



Metabolomic Profile of Posner–Schlossman Syndrome: A Gas Chromatography Time-of-Flight Mass Spectrometry-Based Approach Using Aqueous Humor

Haiyan Wang^{1,2,3†}, Ruyi Zhai^{4,5,6†}, Qian Sun^{1,2,3}, Ying Wu^{1,2,3}, Zhujian Wang^{4,5,6}, Junwei Fang^{1,2,3,7*} and Xiangmei Kong^{4,5,6*}

¹ Department of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, ² Shanghai Key Laboratory of Ocular Fundus Diseases, Shanghai Jiao Tong University, Shanghai, China, ³ Shanghai Engineering Center for Visual Science and Photomedicine, Shanghai, China, ⁴ Department of Ophthalmology and Visual Science, Eye, Ear, Nose and Throat Hospital, Shanghai Medical College, Fudan University, Shanghai, China, ⁵ Key Laboratory of Myopia, Ministry of Health, Fudan University, Shanghai, China, ⁶ Shanghai Key Laboratory of Visual Impairment and Restoration, Fudan University, Shanghai, China, ⁷ College of Basic Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

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*Correspondence:

Junwei Fang
fangjunwei@shsmu.edu.cn
Xiangmei Kong
kongxm95@163.com

[†]These authors have contributed
equally to this work

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The Posner–Schlossman syndrome (PSS) is a disease with clinically recurrent unilateral anterior uveitis with markedly elevated intraocular pressure (IOP) and subsequent progression to optic neuropathy. Retrospective studies have reported increased annual incidence of PSS, especially in China. While currently, the clinical management of PSS is still challenging. Metabolomics is considered to be a sensitive approach for the development of novel targeted therapeutics because of its direct elucidation of pathophysiological mechanisms. Therefore, we adopted gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) technology-based non-targeted metabolomics approach to measure comprehensive metabolic profiles of aqueous humor (AH) samples obtained from patients with PSS, with an aim to demonstrate the underlying pathophysiology, identify potential biomarkers specific to PSS, and develop effective treatment strategies. A comparative analysis was used to indicate the distinct metabolites of PSS. Pathway analysis was conducted using MetaboAnalyst 4.0 to explore the metabolic reprogramming pathways involved in PSS. Logistic regression and receiver-operating characteristic (ROC) analyses were employed to evaluate the diagnostic capability of selected metabolites. Comparative analysis revealed a clear separation between PSS and control groups. Fourteen novel differentiating metabolites from AH samples obtained from patients with PSS were highlighted. Pathway analysis identified 11 carbohydrate, amino acid metabolism and energy metabolism pathways as the major disturbed pathways associated with PSS. The abnormal lysine degradation metabolism, valine–leucine–isoleucine biosynthesis, and citrate circle were considered to weigh the most in the development of PSS. The ROC analysis implied that the combination of glycine and homogentisic acid could serve as potential biomarkers for the discrimination of control and PSS groups. In conclusion, these results revealed for the first time the identity of important metabolites and pathways

contributing to the development/progression of PSS, enabled the better understanding of the mechanism of PSS, and might lead to the development of metabolic biomarkers and novel therapeutic strategies to restrict the development/progression of PSS.

Keywords: Posner–Schlossman syndrome, metabolism, mass spectrometry, gas chromatography time-of-flight mass spectrometry, aqueous humor

INTRODUCTION

Posner–Schlossman syndrome (PSS), also called glaucomatocyclitic crisis (GCC), is a disease with clinically recurrent unilateral anterior uveitis with markedly elevated intraocular pressure (IOP) and subsequent progression to optic neuropathy (Eissler, 1948; Moorthy et al., 1997). Retrospective studies have reported increased annual incidence of PSS, especially in China (Jiang et al., 2017). The clinical management of PSS is still challenging. Interventional measure for PSS is indicated for managing the associated inflammation and elevated IOP in order to prevent optic neuropathy. The use of corticosteroid can relieve the early stage symptoms; however, it fails to prevent the recurrence of PSS. Moreover, many patients develop corticoid dependence over shorter intervals gradually, complicating the management of PSS. Long-term administration of ganciclovir may control the symptoms of certain types of PSS (Miyana et al., 2010; Sobolewska et al., 2014; Su et al., 2014). However, the patients still experience progressive endothelium loss and a high relapse rate after the withdrawal of the drug (Moorthy et al., 1997; Chee and Jap, 2010; Miyana et al., 2010; Sobolewska et al., 2014; Su et al., 2014; Jiang et al., 2017). Eventually, repeated uncontrolled elevation of IOP can result in irreversible optic neuropathy and vision loss. Better understanding of the etiology and pathophysiological mechanism of PSS may aid in blunting the pathogenesis/progression of this disease. Several risk factors for PSS have been reported, such as inflammatory cytokines (Ohira et al., 2016; Pohlmann et al., 2018), vascular endothelial dysfunction (Shen et al., 2010), and primarily, human cytomegalovirus (HCMV) infection (Teoh et al., 2005; Chee and Jap, 2008; Van Gelder, 2008; Shazly et al., 2011; Su et al., 2014), which tops the list. Studies have demonstrated the participation of various metabolites in the inflammation process (Tannahill et al., 2013; Mills and O'Neill, 2014), including the HCMV-induced inflammatory response. Metabolites, as the downstream products of gene transcription, translation, and post-translational protein modification, are directly influenced by the physiological and pathological changes in tissues and accurately reflect the physiological changes (Oldiges et al., 2007; Baharum and Azizan, 2018). Therefore, studies investigating the change in endogenous metabolites and related metabolic pathways, underlying PSS, may provide a more sensitive approach to identify the pathophysiological mechanisms and, hopefully, lead to the identification of metabolic biomarkers for the prognosis of PSS and provide novel hypotheses for developing targeted therapeutics (Baharum and Azizan, 2018; Mayerle et al., 2018).

In metabolomic studies, mass spectrometry (MS) is the most preferred technique. Gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) is perfectly suited for the identification and quantitation of low molecular weight metabolites (Phua et al., 2013; Jaeger et al., 2017; Yin et al., 2017) because of its key features, including higher sensitivity, selectivity, resolution, and accuracy of detection. Therefore, we adopted GC-TOF-MS technology-based non-targeted metabolomics approach and multivariate statistical analysis to measure comprehensive metabolic profiles of aqueous humor (AH) samples obtained from patients with PSS and cataract, with an aim to demonstrate the underlying pathophysiology, identify potential biomarkers specific to PSS, and develop effective treatment strategies.

METHODS

Research Design

The study was prospectively approved by the medical ethics committee of the Eye, Ear, Nose and Throat (EENT) Hospital of Fudan University (2017006–2), and research was conducted in accordance with the Declaration of Helsinki as revised in 2000. Signed informed consent was obtained from all participants enrolled in the study. We conducted this prospective, observational, and internal research project from June 2018 to December 2018 at EENT Hospital of Fudan University.

PSS was diagnosed based on the following clinical features: a) unilateral recurrent mild iridocyclitis; b) nonpigmented keratic precipitates (KPs) and corneal edema; c) cell and flare in the anterior chamber; d) elevated IOP; e) no posterior synechiae or peripheral anterior synechiae and posterior inflammation; and f) a relatively short attack duration. All participant data including gender, age, IOP, best-corrected visual acuity (BCVA), cornea endothelial cell density (CD), KP, Tyndall, and vertical ratio of cup to disk (C/D) were recorded and documented in the electronic case report form. In total, 43 participants were enrolled for this study, including 12 participants with PSS and 12 participants with cataract. All the PSS patients enrolled in this study were naïve patient without any treatment. All the PSS patients were at the acute onset stage.

Sample Collection

Paracentesis of anterior chamber was performed to collect AH sample for MS and anti-virus IgG concentration analysis. Meanwhile, serum sample was obtained in our central laboratory with the use of standardized procedures.

Analysis of Anti-Virus IgG Concentration in AH

Anti-virus IgG in AH and serum were detected by enzyme-linked immunosorbent assay (ELISA) kit (Virion\Serion, Germany). The test procedure was performed according to the kit instructions. Albumin was detected by scattering immunonephelometry (Guosai Biotechnology Co., Ltd, Shenzhen, China). The concentration of anti-virus IgG in AH was presented by a corrected ratio of (AH IgG/serum IgG) to (AH albumin/serum albumin), which was abbreviated as *s/co*. Mann–Whitney *U* test was performed to measure the significance of anti-virus IgG concentration between PSS and control groups.

