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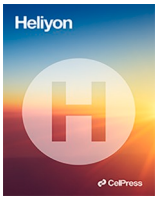

Research article

Differential intestinal microbes and metabolites between Behcet's uveitis and Fuchs syndrome

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ABSTRACT

Objective: Behcet's uveitis (BU) is a type of uveitis with a high rate of blindness, characterized by anterior segment inflammation, vitreous opacity, and retinal vasculitis. Its pathogenesis is still unclear. Fuchs syndrome (Fuchs) is another common type of uveitis, which clinically presents with anterior segment inflammation and vitreous opacity, but rarely causes blindness. This study aims to compare the gut microbiota and metabolites of two different types of uveitis to clarify whether the differences in clinical manifestations are relevant to the alterations in gut microbiota.
Methods: Faecal samples were collected from new-onset BU (n = 11) patients without systemic treatment and other diseases. 16S rRNA and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were performed to analyze gut microbes and metabolites. Fuchs (n = 15) was used as the disease control, and healthy controls (n = 18) without autoimmune diseases and systemic medication were included.
Results: Microbial composition and metabolite profiles differed significantly among the three groups. Compared to controls, Fusicatenibacter and eight metabolites were specifically altered in BU patients, and Pantoea and five metabolites in Fuchs. Pathways involving delta-tocopherol, palmitic acid, and serotonin are significantly disrupted in BU patients. Pathways involving linoleic acid are dysregulated considerably in Fuchs. Microbial markers consisting of 4 genera and 7 metabolites can respectively distinguish BU patients from controls. AUC values of metabolite markers were greater than those of microbial markers. Furthermore, serum zonulin levels were significantly elevated in both types of uveitis, with no difference between them. Correlation analysis revealed correlations between zonulin levels and multiple microbes.
Conclusions: Patients with BU and Fuchs syndrome showed significant differences in gut microbiota and metabolites. Disruption of the intestinal mucosal barrier was observed in both types of uveitis. However, the mechanism of different intestinal microbiota causing different clinical manifestations needs to be studied in the future.

1. Introduction

Behcet's uveitis (BU) is an immune-mediated inflammatory vasculitis marked by recurrent panuveitis, oral and genital ulcers, and multisystem engagement, such as the skin, gastrointestinal tract, vessels, and nervous system [1,2]. The pathogenesis of BU is not completely elucidated, but the disease may be due to autoimmune dysregulation in genetically susceptible individuals after exposure

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to infectious antigens. The main effector cells are T lymphocytes (Th1, Th17, and Treg cells) and neutrophils [3,4]. Members of the IL-1 family and TLRs are crucial in initiating immune responses. These cytokines and TLRs recognize pathogen-associated molecular patterns and bind to specific receptors, activating downstream signaling pathways and triggering inflammatory signalling cascades [5,6]. Evidence is mounting that changes in the intestinal microbiota are also linked to the pathogenesis of BU [4,7–10]. Notably, gut microbiota and metabolites are crucial for maintaining immune homeostasis and inhibiting inflammation [11].

Studies in Chinese populations have proposed a model of the gut microbial community associated with BU, which features diminished numbers of beneficial bacteria (SCFA producers and methanogens) and increased numbers of pathogenic bacteria (opportunistic pathogens and sulfate-reducing bacteria) [8]. The bacterial metabolites SCFAs, which mainly include acetate, propionate, and butyrate, can upregulate tight junction (TJ) proteins and enhance Treg activity, significantly reducing intestinal inflammation [12]. However, diminished numbers of butyrate-producing bacteria in BU result in reduced butyrate levels, impairing Treg regulatory function [4,13]. According to a previous study, a 3-month butyrate diet given to BU patients could significantly improve the blood redox status and reduce disease activity but had no significant effect on the composition of intestinal microorganisms, SCFA production [14]. These findings suggested that short-term nutritional intervention or butyrate supplementation alone could not reverse intestinal microbiota disorders. Further exploration of the gut-related microbiota and metabolites is essential for disease intervention.

Unlike BU, with its multiorgan involvement, Fuchs syndrome, the second most prevalent noninfectious uveitis, is characterized by a chronic, mild inflammation that primarily impacts the anterior uvea and vitreous unilaterally [15,16]. Cellular immune responses against the corneal epithelium occur in 90 % of Fuchs syndrome patients [17]. Metabolomic analysis of the aqueous humour of Fuchs syndrome patients revealed specific metabolites [18]. However, studies on the intestinal microbes and metabolites of Fuchs syndrome patients are lacking.

This study aimed to examine the alterations in gut microbiota and metabolic products in two different types of uveitis, and to further analyze the functional and metabolic pathways affected by differential microbiota and metabolites. Serum zonulin levels were measured to assess intestinal barrier integrity in patients with uveitis. Our results showed that *Fusicatenibacter* and *Pantoea* were specifically depleted in BU and Fuchs syndrome patients, respectively. Metabolites such as palmitic acid (PA) and delta-tocopherol (δT) might participate in the development of BU through fatty acid metabolism and ubiquinone and other terpenoid quinones biosynthesis. Linoleic acid (LA) might lead to the development of Fuchs syndrome through pathways of linoleic acid metabolism.

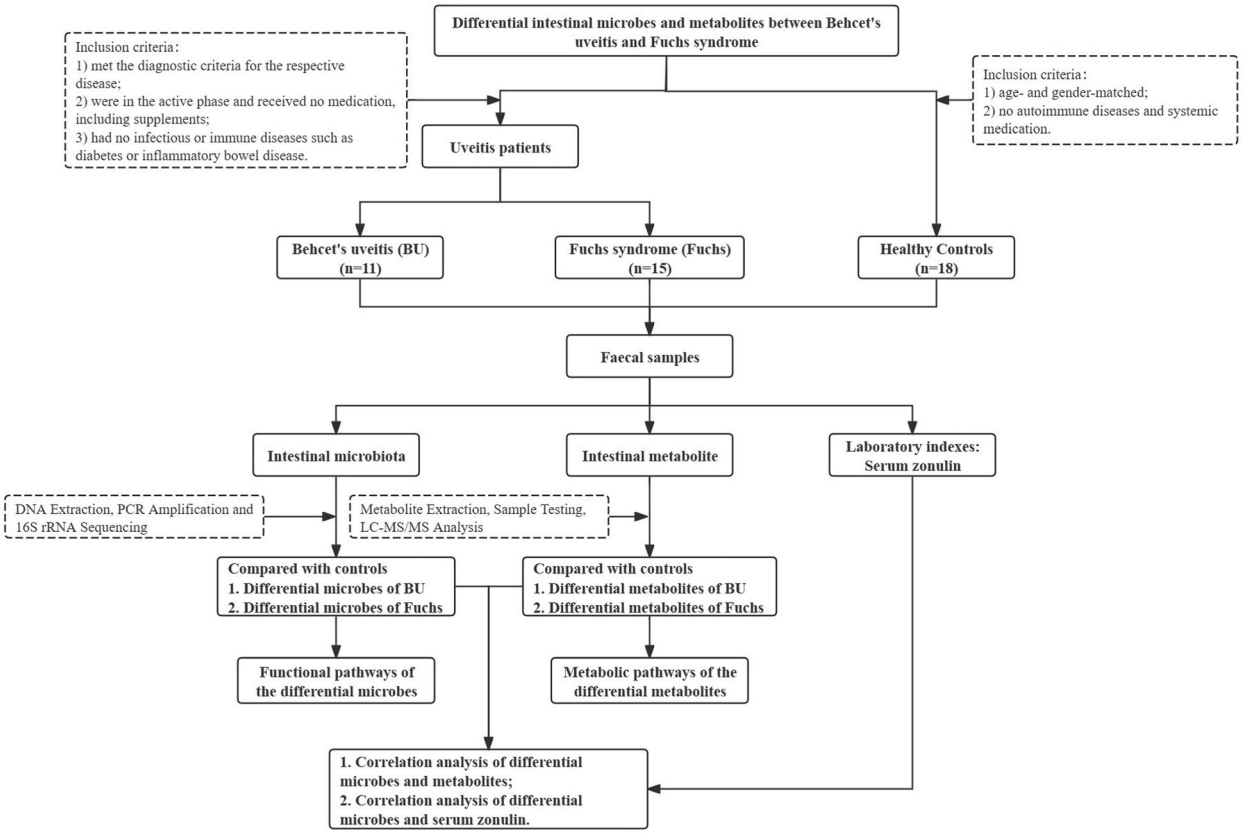

Fig. 1. The study design diagram.

