



# Associations of Gut Microbiota and Fatty Metabolism With Immune Thrombocytopenia

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**Objective:** To determine whether gut microbiota, fatty metabolism and cytokines were associated with immune thrombocytopenia (ITP).

**Methods:** In total, 29 preliminarily diagnosed ITP patients and 33 healthy volunteers were enrolled. Fecal bacterial were analyzed based on 16S rRNA sequencing. Plasma cytokines and metabolites were analyzed using flow cytometry and liquid chromatography-mass spectrometry (LC-MS), respectively.

**Results:** *Bacteroides*, *Phascolarctobacterium*, and *Lactobacillus* were enriched at the genus level in ITP patients, while *Ruminococcaceae* UCG-002, *Eubacterium coprostanoligeues*, *Megamonas*, and *Lachnospiraceae* NC2004 were depleted. At the phylum level, the relative abundance of *Proteobacteria* and *Chloroflexi* increased in ITP patients, while *Firmicutes*, *Actinobacteria*, and the *Firmicutes/Bacteroidetes* ratio decreased. Plasma levels of 5-hydroxyeicosatetraenoic acid (5-HETE), 6-trans-12-epi-leukotriene B4 (6t,12e-LTB<sub>4</sub>), and resolvin D2 (RvD<sub>2</sub>) were upregulated, and stachydrine, dolicide A, dodecanoylcarnitine were downregulated in ITP patients. Furthermore, RvD<sub>2</sub> is positively correlated with order *Bacteroidetes* VC2.1 Bac22, 5-HETE is positively correlated with genus *Azospirillum*, and 6t,12e-LTB<sub>4</sub> is positively correlated with genus *Cupriavidus*. In addition, stachydrine is positively correlated with family *Planococcaceae*, dolicide A is positively correlated with class *MVP-15*, and dodecanoylcarnitine is positively correlated with order *WCHB1-41*. Plasma levels of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were upregulated in ITP patients.

**Conclusion:** Our study revealed a relationship between microbiota and fatty metabolism in ITP. Gut microbiota may participate in the pathogenesis of ITP through affecting cytokine secretion, interfering with fatty metabolism.

**Keywords:** immune thrombocytopenia, gut microbiota, fatty metabolism, 16S rRNA, cytokines

## INTRODUCTION

Immune thrombocytopenia (ITP) is characterized by low peripheral blood platelet count. It is an acquired autoimmune disorder which can lead to easy and excessive bruising and bleeding. The increased platelet clearance arises through diverse mechanisms, such as antiplatelet autoantibodies production, and T lymphocyte dysfunction. It was reported that receptors for the Fc fragment of IgG result in the removal of platelets coated with immunoglobulin (1) and the activation of phagocytosis in the reticuloendothelial system (2). In addition to B cell dependent autoimmune mechanisms, it is well established that cellular immune pathophysiology of ITP is associated with several T-cell abnormalities, including uncontrolled activation of T helper cells, deficiency of T regulatory cells, and increase of CD8 + T cell targeting megakaryocytes, etc. (2, 3). Another mechanism that contributes to the platelet clearance is platelet activation (4). In two pilot studies, it was reported that platelet activation markers, such as P-selectin, CD62 and CD11b expression, were significantly higher in ITP patients (5, 6). In the ITP mouse model, Fc-independent platelet activation was observed, which led to platelet clearance in the liver (7). This partially explained the fact that the risk of venous thromboembolism is twice higher in ITP patients than in healthy population (8). More recently, COVID-19 vaccines have been linked with serious vaccine-induced ITP (9), which is believed to be mediated by activated platelets (10, 11). However, the initial trigger of platelets activation has not yet to be determined.

Emerging data has demonstrated a strong association among platelet function, metabolites of gut microbiota, and lipid metabolism (12). Two gut microbiota derived-metabolites, trimethylamine N-oxide (13) and phenylacetylglutamine (14) were shown to directly contribute to platelet activation related phenotypes. It is worth noticing that the metabolite phenylacetylglutamine was discovered through untargeted metabolomics, a powerful and unbiased platform for discovery of pathways linked to diseases. Using the similar strategy, analysis of a cohort of 534 healthy adult Dutch volunteers provided evidence for the importance of intestinal microbiota in regulating platelet function (15). More than 20% metabolites were significantly associated with platelet activation markers and the majority involved lipids. In a more recent study, clinical findings with experimental data suggested that gut microbiota dysbiosis may be an important mechanism for the high platelet reactivity in patients treated with ticagrelor, a first-line drug for the treatment of acute ST elevation myocardial infarction (16). For example, the abundance of gut microbiota species *Methylobacillus*, *Sphingomonas*, and *Staphylococcus* was negatively correlated with serum salicylate, which was previously found to induce ITP and inhibit platelet production in megakaryocytes (17).

Platelet metabolism and reactivity critically depend on lipid composition (18). For example, arachidonic acid (AA), a polyunsaturated fatty acid constituting an important part of phospholipid-content in cell membranes, is associated with the enzyme-derived oxidized metabolites (19). It enters the cell, activates the platelet and exerts in platelet function and fate.

Up to 25% of phospholipid fatty acids are AA in resting platelets, the concentration of which may influence normal cellular functions and development of platelet diseases (20). Other metabolites like prostacyclin (PGI<sub>2</sub>) and prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) also inhibit platelet activation (21, 22) while 12(S)-HETE produced by 12-LOX was found to impact the platelet function (23). Besides, it was observed that high-density lipoprotein (HDL) reduced platelet aggregation in response to a variety of agonists, while oxidized HDL exerted the opposite effect binding to a similar receptor (24). In general, specific lipid species play important roles in enhancing stability of the domains and stabilizing signaling events in platelets, which permit the feasibility of a systematic assessment on platelet-lipid analysis (18).

To date, the analysis of gut microbes and lipid metabolism in modulation of ITP-associated platelet function has not yet been reported. The present study should provide new insights in the pathogenesis mechanisms associated with gut microbiota, cytokine, fatty metabolism in ITP, and therefore could improve differential diagnosis and support further treatment decision, such as traditional Chinese medicine, for ITP patients.

## MATERIALS AND METHODS

### Subjects and Sample Collection

Fecal and plasma samples were collected from patients with newly diagnosed ITP before systemic treatment at the First Affiliated Hospital of Wenzhou Medical University. None of the recruited patients and healthy volunteers presented any other autoimmune diseases or intestinal and metabolic diseases that could affect the gut microbiota. The protocol was approved by the Ethics Committee of Wenzhou Medical University (KY2021-048), and informed consent was obtained from all participants. ITP was diagnosed according to the criteria established by the American Society of Hematology (ASH), which identifies as an autoimmune disorder characterized by an isolated platelet count  $< 100 \times 10^9/L$ , with or without bleeding manifestations, in the absence of other causes or disorders that may be associated with thrombocytopenia (25). In total, 29 patients were enrolled in the present study. In addition, 33 volunteers were recruited after a routine physical examination. All healthy controls presented no gut problems such as chronic diarrhea, constipation, gas, heartburn, bloating (26), and none had taken any antibiotics or probiotics within 3 months of this study. Moreover, there were no significant differences between the two groups in terms of sex, age, smoking history, and alcohol or dietary intake.

### Cytokines Detected by Flow Cytometry

For cytokine analysis, venous blood (2 mL) was collected and centrifuged to obtain 200  $\mu$ L of serum. Cytokines, including interleukin (IL)-2, IL-4, IL-6, IL-10, TNF- $\alpha$ , and interferon (IFN)- $\gamma$ , were detected by employing a cytometric bead array human inflammation kit according to the manufacturer's instructions (BD Pharmingen, San Diego, CA, United States) and operated using AimPlex<sup>TM</sup> bead-based

multiparametric flow cytometry (EPICS-Elite, Beckman-Coulter, United States). For determining the protein concentration, sample fluorescence signals were compared with those of a standard curve generated from serial dilutions of a known concentration of the analyte.