Correlation Study Between Anti-Virus IgG Concentration and Clinical Variables

Spearman's rank correlation coefficient was performed to evaluate whether a correlation existed between both variables (anti-virus IgG concentration and clinical variables). Spearman's rank correlation coefficient value (Spearman's rho) ranged from 0 (no reliability) to 1 (perfect reliability). *p* values ≤ 0.05 were considered significant. All the analyses were carried out using SPSS 22.0 statistical software.

Mass Spectrometry Analysis

AH sample aliquots of 0.15 ml were transferred into cryovial tubes and stored at -80°C immediately. An Agilent 6890N gas chromatograph (Agilent Technologies) coupled to a Pegasus HT TOF MS (LECO Corp., St. Joseph, MI, USA) was used as the GC-TOF-MS platform. The sample preparation procedure and instrumental analysis were referred in the previously published methods (Qiu et al., 2014) with minor modifications, which was summarized in the **Supplementary Materials**.

Statistical and Data Analysis

For GC/MS data, data processing and identification are in accordance with our previously published work using XploreMET (Pan et al., 2010). The resulting data were exported into Microsoft Excel, and the peaks were normalized to the total sum of spectrum prior to multivariate analyses. The normalized data were analyzed by SIMCA-P (version 14.0 Umetrics AB, Umea, Sweden) for unsupervised principal component analysis (PCA) to obtain a general overview of the variance of metabolic phenotypes among different groups. In addition, supervised orthogonal projection to latent structure-discriminant analysis (OPLS-DA) was performed to obtain information about the variance of metabolic phenotypes that correspond to the classes. Student's *t*-test with Bonferroni's correction and fold change were performed afterwards to evaluate the significance of each metabolite. Significantly changed metabolites were identified by the parameters of OPLS-DA VIP > 1 , *t*-test $p < 0.05$, and fold change > 1.2 or < 0.83 . The correlation between significantly disturbed metabolites and clinical variables of PSS patients was investigated by Spearman's rank correlation analysis using MetaboAnalyst 4.0 (<http://www.metaboanalyst.ca/>) (Xia and Wishart, 2016).

Pathway analysis was conducted using MetaboAnalyst 4.0 as well as the Kyoto Encyclopedia of Genes and Genomes

(KEGG) database (www.genome.jp/kegg/). Former collected metabolites were used as input. A logistic regression analysis and receiver-operating characteristic (ROC) analysis were performed using SPSS software version 18.0 (IBM Corp., Armonk, New York) to evaluate the diagnostic capability of selected metabolites. The area under the ROC curve (AUC) was calculated to quantify the performance of the diagnostic variables.

RESULTS

Sample Characteristics

The clinical characteristics of participants selected for discovery metabolomic profiling are shown in **Table 1**.

Anti-Virus IgG Concentration in AH

There was a significant difference of anti-HCMV IgG concentration between PSS and control groups (p value < 0.001) **Table 2**.

Correlation Analysis Between Anti-Virus IgG Concentration and Clinical Manifestations

In **Table 3**, Spearman's rank correlation coefficient indicated high agreement of HCMV and IOP ($r = 0.458$, $p < 0.05$), HCMV and

TABLE 1 | Clinical characteristics of participants in PSS and control groups.

	PSS	Control	<i>p</i> value
Number	12	12	
Gender (male, %)	5, 41.7	4, 33.3	0.673 Ψ
Age (years)	51.3 \pm 9.9	58.3 \pm 7.9	0.072
IOP (mmHg)	21.6 \pm 9.4	14.3 \pm 2.6	0.02*
BCVA	3.64 \pm 1.9	3.93 \pm 0.7	0.687
CD	2704.6 \pm 190.9	2626.7 \pm 282.3	0.486
KP (y/n)	12/0	0/12	$<0.001\mathcal{P}$ **
Tyndall (y/n)	3/9	0/12	0.064 Ψ
Vertical C/D	0.4 (0.3–0.9)	0.3	$<0.001\#$ ***

PSS, Posner–Schlossman syndrome; BCVA, best-corrected visual acuity; IOP, intraocular pressure; CD, cell density; KP, keratic precipitate; C/D, ratio of cup to disk. # by Mann–Whitney *U* test; Ψ , by χ^2 test. *, p value < 0.05 ; **, p value < 0.01 .

TABLE 2 | Anti-virus IgG concentration in AH from PSS and control groups.

	PSS	Control	<i>p</i> value
Number	12	12	
Anti-HCMV IgG (s/co)	0.45 (0.12–2.39)	0.01 (0–0.02)	<0.001 ***
Anti-rubella virus IgG (s/co)	0.03 (0.01–0.08)	0.01 (0.01–0.03)	<0.001 ***
Anti-VZV IgG (s/co)	0.02 (0.01–5.08)	0.01 (0–0.01)	0.024*
Anti-HSV1 IgG (s/co)	0.01 (0.01–0.04)	0.01 (0.01–0.02)	0.713

PSS, Posner–Schlossman syndrome; HCMV, human cytomegalovirus; VZV, varicella zoster virus; HSV, herpes simplex virus. Data were presented as median (range). Mann–Whitney *U* test was used to compare the anti-virus IgG concentration between PSS group and control group. *, p value < 0.05 ; ***, p value < 0.001 .

TABLE 3 | Spearman's correlation between anti-virus IgG concentration and clinical indexes.

	IOP	CD	CCT	C/D	KP	Tydall
Anti-HCMV IgG (s/co)	0.458*	-0.067	0.600	0.828***	0.879***	0.296
Anti-VZV IgG (s/co)	0.202	-0.174	-0.516	0.426*	0.533**	0.586**
Anti-rubella virus IgG (s/co)	0.233	-0.228	-0.261	0.535*	0.689***	0.563**
Anti-HSV1 IgG (s/co)	0.121	-0.501*	0.131	0.091	0.119	0.167

Correlation coefficient and *p* values are calculated by Spearman's correlation. HCMV, human cytomegalovirus; VZV, varicella zoster virus; HSV, herpes simplex virus; IOP, intraocular pressure; CD, cell density; CCT, central corneal thickness; C/D, ratio of cup to disk; KP, keratic precipitate. *, *p* value < 0.05; **, *p* value < 0.01; ***, *p* value < 0.001.

C/D ($r = 0.828, p < 0.001$), and HCMV and KP ($r = 0.879, p < 0.01$). Statistical correlation was also found between VZV and Tydall ($r = 0.586, p < 0.01$), VZV and C/D ($r = 0.426, p < 0.05$), and VZV and KP ($r = 0.533, p < 0.01$).

Global Metabolomic Profiles Between PSS and Control Groups

Evaluation of metabolomic profiles between PSS and control groups was conducted using unsupervised statistics, PCA. The PCA score plot with the first two (Figure 1A) and three (Figure 1B) principal components demonstrated a clear separation between the PSS and control groups, indicating that PSS caused gradual alterations in metabolism.

Significantly Disturbed Metabolites Between PSS and Control Groups

In the OPLS-DA model, clear differences were obtained for PSS versus control groups: cumulative R²Y at 0.995 and Q²Y at 0.445 (Figure 2A). VIP values and correlation coefficients (i.e., $p[\text{corr}]$) of each metabolite was shown in the V-plot, and 136 variables in red were found to have VIP values > 1 (Figure 2B). Metabolites with significant changes were easily isolated with the help of volcano plot, shown in Figure 2C. The permutation test results of OPLS-DA model are shown in Figure S1. The plot demonstrated as fold difference (*x*-axis) and *p* value (*y*-axis). Fourteen metabolites with significant abundance changes were defined and selected by the parameters of OPLS-DA VIP > 1, *t*-test $p < 0.05$, and fold change > 1.2 or < 0.83 (Table 4).