2. Methods

2.1. Participants

Faecal samples and clinical data were gathered from patients with new-onset BU and Fuchs syndrome from July 2022 to March 2023 at the Department of Ophthalmology, the Second Hospital of Jilin University. All the procedures done within this study were shown in Fig. 1. The standards for inclusion comprised the following: 1) met the diagnostic criteria for the respective disease; 2) were in the active phase and received no medication, including supplements; and 3) had no infectious diseases or immune diseases such as diabetes or inflammatory bowel disease. Patients met the diagnostic criteria for BU as per the International Criteria [19]. The diagnosis of Fuchs syndrome relied primarily on clinical examination, which included three essential findings and five related findings [20]. Healthy controls had no systemic immune disease and no systemic drug treatment. Written informed consent forms have been obtained from all adult participants at the initiation of the study. For participants under the age of 18, written informed consent forms were obtained from their parents or legal guardians, in addition to assent from the participants themselves. All procedures adhered to the principles of the Declaration of Helsinki and received approval from the Medical Ethics Committee of the Second Hospital of Jilin University [No. 2024(142)].

2.2. Faecal sample collection and DNA extraction

Following collection, all samples were promptly preserved at −80 °C. 44 faecal samples were forwarded to Biotree for testing. The extraction of the total microbiome DNA was performed by Cetyltrimethylammonium bromide. Quantitative analysis of DNA was performed through agarose gel electrophoresis. After eluting total DNA with 50 μL elution buffer, it was preserved at −80 °C for PCR amplification [21].

2.3. 16S rDNA sequencing

The 16S rRNA sequencing method has been detailed earlier [21]. Briefly, PCR amplification of the V3-V4 region of the gut bacterial 16S rRNA gene was performed using universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R(5'-GACTACHVGGGTATC-TAATCC-3') [21]. AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) were employed for the purification of PCR products, followed by quantification using Qubit (Invitrogen, USA). The amplicon library's size was analyzed utilizing an Agilent 2100 bioanalyzer (Agilent, USA), and its quantity was determined by the Illumina Library Quantification Kit (Kapa Biosciences, Woburn, MA, USA). Subsequently, sequencing on the NovaSeq PE250 platform was conducted for the libraries.

2.4. Sequence analysis

Sample sequencing was carried out on an Illumina NovaSeq platform. After paired-end read sequencing, samples were trimmed through the elimination of barcodes and primer sequences, and then merged with FLASH. Fqtrim (0.94) and Vsearch (v2.3.4) were used for quality filtering and chimeric sequence screening, respectively. DADA2 was used for dereplication to generate a characteristics table and sequence. QIIME2 was employed to calculate α and β diversity [22]. BLAST-aligned sequences and feature sequences were annotated by the SILVA database.

2.5. Metabolite extraction

LC-MS/MS of human faecal samples was performed as previously described [21]. 25 mg of sample was added to 500 μl of an extract solution, composed of methanol, acetonitrile, and water in a ratio of 2:2:1. After homogenization, the supernatant was sonicated in an ice water bath and then transferred to a dry clean bottle for centrifugation analysis. Equal portions of the supernatants from all samples were mixed to prepare quality control samples.

2.6. LC-MS/MS analysis

LC-MS/MS analyses were carried out via a UHPLC system (Vanquish, Thermo Fisher Scientific) coupled to an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo). The elution mixture comprised water (25 mmol/L ammonium acetate and ammonia, pH = 9.75) and acetonitrile [23]. In the information-dependent acquisition mode of the acquisition software (Xcalibur, Thermo), MS/MS spectra were obtained.

2.7. Data preprocessing, annotation and analysis

Detecting, extracting, aligning, and integrating peaks were carried out using an internal program developed with R and XCMS [24]. An internal MS2 database using a threshold established at 0.3 was utilized to annotate metabolites. Metabolites exhibiting significant inter-group differences were screened using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), and the model's validity was evaluated through permutation testing.

2.8. ELISA for serum zonulin levels

Blood was collected using EDTA-vacuumed blood collection tubes. After centrifugation, the serum was gathered and preserved at −80 °C for further examination [25]. Serum zonulin concentrations were analyzed using an ELISA kit (catalogue no. NBP3-21146, Novus Biologicals, LLC, Centennial, CO, USA).

2.9. Statistical analysis

The difference in age between groups was analyzed using the two-tailed Student's t-test, and the difference in gender between groups was assessed using Pearson's chi-square test ($P < 0.05$). Microbial differences between two groups were measured by the Mann–Whitney *U* test ($P < 0.05$) and Linear Discriminant Analysis (LDA) Effect Size (LEfSe) ($P < 0.05$, LDA>3.0) [26]. The difference in bacterial metabolites between groups was calculated using the two-tailed Student's t-test ($P < 0.01$) and the Variable Importance in the Projection (VIP) > 1.5 based on the OPLS-DA model [27]. The ability to distinguish patients from controls based on a specific microbe or metabolite was determined by the receiver operating characteristic curve. Spearman correlation analysis between microbes and metabolites ($P < 0.05$) was conducted with the psych R package.

3. Results

3.1. Basic information

A cohort of 44 Chinese individuals was included in the study, including 11 BU patients (eight males and three females; average age, 42.64 ± 15.97 years), 14 Fuchs syndrome patients (six females and nine males; average age, 40.07 ± 12.63 years), and 18 controls (eight females and ten males; average age, 39.11 ± 12.86 years). There were no statistically significant differences in age and gender among the three groups. All BU patients (11/11, 100 %) experienced acute uveitis episodes and recurrent oral ulcers. Genital ulcers were observed in three patients (3/11, 27.2 %), cutaneous involvement in seven patients (7/11, 63.6 %), joint involvement in four patients (4/11, 36.3 %), and central nervous system involvement in three patients (3/11, 27.2 %), and none (0/11, 0 %) showed gastrointestinal involvement(Table 1).

3.2. Gut microbial diversity in patients with BU and Fuchs syndrome

The rarefaction curves of the three groups showed a tendency toward a plateau (Fig. 2A), suggesting that the sequencing depth could cover the vast majority of species in the samples. The Shannon, goods_coverage, and pielou_e indices demonstrated no significant differences in α diversity within the three groups (Fig. 2B). β diversity was measured by analysis of similarities (ANOSIM) based on weighted UniFrac distances ($P = 0.143$). No obvious distinction was observed among the three groups, indicating that the distributions of intestinal microbes were similar (Fig. 2C).

