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Metabolomic analysis of the aqueous humor from patients with central retinal vein occlusion using UHPLC-MS/MS

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ABSTRACT

Central retinal vein occlusion (CRVO) is one of the retinal fundus diseases and may result in irreversible visual impairment. Metabolic dysfunction has been proved to play an essential role in the pathogenesis of CRVO. We performed untargeted metabolomic analysis of the aqueous humor (AH) of patients with CRVO and controls using UHPLC-MS/MS.

A total of 248 metabolites were identified in the tested AH samples, 37 of which allowed for the construction of an orthogonal partial least squares discriminant analysis model with good predictive capability (Q2cum = 0.834) and low risk of overfitting. The components contributing the most to the metabolomic signature of CRVO were those related to amino acid metabolism, carbohydrates, and fatty acid metabolites (variable importance on projection>1.0 and $p < 0.05$). The CRVO group appeared to have a lower AH concentration of carbohydrates and amino acids, but a relative higher concentration of carnitine-associated energetic substrates (butyryl carnitine, deoxycarnitine, N6-trimethyl-L-lysine) and osmolytes compared with those of the control group.

These results indicate that patients with CRVO may have ocular aberrations in metabolic pathways involving certain amino acids, fatty acids, and carbohydrates. These metabolite changes might correlate with energy dysfunction and inflammation response in the AH of CRVO patients. This finding may provide insight into the pathophysiology of CRVO for the development of new therapeutic strategies.

1. Introduction

Central retinal vein occlusion (CRVO) is a common retinal disease that may result in irreversible visual impairment. The pathophysiology of CRVO involves thrombotic occlusion of the central retinal vein, which leads to increased intravenous hydrostatic pressure and retinal capillary nonperfusion. In this condition, the mean oxyhemoglobin saturation becomes markedly diminished; insufficient oxygen supply alters retinal blood flow leading to ischemia and metabolic impairment [1]. Thus, ischemia and hypoxia-induced metabolic dysfunction are thought to play an essential role in the pathogenesis of CRVO.

Metabolomics is a study to identify metabolites from tissues or organisms so as to assess pathophysiological changes. Dynamic alterations of endogenous metabolic substances can imply the development of a disease at certain stages of its progression within systems. Metabolomic study has been used to identify the metabolic changes and reveal pathological mechanisms in different eye diseases. The concentration of metabolites involved in osmoprotection, neuroprotectors and amino acid metabolism have been shown to be dysregulated in the aqueous humor (AH) of glaucomatous eyes [2]. Analysis of AH metabolites in myopic eyes demonstrated increased concentrations of 27 significant metabolites compared to controls [3]. Further, differential metabolites have been detected in the AH of patients with diabetic retinopathy (DR) [4] and age-related macular degeneration (AMD) [5]. However, metabolomic studies of CRVO are limited. A previous study showed that abnormal lipid metabolism was accompanied by retinal vein occlusion (RVO); a low level of blood high-density lipoprotein was

Abbreviations: AH, aqueous humor; BCAAs, branched-chain amino acids; CRVO, central retinal vein occlusion; DR, diabetic retinopathy; ESI, electrospray ionization; FC, fold change; FDR, false discovery rate; HILIC, hydrophilic interaction liquid chromatography; iCRVO, ischemic CRVO; OPLS-DA, orthogonal partial least-square discriminant analysis; PCA, principal component analysis; PLS-DA, partial least-square discriminant analysis; QC, quality control; TCA, tricarboxylic acid cycle; TML, 6-N-trimethyllysine; UHPLC-MS/MS, tandem mass spectrometry coupled with ultra-high-performance liquid chromatography; VEGF, vascular endothelial growth factor; VIP, variable importance on projection.

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associated with RVO development [6]. Another study analyzed the metabolite changes in patients with CRVO compared with controls, and found that glucose metabolism might be compromised [7].

The collection of metabolite-containing samples from human retinal tissues for metabolomic analysis is challenging [8]. Although vitreous humor (or even macular tissue) specimens from patients would be ideal, the collection of vitreous samples requires an invasive procedure, which may not be an ethically justifiable approach for clinical research. Additionally, harvesting vitreous fluid carries the risk of vitreous hemorrhage, retinal tears and detachment. These difficulties can be circumvented by using AH samples collected during anterior chamber paracentesis or intraocular procedures. The AH contains factors that provide nutrients to the eye and maintain its homeostasis. The composition of the AH can reflect conditions of the retina, although it is not in direct contact with it [9]. Furthermore, although CRVO originates from and affects the posterior ocular segments, it is associated with blood-aqueous barrier disruption, which could influence the homeostasis of AH metabolism [10]. Metabolomic analysis of the AH from patients with CRVO may, therefore, provide valuable information regarding the disease process. The metabolomic profiles of AH in glaucoma [2] and DR [4] have been previously investigated. However, to the best of our knowledge, the metabolomic profile of the AH from patients with CRVO has not been assessed.

In this study, we hypothesize that disruption of homeostasis in AH metabolism might be associated with the development of CRVO. Hence, we used UHPLC-MS/MS (tandem mass spectrometry coupled with ultra-high-performance liquid chromatography) to analyze alterations in AH metabolites in patients with CRVO, as this method enables rapid detection and monitoring of metabolite variation in biological networks [11].