Spearman's Rank Correlation Analysis Between Disturbed Metabolites and Anti-Virus IgG Concentration in AH

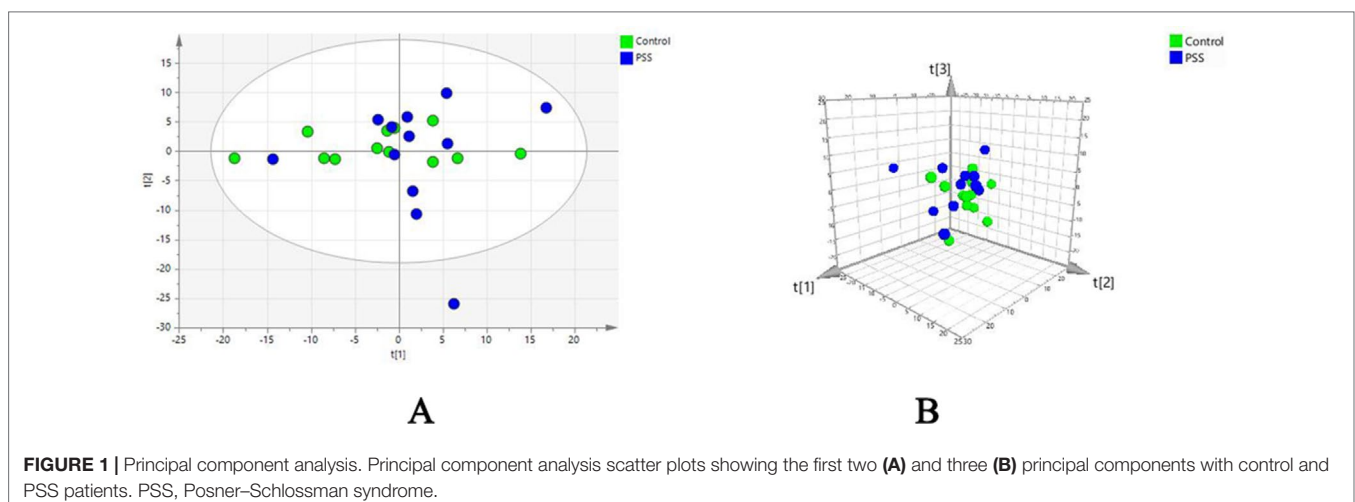
Spearman's rank correlation analysis indicated that there was strong positive correlation between anti-HCMV-IgG and glycine, phenylpyruvic acid, sorbitol, mannitol, aminoadipic acid, fumaric acid (FA), and 3-hydroxybutyric acid. Meanwhile, homogentisic acid and -arabinose exhibited notably negative correlation with anti-HCMV-IgG concentration (Figure 3).

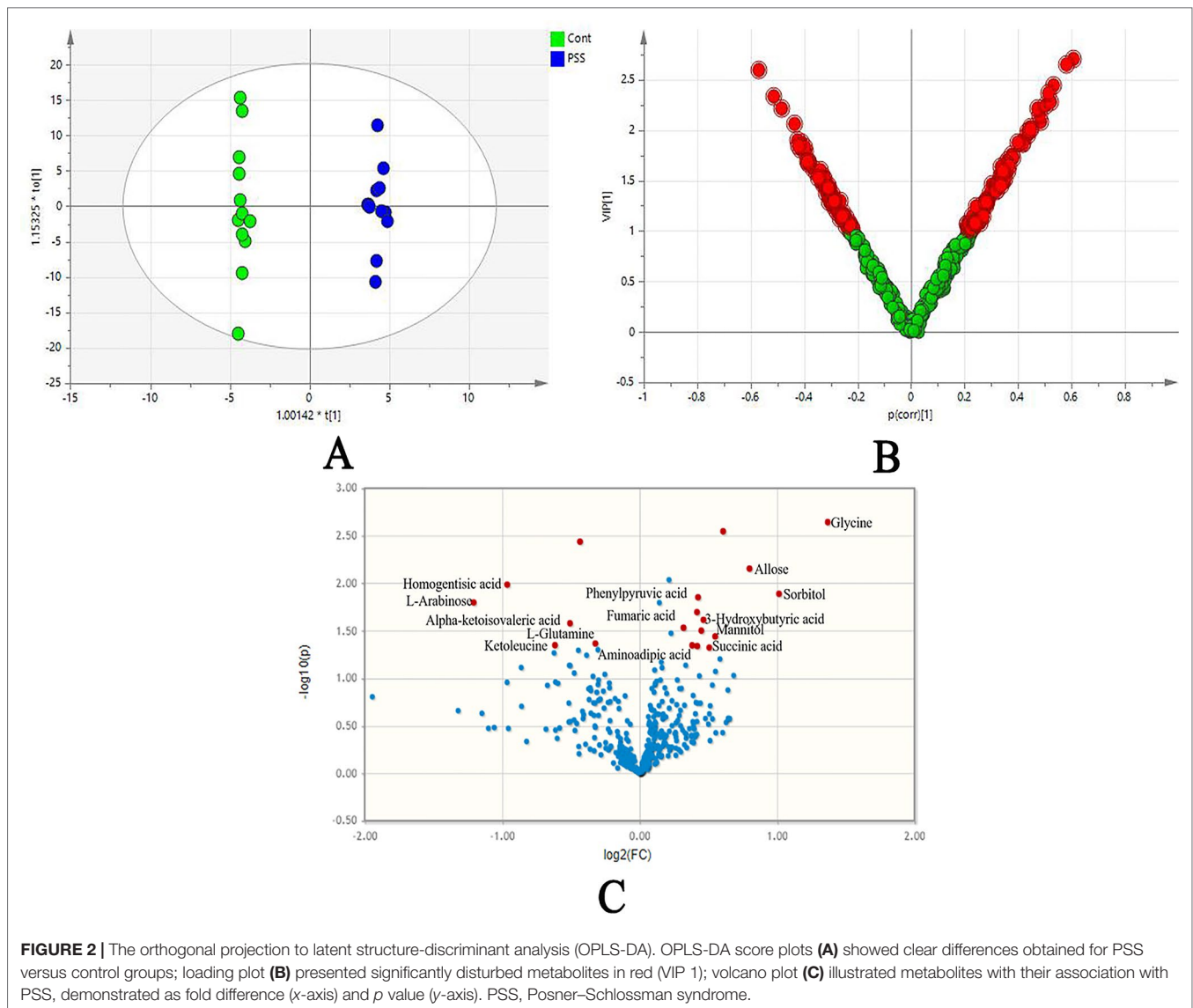
Metabolic Pathway Analysis Associated With PSS

Pathways relevant to 14 differential metabolites in AH are shown in Figure 4 and Table 5. The abnormal of citrate cycle, valine–leucine–isoleucine biosynthesis, valine–leucine–isoleucine degradation, butanoate metabolism, fructose and mannose metabolism, lysine degradation metabolism, alanine, aspartate and glutamate metabolism, nitrogen metabolism, phenylalanine metabolism, tyrosine metabolism, and synthesis and degradation of ketone bodies metabolism were highlighted as the most important pathways in PSS group ($p < 0.05$).

Potential Biomarkers Analysis for Discrimination

We evaluated the impact of multiple metabolites and adopted a forward stepwise-regression selection procedure to select the best combination of potential biomarkers for discrimination. The



**TABLE 4 |** Significantly disturbed metabolites.

Name	VIP	<i>p</i> value	Fold change
3-Hydroxybutyric acid	1.739	0.024	1.38
Allose	1.809	0.007	1.74
Alpha-ketoisovaleric acid	1.531	0.026	0.704
Amino adipic acid	1.539	0.045	1.303
Fumaric acid	1.68	0.02	1.336
Glycine	2.104	0.002	2.582
Homogentisic acid	1.916	0.01	0.513
Ketoleucine	1.519	0.045	0.653
L-Arabinose	1.659	0.016	0.433
L-Glutamine	1.649	0.043	0.8
Mannitol	1.632	0.036	1.464
Phenylpyruvic acid	1.72	0.014	1.343
Sorbitol	1.865	0.013	2.02
Succinic acid	1.425	0.047	1.421

age was adjusted for the logistical regression model. The ROC presentation, on the basis of the logistic regression of metabolic biomarker panel, appears in **Figure 5**. The model containing panel metabolites showed very good discrimination between PSS and control groups. The AUC, sensitivity, and specificity of the logistic regression model established by glycine and homogentisic acid were 1 and 1 (**Figure 5**). Spearman's rank correlation analysis was conducted among 14 different metabolites with clinical variables. Glycine showed strong positive correlation with Tyndall, C/D ratio, and KP (**Figure 6**). Amino adipic acid and allose showed positive correlation with clinical indicators, including KP and IOP (**Figure 6**). Alpha-ketoisovaleric acid (KIV), -arabinose, ketoleucine, homogentisic, and allose showed negative correlation with clinical indicators, including IOP, KP, and C/D ratio (**Figure 6**).