3.3. Differences in the gut microbial composition in patients with BU and Fuchs syndrome

First, we investigated the phylum- and genus-level bacterial taxonomic differences among the three groups. At the level of phyla, the abundances of Firmicutes and Proteobacteria were lower in the disease groups than in the control group (Fig. 3A). Bacteroidetes abundance was highest in the Fuchs group (Fuchs: 32.49 %, BU: 27.95 %, Controls: 27.36 %), while Actinobacteria was highest in the BU group (BU: 14.74 %, Fuchs: 6.62 %, Controls: 3.87 %). In terms of the genus, *Bacteroides*, *Faecalibacterium*, and *Escherichia-Shigella* were depleted in the BU and Fuchs syndrome patients (Fig. 3B). The BU group had the highest average abundance of Bifidobacterium (BU: 11.18 %, Fuchs: 4.99 %, Controls: 2.65 %), while the Fuchs group had the highest average abundance of Prevotella_9 (Fuchs:

Table 1
The characteristics of patients with BU and Fuchs and controls.

	BU (n = 11)	Fuchs (n = 14)	Controls (n = 18)	P-value
Age (years)	42.64 ± 15.97	40.07 ± 12.63	39.11 ± 12.86	0.500[a], 0.832[b], 0.420[c]
Gender (F/M)	3/8	6/9	8/10	0.355[a], 0.797[b], 0.500[c]
Organ involvement				
Acute uveitis	11 (100 %)	11 (100 %)	–	–
Oral ulcers	11 (100 %)	–	–	–
Genital ulcers	3 (27.2 %)	–	–	–
Cutaneous involvement	7 (63.6 %)	–	–	–
Joint involvement	4 (36.3 %)	–	–	–
Central nervous system involvement	3 (27.2 %)	–	–	–
Gastrointestinal involvement	0	–	–	–
Systemic medications	no	no	no	–

[a] P, BU vs. Controls.
[b] P, Fuchs vs. Controls.
[c] P, BU vs. Fuchs.

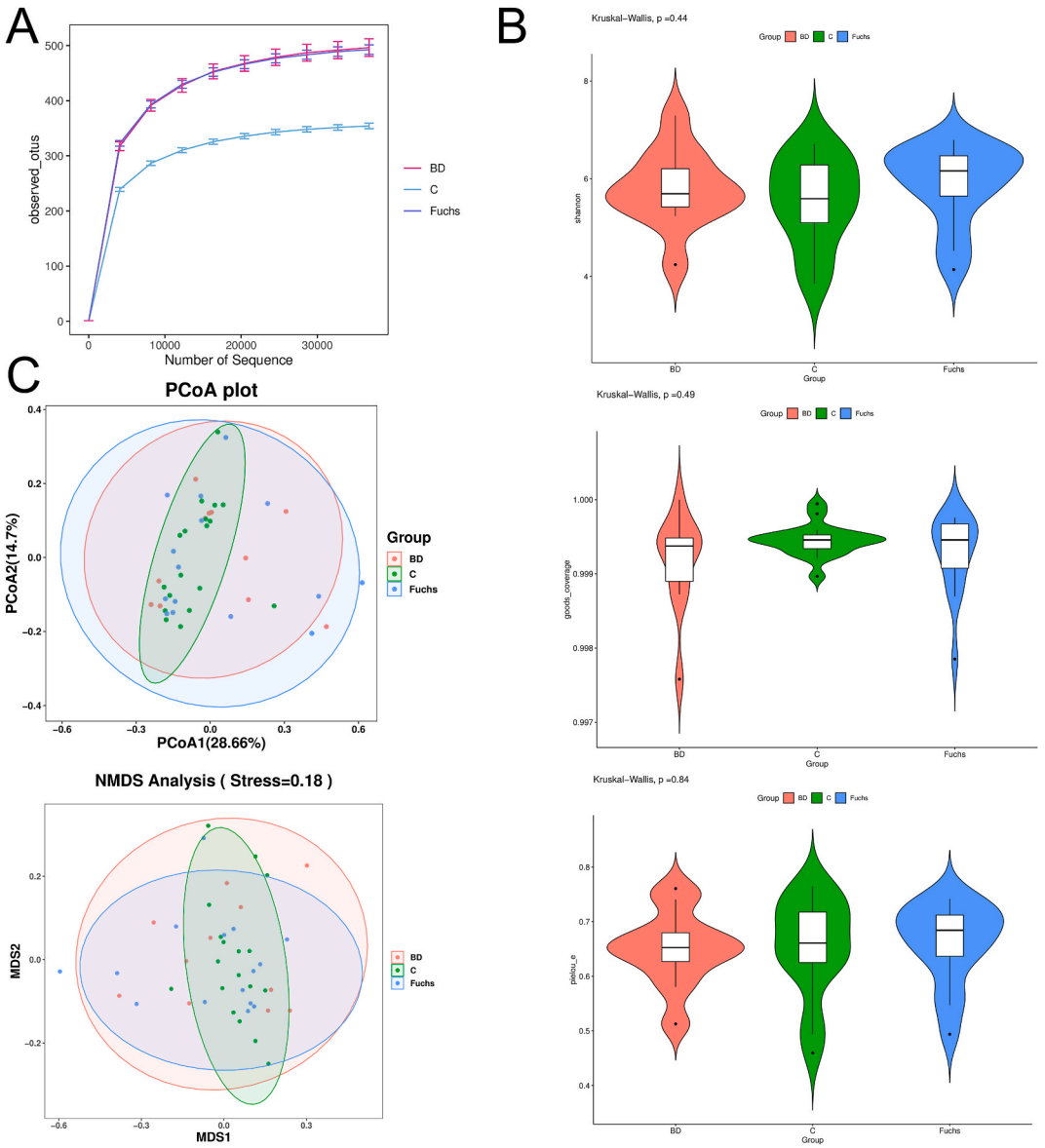

Fig. 2. Gut microbial diversity in patients with BU or Fuchs syndrome compared to controls. (A) Rarefaction curves. (B) The α diversity was estimated by the Shannon, goods_coverage, and pielou_e indices. (C) The β diversity was assessed by PCoA and NMDS of the weighted UniFrac distance matrix among the three groups.

10.15 %, BU: 6.31 %, Controls: 2.21 %).

We further screened 30 differential genera with the highest abundance. Compared with controls, BU patients had 17 more abundant genera and 13 less abundant genera (Fig. 3C). Fuchs syndrome patients had 15 more abundant and 15 less abundant genera (Fig. 3D). Notably, *Moraxella* was detected only in the disease groups, while neither disease group showed the presence of *Lawsonia* and *Photobacterium*.

LEfSe was performed to investigate the differential microbial features between the BU or Fuchs syndrome patients and controls ($P < 0.05$, LDA>3.0). The results indicated that BU patients had 31 enriched bacterial taxa, including *Prevotella*, *Desulfovibrio*, and *Alloprevotella* genera, while controls had 26 increased bacterial taxa, including *Escherichia-Shigella*, Erysipelotrichaceae_UCG-003, and *Streptococcus* genera (Fig. 3E). In contrast with controls, Fuchs syndrome patients had 30 enriched and 29 deleted bacterial taxa (Fig. 3F). Among them, ten genera, including *Megasphaera*, *Streptococcus*, and *Lachnoclostridium*, were Fuchs syndrome-enriched genera, and 11 genera, including *Escherichia-Shigella*, *Klebsiella*, *Clostridium*_sensu_stricto_1, and the *Ruminococcus gnavus* group, were Fuchs syndrome-depleted genera.