2. Materials and methods

2.1. Participants

The current project was discussed and certified by the Human Ethics committee of Tianjin Eye Hospital. This project protocol was conducted in accordance with the tenets of the Declaration of Helsinki. It was registered online with Chinese Clinical Trial Registry (ChiCTR1900022442). All participants enrolled provided their informed consent prior to inclusion at the beginning of the examination.

Thirty-five participants were divided into two groups: fifteen patients (fifteen eyes) first diagnosed with central retinal vein occlusion who underwent intravitreal anti-VEGF injection, and twenty patients (twenty eyes) with cataract surgery. The two groups were closely matched in terms of sex and age. The participants met the following criteria: patients with CRVO underwent comprehensive eye examination, including visual acuity, slit-lamp biomicroscopy, spectral-domain optical coherence tomography (central macular thickness> 300μm), and fluorescein angiography (FA) before surgical intervention. Patients with cataract in the operated eye had visual acuity less than 20/50, nuclear hardness grade classification ≥III, and consented to the cataract surgery.

The exclusion criteria were: presence of any other fundus retinopathy, anterior chamber disease, ocular infection, or high myopia; past history of eye trauma and intraocular laser or injections; past history of any other ocular surgery; and diabetes mellitus.

2.2. Sample collection

Approximately 80–100μl of aqueous humor was collected under the surgical microscope before cataract surgery and anti-VEGF intravitreal injection. The AH specimens were immediately transferred into Eppendorf containers and centrifuged at 15,000 *g* for 5 min before storage at −80 °C until further analysis. AH samples were thoroughly dissolved in methanol/acetonitrile and sonicated for one hour. AH supernatants were then collected before being centrifuged at 14,000 *g* at 4 °C. Quality control (QC) samples were performed following the same protocol as previous analysis for the CRVO and cataract group in each batch.

2.3. UHPLC-MS/MS analysis

UHPLC-MS/MS analysis was acquired with the UPLC system (UHPLC, 1290 Infinity LC, Agilent Technologies, Santa Clara, CA, USA) equipped with tandem mass spectrometry on a TripleTOF 5600 Plus (AB Sciex, Framingham, MA, USA).

Hydrophilic interaction liquid chromatography (HILIC) separation of AH specimens was performed using an ACQUIY UPLC BEH Amide column (Waters Corp., Milford, MA, USA). MS data were acquired via the electrospray ionization (ESI) positive-mode (Ion Spray Voltage Floating 5500 V) and negative-mode (Ion Spray Voltage Floating -5500 V). A high sensitivity mode was used for information-dependent acquisition during product ion scanning. Collision energy was set at 30 eV, and declustering potential was fixed as 60 V in both the positive and negative-modes.

For data normalization, quality control specimens were performed by pooling aliquots of all representatives AH samples. Blank (75 % ACN in water) and quality control specimens were injected every 6 samples when performing data acquisition.

2.4. Data pre-processing and filtering

The processing software packages ProteoWizard and XCMS (version 3.2) were used for retention time correction, feature extraction, and alignment. As described before [5], a combination of accurate mass (<25 ppm) and experimental MS/MS matched against our in-house tandem MS spectral library and other public databases (NIST, and MassBank) was used for metabolite identification. Metabolic peaks detected with a signal less than 50 % greater than those in quality control specimens were excluded in the extracted-ion features. Variable ion peaks displaying over fifty percentage of the nonzero measurement data in at least one group were included for further statistical analysis.

2.5. Multivariate statistical analysis

SIMCA-P software (Version 14.0, Umetrics, Umea, Sweden) was utilized for multivariate pattern recognition analysis and model establishment. The peak area in chromatograms were used for multivariate statistical analysis in current study. PCA was used in the model for the analyze of specimen separation and outliers. Subsequently, OPLS-DA and PLS-DA were both performed to displace variation between control and experimental groups. Evaluated models were calculated for over-fitting with methods of permutation tests. R^2X and R^2Y values were used to described the performance of the models while Q2 and permutation test (n = 200) were performed to evaluate model prediction performance. The variable importance on projection (VIP) values more than 1.0 and *p*-values less than 0.05 were considered as statistically significant in this model. Fold change was derived from the logarithm of the average mass response (area) ratio between two arbitrary classes.

2.6. KEGG enrichment analysis

KEGG analysis using the KEGG database (https://www.kegg.jp/) was performed with MetaboAnalyst 3.0 to identify potential path-

Table 1
The clinical futures of the CRVO and cataract patients in current study (Mean ± SD).