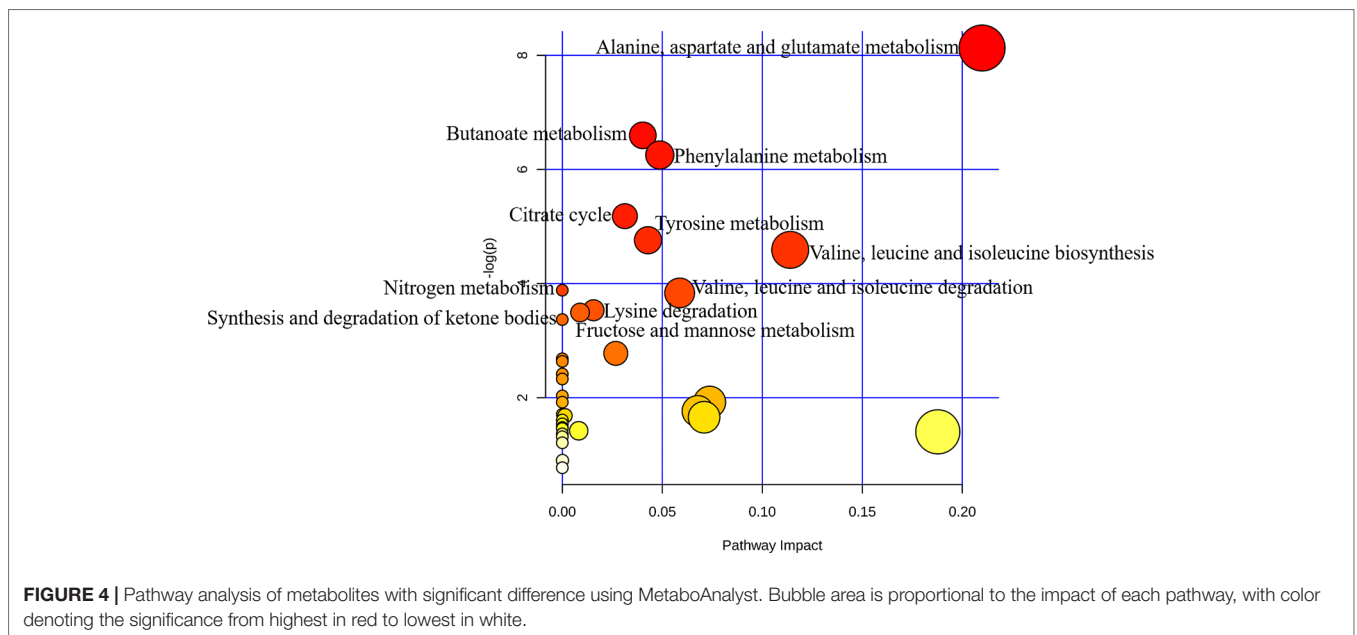
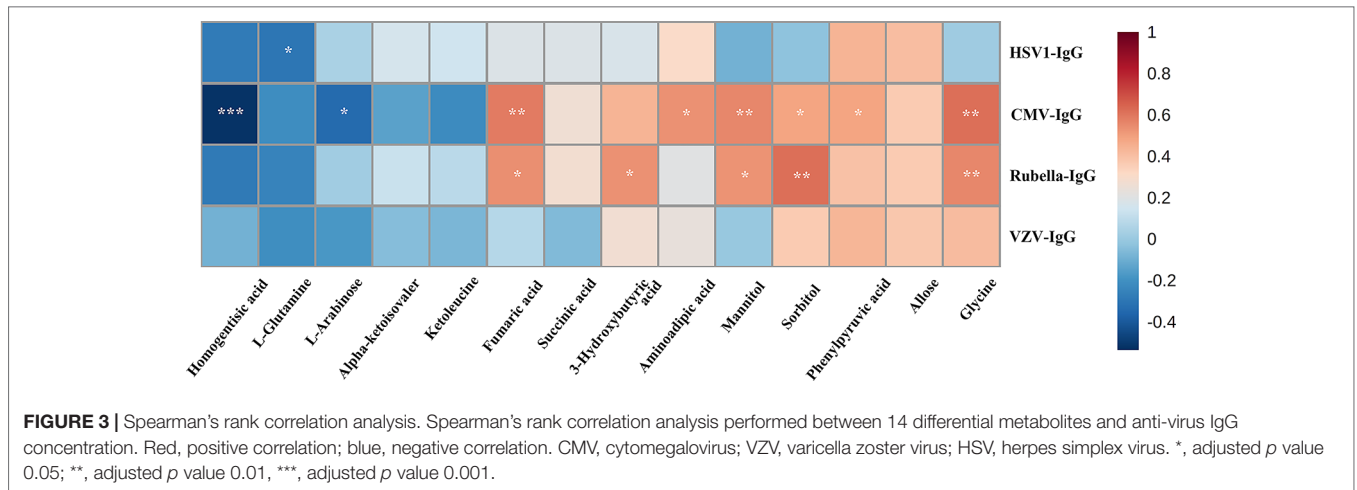


TABLE 5 | Pathways relevant to 14 differential metabolites.

Pathway	Metabolites	Total	Impact	Hits	<i>p</i> value
Alanine, aspartate, and glutamate metabolism	L-Glutamine, fumaric acid, succinic acid	24	0.1396	3	0.0003
Butanoate metabolism	(<i>R</i>)-3-Hydroxybutyric acid, succinic acid, fumaric acid	40	0.2327	3	0.0014
Citrate cycle (TCA cycle)	Succinic acid, fumaric acid	20	0.1163	2	0.0056
Fructose and mannose metabolism	Sorbitol, mannitol	48	0.2792	2	0.0304
Lysine degradation	Amino adipic acid, glycine	47	0.2734	2	0.0293
Nitrogen metabolism	L-Glutamine	39	0.2268	2	0.0206
Phenylalanine metabolism	Phenylpyruvic acid, succinic acid, fumaric acid	45	0.2617	3	0.0019
Synthesis and degradation of ketone bodies	(<i>R</i>)-3-Hydroxybutyric acid	6	0.0349	1	0.0344
Tyrosine metabolism	Homogentisic acid, fumaric acid, succinic acid	76	0.442	3	0.0086
Valine, leucine, and isoleucine biosynthesis	Alpha-ketoglutaric acid, 4-Methyl-2-oxopentanoate	27	0.157	2	0.0102
Valine, leucine, and isoleucine degradation	Alpha-ketoglutaric acid, 4-Methyl-2-oxopentanoate	40	0.2327	2	0.0216

TCA, tricarboxylic acid.