## 16S rRNA Gene Sequencing

### DNA Extraction and Polymerase Chain Reaction Amplification

Genomic DNA was extracted based on the V4 hypervariable region of 16S rDNA using a fecal genomic DNA extraction kit (QIAGEN), with 0.8% agarose gel electrophoresis performed to separate DNA. Then, to amplify the bacterial 16S rRNA from diluted DNA extracts, we used template-specific sequences 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'GGACTACHVGGGTWTCTAAT-3'). Polymerase chain reaction (PCR) amplification was performed using a 25  $\mu$ L reaction volume with the following composition: 1  $\times$  PCR buffer, 1.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M dNTPs, 1.0  $\mu$ L forward primer, 1.0  $\mu$ L reverse primer, 0.5 U KOD-Plus-Neo enzyme (TOYOBO), and 10 ng template. The reaction was initiated at 94°C for 1 min, followed by 30 cycles of denaturation at 94°C for 20 s, annealing at 54°C for 30 s, and elongation at 72°C for 30 s, with a final extension step at 72°C for 5 min. Each sample was assessed in triplicate. PCR products were mixed with 1/6 volume 6  $\times$  loading buffer and detected by 2% agarose gel electrophoresis. These amplicons were recycled using QIAquick Gel Extraction Kit (QIAGEN), quantified using Qubit@ 2.0 Fluorometer (Thermo Scientific), and finally mixed equally. The library was prepared using TruSeq DNA PCR-Free Sample Prep Kit (FC-1213001/3003) (Illumina Inc., San Diego, CA). After quantification and detection, the sequencing reaction was conducted using the PE250 pattern of HiSeq Rapid SBS Kit v2 (FC-402-4023 500 Cycle) (Illumina Inc., San Diego, CA).

### Microbial Diversity Analysis

After sequencing, high-quality sequences were filtered for subsequent analysis and assembled from raw data. Alpha diversity [Chao1, phylogenetic diversity (PD), Simpson, Shannon] and beta diversity (PCoA) were analyzed using Usearch and QIIME. Statistics and plotting were completed using R6, Python, and Java. Next, to determine the species composition and diversity, high-quality sequences were clustered with a cut-off of 97% similarity, resulting in a total of 6,327 operational taxonomic units (OTUs). For each OTU, the sequence that occurred most frequently was selected as the representative OTU sequence. Annotation analysis was performed using UCLUST and SILVA databases. Multiple sequence alignment was performed using PyNAST, with the phylogenetic tree constructed using FastTree. Samples were homogenized and resampled with the standard of the least amount.

## Metabolomics Analysis

### Metabolite Extraction

After plasma samples were thawed on ice, 120  $\mu$ L of precooled 50% methanol buffer was added to 20  $\mu$ L of samples. Then, samples were vortexed for 1 min, incubated for 10 min at room

temperature and stored overnight at  $-20^{\circ}\text{C}$ . The mixture of metabolites was centrifuged at  $4,000 \times g$  for 20 min to obtain the supernatant. Subsequently, supernatants were transferred into new 96-well plates and stored at  $-80^{\circ}\text{C}$  for subsequent liquid chromatography-mass spectrometry (LC-MS) analysis. For pooled quality control (QC) samples, 10  $\mu$ L of each extraction mixture were combined and mixed.

### Liquid Chromatography-Mass Spectrometry Analysis

All samples were analyzed using the TripleTOF 5600 Plus high-resolution tandem mass spectrometer (SCIEX, Warrington, United Kingdom), utilizing both positive and negative ion modes. Chromatographic separation was performed in an ultra-performance liquid chromatography (UPLC) system (SCIEX, United Kingdom) using an ACQUITY UPLC T3 column (100 mm  $\times$  2.1 mm, 1.8  $\mu$ m; Waters, United Kingdom) for reversed-phase separation, with a constant column temperature of 35°C. The mobile phase consisted of solvent A (water, 0.1% formic acid) and solvent B (acetonitrile, 0.1% formic acid). The UPLC elution conditions were optimized and set as follows: flow rate, 0.4 mL/min; 0–0.5 min, 5% solvent B; 0.5–7 min, 5–100% solvent B; 7–8 min, 100% solvent B; 8–8.1 min, 100–5% solvent B; 8.1–10 min, 5% solvent. The TripleTOF 5600 Plus System was used to detect eluted metabolites. The curtain gas was set at 30 PSI, with ion source gases 1 and 2 at 60 PSI. The temperature of the interface heater was 650°C. The ion spray floating voltage was set at 5 kV for the positive ion mode and  $-4.5$  kV for the negative ion mode.

MS data were acquired in Information Dependent Acquisition mode. The time-of-flight mass ranged between 60 and 1,200 Da. Survey scans were acquired over 0.15 s, and up to 12 product ion scans were collected if the 100 counts/s threshold was exceeded, with a 1 + charge state. The total cycle time was fixed at 0.56 s. Four-time bins were summed for each scan at an impulsator frequency of 11 kHz by monitoring the 40 GHz multichannel TDC detector with four-anode/channel detectors. Dynamic exclusion was performed for 4 s. During the entire acquisition period, the mass accuracy was calibrated for every 20 samples. In addition, a QC sample was analyzed every 10 samples to evaluate the LC-MS stability.

### Database Searches and Bioinformatics Analysis

The acquired LC-MS data was pretreated using XCMS software (SCIEX). Raw data was converted into mzXML format and processed using the XCMS, CAMERA, and metaX toolboxes included in R software. Each ion was identified using comprehensive information regarding the retention time and m/z. The intensity of each peak was recorded, and a three-dimensional matrix containing arbitrarily assigned peak indices (retention time  $-m/z$  pairs), sample names (observations), and ion intensity information (variables) were generated. Then, the information was matched to in-house and public databases. The open-access databases, KEGG (Kyoto Encyclopedia of Genes and Genomes)<sup>1, 2</sup> and HMDB (Human Metabolome Database)<sup>3</sup>,

<sup>1</sup><http://www.kegg.jp>

<sup>2</sup><http://www.genome.jp/kegg/>

<sup>3</sup><http://www.hmdb.ca>

were employed to annotate metabolites by matching the exact molecular mass data ( $m/z$ ) to those from the database, within a threshold of 10 ppm. Peak intensity data was further pre-processed using metaX software. Features detected in 30% of samples were removed. Group datasets were normalized before analysis. Data normalization was performed for all samples using a probabilistic quotient normalization algorithm. Then, QC-robust spline batch correction was performed using QC samples. The  $P$ -value was analyzed using the Student's  $t$ -test, then adjusted for multiple tests using a false discovery rate (Benjamini–Hochberg) to select different metabolites. Supervised partial least-squares discriminant analysis was performed using metaX to identify variables that differed between groups. The variable importance in projection was calculated, and a cut-off value of 1.0 was set to select important features.

## Statistics Analysis

The results were statistically analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, United States). Data are expressed as mean  $\pm$  standard error of the mean. Significant differences in clinical characteristics and cytokine levels between healthy controls and ITP patients were analyzed by independent-sample  $t$ -test. A  $P$ -value of  $<0.05$  was considered statistically significant. We used Spearman correlation analysis to filter differential metabolites and gut microbiota.

## RESULTS

### Demographic, Clinical, and Metabolite Parameters of Study Subjects

The present study recruited 62 subjects, including 29 ITP patients (13 men, 16 women) and 33 healthy controls (17 men, 16 women) (Table 1). No statistically significant difference was observed between the patient group and control group in terms of gender (male: 45% vs. male: 52%,  $P = 0.599$ ) and age ( $39.50 \pm 15.98$  vs.  $39.60 \pm 15.87$ ,  $P = 0.989$ ) (Table 1). Serum levels of total protein (TP), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), glucose (GLU), urea, total cholesterol (TC), total triglyceride (TG), and low-density lipoprotein (LDL) were similar between patients and healthy controls. Levels of total bilirubin (TB,  $9.93 \pm 4.17$  vs.  $13.11 \pm 4.33$ ,  $P = 0.007$ ), indirect bilirubin (IB,  $5.86 \pm 2.66$  vs.  $10.37 \pm 3.67$ ,  $P < 0.001$ ), albumin (ALB,  $40.78 \pm 4.94$  vs.  $45.21 \pm 2.62$ ,  $P < 0.001$ ), creatinine (Crea,  $58.62 \pm 16.92$  vs.  $69.93 \pm 15.70$ ,  $P = 0.012$ ), uric acid (UA,  $287.24 \pm 87.61$  vs.  $345.37 \pm 91.31$ ,  $P = 0.018$ ), and high-density lipoprotein (HDL,  $1.11 \pm 0.35$  vs.  $1.34 \pm 0.27$ ,  $P = 0.011$ ) were lower in ITP patients compared to healthy controls (Table 1). In addition, levels of direct bilirubin (DB,  $4.07 \pm 1.85$  vs.  $2.74 \pm 0.90$ ,  $P = 0.001$ ), globulin (GLB,  $32.67 \pm 6.85$  vs.  $29.23 \pm 2.48$ ,  $P = 0.016$ ), and aspartate aminotransferase (AST,  $21.57 \pm 5.89$  vs.  $18.33 \pm 3.28$ ,  $P = 0.015$ ) were higher in ITP patients than healthy controls (Table 1).