Characteristic	CRVO Group (N = 15)	Control Group (N = 20)	p Value
Age (year)	64.7 ± 3.1	69.6 ± 6.8	0.128
Gender			
Male (%)	8 (53.3 %)	13 (65 %)	0.486
Female (%)	7 (46.7 %)	7 (35 %)	0.486
Right eye (%)	6 (40 %)	11 (55 %)	0.380
Smoking history (%)	1 (6.7 %)	1 (5%)	0.833
History of hypertension (%)	8 (40 %)	7 (46.7 %)	0.693
CMT (μm)	617.0 ± 172.1	227.7 ± 48.5	<0.001
Duration from onset(week)	6.6 ± 4.4		
Clinical subgroup			
Ischemic (N)	8		
Nonischemic (N)	7		

Clinical characteristics were compared between CRVO patients and controls using independent *t*-test for continuous variables and a $\chi 2$ test for categorical variables; CMT: Central Macular Thickness.

ways affected by CRVO. A p-value less than 0.05 and a count more than 3 was defined as the significant changed pathways. Analysis of KEGG enrichment was performed with Fisher's exact test while false discovery rate (FDR) correction for multiple testing was also performed.

3. Results and discussion

3.1. Clinical characteristics of the participants

A total of thirty-five patients were enrolled in this study, among them comprising fifteen were diagnosed with CRVO and twenty controls. The control group comprised patients with cataracts without retinal pathology. The clinical futures of the CRVO and cataract patients are displayed in Table 1. Average duration from onset in CRVO group was 6.6 ± 4.4 weeks.

3.2. Changes in the metabolic profiles in patients from CRVO and control groups

Metabolites concentration in patients from control and CRVO groups were firstly analyzed and compared using unsupervised statistics. The PCA score plots of the first two principal components showed that individuals in the control group were clustered in the bottom left part, while CRVO individuals were clustered near the upper right part **(Fig. A.2);** typical total ion chromatograms(TIC) of aqueous humor specimens from patients with CRVO are shown in **Figure A.1**). Subsequently, we used supervised pattern recognition techniques, i.e. OPLS-DA, demonstrating clear separation within the two groups (Fig. 1A). The cross-validation and permutation testing showed a cumulative R^2Y of 0.998 and a Q^2 of 0.834 (Fig. 1B). The permutation test showed that the models fit well.

CRVO was classified into ischemic and non-ischemic forms according to the fluorescein fundus angiography findings. The principal component analysis (PCA) showed a partial overlap between ischemic CRVO (iCRVO) and non-ischemic CRVO. When comparing these two groups, only one differential metabolite was found. Therefore, we did not show any correlation results in the manuscript (data not shown). We speculate that in the acute period, the short onset of the disease might not lead to significant metabolic changes within the iCRVO and non-ischemic CRVO groups. For instance, a previous study has shown that homocysteine levels did not significantly differ between iCRVO and nonischemic CRVO patients within three months of onset [12]. In this study, the average duration from onset was within two months; therefore, it might not have been possible to distinguish the two patient groups metabolically.

3.3. Metabolic network variation based on the altered metabolites

A total of 248 metabolites were identified in the tested AH samples. Among these, the concentrations of 37 metabolites were significantly different between the CRVO and control groups (VIP > 1 and $p < 0.05$). Twenty-six metabolites were significantly downregulated in the CRVO group compared with the control group; twelve of these were proteogenic amino acids and amino acid analogues. Certain carbohydrates (D-fructose and glucose) had decreased concentrations in the CRVO group compared to the control group. Certain osmolytes (taurine, creatinine, and betaine) and fatty acid conjugates (butyryl carnitine and deoxycarnitine) were upregulated in the CRVO group compared with the control group (Fig. 2). The MS/MS metabolite data has been shown in Table 2. Heatmaps to better visualize metabolite concentration differences and to distinguish the increased and decreased metabolites are presented in Fig. 3.

Metabolomics could be used as a tool to reveal the pathogenesis of diseases at the molecular level. The metabolic constituents in the AH reflect the physiological condition of the eyes [4]. Although the AH is not directly in contact with the retina, it has been shown that changes in metabolites in the AH can mark retinopathy [9]. In our study, the AH-based metabolomic signature of CRVO was characterized by decreased concentrations of glucose and amino acids and increased concentrations of osmolytes (taurine and betaine) and certain carnitine-associated energetic substrates. This profile of differential amino acids, lipids, and carbohydrates were partially similar to the results of previous metabolomic studies of the AH in DR and glaucoma [2,4]. For instance, in glaucoma, changes in the concentrations of amino acids, including taurine and glutamate, pointed to ganglion cell damage induced by high intraocular pressure and neuroprotectors [2]. In DR, changes to the levels of amino acids and carbohydrates, i.e. lactate and glutamate, might be related to high-glucose-induced mitochondrial dysfunction and oxidative stress damage [4]. Comparing with these two chronic diseases, amino acids and carbohydrates are also impaired in CRVO, which is characterized by ischemia and hypoxia in the retina, disrupt not only energy metabolism but also inflammation response in the AH of CRVO. A schematic diagram of the specific metabolites and their effect in the AH is shown in Fig. 4.

3.4. Metabolic pathway analysis associated with CRVO

Our results showed that 10 metabolic pathways were influenced by CRVO. These included pathways involved in the metabolism of amino acids, carbohydrate, and lipid; such as valine, leucine, and isoleucine biosynthesis; aminoacyl-tRNA biosynthesis; arginine and proline metabolism; and 2-oxocarboxylic acid metabolism (Fig. 5).