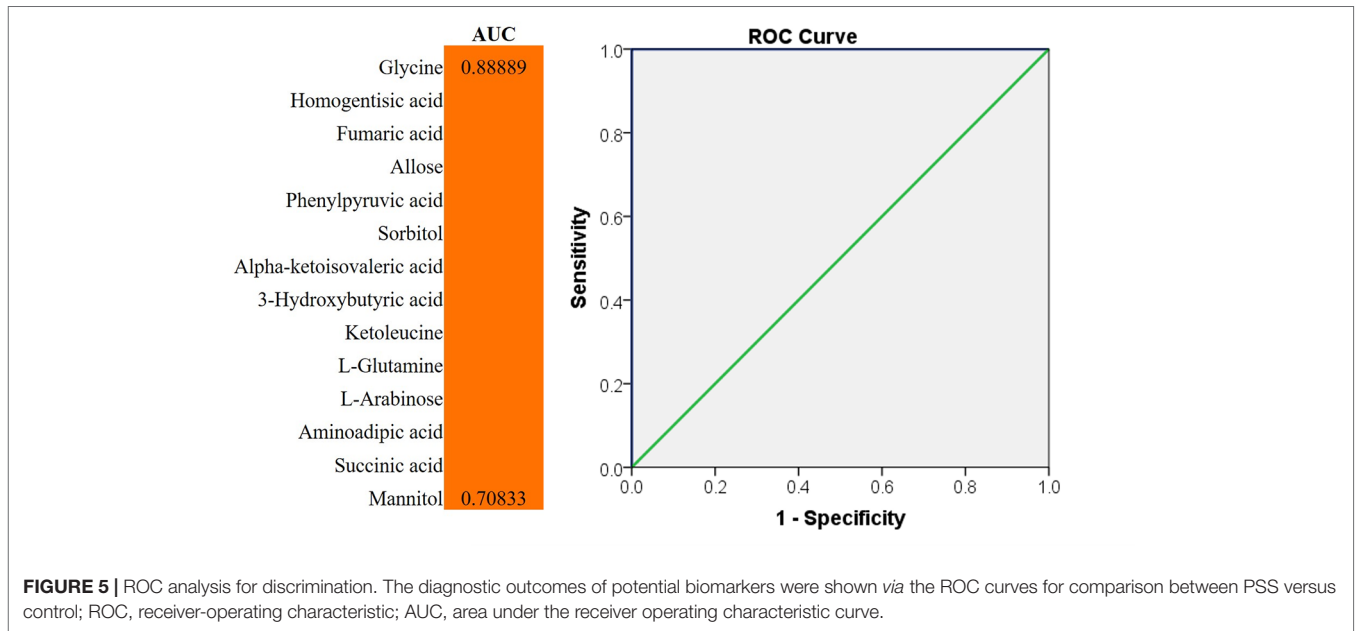


FIGURE 5 | ROC analysis for discrimination. The diagnostic outcomes of potential biomarkers were shown *via* the ROC curves for comparison between PSS versus control; ROC, receiver-operating characteristic; AUC, area under the receiver operating characteristic curve.

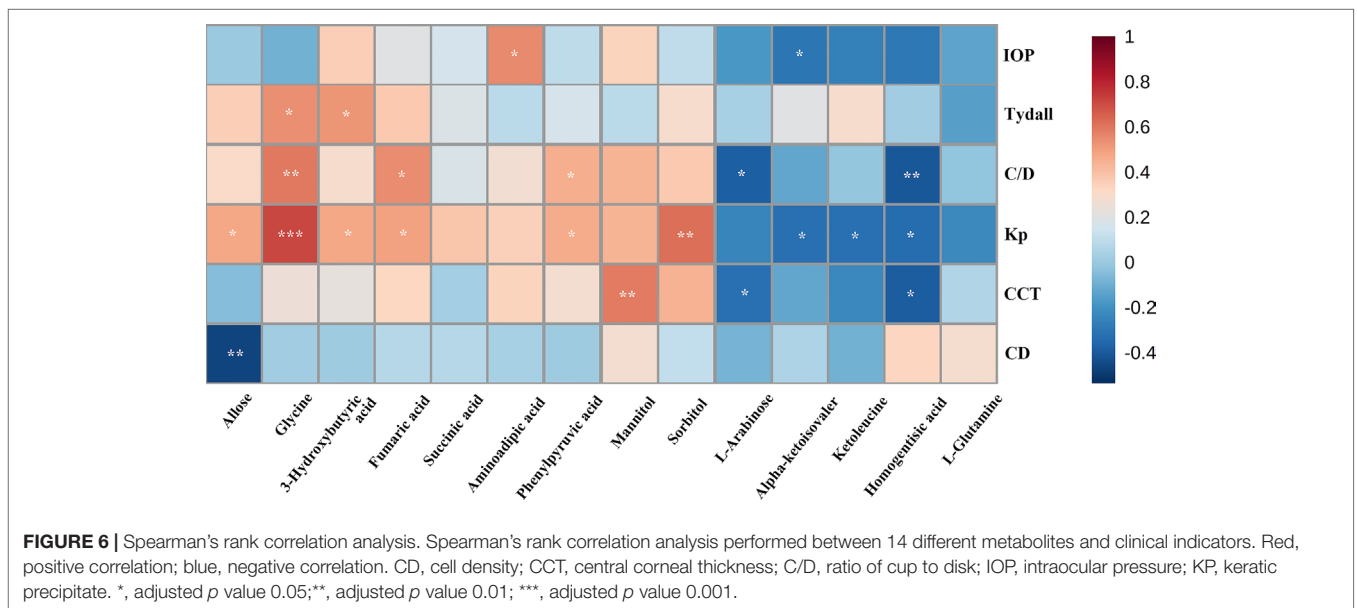


FIGURE 6 | Spearman's rank correlation analysis. Spearman's rank correlation analysis performed between 14 different metabolites and clinical indicators. Red, positive correlation; blue, negative correlation. CD, cell density; CCT, central corneal thickness; C/D, ratio of cup to disk; IOP, intraocular pressure; KP, keratic precipitate. *, adjusted *p* value 0.05; **, adjusted *p* value 0.01; ***, adjusted *p* value 0.001.

DISCUSSIONS

Metabolomics is a promising approach for the exploration of disease pathogenesis. The metabolomic profile of AH can directly indicate the physiological status of the eyes (Haines et al., 2018). In our study, 14 metabolites were discovered for the first time from AH samples obtained from patients with PSS—3-hydroxybutyric acid, allose, alpha-ketoisovaleric acid, amino adipic acid, FA, glycine, homogentisic acid, ketoleucine, -arabinose, -glutamine, mannitol, phenylpyruvic acid, sorbitol, and succinic acid. Some of these metabolites have been recognized as node molecules in pathways associated with inflammation or neuroprotection (Effenberger-Neidnicht et al., 2014; Chen et al., 2016; Xu et al.,

2018; Liu et al., 2019). To advance our understanding of the pathophysiological patterns of these identified metabolites, we performed metabolic pathway analysis to identify pathway-based metabolomic features of these metabolites in PSS.

In our research, the lysine degradation metabolic pathway was significantly enriched in PSS (https://www.kegg.jp/kegg-bin/show_pathway?map00310). This enrichment was associated with the concomitant increase of glycine in the samples. Glycine, as a nutritionally important amino acid (AA), is highly essential for fetal and neonatal growth and development (Wang et al., 2014; Wu, 2014). Besides the generation of energy, studies have indicated that glycine can prevent the overproduction of pro-inflammatory cytokines (Li et al., 2001; Stoffels et al., 2011;

Effenberger-Neidnicht et al., 2014). Exogenous administration of glycine can modulate the toll-like receptor-4 (TLR4) and nucleotide-binding oligomerization domain protein inflammatory signaling pathway and subsequently downregulate the mRNA expression of related cytokines, such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), nuclear factor kappa-B (NF- κ B) p65, and, especially, the mediator of macrophage recruitment, intercellular adhesion molecule-1 (ICAM-1), which regulates macrophage polarization and recruitment (Wheeler et al., 2000; Stoffels et al., 2011; Xu et al., 2018). We speculated that modulation of the unfavorable local resident macrophages by exogenous administration or endogenous upregulating of glycine might provide a valuable approach to mitigate macrophage-mediated inflammation in PSS. We also know that certain kinds of PSS are caused by HCMV infection. Researches have demonstrated that HCMV infection can induce the activation of the transcription factor NF- κ B, which is critical for transactivation of the major immediate-early promoter for HCMV[36]. Hence, downregulation of NF- κ B by glycine might be more efficient for treating HCMV-positive PSS. Spearman's rank correlation analysis confirmed significant strong positive correlation between anti-HCMV IgG and glycine (Figure 3). Based on the significant positive correlation between anti-HCMV IgG and glycine, we speculated that glycine might be a bioprobe for HCMV-derived PSS. In this circumstance, combined anti-HCMV therapy might be taken into consideration. Moreover, glycine has been well-documented as having the best neuroprotective effect among all AAs. Accumulated evidence indicates a direct role of glycine in neuroprotection and amelioration of hypoxia-ischemic brain injuries in adult and neonatal animals (Wheeler et al., 2000; Li et al., 2001; DeMeritt et al., 2004; Stoffels et al., 2011; Wang et al., 2014; Wu, 2014; Chen et al., 2016; Mori et al., 2017; Liu et al., 2019). This neuroprotective effect of glycine involved the suppression of inflammatory pathway associated with TNF- α or NF- κ B p65/hypoxia inducible factor-1 α (Hif-1 α) signalling (Wheeler et al., 1999; Liu et al., 2019). Stroke patients clinically treated with glycine-based modalities demonstrated favorable outcome and a reduced tendency for 30-day mortality (Gusev et al., 2000). Similar to cerebrospinal fluid, increased amount of glycine was detected in the AH of patients with PSS, which might confer a neuroprotection from optic neuropathy caused by marked elevation of IOP during the acute phase of PSS.