We further developed gut microbial markers to test whether the above differential genera could distinguish BU or Fuchs syndrome patients from controls. After removing genera with an average abundance <0.1 in any group, *Alloprevotella*,

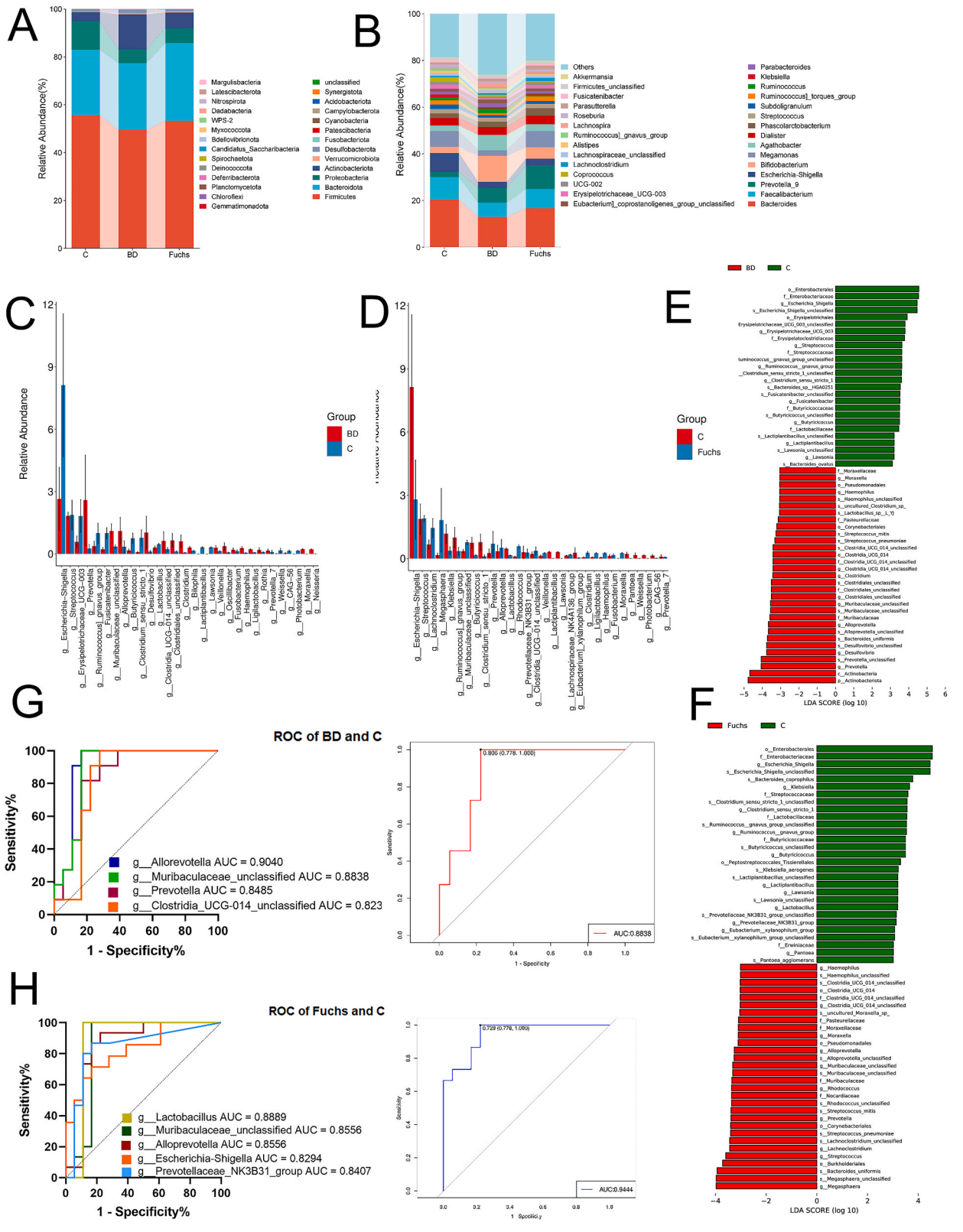

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Fig. 3. Gut microbial composition and microbial markers in patients with BU or Fuchs syndrome compared to controls. Relative abundance of predominant bacteria at the phylum (A) and genus (B) levels. The top 30 altered genera with the highest abundance in BU (C) and Fuchs syndrome (D) patients compared to controls. LEfSe (LDA>3.0) analysis of the major differences in bacterial taxa between BU patients and controls (E) and between Fuchs syndrome patients and controls (F). Microbial biomarkers, alone or in combination, that distinguished BU (G) and Fuchs syndrome (H) patients from controls.

Muribaculaceae_unclassified, *Prevotella*, and Clostridia_UCG-014_unclassified could effectively distinguish the BU patients from the controls (AUC>80 %). A gut microbial marker profile comprising the four genera also had predictive value (AUC = 88.38 %) (Fig. 3G). Using the same method, five genera (*Lactobacillus*, Muribaculaceae_unclassified, *Alloprevotella*, *Escherichia–Shigella*, and Prevotellaceae_NK3B31_group) could distinguish the Fuchs syndrome patients from the controls (AUC>80 %). The combined index comprised the five genera had higher stability and accuracy (AUC = 94.44 %) (Fig. 3H).

3.4. Specific microbes in patients with BU and Fuchs syndrome

The genera that were significantly different in BU vs. C and BU vs. Fuchs but not in Fuchs vs. C were selected as BU-specific microorganisms, including increased Peptostreptococcaceae_unclassified and *Eikenella*, and decreased *Fusicatenibacter* and *Stenotrophomona*. *Fusicatenibacter* was the most abundant of these four genera. Employing the method similar to screening BU-specific microorganisms, Fuchs syndrome-specific microorganisms comprised five increased and three decreased genera. *Pantoea* was the most abundant of these eight genera. (Fig. 4A).

3.5. Functional analysis of the altered microbiota in patients with BU and Fuchs syndrome

We further investigated the differential functional pathways of the differential microbes identified in the BU and Fuchs syndrome patients. Compared with controls, the 30 pathways exhibiting the most pronounced disparities in BU patients, included 20 upregulated and 10 downregulated pathways (Fig. 4B), such as the L-methionine biosynthesis pathway. There were 26 enriched and four depleted pathways between the Fuchs syndrome patients and controls (Fig. 4C), for instance, L-tryptophan, L-tyrosine, and nicotinate degradation pathways. Pathways were significantly different in both BU vs C and BU vs Fuchs but not in Fuchs vs C. BU-specific functional pathways included two upregulated (nitrate reduction I, protein N-glycosylation) and two downregulated (chlorophyllide a biosynthesis I, glucose degradation). There were no Fuchs-specific pathways.

3.6. Metabolic diversity in patients with BU and Fuchs syndrome

The plot of PCA score indicated a clear separation between the BU patients and controls (Fig. 5A). The OPLS-DA model (R^2Y = 0.991 and Q^2 = 0.782) indicated meaningful distinctions in metabolic phenotypes across the two groups. The permutation test suggested that the model was not overfitted and had high reliability and predictability. Similarly, the metabolic phenotypes of the Fuchs syndrome patients and controls were also significantly different (Fig. 5B).

3.7. Metabolic phenotypes in patients with BU and Fuchs syndrome

The metabolites with VIP>1.5, $P < 0.01$, and a fold change ≥ 2 or ≤ 0.5 are shown in Table 2, including 11 increased metabolites and eight decreased metabolites in the BU patients, as well as 26 increased metabolites and six decreased metabolites in the Fuchs syndrome patients.