3.4.1. Amino acid metabolism

Certain amino acids were decreased in CRVO patients compared with controls, which may be because CRVO causes retinal ischemia, which limits aerobic respiration and leads to increased catabolism of amino acids for energy production [13]. All the amino acids that were lowered in the AH of CRVO patients can be catabolized for energy. Some amino acids (glutamine, proline, methionine, and serine) can be converted to precursors of gluconeogenesis, while others (tryptophan, leucine, and tyrosine) are normally used as substrates for ketogenesis [14].

Glutamine can act as a nitrogen donor in the biosynthesis of amino sugars, nucleotides, and other amino acids. A decrease in the concentration of glutamine has been observed in the aqueous humor from patients with RAO (retinal artery occlusion) [15]. Accordingly, our study also revealed that the concentration of glutamine was less than that in the CRVO group. Several reasons may

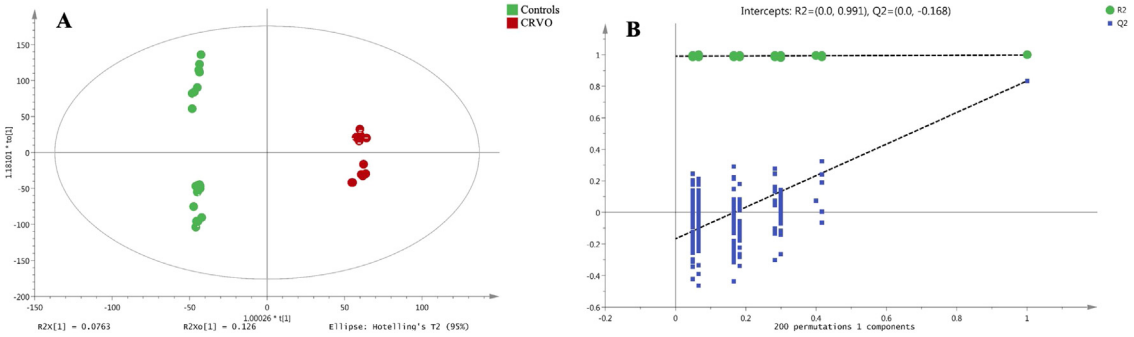

Fig. 1. Significant disturbed metabolites analysis. A: The orthogonal projection to latent structure discriminant analysis (OPLS-DA) between the control and CRVO groups. Samples from the CRVO group are marked with red circles and samples from controls are marked with green circles. B: Permutation test of the OPLS-DA model. The values of R^2Y and $Q2$ represent the goodness of fit and predictability of the model, respectively.