**TABLE 1 |** Demographic, clinical and metabolite characteristics of the study participants.

Characteristic	ITP (n = 29)	HC (n = 33)	P-value
Sex (male)	13 (45%)	17 (52%)	0.599
Age (year)	$39.50 \pm 15.98$	$39.60 \pm 15.87$	0.989
TB ( $\mu\text{mol/L}$ )	$9.93 \pm 4.17$	$13.11 \pm 4.33$	0.007
DB ( $\mu\text{mol/L}$ )	$4.07 \pm 1.85$	$2.74 \pm 0.90$	0.001
IB ( $\mu\text{mol/L}$ )	$5.86 \pm 2.66$	$10.37 \pm 3.67$	$<0.001$
TP (g/L)	$73.45 \pm 5.18$	$74.43 \pm 3.66$	0.420
ALB (g/L)	$40.78 \pm 4.94$	$45.21 \pm 2.62$	$<0.001$
GLB (g/L)	$32.67 \pm 6.85$	$29.23 \pm 2.48$	0.016
ALT (U/L)	$22.86 \pm 15.24$	$19.52 \pm 10.39$	0.349
AST (U/L)	$21.57 \pm 5.89$	$18.33 \pm 3.28$	0.015
ALP (U/L)	$71.59 \pm 25.94$	$65.48 \pm 18.05$	0.315
GGT (U/L)	$25.18 \pm 16.59$	$21.22 \pm 11.40$	0.309
GLU (mmol/L)	$6.06 \pm 1.71$	$5.42 \pm 0.89$	0.088
Urea (mg/dl)	$5.30 \pm 1.36$	$4.69 \pm 1.33$	0.094
Crea ( $\mu\text{mol/L}$ )	$58.62 \pm 16.92$	$69.93 \pm 15.70$	0.012
UA ( $\mu\text{mol/L}$ )	$287.24 \pm 87.61$	$345.37 \pm 91.31$	0.018
TC (mmol/L)	$4.51 \pm 1.16$	$4.80 \pm 0.92$	0.316
TG (mmol/L)	$1.33 \pm 0.76$	$1.37 \pm 0.60$	0.822
HDL (mmol/L)	$1.11 \pm 0.35$	$1.34 \pm 0.27$	0.011
LDL (mmol/L)	$2.63 \pm 0.77$	$2.72 \pm 0.71$	0.654

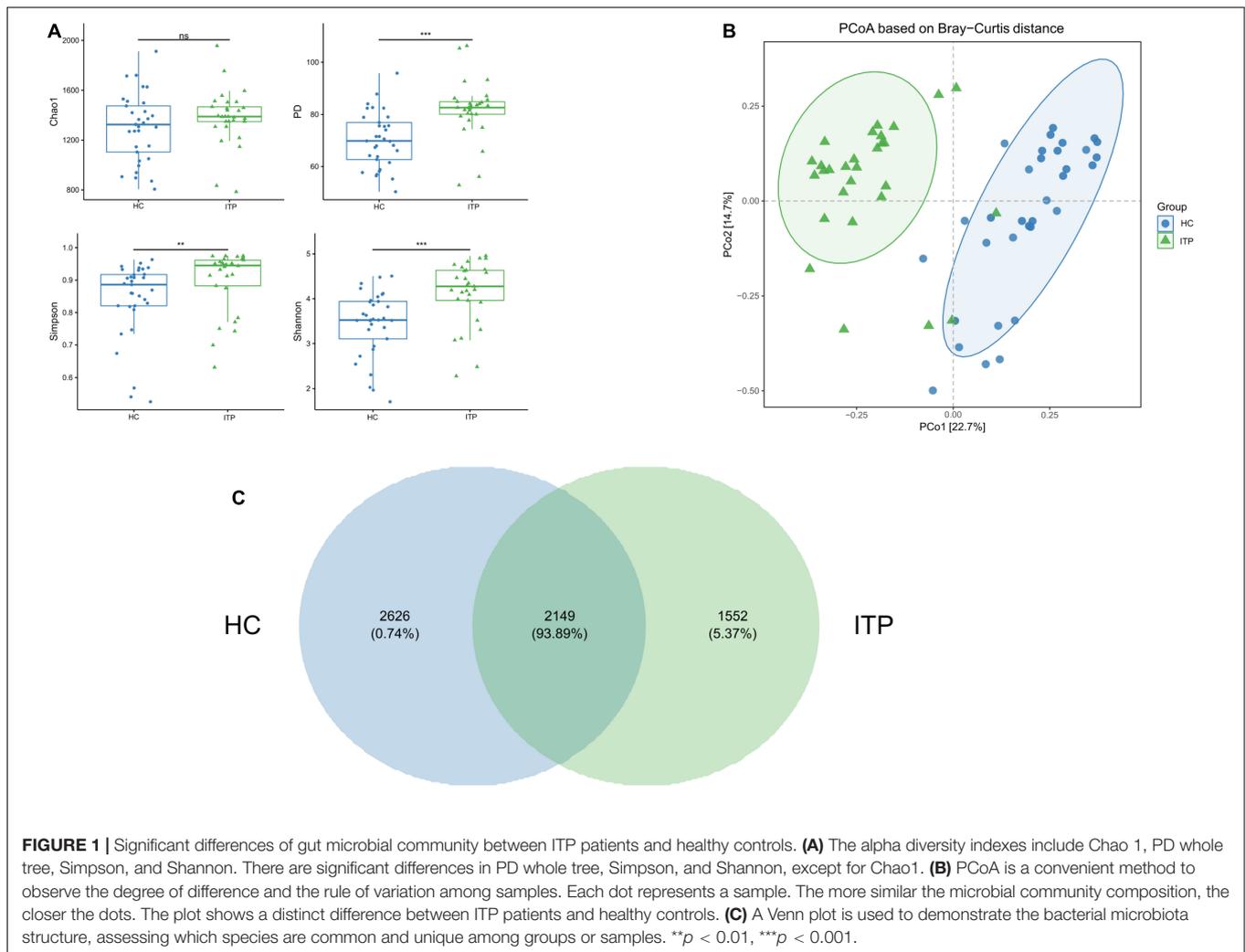
TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin; TP, total protein; ALB, albumin; GLB, globulin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; GLU, glucose; UA, uric acid; TC, total cholesterol; TG, total triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein.

### Structure and Composition of Gut Microbiome in Immune Thrombocytopenia

Alpha and beta diversities were calculated to evaluate the structural differences of gut microbial community between ITP patients and healthy controls. Chao 1 is a measure of total richness and is particularly useful owing to a valid variance, which can be employed to calculate confidence intervals. PD whole tree reflects the sum of all branch-lengths on the constructed phylogenetic tree from all taxa. Simpson considers both species abundance (Richness) and uniformity (Evenness). Shannon reflects species numbers and evenness of species abundance. As shown in Figure 1A, PD whole tree, Simpson, and Shannon had a significant difference between two groups, which represent the richness and diversity of gut microbiome community. The beta diversity in Figure 1B was also showed differences according to the principal coordinates analysis (PCoA). A total of 2,149 OTUs were found to be shared between ITP patients and healthy controls, accounting for a similarity level of more than 93% (Figure 1C).

### Immune Thrombocytopenia-Associated Microbiome Changes

The top 10 ITP-associated bacterium at phylum level were shown in Figure 2A. *Proteobacteria* ( $P = 0.002$ ), *Chloroflexi*



( $P < 0.001$ ) were significantly high in ITP patients, while *Firmicutes* ( $P < 0.001$ ), *Actinobacteria* ( $P < 0.001$ ) were lower compared to healthy controls. Correspondingly, the *Firmicutes/Bacteroidetes* ratio was decreased. Next, we explored ITP-associated microbiome changes at genus level. As shown in **Figure 2B**, *Bacteroides* ( $P = 0.020$ ), *Phascolarctobacterium* ( $P = 0.008$ ), and *Lactobacillus* ( $P < 0.001$ ) were significant high in ITP patients. While the *Ruminococcaceae* UCG-002 ( $P < 0.001$ ), *Eubacterium coprostanoligeus* ( $P = 0.011$ ), *Megamonas* ( $P < 0.001$ ), and *Lachnospiraceae* NC2004 ( $P = 0.002$ ) were significantly decreased.