We further screened the metabolites from Table 2 to identify biomarkers that could distinguish uveitis patients from controls. The AUC values of 19 differential metabolites between the BU patients and controls were all greater than 80 %, with seven metabolites going beyond 90 %. Except for androstanediol, the AUC values of 31 distinct metabolites between Fuchs syndrome patients and controls exceeded 80 %, with nine metabolites surpassing 90 % (Table 3).

3.8. Specific metabolites in patients with BU and Fuchs syndrome

In contrast with those in Fuchs syndrome patients and controls, BU patients displayed specific alterations in the levels of eight metabolites, including two enriched metabolites and six depleted metabolites. Five metabolites, namely (S)-2-hydroxyglutarate, maleic acid, 4-acetyl-2(3H)-benzoxazolone, acetone cyanohydrin, and androstanediol, were specifically elevated in the Fuchs syndrome patients in comparison with the BU patients and controls (Fig. 5C).

3.9. Metabolic pathways associated with altered metabolites in patients with BU and Fuchs syndrome

We performed enrichment and topological analyses to identify key pathways correlated with differential metabolites. In contrast with controls, the pathways of tyrosine metabolism (4-Hydroxyphenylpyruvic acid, N-Methyltyramine, tyrosol), ubiquinone and other terpenoid-quinone biosynthesis (4-Hydroxyphenylpyruvic acid, δT), tryptophan metabolism (serotonin), ascorbate and aldarate metabolism (ascorbic acid), and fatty acid metabolism (palmitic acid) (from high to low) were disrupted in the BU patients (Fig. 6A).

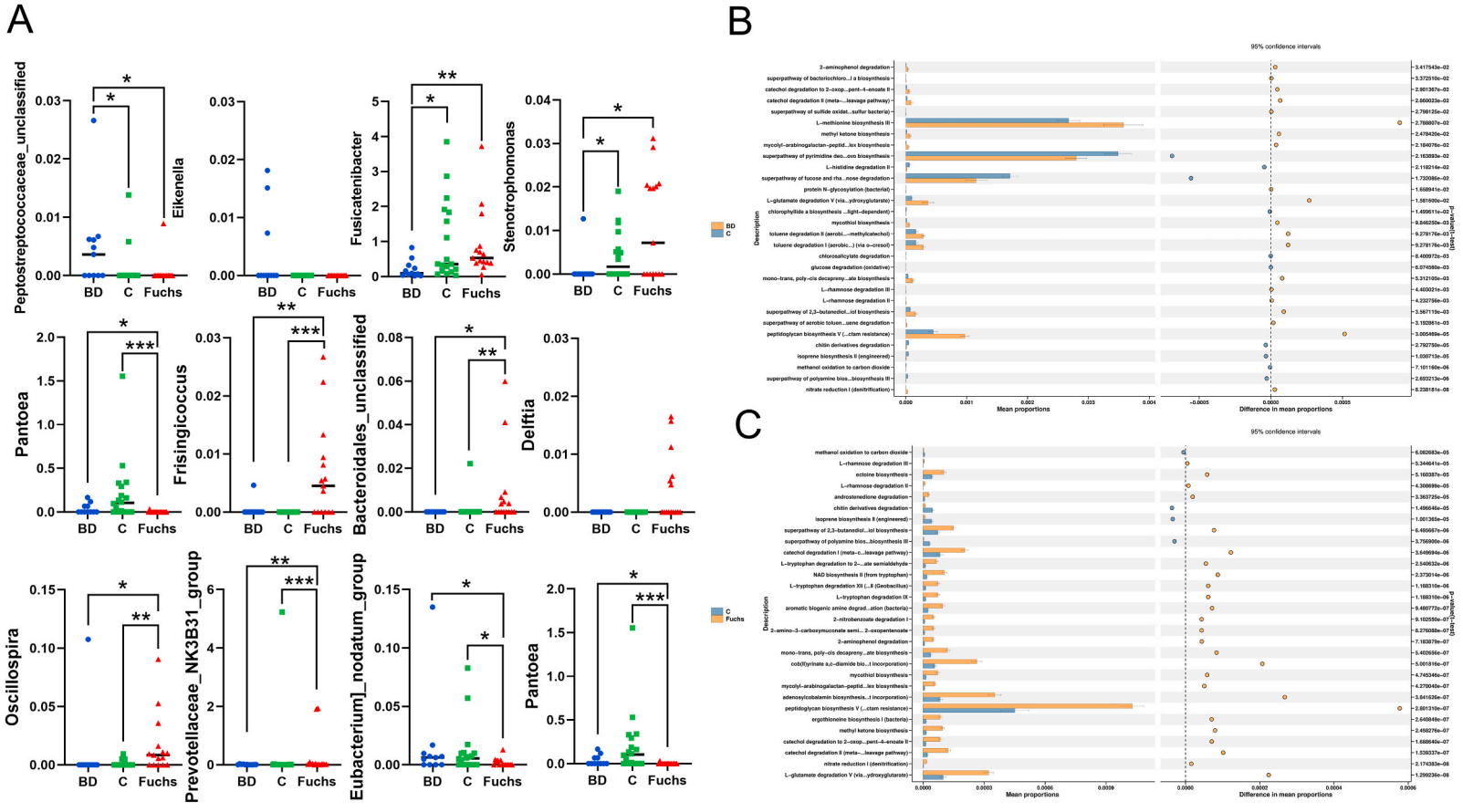

Fig. 4. Specific genera and differential functional pathways in patients with BU and Fuchs syndrome. (A) Four specific genera in BU patients and eight specific genera in Fuchs syndrome patients (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). 30 differential functional pathways in BU (B) and 30 in Fuchs syndrome (C) patients.