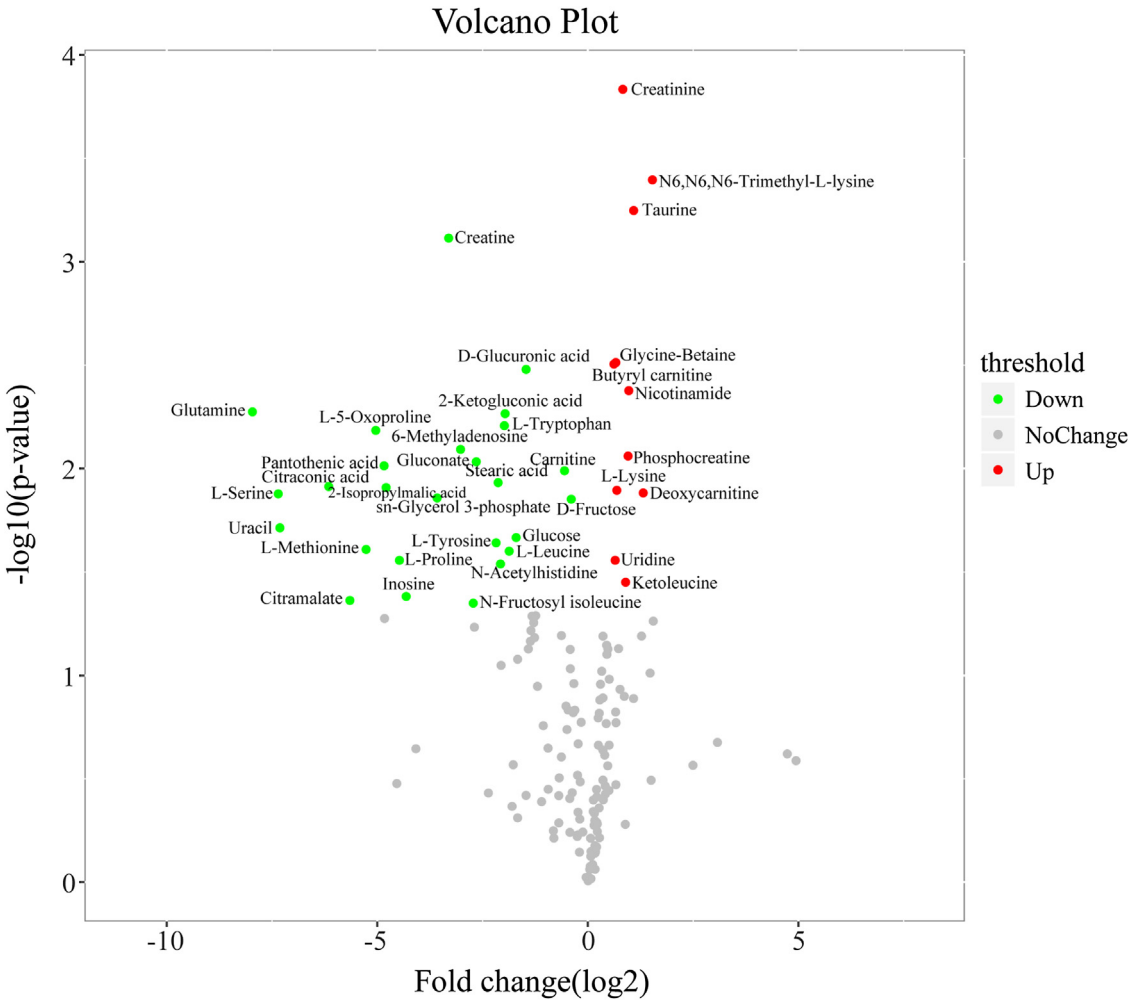

Fig. 2. Volcano plot representing the relationship between fold change and significance of the metabolic features forwarded for statistical analysis.

contribute to the decreased glutamine levels in AH. Our results indicated that the synthesis of glutamine by the enzyme glutamine synthetase might be disrupted in CRVO patients with impaired outflow of the central retinal vein [16]. Furthermore, glutamine plays a crucial role in maintaining energy balance, which may be altered under retinal ischemia conditions [13].

In our study, methionine was significantly decreased in patients with CRVO. Methionine is a sulfur-containing amino acid that is important for protein synthesis and transmethylation reactions. It functions as an antioxidant and as a key component for the regulation of cellular metabolism through reversible oxidation and reduction [17]. A previous study showed that the mean methionine level was reduced in the plasma of CRVO individuals, and the authors concluded that low methionine was a risk factor for CRVO [18]. Since methionine deficiency has been reported to be associated with microvascular damage to the retina [19], the decreased concentration in the AH may take an essential role in the mechanism of CRVO pathogenesis.

The branched-chain amino acids (BCAAs) leucine and isoleucine were significantly downregulated in the CRVO group. Previous

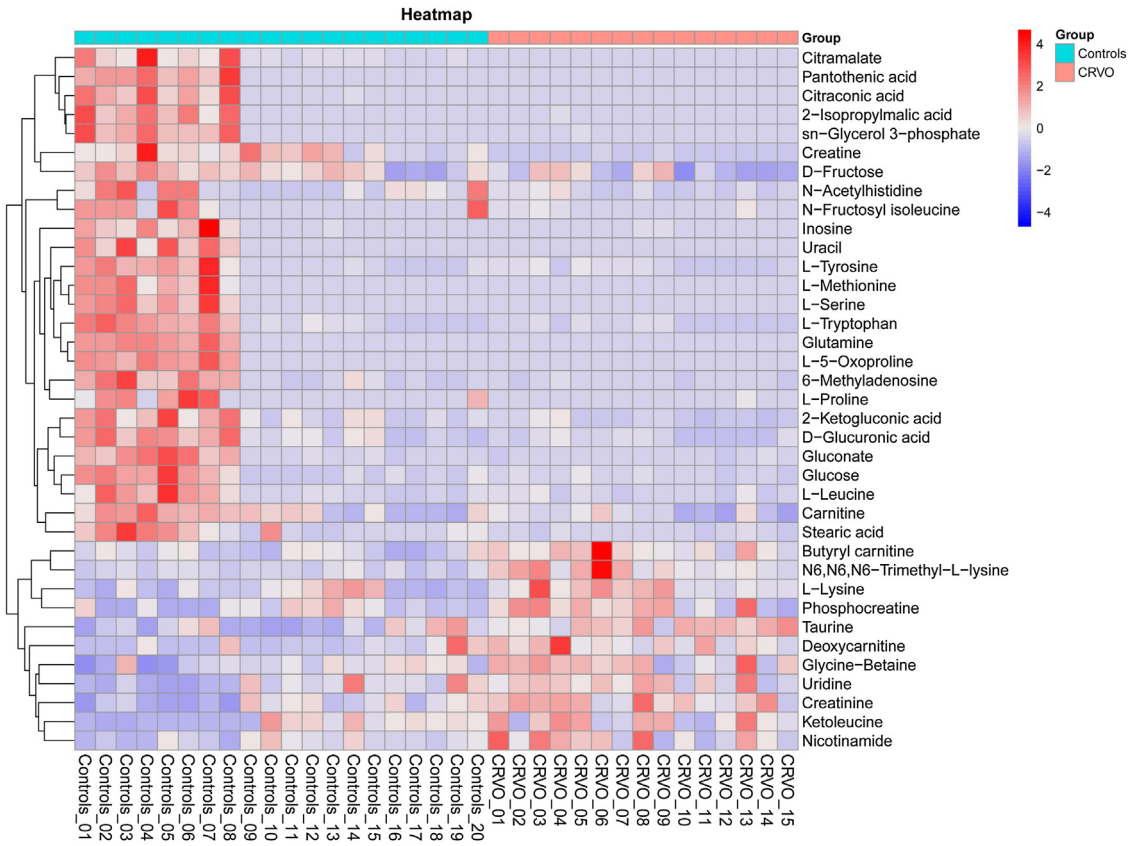

Fig. 3. Heatmap representation of 37 metabolites across 35 samples. Each line in the heat map represents a metabolite. The deeper the red colour, the higher its content in the tested sample; similarly, the deeper the blue colour, the lower its content in the tested sample.