### Significant Differences in Gut Microbial Taxa Between Immune Thrombocytopenia Patients and Healthy Controls

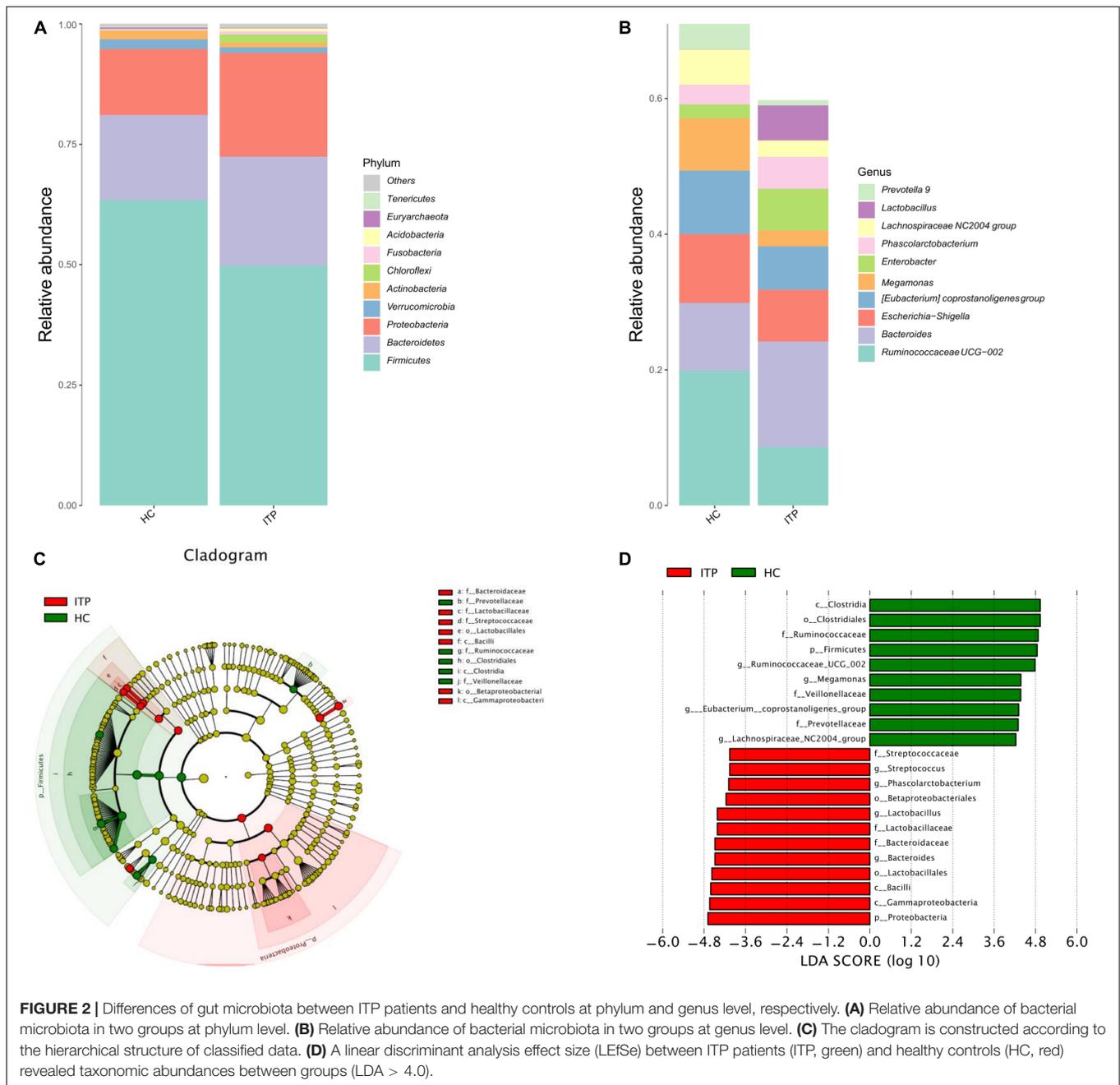
The cladogram was used for discovering the high-dimensional biomarkers that substantially contributed to the differences between groups. The main taxonomic groups of ITP patients and healthy controls were highlighted, respectively. Accordingly,

*Lactobacillaceae* (family) and *Streptococcaceae* (family) belong to *Lactobacillales* (order), which belong to *Bacilli* (class) is highlighted (**Figure 2C**).

Next, we compared taxonomic abundances between ITP patients and healthy controls by employing linear discriminant analysis effect size (LEfSe), which was used to quantitatively analyze the important species that significantly differed among groups. The linear discriminant analysis (LDA) score correlates taxa abundance which have the potential to be a biomarker. We listed those taxa which were meeting a significant LDA threshold more than 4 (LDA score  $> 4.0$ ,  $P < 0.05$ ). In this study, LEfSe analysis revealed more abundant *Proteobacteria* in ITP patients at phylum level, and more abundant *Bacteroides*, *Lactobacillus*, *Phascolarctobacterium*, and *Streptococcus* at genus level (**Figure 2D**).

### Differential Metabolite Expression

Next, we explored the alteration of metabolites from gut microbiota in ITP. Specific diversity of metabolites was found between ITP patients and healthy controls. The metabolite expression levels were identified by volcano plot. As shown



in **Figure 3A**, totally 423 metabolites which had a fold change more than 2.0 ( $P < 0.05$ ) were identified from 4,786 metabolites. Among them, 337 were upregulated and 86 were downregulated (**Figure 3B**).

