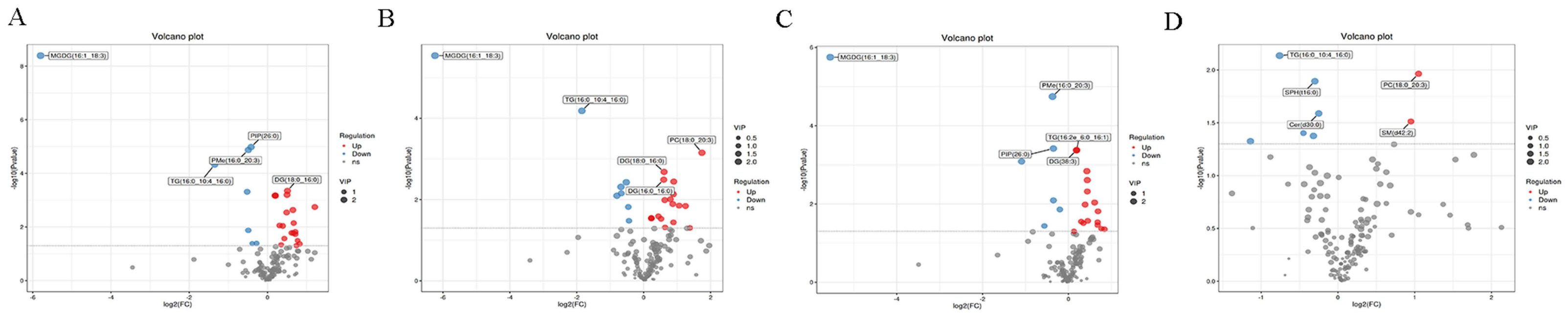

Figure 4 Volcano plots of aqueous humor metabolomic. (**A**) Control vs RVO-ME, (**B**) Control vs CRVO-ME, (**C**) Control vs BRVO-ME, (**D**) CRVO-ME vs BRVO-ME.

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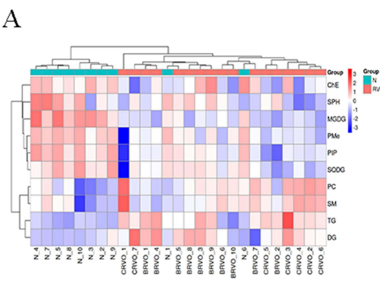

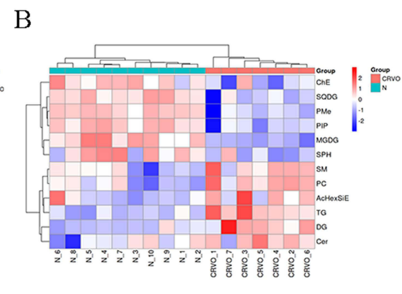

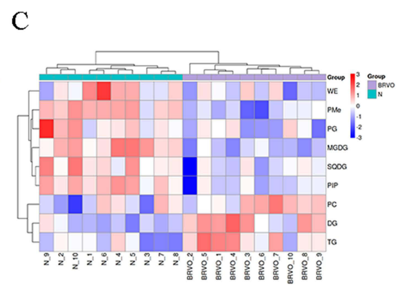

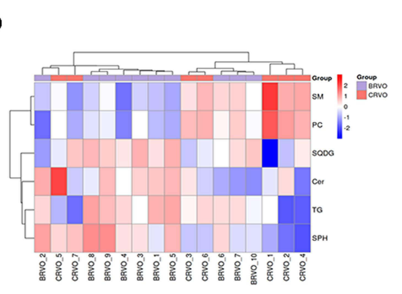

Figure 5 Heat plot of aqueous humor metabolomic. (**A**) Control vs RVO-ME, (**B**) Control vs CRVO-ME, (**C**) Control vs BRVO-ME, (**D**) CRVO-ME vs BRVO-ME.