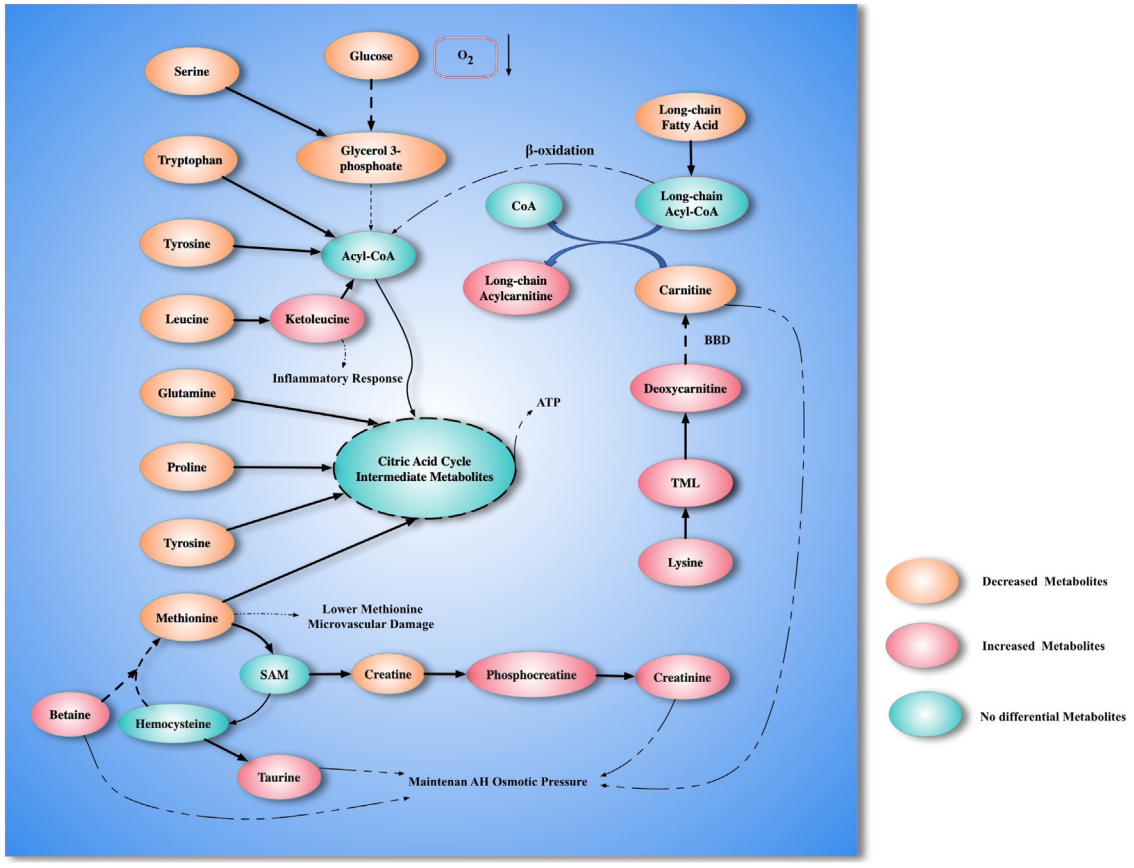

Fig. 4. The schematic diagram of the specific metabolites and their effect in the AH; Abbreviations: TML, 6-*N*-trimethyllysine; BBD, γ-butyrobetaine dioxygenase.

Table 2
List of significantly different metabolites in AH from CRVO compared with controls.