KIV was found decreased in the AH of patients with PSS. The associated valine–leucine–isoleucine biosynthesis pathway was highlighted during the *in silico* pathway analysis as an important pathway in PSS (https://www.kegg.jp/kegg-bin/show_pathway?map00290). Valine, leucine, and isoleucine from this pathway constitute the branched-chain AAs (BCAAs) family. BCAAs are strong nutritional stimuli that are able to regulate the inflammatory status by the regulation of glutamine production (Nicastro et al., 2012; Gameiro and Struhl, 2018). Under inflammatory circumstances, BCAAs can be transaminated to glutamate to meet the high requirement of glutamate in inflammatory cells, such as macrophages, and help maintain the cellular function (Pithon-Curi et al., 2004). Moreover, BCAAs have been widely known to activate mammalian target of rapamycin (mTOR), which can modulate autophagy and inflammatory

responses (Xu and Brink, 2016; Saxton and Sabatini, 2017; Sinclair et al., 2017). Reports have demonstrated that mTOR can facilitate PI3K/Akt-induced NF- κ B activation (Peppenelli et al., 2018; Altman et al., 2019), an important step in all the three stages of HCMV infection (Li et al., 2015; Peppenelli et al., 2018; Altman et al., 2019) (https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map05163&keyword=human%20herpesvirus%203). Consequently, targeting the PIK/Akt/mTOR cascade might be more efficient for treating HCMV-positive PSS. Significantly decreased KIV was found in the AH of PSS. We speculated that KIV uptake increased significantly during the course of HCMV infection. Mammalian mitochondrial branched-chain α -keto acid dehydrogenase complex can catalyze the oxidative decarboxylation of KIV to yield the BCAA product, valine (Funchal et al., 2007). The metabolic stimulation of this step may be responsible for the decrease in KIV and accumulation of BCAA. This information provides a target for the metabolic inhibition of this step, which might reverse the result and diminish the activity of BCAAs and PIK/Akt/mTOR cascade.

Besides the PIK/Akt/mTOR pathway, BCAAs-induced mTOR activation can also significantly contribute to the upregulation of vascular endothelial growth factor (VEGF) level (Brugarolas et al., 2003; Klos et al., 2006), which has been found to be highly expressed in the AH of patients with uveitic glaucoma (Ohira et al., 2016). Recently, emerging evidences indicate that the VEGF–VEGF receptor (R) system is significantly involved in inflammation (Shibuya, 2015). Among the VEGFRs, VEGFR1 is expressed on the membrane of macrophage lineage cells and mediates the transduction of important cytokine/chemokine signaling in these cells (Shibuya, 2015). This VEGFR1–macrophage axis can trigger inflammatory responses in various tissues and might be a potential target for managing inflammation. Taken together, we surmised that the inhibition of BCAA/mTOR pathway could have beneficial therapeutic effects by preventing inflammation-mediated pathogenesis.

Marked increase in succinic acid and the associated pathways, citrate acid cycle (tricarboxylic acid [TCA] cycle) (https://www.kegg.jp/kegg-bin/show_pathway?map00020) was detected in the PSS group. TCA cycle has been considered to interact with the frontline innate leukocytes, such as macrophages and monocytes (Jha et al., 2015; Kelly and O'Neill, 2015; Murphy and O'Neill, 2018). The mechanism behind this process might involve the metabolic reprogramming of endogenous TCA cycle intermediates, namely, citric acid, which adopts regulatory features to harness inflammatory response. Typically, in the TCA cycle, citrate is converted to isocitrate, which is then diverted to succinate, a key marker of macrophage activation. Succinate has been found to be elevated in metabolic disorders associated with inflammation (Tannahill et al., 2013; Mills and O'Neill, 2014). Intracellular succinate accumulation is postulated to regulate the IL-1 β –HIF-1 α axis to exert pro-inflammatory function (Tannahill et al., 2013; Mills and O'Neill, 2014; Lampropoulou et al., 2016; Xiao et al., 2017). Meanwhile, we also detected increased FA in PSS AH, another well-known intermediate product of TCA. Recently, FA has shown its therapeutic potentials for the management of inflammatory clinical diseases, such as autoimmune myocarditis and multiple sclerosis (Wakkee

and Thio, 2007; Meili-Butz et al., 2008; Moharreg-Khiabani et al., 2009; Loewe et al., 2011). Hence, the regulation of TCA to generate favorable metabolites may have important clinical utility for the treatment of PSS.

Besides pathway analysis, ROC analysis was conducted to evaluate the diagnostic potential of selected metabolites. The results of ROC analysis implied that the combination of glycine and homogentisic acid could serve as potential biomarkers for the discrimination of control and PSS groups. Spearman's rank correlation analysis simultaneously revealed that these two metabolites, differentially expressed in patients with PSS, presented significant correlation with IOP, C/D, and anti-HCMV IgG titer in AH (**Figures 3 and 6**). This discovery demonstrated immense potential for PSS diagnosis. Most importantly, these metabolites could also provide valuable information regarding subtype identification and allow us to perform specific intervention for patients with PSS.

In conclusion, these results revealed for the first time the identity of important metabolites and pathways contributing to the development/progression of PSS. These discoveries enabled the better understanding of the mechanism of PSS and might lead to the development of metabolic biomarkers and novel therapeutic strategies to restrict the development/progression of PSS. Further investigations are needed to validate the role of these metabolites and relevant metabolic reprogramming pathways. We have realized there are limitations in our research. The following studies will recruit more participants and choose more suitable control group to support our results.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the medical ethics committee of the Eye, Ear, Nose and Throat (ENT) Hospital of Fudan University (2017006–2). The patients/participants provided their written informed consent to participate in this study.

REFERENCES

- Altman, A. M., Mahmud, J., Nikolovska-Coleska, Z., and Chan, G. (2019). HCMV modulation of cellular PI3K/AKT/mTOR signaling: new opportunities for therapeutic intervention? *Antiviral. Res.* 163, 82–90. doi: 10.1016/j.antiviral.2019.01.009
- Baharum, S. N., and Azizan, K. A. (2018). Metabolomics in systems biology. *Adv. Exp. Med. Biol.* 1102, 51–68. doi: 10.1007/978-3-319-98758-3_4
- Brugarolas, J. B., Vazquez, F., Reddy, A., Sellers, W. R., and Kaelin, W. G. Jr. (2003). TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* 4 (2), 147–158. doi: 10.1016/S1535-6108(03)00187-9

AUTHOR CONTRIBUTIONS

HW designed the study, evaluated of the results, and wrote the draft and final manuscript. RZ collected the clinical data of the subjects and analyzed statistical data. QS clinically evaluated the subjects and collected the AH sample. YW collected the metabolomic data. ZW performed the laboratory exams. JF evaluated of the original metabolomic data and edited the draft manuscript. XK evaluated the clinical characteristics of the subjects to establish the diagnosis, performed clinical procedures of the study, and edited the final manuscript. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2019.01322/full#supplementary-material>

FIGURE S1 | The validation plot from the 200-permutation test model. The Q2_y of the original model on the right were higher than the corresponding values of the permutation test models on the left.