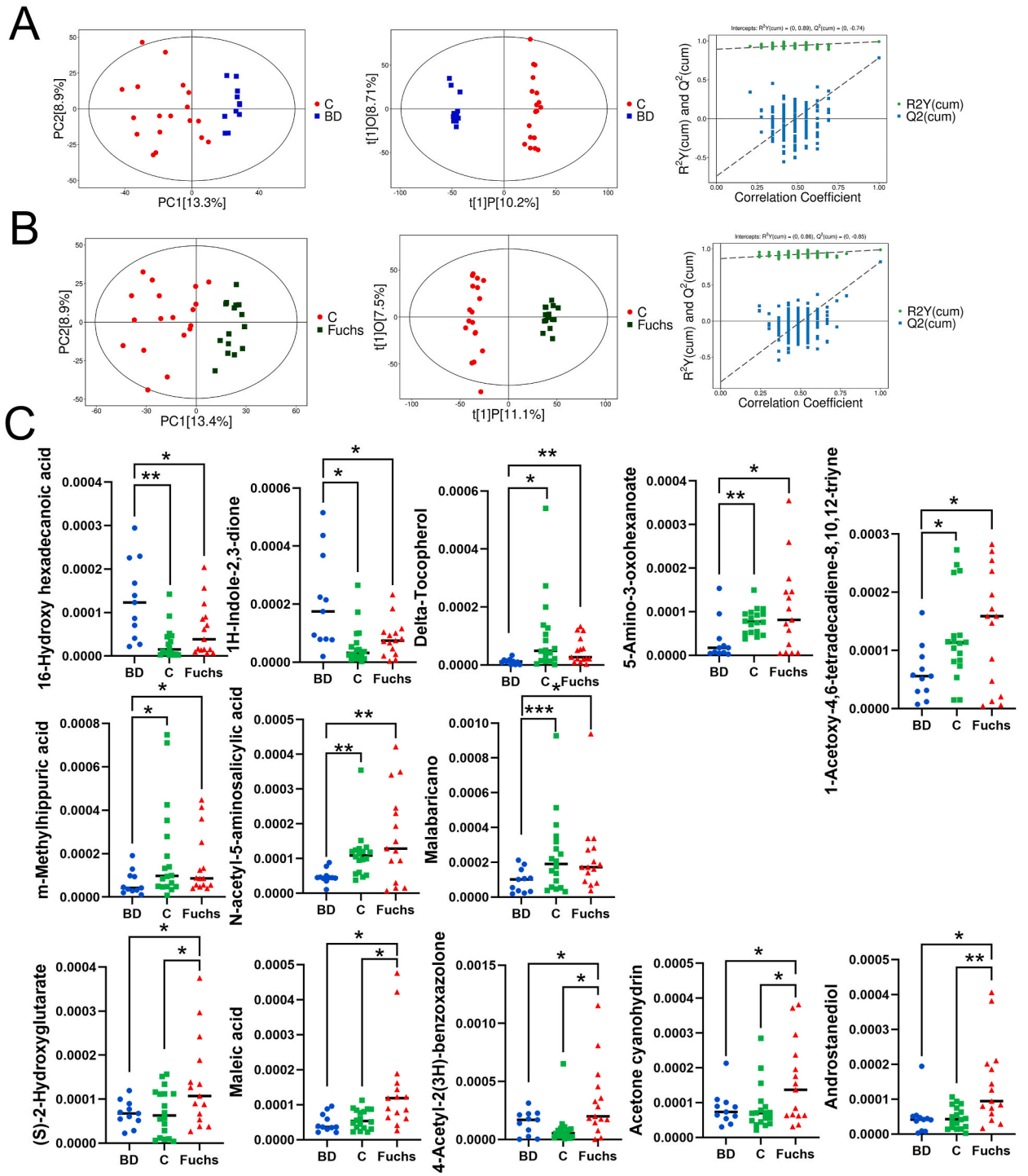

Fig. 5. Diversity of metabolites and specific metabolites in patients with BU or Fuchs syndrome compared to controls. The diversity of metabolites in BU patients (blue squares) (A) and Fuchs syndrome patients (green squares) (B) compared to controls (red circles). (C) Eight specific metabolites in BU patients and five specific metabolites in Fuchs syndrome patients (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Pathways associated with glycine, serine and threonine metabolism (L-Serine, L-Threonine, D-Serine); linoleic acid metabolism (linoleic acid); caffeine metabolism (paraxanthine); vitamin B6 metabolism (pyridoxine); ascorbate and aldarate metabolism (ascorbic acid) were disrupted in the Fuchs syndrome patients (Fig. 6B).

Table 2
The metabolites with VIP>1.5, P < 0.01, and a fold change ≥2 or ≤0.5 in BU and Fuchs syndrome patients.

	Class	VIP	P-value	Fold_change	label
Metabolites in BU					
Palmitic acid	Fatty Acyls	2.3514	0.0000	2.7255	up
3-Hydroxykynurenamine	Organooxygen compounds	2.2836	0.0002	3.3000	up
(13E)-11a-Hydroxy-9,15-dioxoprost-13-enoic acid	Fatty Acyls	2.1277	0.0020	3.8456	up
8-(1,2-dihydroxy-3-methylbut-3-en-1-yl)-7-methoxy-2H-chromen-2-one	Coumarins and derivatives	2.1046	0.0000	2.1434	up
Homocitrulline	Carboxylic acids and derivatives	2.0941	0.0066	6.8209	up
L-Saccharopine		2.0514	0.0094	2.5083	up
16-Hydroxy hexadecanoic acid	Fatty Acyls	1.9905	0.0050	4.2391	up
Succinic anhydride	Carboxylic acids and derivatives	1.9354	0.0008	2.8410	up
Myristic acid	Fatty Acyls	1.8206	0.0002	2.7422	up
L-alpha-Aspartyl-L-hydroxyproline	Carboxylic acids and derivatives	1.8078	0.0053	2.0261	up
(−)-Matairesinol	Furanoid lignans	1.6452	0.0051	2.6722	up
5-Amino-3-oxohexanoate		2.1880	0.0039	0.4280	down
Guanine	Imidazopyrimidines	2.1179	0.0000	0.2024	down
Procurcumenol	Prenol lipids	2.0715	0.0015	0.2029	down
Butyrylcarnitine	Fatty Acyls	2.0191	0.0017	0.0458	down
N-acetyl-5-aminosalicylic acid	Benzene and substituted derivatives	1.9044	0.0015	0.4311	down
Ethylparaben	Benzene and substituted derivatives	1.8273	0.0021	0.2652	down
Carvyl propionate	Prenol lipids	1.7525	0.0093	0.2490	down
Cholesterol sulfate	Steroids and steroid derivatives	1.6134	0.0003	0.2660	down
Metabolites in Fuchs					
Serotonin	Indoles and derivatives	2.4644	0.0005	7.6033	up
Palmitic acid	Fatty Acyls	2.4119	0.0000	2.8413	up
Piperolein B	Benzodioxoles	2.4084	0.0000	6.8479	up
Isopalmitic acid	Fatty Acyls	2.3461	0.0000	2.0581	up
gamma-Aminobutyric acid		2.0339	0.0001	3.6757	up
Acetylhydrazine	Carboxylic acids and derivatives	2.0175	0.0000	2.0730	up
Gallic acid	Benzene and substituted derivatives	1.9560	0.0018	4.8684	up
L-alpha-Aspartyl-L-hydroxyproline	Carboxylic acids and derivatives	1.9346	0.0004	2.1874	up
Lysyl-Asparagine	Carboxylic acids and derivatives	1.9342	0.0024	3.6882	up
4-Hydroxyphenylpyruvic acid	Benzene and substituted derivatives	1.9314	0.0000	2.4457	up
Succinic anhydride	Carboxylic acids and derivatives	1.9225	0.0001	3.2601	up
Ascorbic acid	Dihydrofurans	1.9201	0.0000	2.0137	up
LysoPC(P-18:1(9Z))	Glycerophospholipids	1.9019	0.0063	5.1368	up
DG(18:3(6Z,9Z,12Z)/20:3(5Z,8Z,11Z)/0:0)	Glycerolipids	1.8950	0.0015	3.1033	up
8-Acetoxy-4'-methoxypinoresinol	Furanoid lignans	1.8677	0.0019	2.7608	up
Camellenodiol	Prenol lipids	1.8419	0.0003	3.2348	up
(4E)-1,7-bis(4-hydroxyphenyl)hept-4-en-3-one	Diarylheptanoids	1.8312	0.0077	6.2770	up
NAD	(5'->5')-dinucleotides	1.8207	0.0042	3.6376	up
Isoniazid pyruvate	Pyridines and derivatives	1.7802	0.0091	2.0735	up
LysoPE(18:3(6Z,9Z,12Z)/0:0)	Glycerophospholipids	1.7391	0.0001	2.6919	up
3-Hydroxykynurenamine	Organooxygen compounds	1.7251	0.0026	2.7792	up
Momordenol		1.7006	0.0015	3.4719	up
Myristic acid	Fatty Acyls	1.5995	0.0037	2.6067	up
Androstanediol	Glycerophospholipids	1.5949	0.0086	3.0113	up
N-Acetyl-D-neuraminic acid		1.5710	0.0044	2.5608	up
Ricinoleic acid	Fatty Acyls	1.5421	0.0039	2.3254	up
Sinapyl alcohol	Phenols	2.0361	0.0013	0.2034	down
Carvyl propionate	Prenol lipids	1.9802	0.0048	0.1822	down
Guanine	Imidazopyrimidines	1.9359	0.0000	0.2345	down
Ethylparaben	Benzene and substituted derivatives	1.7255	0.0025	0.2875	down
Cholesterol sulfate	Steroids and steroid derivatives	1.7249	0.0003	0.2605	down
Tyrosol	Phenols	1.6567	0.0054	0.2076	down