Metabolite	m/z	RT (s)	VIP	Fold Change	P.value
L-Serine	104.0366	9.32	1.49192	163.388956	0.01325406
L-5-Oxoproline	128.0353	9.25	1.64877	32.8366953	0.00653962
Citraconic acid	129.0209	11.24	1.56488	71.1868065	0.01222093
Ketoleucine	129.0574	1.45	1.29733	0.53715354	0.03540566
L-Glutamine	145.0632	9.26	1.67838	249.774873	0.00531798
Citramalate	147.0313	9.61	1.29876	50.2273314	0.04334647
L-Methionine	148.0442	6.71	1.35924	38.468369	0.02458461
sn-Glycerol 3-phosphoate	171.0068	10.94	1.53703	11.9469711	0.01387368
2-Isopropylmalic acid	175.0624	6.09	1.56037	27.665408	0.01235329
D-Fructose	179.0557	9.98	1.50629	1.3144555	0.01406429
Glucose	179.0574	7.4	1.40646	3.25505791	0.02153302
L-Tyrosine	180.0655	7.32	1.3882	4.52722673	0.02283082
D-Glucuronic acid	193.0361	9.75	1.7733	2.76437644	0.00331803
2-Ketogluconic acid	193.0366	9.82	1.6892	3.90508915	0.00542965
Gluconate	195.0512	9.23	1.57814	6.27332187	0.00927263
L-Tryptophan	203.081	6.21	1.64743	3.95393183	0.00620492
Inosine	267.0739	4.96	1.25945	19.8881252	0.04146356
Stearic acid	283.2634	1.61	1.51094	4.37970391	0.01169461
Uracil	113.035	3.52	1.41753	158.954536	0.01933331
Creatinine	114.0657	4.01	2.19936	0.56288676	0.0001467
L-Proline	116.071	7.83	1.32692	22.2455607	0.0277473
Glycine-Betaine	118.0863	11.05	1.78747	0.63118708	0.00307251
Nicotinamide	123.0544	1.72	1.71716	0.51018137	0.00420177
Taurine	126.0223	7.29	2.007	0.47071384	0.00056558
Creatine	132.0768	8.78	1.97267	9.88226218	0.00076916
L-Leucine	132.102	6.32	1.35472	3.65266722	0.02505421
L-Lysine	147.1118	12.43	1.52583	0.6209882	0.0127452
Carnitine	162.1125	8.93	1.53882	1.46931456	0.01025062
L-Tyrosine	182.0802	7.25	1.40142	104.04149	0.01958362
N6,N6,N6-Trimethyl-L-lysine	189.1604	12.5	2.05113	0.34560074	0.00040198
N-Acetylhistidine	198.0853	7.99	1.3303	4.21238643	0.02888354
Phosphocreatine	212.0431	11.53	1.58785	0.51602384	0.0087028
Pantothenic acid	220.1168	6.15	1.59405	28.631322	0.00970029
Butyryl carnitine	232.1541	6.32	1.77061	0.65115781	0.00312533
Uridine	245.074	3.86	1.34914	0.63820405	0.02773204
6-Methyladenosine	282.1197	7.18	1.5957	8.12609802	0.00808041
N-Fructosyl isoleucine	294.1551	7.92	1.24283	6.59720056	0.04469792

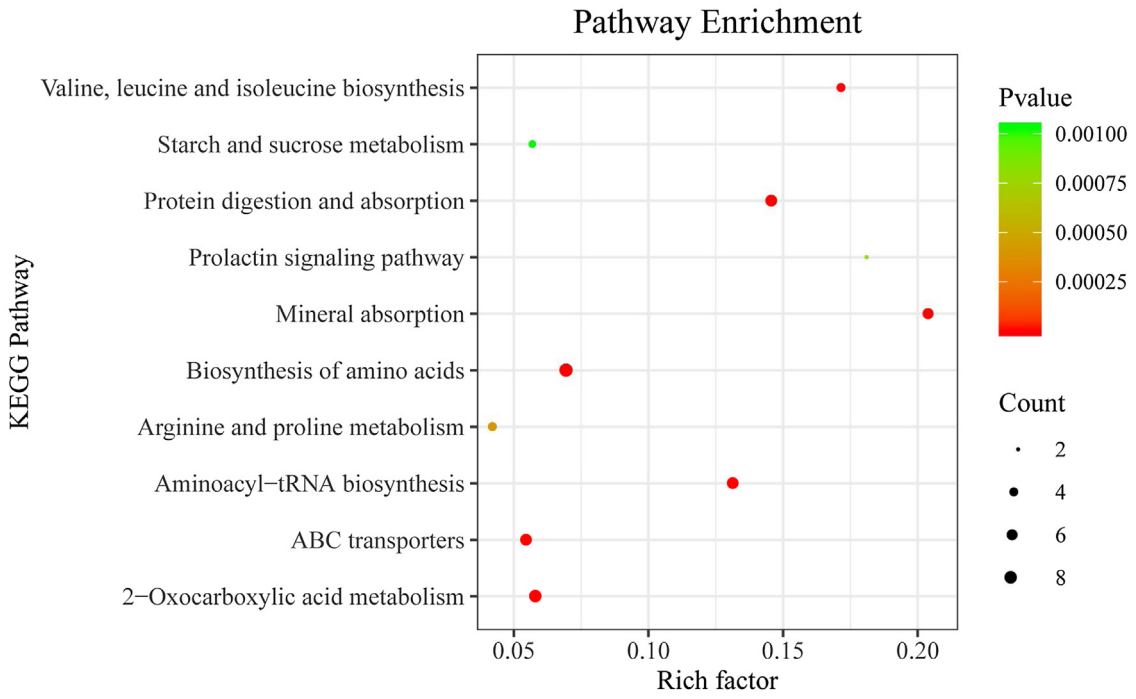

Fig. 5. Metabolic pathway analysis was performed with MetaboAnalyst 3.0. The calculated p-value was established based on the pathway enrichment analysis, while the pathway impact value was based on the pathway topology analysis; the count is the matched number from the user uploaded data. ABC transporters: ATP-binding cassette (ABC) transporters.