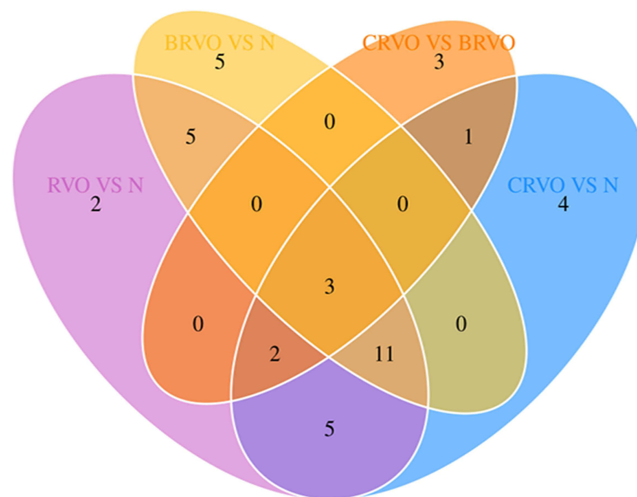

Figure 6 Venn diagram showing the metabolite are commonly expressed differently among the groups.

In summary, we investigated the metabolic changes in the aqueous humor (AH) of patients with RVO-related macular edema (RVO-ME). We analyzed the metabolic profiles of RVO-ME, central retinal vein occlusion-related macular edema (CRVO-ME), and branch retinal vein occlusion-related macular edema (BRVO-ME) in comparison to a control group. Additionally, we compared the metabolic profiles between CRVO-ME and BRVO-ME. To our knowledge, this is the first study to utilize LC-MS to analyze the differences in AH metabolomics between BRVO-ME and the control group, as well as between CRVO-ME and BRVO-ME. After correction, we identified 28 differentially expressed metabolites in RVO-ME compared to the control group. Specifically, CRVO-ME and BRVO-ME exhibited 26 and 24 differentially expressed metabolites, respectively, relative to the control group. Furthermore, we found 9 metabolites with differential expression between CRVO-ME and BRVO-ME. These biomarkers can distinguish RVO-ME, CRVO-ME, and BRVO-ME patients from the control group, as well as differentiate CRVO-ME patients from BRVO-ME patients. Interestingly, we observed differential expression of metabolites such as PC (18:0_20:3), SQDG (33:2), and TG (16:0_10:4_16:0) across the various groups. The discovery of novel metabolite biomarkers in the AH from patients with RVO-ME offers unprecedented insights into the pathogenic mechanisms underlying this visually debilitating condition. These findings not only contribute to a deeper understanding of the disease's etiology but also pave the way for potential novel therapeutic interventions. Furthermore, the unique metabolic profiles identified in the AH of patients with CRVO-ME and BRVO-ME enhance diagnostic precision, allowing for more accurate differentiation between these entities. This enhanced accuracy is crucial for tailoring individualized treatment regimens that target the specific metabolic perturbations present in each patient, thereby optimizing treatment outcomes and potentially improving visual prognosis.

There are several limitations to our study. Firstly, the small sample size poses a major limitation, potentially restricting the detection of significant changes in some metabolites. Future research will involve collecting a larger number of samples to address this issue. Additionally, the control group comprised patients undergoing cataract surgery rather than truly "healthy" individuals, which may introduce bias into the study results. Ideally, obtaining AH samples from individuals without any ocular diseases would provide a more accurate baseline for comparison.

Conclusion

Our study offers the first comprehensive understanding of the metabolomics of aqueous humor (AH) in patients with RVO-related macular edema (RVO-ME), central retinal vein occlusion-related macular edema (CRVO-ME), and branch retinal vein occlusion-related macular edema (BRVO-ME). The results reveal a complex and significant metabolic disruption occurring in the AH of these patients. Moreover, we identified notable differences in metabolites between CRVO-ME and BRVO-ME patients. Importantly, intraocular angiogenic factors, inflammatory mechanisms, and oxidative stress responses may play crucial roles in the onset and progression of RVO-ME. These findings could illuminate potential prognostic metabolic biomarkers and novel therapeutic strategies for the prevention or delay of RVO-ME development.

Ethics Approval and Consent to Participate

All procedures of this study were in accordance with the tenets of the Declaration of Helsinki and approved by the medical ethics committee. Samples were collected after patients' written informed consent to participate in the study.

Patient Consent for Publication

All Patients declare that they consent for publication.

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Disclosure

The authors declare that they have no competing interests. This paper has been uploaded to SSRN as a preprint: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4599037.

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