3.10. Correlations between gut microbes and metabolites in patients with BU and Fuchs syndrome

The metabolites hit in metabolic pathways were correlated with the differential genera identified by LEfSe analysis (Fig. 6C and D). δT, tyrosol, and N-methyltyramine were decreased, while ascorbic acid, serotonin, PA, and 4-hydroxyphenylpyruvic acid were increased in the BU patients. δT positively correlated with three genera and inversely correlated with seven elevated genera, including *Moraxella*, Clostridia_UCG-014_unclassified, and *Alloprevotella*. PA was significantly negatively correlated with six deleted genera, including *Fusicatenibacter*, and positively correlated with seven elevated genera, including *Moraxella*, Clostridia_UCG-014_unclassified, and *Alloprevotella*. Seven metabolites were all enriched in the Fuchs syndrome patients. LA was positively correlated with six increased genera, including *Moraxella* and *Rhodococcus*, and negatively correlated with four decreased genera, including *Pantoea*.

Table 3
Metabolites with AUC values greater than 90 % in BU and Fuchs syndrome patients.

	AUC (95 % CI)	Specificity	Sensitivity	P value
Metabolites in BU patients				
Palmitic acid	97.47 % (92.09%–100.0 %)	1.000	0.909	<0.0001
Guanine	90.91 % (79.54%–100.0 %)	0.909	0.889	0.0003
8-(1,2-Dihydroxy-3-methylbut-3-en-1-yl)-7-methoxy-2H-chromen-2-one	92.93 % (83.02%–100.0 %)	0.889	0.909	0.0001
3-Hydroxykynurenamine	91.41 % (79.49%–100.0 %)	1.000	0.818	0.0002
N-Acetyl-5-aminosalicylic acid	90.91 % (79.99%–100.0 %)	1.000	0.722	0.0003
(13E)-11a-Hydroxy-9,15-dioxoprost-13-enoic acid	93.43 % (85.01%–100.0 %)	0.833	0.909	0.0001
L-Saccharopine	90.91 % (80.65%–100.0 %)	0.722	1.000	0.0003
Metabolites in Fuchs syndrome patients				
Isopalmitic acid	100.0 % (100.0%–100.0 %)	1.000	1.000	<0.0001
Palmitic acid	99.63 % (98.42%–100.0 %)	0.944	1.000	<0.0001
Ascorbic acid	98.15 % (94.69%–100.0 %)	1.000	0.867	<0.0001
Piperolein B	99.26 % (97.32%–100.0 %)	1.000	0.933	<0.0001
4-Hydroxyphenylpyruvic acid	91.48 % (80.16%–100.0 %)	0.889	0.867	<0.0001
gamma-Aminobutyric acid	92.59 % (82.86%–100.0 %)	0.944	0.867	<0.0001
Succinic anhydride	91.48 % (80.30%–100.0 %)	0.944	0.867	<0.0001
L-alpha-Aspartyl-L-hydroxyproline	90.00 % (78.24%–100.0 %)	0.722	1.000	<0.0001
Serotonin	96.67 % (90.03%–100.0 %)	0.944	1.000	<0.0001

3.11. Increased intestinal permeability in patients with BU and Fuchs syndrome

Previous studies suggested that increased intestinal permeability is one of the hypothesized mechanisms of intestinal microbiota disorders in the onset of uveitis. Zonulin is secreted by intestinal epithelial cells and is a non-invasive biomarker of intestinal permeability. To test the above hypothesis, we measured the serum zonulin concentrations in nine BU patients, four Fuchs syndrome patients, and eight controls by ELISA. The results indicated that the zonulin levels in BU and Fuchs syndrome patients were markedly greater than those in the controls (BU: $P = 0.0382$; Fuchs: $P = 0.0275$) (Fig. 6E). Next, to analyze the effect of the major imbalance of the intestinal microbes on the gut mucosal barrier, we correlated the abundances of the differential microbes identified by LEfSe with zonulin levels in the BU and Fuchs syndrome groups (Table 4). In the BU patients, zonulin levels were inversely related to the abundance of *Fusicatenibacter* and positively linked to those of *Moraxella*, Clostridia_UCG-014_unclassified, and *Alloprevotella*. In the Fuchs syndrome patients, zonulin levels were positively related to six genera and negatively correlated with the *Ruminococcus gnavus* group. Conversely, there was no correlation between the abundance of *Pantoea* (a Fuchs syndrome-specific genus) and zonulin concentrations ($P = 0.327$).

4. Discussion

In this study, we found four specific genera and eight specific metabolites in BU patients compared to Fuchs syndrome patients and controls. Fuchs syndrome patients had eight specific genera and six specific metabolites. Serum zonulin levels were markedly greater in BU and Fuchs syndrome patients than that in controls ($P < 0.05$). Correlation analysis revealed correlations between zonulin levels and multiple microbes. For instance, zonulin levels were inversely correlated with *Fusicatenibacter* abundance in BU patients. These findings imply that the intestinal microbes and metabolites may result in uveitis by disrupting the intestinal barrier.

In BU patients, Fusicatenibacter notably decreased, while *Moraxella*, Clostridia_UCG-014_unclassified, and *Alloprevotella* increased. Essex et al. reported that the abundance of *Fusicatenibacter* was inversely correlated with the levels of C-reactive protein [28], which was depleted in patients with inflammatory bowel disease [29,30] and acute anterior uveitis [28]. In vitro, *Fusicatenibacter* can alleviate colitis by promoting IL-10 production in the lamina propria cells of the gut mucosa [31]. However, it is unclear which cell type is stimulated by *Fusicatenibacter* to increase IL-10 production. Furthermore, *Fusicatenibacter* can produce SCFAs [31]. *Moraxella* was altered in cows with clinical mastitis, impacting the blood-milk barrier [32]. The above differential microbes may influence the expression of inflammatory mediators and the levels of metabolites such as SCFAs, which in turn affect intestinal permeability.

The level of PA was strongly positively linked to the abundance of *Moraxella*, and inversely related to the abundance of *Fusicatenibacter*. PA is a long-chain saturated fatty acid. Studies have indicated increased PA levels in both the aqueous humour [33] and urine [34] of BU patients. Cultivation of murine colonic epithelial cells with PA alone or in combination with TNF-α resulted in the overproduction of reactive oxygen species (ROS). This activated the myosin light chain kinase pathway (a key regulator of intestinal barrier permeability) and induced alterations in the expression of occludin and claudin-2. High concentrations of PA in the blood can activate neutrophil autophagy, leading to increased vacuolization of neutrophils, which is an indicator of immune activation [35]. Neutrophils with T cell regulatory features amplify Treg cells and attenuate Th1/Th17 cells in BU patients and EAU [36]. The above findings suggested that fatty acids and inflammation were key factors driving oxidative stress and impairing the gut barrier. The differential microbiota and metabolites may be implicated in the onset of BU by influencing pathways of fatty acid metabolism.