studies showed that the administration of BCAAs to retinitis pigmentosa and glaucoma mouse models significantly attenuated photoreceptor and retinal ganglion cell death [20], indicating that BCAA depletion could lead to the retinal pathology seen in CRVO. Furthermore, BCAA metabolism is significantly altered in metabolic disorders such as diabetes mellitus as well as in cardiovascular diseases [21]. Each of these conditions shares risk factors with CRVO, suggesting that they share common disease mechanisms. In the presence of ischemia, BCAAs have the potential to act as alternative energy sources to glucose. In support of this, our study found that ketoleucine was upregulated in the AH of the CRVO group. Ketoleucine is an intermediate in the first step of BCAA catabolism by transamination, and is then decarboxylated into acetyl-CoA to fuel other metabolic pathways. Alternatively, ketoleucine was shown to be positively correlated with the cell count in the AH of anterior uveitis [22]. Moreover, patients with CRVO were shown to have higher levels of cytokines in their AH [23]. Considering this, we speculate that a higher concentration of ketoleucine in AH might be associated with an inflammatory response in CRVO.

3.4.2. Fatty acid metabolism

The carnitine shuttle pathway is responsible for the β-oxidation of fatty acids. Most mammals can synthesize carnitine endogenously from lysine degradation. The biosynthesis of carnitine starts from its substrate 6-*N*-trimethyllysine (TML); it then gets oxidized to deoxycarnitine, which is hydroxylated to yield L-carnitine [24]. Interestingly, in our study, the level of lysine, TML, and deoxycarnitine were dramatically upregulated in the CRVO group, and a concomitant downregulated L-carnitine levels. Dysregulated carnitine metabolism has been shown to be associated with wet age-related macular degeneration [25]. Additionally, according to a retinal model of mitochondrial impairment, disorders in carnitine metabolism and in fatty acid β-oxidation may lead to retinopathy [26]. It is tempting to speculate that the decrease in carnitine leads to compromised mitochondrial function, which may be involved in CRVO pathogenesis. This is substantiated by several experimental studies showing that carnitine could improve carbohydrate metabolism, decrease oxidative stress, and prevent subsequent cell death during ischemia [27].

Despite its primary role in lipid metabolism, carnitine in the AH may also play a role in maintaining AH homeostasis and osmosis [28]. In the current study, the levels of other osmolytes including betaine, taurine, and creatinine were increased in the AH of patients with CRVO. A similar alteration of osmolytes was found in the AH of patients with glaucoma [2]. These metabolites in the AH are essential for its formation and the maintenance of its osmotic pressure [29]. It has been shown that the concentration of free carnitine is highest in the ciliary body [30], a crucial tissue for AH secretion. Based on this, the alteration in the level of osmolytes indicated their important role in the CRVO pathogenesis.

3.4.3. Carbohydrate metabolism

Glucose was dramatically decreased in the aqueous humor of CRVO patients. Under physiological conditions, glucose is essential for normal visual function and maintaining ocular homeostasis [16]; glucose and oxygen are pivotal for the metabolism of the retina [16]. Oxygen deficiency in the retina of CRVO patients lead to mitochondrial dysfunction and ATP depletion by impairing ATP production from glucose metabolism [1]. Decreased glucose concentrations in the AH of CRVO patients may indicate an energy shortage or a relative increase in fuel consumption. Our findings suggest that a subtle alteration in carbohydrate metabolism, in particular, of glucose metabolism, may contribute to the development of CRVO.

The current study was limited by its relatively small population cohorts. The sample size was reduced because we excluded patients with a history of diabetes mellitus in the CRVO group. This was necessary as diabetes mellitus may significantly influence metabolite levels in the AH. Further, in metabolomics, a previous study has suggested that controlled clinical studies should be carried out with as few as 10–20 patients and carefully matched controls [9]. Moreover, the AH is not in direct contact with the retina and may only partially reflect retinal changes. One should also consider that because the metabolomic profiles of individuals can only be obtained at one point in time, the metabolomic analysis can only establish association, not causality. Further investigation is needed to determine whether the metabolomic differences between the two groups contribute to the pathology seen in CRVO.

4. Conclusions

The retina, having tissues with the highest energy consumption, is vulnerable to metabolic defects. When central retinal vein was blocked, the normal metabolism of the retina could be disrupted, altering its key metabolic pathways and depleting energy. Hence, understanding the metabolomic changes is fundamental for the development of effective treatments for retinal diseases. In this study, we obtained a comprehensive metabolomic profile of the AH of patients with CRVO. This revealed possible alterations in the metabolism of lipids, carbohydrates, and amino acids in these patients. Given the importance of these metabolites, these differences could shed light on disease pathogenesis and lead to the discovery of new therapeutic targets for CRVO.

CRediT authorship contribution statement

Pinghui Wei: Data curation, Writing - original draft, Software, Validation, Writing - review & editing. **Meiqin He:** Visualization, Investigation. **He Teng:** Funding acquisition, Visualization, Investigation. **Guoge Han:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

None of the authors has a financial or proprietary interest in any material or method mentioned.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2020.113448.

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