- Chee, S. P., and Jap, A. (2008). Presumed fuchs heterochromic iridocyclitis and posner-schlossman syndrome: comparison of cytomegalovirus-positive and negative eyes. *Am. J. Ophthalmol.* 146 (6), 883–9 e1. doi: 10.1016/j.ajo.2008.09.001
- Chee, S. P., and Jap, A. (2010). Cytomegalovirus anterior uveitis: outcome of treatment. *Br. J. Ophthalmol* 94 (12), 1648–1652. doi: 10.1136/bjo.2009.167767
- Chen, J., Zhuang, Y., Zhang, Z. F., Wang, S., Jin, P., He, C., et al. (2016). Glycine confers neuroprotection through microRNA-301a/PTEN signaling. *Mol. Brain* 9 (1), 59. doi: 10.1186/s13041-016-0241-3
- DeMeritt, I. B., Milford, L. E., and Yurochko, A. D. (2004). Activation of the NF-kappaB pathway in human cytomegalovirus-infected cells is necessary for

- efficient transactivation of the major immediate-early promoter. *J. Virol.* 78 (9), 4498–4507. doi: 10.1128/JVI.78.9.4498-4507.2004
- Effenberger-Neidnisch, K., Jägers, J., Verhaegh, R., and de Groot, H. (2014). Glycine selectively reduces intestinal injury during endotoxemia. *J. Surg. Res.* 192 (2), 592–598. doi: 10.1016/j.jss.2014.06.016
- Eissler, R. (1948). Atrophy of the optic nerve due to malnutrition; report of two cases. *Arch. Ophthalmol.* 39 (4), 465–470. doi: 10.1001/archophth.1948.00900020473002
- Funchal, C., Tramontina, F., Quincozes dos Santos, A., Fraga de Souza, D., Gonçalves, C. A., Pessoa-Pureur, R., et al. (2007). Effect of the branched-chain alpha-keto acids accumulating in maple syrup urine disease on S100B release from glial cells. *J. Neurol. Sci.* 260 (1–2), 87–94. doi: 10.1016/j.jns.2007.04.011
- Gameiro, P. A., and Struhl, K. (2018). Nutrient deprivation elicits a transcriptional and translational inflammatory response coupled to decreased protein synthesis. *Cell Rep.* 24 (6), 1415–1424. doi: 10.1016/j.celrep.2018.07.021
- Gusev, E. I., Skvortsova, V. I., Dambinova, S. A., Raevskiy, K. S., Alekseev, A. A., Bashkatova, V. G., et al. (2000). Neuroprotective effects of glycine for therapy of acute ischaemic stroke. *Cerebrovasc. Dis.* 10 (1), 49–60. doi: 10.1159/000016025
- Haines, N. R., Manoharan, N., Olson, J. L., D'Alessandro, A. Z., and Reisz, J. A. (2018). Metabolomics analysis of human vitreous in diabetic retinopathy and rhegmatogenous retinal detachment. *J. Proteome Res.* 17 (7), 2421–2427. doi: 10.1021/acs.jproteome.8b00169
- Jaeger, C., Méret, M., Schmitt, C. A., and Lisec, J. (2017). Compound annotation in liquid chromatography/high-resolution mass spectrometry based metabolomics: robust adduct ion determination as a prerequisite to structure prediction in electrospray ionization mass spectra. *Rapid Commun. Mass Spectrom.* 31 (15), 1261–1266. doi: 10.1002/rcm.7905
- Jha, A. K., Huang, S. C., Sergushichev, A., Lampropoulou, V., Ivanova, Y., Loginicheva, E., et al. (2015). Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 42 (3), 419–430. doi: 10.1016/j.immuni.2015.02.005
- Jiang, J. H., Zhang, S. D., Dai, M. L., Yang, J. Y., Xie, Y. Q., Hu, C., et al. (2017). Posner-Schlossman syndrome in Wenzhou, China: a retrospective review study. *Br. J. Ophthalmol.* 101 (12), 1638–1642. doi: 10.1136/bjophthalmol-2016-309863
- Kelly, B., and O'Neill, L. A. (2015). Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* 25 (7), 771–784. doi: 10.1038/cr.2015.68
- Klos, K. S., Wyszomierski, S. L., Sun, M., Tan, M., Zhou, X., Li, P., et al. (2006). ErbB2 increases vascular endothelial growth factor protein synthesis via activation of mammalian target of rapamycin/p70S6K leading to increased angiogenesis and spontaneous metastasis of human breast cancer cells. *Cancer Res.* 66 (4), 2028–2037. doi: 10.1158/0008-5472.CAN-04-4559
- Lampropoulou, V., Sergushichev, A., Bambouskova, M., Nair, S., Vincent, E. E., Loginicheva, E., et al. (2016). Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab.* 24 (1), 158–166. doi: 10.1016/j.cmet.2016.06.004
- Li, X., Bradford, B. U., Wheeler, M. D., Stimpson, S. A., Pink, H. M., Brodie, T. A., et al. (2001). Dietary glycine prevents peptidoglycan polysaccharide-induced reactive arthritis in the rat: role for glycine-gated chloride channel. *Infect. Immun.* 69 (9), 5883–5891. doi: 10.1128/IAI.69.9.5883-5891.2001
- Li, Q., Wilkie, A. R., Weller, M., Liu, X., and Cohen, J. I. (2015). THY-1 cell surface antigen (CD90) has an important role in the initial stage of human cytomegalovirus infection. *PLoS Pathog.* 11 (7), e1004999. doi: 10.1371/journal.ppat.1004999
- Liu, R., Liao, X. Y., Pan, M. X., Tang, J. C., Chen, S. F., Zhang, Y., et al. (2019). Glycine exhibits neuroprotective effects in ischemic stroke in rats through the inhibition of M1 microglial polarization via the NF-kappaB p65/Hif-1alpha signaling pathway. *J. Immunol.* 202 (6), 1704–1714. doi: 10.4049/jimmunol.1801166
- Loewe, R., Pillinger, M., de Martin, R., Mrowietz, U., Gröger, M., Holthöner, W., et al. (2011). Dimethylfumarate inhibits tumor-necrosis-factor-induced CD62E expression in an NF-kB-dependent manner. *J. Invest. Dermatol.* 117 (6), 1363–1368. doi: 10.1046/j.0022-202x.2001.01576.x
- Mayerle, J., Kalthoff, H., Reszka, R., Kamlage, B., Peter, E., Schniewind, B., et al. (2018). Metabolic biomarker signature to differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis. *Gut* 67 (1), 128–137. doi: 10.1136/gutjnl-2016-312432
- Meili-Butz, S., Niermann, T., Fasler-Kan, E., Barbosa, V., Butz, N., John, D., et al. (2008). Dimethyl fumarate, a small molecule drug for psoriasis, inhibits Nuclear Factor-kB and reduces myocardial infarct size in rats. *Eur. J. Pharmacol.* 586 (1–3), 251–258. doi: 10.1016/j.ejphar.2008.02.038
- Mills, E., and O'Neill, L. A. (2014). Succinate: a metabolic signal in inflammation. *Trends Cell Biol.* 24 (5), 313–320. doi: 10.1016/j.tcb.2013.11.008
- Miyayama, M., Sugita, S., Shimizu, N., Morio, T., Miyata, K., Maruyama, K., et al. (2010). A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis. *Br. J. Ophthalmol.* 94 (3), 336–340. doi: 10.1136/bjo.2008.156422
- Moharrehg-Khiabani, D., Linker, R. A., Gold, R., and Stangel, M. (2009). Fumaric acid and its esters: an emerging treatment for multiple sclerosis. *Curr. Neuropharm.* 7 (1), 60–64. doi: 10.2174/157015909787602788
- Moorthy, R. S., Mermoud, A., Baerveldt, G., Minckler, D. S., Lee, P. P., and Rao, N. A. (1997). Glaucoma associated with uveitis. *Surv. Ophthalmol.* 41 (5), 361–394. doi: 10.1016/S0039-6257(97)00006-4
- Mori, H., Momosaki, K., Kido, J., Naramura, T., Tanaka, K., Matsumoto, S., et al. (2017). Amelioration by glycine of brain damage in neonatal rat brain following hypoxia-ischemia. *Pediatr. Int.* 59 (3), 321–327. doi: 10.1111/ped.13164
- Murphy, M. P., and O'Neill, L. A. J. (2018). Krebs cycle reimaged: the emerging roles of succinate and itaconate as signal transducers. *Cell* 174 (4), 780–784. doi: 10.1016/j.cell.2018.07.030
- Nicastro, H., da Luz, C. R., Chaves, D. F., Bechara, L. R., Voltarelli, V. A., Rogero, M. M., et al. (2012). Does branched-chain amino acids supplementation modulate skeletal muscle remodeling through inflammation modulation? possible mechanisms of action. *J. Nutr. Metab.* 2012, 136937. doi: 10.1155/2012/136937
- Ohira, S., Inoue, T., Iwao, K., Takahashi, E., and Tanihara, H. (2016). Factors influencing aqueous proinflammatory cytokines and growth factors in uveitic glaucoma. *PLoS One* 11 (1), e0147080. doi: 10.1371/journal.pone.0147080
- Oldiges, M., Lütz, S., Pflug, S., Schroer, K., Stein, N., and Wiendahl, C. (2007). Metabolomics: current state and evolving methodologies and tools. *Appl. Microbiol. Biotechnol.* 76 (3), 495–511. doi: 10.1007/s00253-007-1029-2
- Pan, L., Qiu, Y., Chen, T., Lin, J., Chi, Y., Su, M., et al. (2010). An optimized procedure for metabolomic analysis of rat liver tissue using gas chromatography/time-of-flight mass spectrometry. *J. Pharm. Biomed. Anal.* 52 (4), 589–596. doi: 10.1016/j.jpba.2010.01.046
- Peppenelli, M. A., Miller, M. J., Altman, A. M., Cojohari, O., and Chan, G. C. (2018). Aberrant regulation of the Akt signaling network by human cytomegalovirus allows for targeting of infected monocytes. *Antiviral Res.* 158, 13–24. doi: 10.1016/j.antiviral.2018.07.015
- Phua, L. C., Koh, P. K., Cheah, P. Y., Ho, H. K., and Chan, E. C. (2013). Global gas chromatography/time-of-flight mass spectrometry (GC/TOFMS)-based metabolomic profiling of lyophilized human feces. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 937, 103–113. doi: 10.1016/j.jchromb.2013.08.025
- Pithon-Curi, T. C., De Melo, M. P., and Curi, R. (2004). Glucose and glutamine utilization by rat lymphocytes, monocytes and neutrophils in culture: a comparative study. *Cell Biochem. Funct.* 22 (5), 321–326. doi: 10.1002/cbf.1109
- Pohlmann, D., Schlickeiser, S., Metzner, S., Lenglinger, M., Winterhalter, S., Pleyer, U. (2018). Different composition of intraocular immune mediators in Posner-Schlossman-Syndrome and Fuchs' Uveitis. *PLoS One* 13 (6), e0199301. doi: 10.1371/journal.pone.0199301
- Qiu, Y., Cai, G., Zhou, B., Li, D., Zhao, A., Xie, G., et al. (2014). A distinct metabolic signature of human colorectal cancer with prognostic potential. *Clin. Cancer Res.* 20 (8), 2136–2146. doi: 10.1158/1078-0432.CCR-13-1939
- Saxton, R. A., and Sabatini, D. M. (2017). mTOR signaling in growth, metabolism, and disease. *Cell* 168 (6), 960–976. doi: 10.1016/j.cell.2017.02.004
- Shazly, T. A., Aljajeh, M., and Latina, M. A. (2011). Posner-Schlossman glaucomatocyclitic crisis. *Semin. Ophthalmol.* 26 (4–5), 282–284. doi: 10.3109/08820538.2011.605821
- Shen, S. C., Ho, W. J., Wu, S. C., Yu, K. H., Lin, H. C., Lin, Y. S., et al. (2010). Peripheral vascular endothelial dysfunction in glaucomatocyclitic crisis: a preliminary study. *Invest. Ophthalmol Vis Sci.* 51 (1), 272–276. doi: 10.1167/iovs.09-3849
- Shibuya, M. (2015). VEGF-VEGFR System as a target for suppressing inflammation and other diseases. *Endocr. Metab. Immune Disord. Drug Targets* 15 (2), 135–144. doi: 10.2174/1871530315666150316121956