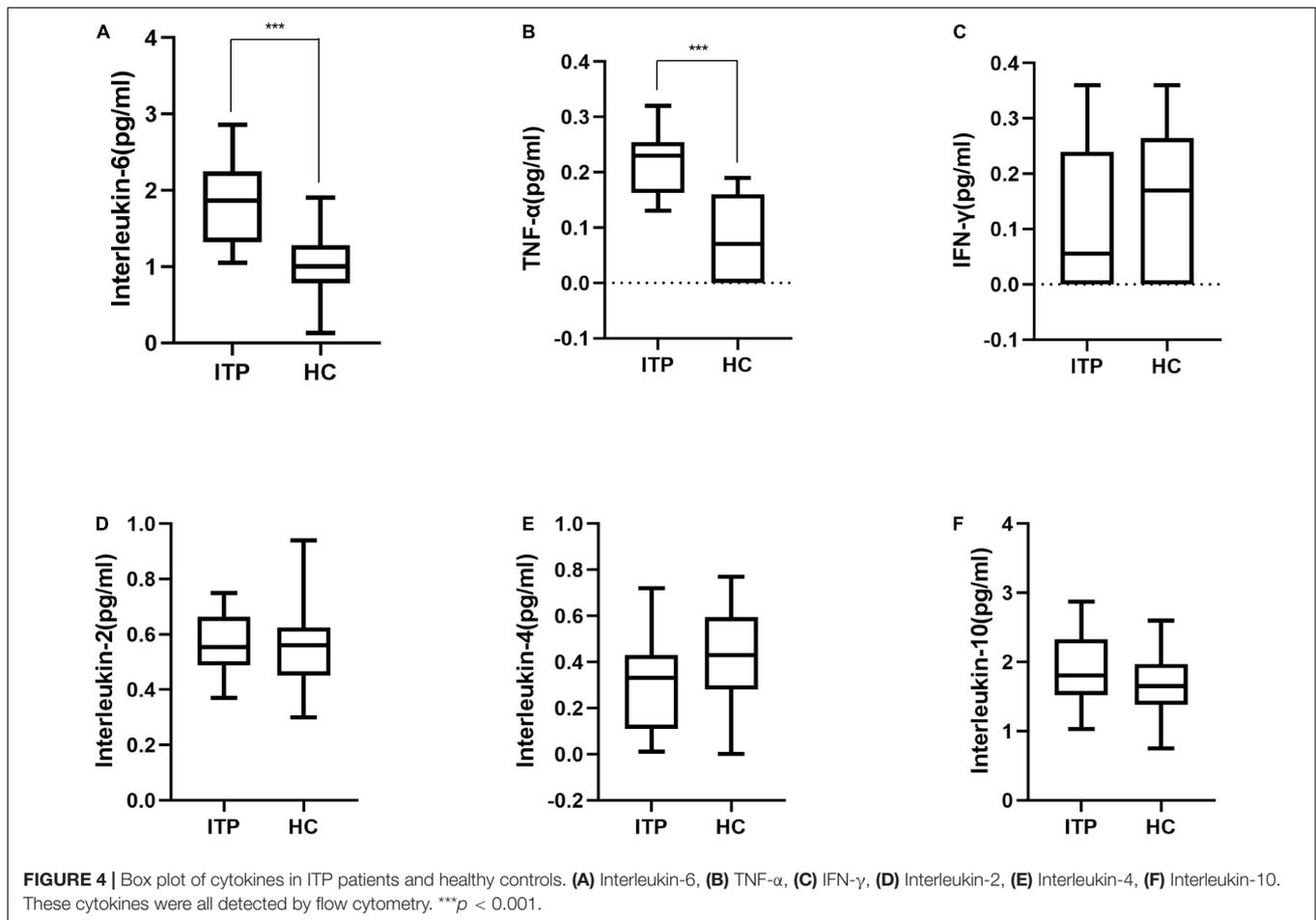
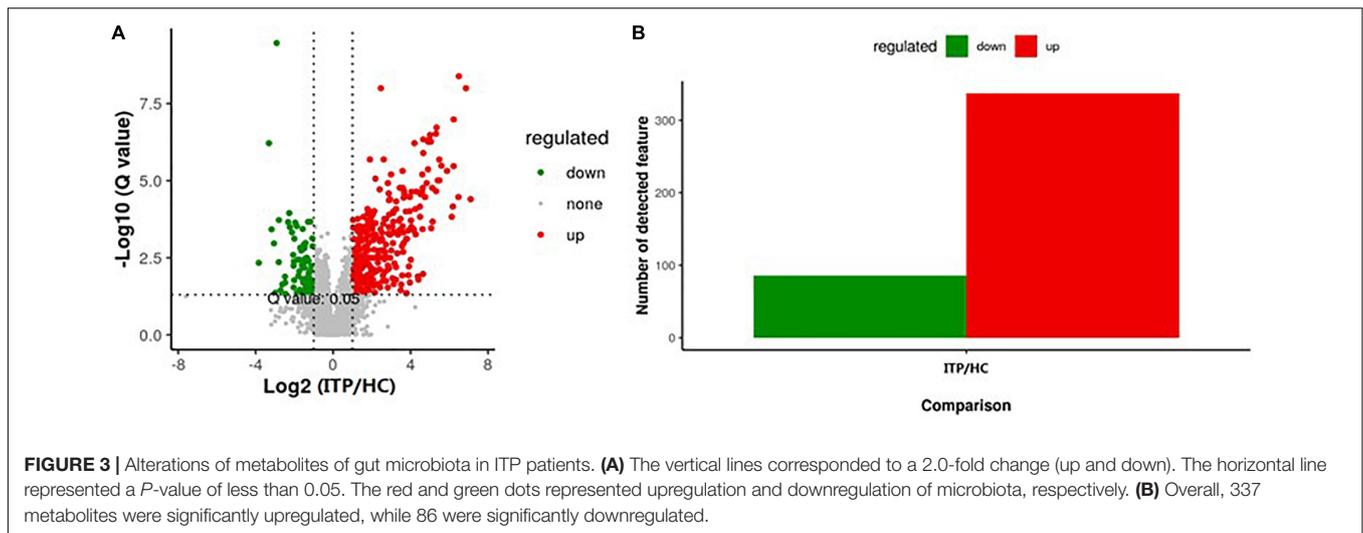
### Changes in Serum Cytokine Levels

Furthermore, serum IL-2, IL-6, IL-4, IL-10, TNF- $\alpha$ , and IFN- $\gamma$  were detected to reflect the immune response of ITP patients which were shown in **Figure 4**. IL-6 and TNF- $\alpha$  were significant higher in ITP patients compared to the healthy controls ( $P < 0.001$ ,  $P < 0.001$ , respectively). While IL-2, IL-4, IL-10, and IFN- $\gamma$  had no significant difference between two

groups. However, a trend of down regulation of IL-4 and IFN- $\gamma$  were observed in ITP patients, even they failed to reach a statistical significance.

### Potential of Metabolic Parameters as a Possible Indicator of Immune Thrombocytopenia

We then investigated whether the metabolic pathway could help predict the clinical prognosis of ITP. The PCA plot showed metabolites of ITP patients are different from healthy controls. Univariate analysis, fold change



(Ratio  $\geq 2$  or  $\leq 1/2$ ), *t*-test (*P* value < 0.05), and Variable Important for the Projection (VIP  $\geq 1$ ) were obtained by multivariate analysis, which were employed to filter the representative metabolic parameters. Compared with healthy controls, fatty acyls compounds including 5-HETE,

RvD<sub>2</sub>, and 6t,12e-LTB<sub>4</sub> were significantly upregulated. While stachydrine, dowicide and dodecanoylcarnitine were significantly downregulated. Hence, we speculated that lipid metabolism dysfunction might be occurred in the pathogenesis of ITP (Table 2).



## Correlation Between Differential Metabolites and Gut Microbiota

In present study, interactions between gut microbiota and metabolites were investigated through metabolic and 16S sequencing analyses. Spearman correlation was performed to filter differential metabolites and gut microbiota. The Spearman's rank correlation coefficient was showed as heatmaps of **Figure 5**. It was showed that RvD<sub>2</sub>, 5-HETE, 6t,12e-LTB<sub>4</sub>, stachydrine, dolicide A and dodecanoylcarnitine were highly correlated with order *Bacteroidetes* VC2.1 Bac22 ( $r = 0.91, P < 0.05$ ), genus *Azospirillum* ( $r = 0.91, P < 0.05$ ), genus *Cupriavidus* ( $r = 0.90, P < 0.05$ ), family *Planococcaceae* ( $r = 0.88, P < 0.05$ ), class *MVP-15* ( $r = 0.90, P < 0.05$ ), order *WCHB1-41* ( $r = 0.85, P < 0.05$ ), respectively (**Table 3**).

## DISCUSSION

Herein, we investigated the relationship among the gut microbiome, serum metabolism, cytokines in both the healthy people ( $n = 33$ ) and ITP patients ( $n = 29$ ), an autoimmune disorder. In addition to genetic and environmental triggers, accumulating evidence has suggested gut microbiota as another important causative element influencing the development of autoimmunity (27) as well as hematologic diseases (28). For example, there was evidence showing that gut bacterium contribute to type 1 diabetes development probably through translocation into pancreatic lymph nodes (28). For another example, inflammatory bowel disease with intestinal inflammation was reported to result in thrombocytopenia (29) and treatment led to recovery of the platelet number (30) indicating the link between hematology and gut microbiome. Indeed, Malnick et al. reported a case, in which the patient underwent fecal microbial transplantation twice from the same donor (31). Each time the patient developed transient ITP, with the platelet level decreasing and antiplatelet antibodies increasing rapidly after transplantation. These results, especially the recurrence of ITP after the second transplantation, strongly indicated that the gut microbiota is associated with the pathogenesis of ITP.

However, the causality has not yet been established, with some arguments that the change of gut microbiome is only an outcome of disease progression rather than an initiator or exacerbator of disease.

Our results revealed that gut microbiota (diversity, richness, evenness, and PCA plot) and metabolites of fatty acids in ITP patients significantly differed from those in normal controls. Specifically, *Bacteroides*, *Phascolarctobacterium* and *Lactobacillus* were increased at the genus level, while *Ruminococcaceae* UCG-002, *Eubacterium coprostanoligeus*, *Megamonas* and *Lachnospiraceae* NC2004 were decreased. At the phylum level, the relative abundance of *Proteobacteria* and *Chloroflexi* was increased in ITP patients, while *Firmicutes*, *Actinobacteria* and the *Firmicutes/Bacteroidetes* ratio were decreased. It was reported that *Firmicutes* (32) and *Firmicutes/Bacteroidetes* ratio (33) were associated with Th17-Tregs trans-differentiation, which is regulated by IL-6 (34). Interestingly, we found that IL-6 levels were significantly elevated in ITP patients when compared with healthy controls, which is consistent to the other reports showing that IL-6 upregulation is related to autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and asthma (35). TNF- $\alpha$  is another cytokine that correlates with Treg frequencies in chronic ITP (36) and its secretion is stimulated by *Bacteroides* (37). It was evident that fecal microbiota transplantation reduced TNF- $\alpha$  signaling (38). Our results also showed that the *Bacteroides* composition and TNF- $\alpha$  level were elevated in ITP patients. For the fatty metabolites, those that were upregulated in ITP patients included RvD<sub>2</sub>, eicosatetraenoic acids, Monolinolein, and phosphatidylcholine, all of which can be regulated by gut microbiota (39). Interestingly, RvD<sub>2</sub> was recently shown to reduce platelet activation and preserve platelet function (40). Eicosatetraenoic acid, also named arachidonic acid, is considered to regulate platelet homeostasis (41). In addition, eicosatetraenoic acids (41), Monolinolein (42), phosphatidylcholine (43), are all proposed to be the key compounds contributing to platelet activation. Nevertheless, at this moment, our understanding is still at its relative infancy regarding the exact roles of gut microbiota and fatty metabolism in the development of ITP. Apparently, this requires additional and longer-term studies in the future.

**TABLE 3** | The correlation of the differential metabolites and differential gut microbiota.

Metabolite	Microflora	Rho-value	P-value
Resolvin D2	k__Bacteria  p__Bacteroidetes  c__Bacteroidia  o__Bacteroidetes VC2.1 Bac22  f__  g__	0.91	< 0.05
5S-Hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid	k__Bacteria  p__Proteobacteria  c__Alphaproteobacteria  o__Azospirillales  f__Azospirillaceae  g__Azospirillum	0.91	< 0.05
6-trans-12-epi-Leukotriene B4	k__Bacteria  p__Proteobacteria  c__Gammaproteobacteria  o__Betaproteobacteriales  f__Burkholderiaceae  g__Cupriavidus	0.90	< 0.05
1-Methylene-5.alpha.-androstan-3.alpha.-ol-17-one	k__Bacteria  p__Proteobacteria  c__Alphaproteobacteria  o__Azospirillales  f__Azospirillaceae  g__Azospirillum	0.89	< 0.05
Cinnassiol E	k__Bacteria  p__Bacteroidetes  c__Bacteroidia  o__Bacteroidetes VC2.1 Bac22  f__  g__	0.91	< 0.05
Octanoylcarnitine	k__Bacteria  p__Kiritimatiellaeota  c__Kiritimatiellae  o__WCHB1-41  f__  g__	0.77	< 0.05
Decanoyl-L-carnitine	k__Bacteria  p__Proteobacteria  c__Gammaproteobacteria  o__Aeromonadales  f__Succinivibrionaceae  g__Succinivibrionaceae UCG-002	0.83	< 0.05
Dodecanoylcarnitine	k__Bacteria  p__Kiritimatiellaeota  c__Kiritimatiellae  o__WCHB1-41  f__  g__	0.85	< 0.05
Dolicide A	k__Bacteria  p__Spirochaetes  c__MVP-15  o__  f__  g__	0.90	< 0.05
Stachydrine	k__Bacteria  p__Firmicutes  c__Bacilli  o__Bacillales  f__Planococcaceae  g__	0.88	< 0.05

**TABLE 4** | Roles of gut microbiota on toxicity of TCMs.

Gut microbiota	Specific mechanism	Influence on toxicity	Possibly affected TCM formulae/herbs	References
<i>Bacteroidetes</i>	Metabolizes geniposide to genipin with stronger hepatic toxic effect.	Increase	Qing-Kai-Ling Injection, Huang-Lian-Jiee-Dwu Tang, In-Chern-Hau Tang, Gardenia jasminoides Elll, Eucommia ulmoides Oliv., Rehmannia glutinosa Libosch., and Achyranthes bidentata Bl	(45)
	Metabolizes phorbol to isophorbol that induces ventricular fibrillation.	Increase	Croton tiglium, and thymelaeaceae	(46, 47)
	Metabolizes ginsenoside Rg3 to protopanaxadiol that exhibited the most potent cytotoxicity.	Increase	Panax ginseng	(48)
	Metabolizes rhein to rheinanthrone that is far more pronounced in laxative effect.	Increase	Yin-Chen-Hao Tang, Da-Cheng-Qi Tang, Rheum palmatum, and Duhaldea nervosa	(49)
	Metabolizes shikonin to its derivatives that are thought to have strong cytotoxicity	Increase	Lithospermum erythrorhizon, Arnebia euchroma, and Arnebia guttata	(50)
<i>Lactobacillus</i>	Transforms ginsenoside Rc into ginsenosides 20(S)-Rg <sub>2</sub> and Rd, induces nephrotoxicity	Increase	Du-Shen-Tang	(51, 52)
	Ketonizes ginsenoside F1 and CK to new ketonized compounds with more potent inhibitory effects against mushroom tyrosinase	Increase	Panax ginseng	(53)
	Intervenes the tryptophan metabolism and causes diarrhea		Folium sennae	(54)
<i>Ruminococcus</i>	Enhances production of butyrate and G-protein-coupled receptors (GPRs) to support indigo naturalis to protect gut barrier	Decrease	Realgar-Indigo Naturalis	(55)
	Produces short chain fatty acids to assist schisandra chinensis to improve the gut micro-ecology	Decrease	Shegan-Mahuang Tang, Shengmai San, Fuzheng Huayu formula, Jian-Gan-Bao, CKBM, Shuang-Di-Shou-Zhen Tablets, Yangfei Huoxue Decoction, YiQiFuMai lyophilized injection powder, Wei Kang Su, Bakumijijogan	(56)
	Plays a crucial role in host energy utilization to promote Sophora flavescens ETOAc extract to rectify the abnormal energy metabolism	Decrease	Sophora flavescens, Qu-Yu-Jie-Du Decoction, San Wu Huangqin Decoction, ASHMI	(57)
	Increases short chain fatty acid level to support Gegen Qinlian Decoction to repair intestinal mucosa, ameliorates permeability, and alleviates diarrhea	Decrease	Gegen Qinlian Decoction	(58)
<i>Eubacterium</i>	Utilize lysine and arginine to produce acetate and butyrate to help Scutellariae radix and Coptidis rhizome to ameliorate the glycolipid metabolism	Decrease	Scutellariae radix, Coptidis rhizome, Huanglian Jiedu Decoction, Gegen Qinlian Decoction, San-Huang-Xie-Xin-Tang, Sanhuang-Siwu-Tang	(59)
	Produces butyrate to assist Xiexin tang to enhance epithelial barrier function, improve gut permeability, inhibit the inflammation and then exert systemically hypoglycemic activities	Decrease	Xiexin Tang	(60)

In summary, we attempt to obtain a comprehensive insight of altered gut microbiota and fatty metabolism in ITP patients. Our study may provide information for the treatment of ITP. Indeed, ITP is considered as purpura disease and differentiated as spleen deficiency in the TCMs. Nowadays in China, Many TCM drugs are routinely used to treat ITP *via* tonifying the spleen and qi, which may reduce the frequency and severity of bleeding (44) (Table 4). Meanwhile, TCM treatments were proven to be associated with changes in gut microbiota (61), and serum metabolic (62). Our data along with other literatures suggested that changes of gut microbiota in ITP patients may increase the toxicity of specific TCM drugs. For example, *Bacteroides* metabolizes geniposide to genipin with stronger hepatic toxic effect (45) and thus, geniposide-containing TCM formulations should be cautiously used to treat ITP. Further studies are needed with larger number of patients and animal

experiments to establish the causality among gut microbiota, fatty metabolism and ITP.

## CONCLUSION

Over the years, several researchers have explored the relationship between gut microbiota, metabolites, traditional Chinese medicine and autoimmune diseases. Advances in sequencing technology have made it possible to further explore the role of gut microbiota in autoimmune diseases. Our research revealed that gut microbiota and metabolites are associated with the pathogenesis of ITP and the gut microbiota might influence the metabolism of TCMs in ITP patients. Further experiments are required to determine the mechanisms of microbial species, the pathway of metabolome, the interaction between the gut microbiota and TCMs, and the pathogenesis of ITP.

## DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in NCBI Sequence Read Archive, accession number PRJNA816310.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee in Clinical Research (ECCR) of the First Affiliated Hospital of Wenzhou Medical University. The patients/participants provided their written informed consent to participate in this study.