- Sinclair, C., Bommakanti, G., Gardinassi, L., Loebbermann, J., Johnson, M. J., Hakimpour, P., et al. (2017). mTOR regulates metabolic adaptation of APCs in the lung and controls the outcome of allergic inflammation. *Science* 357 (6355), 1014–1021. doi: 10.1126/science.aaj2155
- Sobolewska, B., Deuter, C., Doycheva, D., Zierhut, M. (2014). Long-term oral therapy with valganciclovir in patients with Posner-Schlossman syndrome. *Graefes. Arch. Clin. Exp. Ophthalmol.* 252 (1), 117–124. doi: 10.1007/s00417-013-2535-9
- Stoffels, B., Türler, A., Schmidt, J., Nazir, A., Tsukamoto, T., Moore, B. A., et al. (2011). Anti-inflammatory role of glycine in reducing rodent postoperative inflammatory ileus. *Neurogastroenterol. Motil.* 23 (1), 76–87, e8. doi: 10.1111/j.1365-2982.2010.01603.x
- Su, C. C., Hu, F. R., Wang, T. H., Huang, J. Y., Yeh, P. T., Lin, C. P., et al. (2014). Clinical outcomes in cytomegalovirus-positive Posner-Schlossman syndrome patients treated with topical ganciclovir therapy. *Am. J. Ophthalmol.* 158 (5), 1024–1031 e2. doi: 10.1016/j.ajo.2014.08.007
- Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., et al. (2013). Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature* 496 (7444), 238–242. doi: 10.1038/nature11986
- Teoh, S. B., Thean, L., and Koay, E. (2005). Cytomegalovirus in aetiology of Posner-Schlossman syndrome: evidence from quantitative polymerase chain reaction. *Eye (Lond)* 19 (12), 1338–1340. doi: 10.1038/sj.eye.6701757
- Van Gelder, R. N. (2008). Idiopathic no more: clues to the pathogenesis of Fuchs heterochromic iridocyclitis and glaucomatocyclitic crisis. *Am. J. Ophthalmol.* 145 (5), 769–771. doi: 10.1016/j.ajo.2008.02.010
- Wakke, M., and Thio, H. B. (2007). Drug evaluation: BG-12, an immunomodulatory dimethylfumarate. *Curr. Opin. Invest. Drugs* 8 (11), 955–962.
- Wang, W., Dai, Z., Wu, Z., Lin, G., Jia, S., Hu, S., et al. (2014). Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs. *Amino Acids* 46 (8), 2037–2045. doi: 10.1007/s00726-014-1758-3
- Wheeler, M. D., Ikejima, K., Enomoto, N., Stacklewitz, R. F., Seabra, V., Zhong, Z., et al. (1999). Glycine: a new anti-inflammatory immunonutrient. *Cell Mol. Life Sci.* 56 (9–10), 843–856. doi: 10.1007/s000180050030
- Wheeler, M. D., Rose, M. L., Yamashima, S., Enomoto, N., Seabra, V., Madren, J., et al. (2000). Dietary glycine blunts lung inflammatory cell influx following acute endotoxin. *Am. J. Physiol. Lung Cell Mol. Physiol.* 279 (2), L390–L398. doi: 10.1152/ajplung.2000.279.2.L390
- Wu, G. (2014). Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. *J. Anim. Sci. Biotechnol.* 5 (1), 34. doi: 10.1186/2049-1891-5-34
- Xia, J., and Wishart, D. S. (2016). Using metaboanalyst 3.0 for comprehensive metabolomics data analysis. *Curr. Protoc. Bioinf.* 55, 14 10 1–14 10 91. doi: 10.1002/cpbi.11
- Xiao, N., Lou, M. D., Lu, Y. T., Yang, L. L., Liu, Q., Liu, B., et al. (2017). Ginsenoside Rg5 attenuates hepatic glucagon response via suppression of succinate-associated HIF-1 α induction in HFD-fed mice. *Diabetologia* 60 (6), 1084–1093. doi: 10.1007/s00125-017-4238-y
- Xu, L., and Brink, M. (2016). mTOR, cardiomyocytes and inflammation in cardiac hypertrophy. *Biochim. Biophys. Acta.* 1863 (7), 1894–1903. doi: 10.1016/j.bbamcr.2016.01.003
- Xu, X., Wang, X., Wu, H., Zhu, H., Liu, C., Hou, Y., et al. (2018). Glycine relieves intestinal injury by maintaining mtor signaling and suppressing AMPK, TLR4, and NOD Signaling in Weaned piglets after lipopolysaccharide challenge. *Int. J. Mol. Sci.* 19 (7), 1980–1998. doi: 10.3390/ijms19071980
- Yin, S., Guo, P., Hai, D., Xu, L., Shu, J., Zhang, W., et al. (2017). Optimization of GC/TOF MS analysis conditions for assessing host-gut microbiota metabolic interactions: Chinese rhubarb alters fecal aromatic amino acids and phenol metabolism. *Anal. Chim. Acta* 995, 21–33. doi: 10.1016/j.aca.2017.09.042

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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