## REFERENCES

- Yu X, Menard M, Prechl J, Bhakta V, Sheffield WP, Lazarus AH. Monovalent Fc receptor blockade by an anti-Fcγ receptor/albumin fusion protein ameliorates murine ITP with abrogated toxicity. *Blood*. (2016) 127:132–8. doi: 10.1182/blood-2015-08-664656
- Chow L, Aslam R, Speck ER, Kim M, Cridland N, Webster ML, et al. A murine model of severe immune thrombocytopenia is induced by antibody- and CD8+ T cell-mediated responses that are differentially sensitive to therapy. *Blood*. (2010) 115:1247–53. doi: 10.1182/blood-2009-09-244772
- Swinkels M, Rijkers M, Voorberg J, Vidarsson G, Leebeek FWG, Jansen AJG. Emerging concepts in immune thrombocytopenia. *Front Immunol*. (2018) 9:880. doi: 10.3389/fimmu.2018.00880
- Sun S, Urbanus RT, Ten Cate H, de Groot PG, de Laat B, Heemskerk JWM, et al. Platelet activation mechanisms and consequences of immune thrombocytopenia. *Cells*. (2021) 10:3386. doi: 10.3390/cells10123386
- Bonnard G, Babuty A, Collot R, Costes D, Drillaud N, Evellard M, et al. Platelet features allow to differentiate immune thrombocytopenia from inherited thrombocytopenia. *Ann Hematol*. (2021) 100:2677–82. doi: 10.1007/s00277-021-04651-4
- Lozano ML, Garabet L, Fernandez-Perez MP, De Los Reyes-García AM, Diaz-Lozano P, Garcia-Barbera N, et al. Platelet activation and neutrophil extracellular trap (NET) formation in immune thrombocytopenia: is there an association?. *Platelets*. (2020) 31:906–12. doi: 10.1080/09537104.2019.1696456
- Li J, van der Wal DE, Zhu G, Xu M, Yougbare I, Ma L, et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. *Nat Commun*. (2015) 6:7737. doi: 10.1038/ncomms8737
- Rodeghiero F. Is ITP a thrombophilic disorder?. *Am J Hematol*. (2016) 91:39–45. doi: 10.1002/ajh.24234
- Greinacher A, Selleng K, Palankar R, Wesche J, Handtke S, Wolff M, et al. Insights in ChAdOx1 nCoV-19 vaccine-induced immune thrombotic thrombocytopenia. *Blood*. (2021) 138:2256–68. doi: 10.1182/blood.2021013231
- Ostrowski SR, Søgaard OS, Tolstrup M, Stærke NB, Lundgren J, Østergaard L, et al. Inflammation and platelet activation after COVID-19 vaccines - possible mechanisms behind vaccine-induced immune thrombocytopenia and thrombosis. *Front Immunol*. (2021) 12:779453. doi: 10.3389/fimmu.2021.779453
- Uzun G, Pelzl L, Singh A, Bakchoul T. Immune-mediated platelet activation in COVID-19 and vaccine-induced immune thrombotic thrombocytopenia. *Front Immunol*. (2022) 13:837629. doi: 10.3389/fimmu.2022.837629
- Duttaroy AK. Role of gut microbiota and their metabolites on atherosclerosis, hypertension and human blood platelet function: a review. *Nutrients*. (2021) 13:144. doi: 10.3390/nu13010144
- Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell*. (2016) 165:111–24. doi: 10.1016/j.cell.2016.02.011
- Nemet I, Saha PP, Gupta N, Zhu W, Romano KA, Skye SM, et al. A cardiovascular disease-linked gut microbial metabolite acts via adrenergic receptors. *Cell*. (2020) 180:862–77.e22. doi: 10.1016/j.cell.2020.02.016

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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- Vadaq N, Schirmer M, Tunjungputri RN, Vlamakis H, Chiriac C, Ardiansyah E, et al. Untargeted plasma metabolomics and gut microbiome profiling provide novel insights into the regulation of platelet reactivity in healthy individuals. *Thromb Haemost*. (2021). [Epub ahead of print]. doi: 10.1055/a-1541-3706
- Zhang X, Zhang X, Tong F, Cai Y, Zhang Y, Song H, et al. Gut microbiota induces high platelet response in patients with ST segment elevation myocardial infarction after ticagrelor treatment. *Elife*. (2022) 11:e70240. doi: 10.7554/eLife.70240
- Kazama I, Baba A, Endo Y, Toyama H, Ejima Y, Matsubara M, et al. Salicylate inhibits thrombopoiesis in rat megakaryocytes by changing the membrane micro-architecture. *Cell Physiol Biochem*. (2015) 35:2371–82. doi: 10.1159/000374039
- Peng B, Geue S, Coman C, Münzer P, Kopczynski D, Has C, et al. Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome. *Blood*. (2018) 132:e1–12. doi: 10.1182/blood-2017-12-822890
- Ramström S. Arachidonic acid causes lysis of blood cells and ADP-dependent platelet activation responses in platelet function tests. *Platelets*. (2019) 30:1001–7. doi: 10.1080/09537104.2018.1557614
- Paes AMA, Gaspar RS, Fuentes E, Wehinger S, Palomo I, Trostchansky A. Lipid metabolism and signaling in platelet function. *Adv Exp Med Biol*. (2019) 1127:97–115. doi: 10.1007/978-3-030-11488-6\_7
- Midgett C, Stitham J, Martin K, Hwa J. Prostacyclin receptor regulation—from transcription to trafficking. *Curr Mol Med*. (2011) 11:517–28. doi: 10.2174/156652411800615144
- Stitham J, Midgett C, Martin KA, Hwa J. Prostacyclin: an inflammatory paradox. *Front Pharmacol*. (2011) 2:24. doi: 10.3389/fphar.2011.00024
- Van Doren L, Nguyen N, Garzia C, Fletcher EK, Stevenson R, Jaramillo D, et al. Lipid receptor GPR31 (G-Protein-Coupled Receptor 31) regulates platelet reactivity and thrombosis without affecting hemostasis. *Arterioscler Thromb Vasc Biol*. (2021) 41:e33–45. doi: 10.1161/ATVBAHA.120.315154
- Valiyaveetil M, Kar N, Ashraf MZ, Byzova TV, Febbraio M, Podrez EA. Oxidized high-density lipoprotein inhibits platelet activation and aggregation via scavenger receptor BI. *Blood*. (2008) 111:1962–71. doi: 10.1182/blood-2007-08-107813
- Rodeghiero F, Ruggeri M. ITP and international guidelines: what do we know, what do we need?. *Presse Med*. (2014) 43:e61–7. doi: 10.1016/j.lpm.2014.02.004
- De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazanetti DI, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One*. (2013) 8:e76993. doi: 10.1371/journal.pone.0076993
- Mu Q, Kirby J, Reilly CM, Luo XM. Leaky gut as a danger signal for autoimmune diseases. *Front Immunol*. (2017) 8:598. doi: 10.3389/fimmu.2017.00598
- Manzo VE, Bhatt AS. The human microbiome in hematopoiesis and hematologic disorders. *Blood*. (2015) 126:311–8. doi: 10.1182/blood-2015-04-574392
- Millstead J, Kamat A, Duffner U, Abdel-Mageed A, Freswick P, Dickens D, et al. WD repeat domain 1 (WDR1) deficiency presenting as a cause of infantile

- inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* (2020) 71:e113–7. doi: 10.1097/MPG.0000000000002826
30. Uzzan M, Galicier L, Gornet JM, Oksenhendler E, Fieschi C, Allez M, et al. Autoimmune cytopenias associated with inflammatory bowel diseases: insights from a multicenter retrospective cohort. *Dig Liver Dis.* (2017) 49:397–404. doi: 10.1016/j.dld.2016.12.006
  31. Malnick SD, Oppenheim A, Melzer E. Immune thrombocytopenia caused by fecal microbial transplantation in a patient with severe recurrent Clostridium difficile infection. *J Clin Gastroenterol.* (2015) 49:888–9. doi: 10.1097/MCG.0000000000000404
  32. Johnson BM, Gaudreau MC, Al-Gadban MM, Gudi R, Vasu C. Impact of dietary deprivation on disease progression and gut microbiome composition in lupus-prone SNF1 mice. *Clin Exp Immunol.* (2015) 181:323–37. doi: 10.1111/cei.12609
  33. Ruiz L, López P, Suárez A, Sánchez B, Margolles A. The role of gut microbiota in lupus: what we know in 2018?. *Expert Rev Clin Immunol.* (2018) 14:787–92. doi: 10.1080/1744666X.2018.1519395
  34. Zheng X, Wang D. The adenosine A2A receptor agonist accelerates bone healing and adjusts Treg/Th17 cell balance through interleukin 6. *Biomed Res Int.* (2020) 2020:2603873. doi: 10.1155/2020/2603873
  35. Zhu XM, Shi YZ, Cheng M, Wang DF, Fan JF. Serum IL-6, IL-23 profile and Treg/Th17 peripheral cell populations in pediatric patients with inflammatory bowel disease. *Pharmazie.* (2017) 72:283–7. doi: 10.1691/ph.2017.6957
  36. Zhong H, Bussel J, Yazdanbakhsh K. *In vitro* TNF blockade enhances ex vivo expansion of regulatory T cells in patients with immune thrombocytopenia. *Br J Haematol.* (2015) 168:274–83. doi: 10.1111/bjh.13126
  37. Lin CH, Chen CC, Chiang HL, Liou JM, Chang CM, Lu TP, et al. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J Neuroinflammation.* (2019) 16:129. doi: 10.1186/s12974-019-1528-y
  38. Sun MF, Zhu YL, Zhou ZL, Jia XB, Xu YD, Yang Q, et al. Neuroprotective effects of fecal microbiota transplantation on MPTP-induced Parkinson's disease mice: gut microbiota, glial reaction and TLR4/TNF- $\alpha$  signaling pathway. *Brain Behav Immun.* (2019) 70:48–60. doi: 10.1016/j.bbi.2018.02.005
  39. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. *Science.* (2012) 336:1262–7. doi: 10.1126/science.1223813
  40. Reddoch-Cardenas KM, Sharma U, Salgado CL, Cantu C, Darlington DN, Pidcoke HF, et al. Use of specialized pro-resolving mediators to alleviate cold platelet storage lesion. *Transfusion.* (2020) 60:S112–8. doi: 10.1111/trf.15750
  41. Djuricic I, Calder PC. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: an update for 2021. *Nutrients.* (2021) 13:2421. doi: 10.3390/nu13072421
  42. Trostchansky A, Moore-Carrasco R, Fuentes E. Oxidative pathways of arachidonic acid as targets for regulation of platelet activation. *Prostaglandins Other Lipid Mediat.* (2019) 145:106382. doi: 10.1016/j.prostaglandins.2019.106382
  43. Mao G, Songdej N, Voora D, Goldfinger LE, Del Carpio-Cano FE, Myers RA, et al. Transcription Factor RUNX1 regulates platelet PCTP (Phosphatidylcholine Transfer Protein): implications for cardiovascular events: differential effects of RUNX1 variants. *Circulation.* (2017) 136:927–39. doi: 10.1161/CIRCULATIONAHA.116.023711
  44. Zhang Y, Wang J, Gao J, Guo M, Chen H, Liu J, et al. Jianpi Yiqi Shexue ameliorates immune thrombocytopenia related fatigue by regulating mitochondrial function. *Ann Palliat Med.* (2021) 10:156–68. doi: 10.21037/apm-20-2153
  45. Kang MJ, Khanal T, Kim HG, Lee DH, Yeo HK, Lee YS, et al. Role of metabolism by human intestinal microflora in geniposide-induced toxicity in HepG2 cells. *Arch Pharm Res.* (2012) 35:733–8. doi: 10.1007/s12272-012-0418-y
  46. Abdel-Hafez AA, Nakamura N, Hattori M. Biotransformation of phorbol by human intestinal bacteria. *Chem Pharm Bull.* (2002) 50:160–4. doi: 10.1248/cpb.50.160
  47. Mills SW, Montgomery SH, Morck DW. Evaluation of the effects of short-chain fatty acids and extracellular pH on bovine neutrophil function *in vitro*. *Am J Vet Res.* (2006) 67:1901–7. doi: 10.2460/ajvr.67.11.1901
  48. Bae EA, Shin JE, Kim DH. Metabolism of ginsenoside Re by human intestinal microflora and its estrogenic effect. *Biol Pharm Bull.* (2005) 28:1903–8. doi: 10.1248/bpb.28.1903
  49. Cyong J, Matsumoto T, Arakawa K, Kiyohara H, Yamada H, Otsuka Y. Anti-Bacteroides fragilis substance from rhubarb. *J Ethnopharmacol.* (1987) 19:279–83. doi: 10.1016/0378-8741(87)90005-5
  50. Zhao Q, Kretschmer N, Bauer R, Efferth T. Shikonin and its derivatives inhibit the epidermal growth factor receptor signaling and synergistically kill glioblastoma cells in combination with erlotinib. *Int J Cancer.* (2015) 137:1446–56. doi: 10.1002/ijc.29483
  51. Zhou SS, Xu J, Zhu H, Wu J, Xu JD, Yan R, et al. Gut microbiota-involved mechanisms in enhancing systemic exposure of ginsenosides by coexisting polysaccharides in ginseng decoction. *Sci Rep.* (2016) 6:22474. doi: 10.1038/srep22474
  52. Gao Y, Wang G, Wang T, Li G, Lin J, Sun L, et al. A 26-week 20(S)-ginsenoside Rg3 oral toxicity study in Beagle dogs. *Regul Toxicol Pharmacol.* (2020) 110:104522. doi: 10.1016/j.yrtph.2019.104522
  53. Jin Y, Jung SY, Kim YJ, Lee DY, Min JW, Wang C, et al. Microbial ketonization of ginsenosides F1 and C-K by *Lactobacillus brevis*. *Antonie Van Leeuwenhoek.* (2014) 106:1215–21. doi: 10.1007/s10482-014-0291-4
  54. Zhang C, Shao H, Li D, Xiao N, Tan Z. Role of tryptophan-metabolizing microbiota in mice diarrhea caused by Folium sennae extracts. *BMC Microbiol.* (2020) 20:185. doi: 10.1186/s12866-020-01864-x
  55. Sun Z, Li J, Dai Y, Wang W, Shi R, Wang Z, et al. Indigo naturalis alleviates dextran sulfate sodium-induced colitis in rats via altering gut microbiota. *Front Microbiol.* (2020) 11:731. doi: 10.3389/fmicb.2020.00731
  56. Qi Y, Chen L, Gao K, Shao Z, Huo X, Hua M, et al. Effects of *Schisandra chinensis* polysaccharides on rats with antibiotic-associated diarrhea. *Int J Biol Macromol.* (2019) 124:627–34. doi: 10.1016/j.ijbiomac.2018.11.250
  57. Shao J, Liu Y, Wang H, Luo Y, Chen L. An integrated fecal microbiome and metabolomics in T2DM rats reveal antidiabetes effects from host-microbial metabolic axis of EtOAc extract from *Sophora flavescens*. *Oxid Med Cell Longev.* (2020) 2020:1805418. doi: 10.1155/2020/1805418
  58. Liu CS, Liang X, Wei XH, Jin Z, Chen FL, Tang QF, et al. Gegen Qinlian decoction treats diarrhea in piglets by modulating gut microbiota and short-chain fatty acids. *Front Microbiol.* (2019) 10:825. doi: 10.3389/fmicb.2019.00825
  59. Xiao S, Liu C, Chen M, Zou J, Zhang Z, Cui X, et al. Scutellariae radix and coptidis rhizoma ameliorate glycolipid metabolism of type 2 diabetic rats by modulating gut microbiota and its metabolites. *Appl Microbiol Biotechnol.* (2020) 104:303–17. doi: 10.1007/s00253-019-10174-w
  60. Wei X, Tao J, Xiao S, Jiang S, Shang E, Zhu Z, et al. Xiexin Tang improves the symptom of type 2 diabetic rats by modulation of the gut microbiota. *Sci Rep.* (2018) 8:3685. doi: 10.1038/s41598-018-22094-2
  61. Zhu LQ, Zhang L, Zhang J, Chang GL, Liu G, Yu DD, et al. Evodiamine inhibits high-fat diet-induced colitis-associated cancer in mice through regulating the gut microbiota. *J Integr Med.* (2021) 19:56–5. doi: 10.1016/j.joim.2020.11.001
  62. Tang SQ, Wang YL, Xie ZY, Zhang Y, Guo Y, Gao KL, et al. Serum metabolic profiling of traditional Chinese medicine syndromes in patients with diarrhea-predominant irritable bowel syndrome. *J Integr Med.* (2021) 19:274–81. doi: 10.1016/j.joim.2021.03.002

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