The abundances of *Moraxella* were inversely correlated with δT level and positively correlated with ascorbic acid level, while the abundance of *Fusicatenibacter* was inversely related to the level of ascorbic acid. Although alpha-tocopherol (αT) is the primary form of vitamin E in tissues, non-αT forms, such as δT, exhibit superior anti-inflammatory and antioxidant activities compared with αT [37,38]. The non-αT forms are metabolized into 13'-carboxychromanols, with enhanced anti-inflammatory capabilities [37]. Moreover, δT

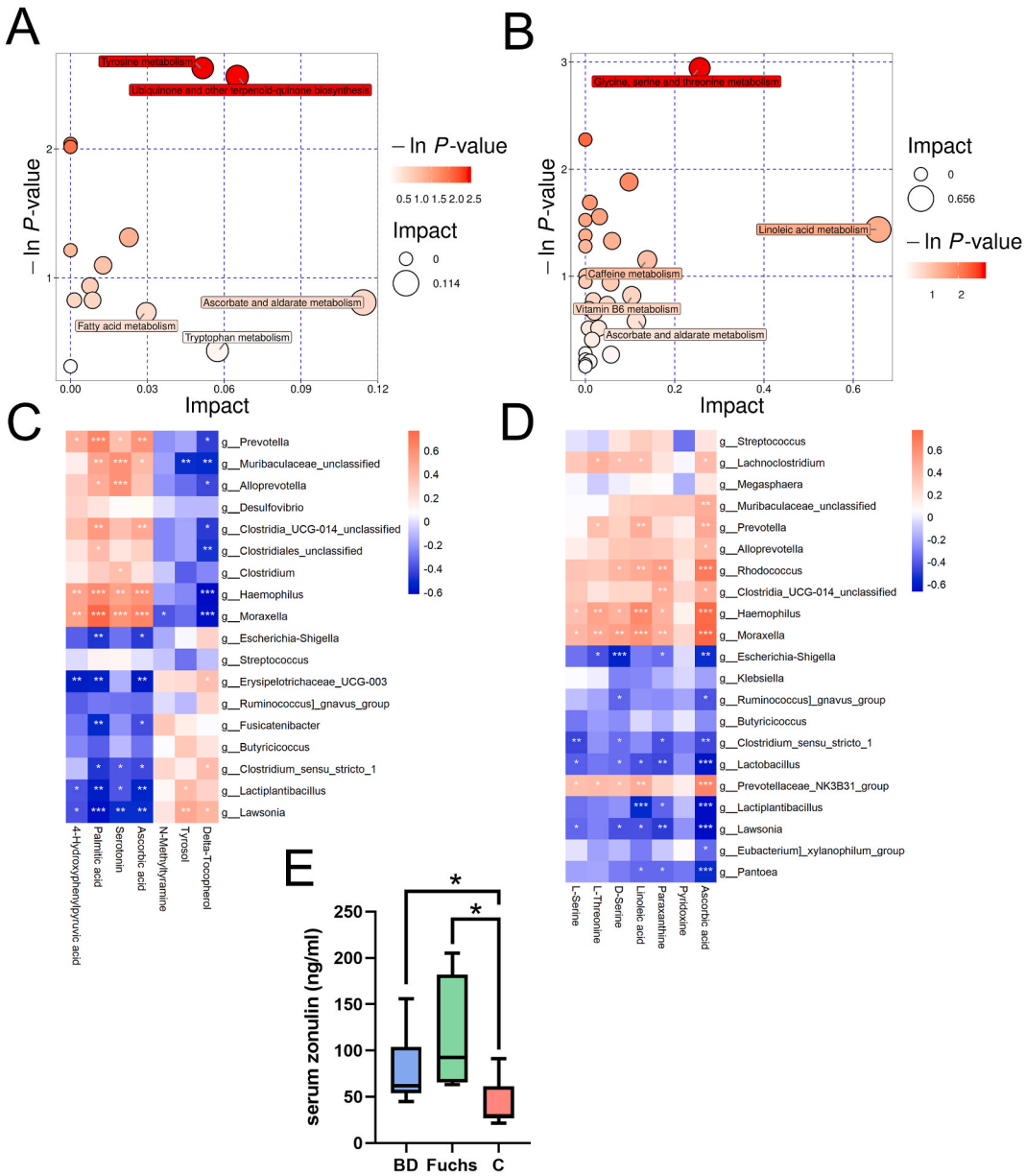

Fig. 6. (A) Five differential metabolic pathways between BU patients and controls. (B) Five differential metabolic pathways between Fuchs syndrome patients and controls. Correlation heatmaps between the microbiota obtained by LEfSe and metabolites that hit metabolic pathways in patients with BU (C) and with Fuchs syndrome (D). (E) Serum zonulin concentrations in BU patients (n = 9), Fuchs syndrome patients (n = 4), and controls (n = 8) (*, $P < 0.05$).

inhibits ERK phosphorylation and 5-lipoxygenase nuclear translocation by blocking the increase of intracellular calcium, making it more effective than αT in suppressing neutrophil-mediated leukotriene B4 production, which is an important lipid regulator of inflammation [39]. Ascorbic acid interacts with δT and can regenerate tocopherol free radicals into tocopherols, thereby extending the half-life of tocopherols. Smokers experience higher levels of oxidative stress. Supplementation of ascorbic acid elevated plasma levels of tocopherol and effectively reduced its disappearance rate. However, this supplementation could not reverse the depletion of tocopherols [40]. Our results also indicated a negative correlation between the δT and ascorbic acid levels ($P = 0.016$, $r = -0.444$). We speculated that there was excessive oxidative stress in BU patients, leading to the disruption of ubiquinone and other terpenoid-quinone biosynthesis metabolic pathways and excessive consumption of δT, as evidenced by a notable decrease in the faecal level of δT. Nevertheless, gut microbiota may compensate for the reduced δT level by enhancing the pathway of ascorbate and aldarate metabolism. This ongoing compensation aids in mobilizing the host's antioxidant capacity to maintain sustainable homeostasis.

This study is the inaugural exploration of the composition of intestinal microbiota and metabolites in Fuchs syndrome patients.

Table 4
Correlation analysis between the differential bacterial genera determined by LEfSe and serum zonulin.

Genus	Zonulin	
	R	P-value
BU		
Fusicatenibacter↓	−0.612	0.012
Moraxella↓	0.550	0.027
Clostridia_UCG-014_unclassified↑	0.499	0.049
Alloprevotella↑	0.553	0.026
Fuchs		
Ruminococcus]_gnavus_group↓	−0.736	0.010
Moraxella↓	0.705	0.015
Clostridia_UCG-014_unclassified↓	0.670	0.024
Rhodococcus↑	0.655	0.029
Alloprevotella↑	0.618	0.043
Muribaculaceae_unclassified↑	0.673	0.023
Megasphaera↑	0.706	0.015

↓, decreased genus; ↑, increased genus.

Sublingual administration of lipopolysaccharides produced by *Pantoea agglomerans* can enhance the development and expansion of innate immune cells, triggering systemic IgG and mucosal IgA responses [41]. Despite more pronounced differences in intestinal microbiota between Fuchs syndrome patients and controls than those between BU and controls, there was a partial overlap in microbial alterations between the two uveitis conditions. In Fuchs syndrome patients, the abundance of potentially pathogenic bacteria, such as the *Ruminococcus gnavus* group, was reduced, while that of SCFA-producing bacteria, such as Muribaculaceae_unclassified, was increased, possibly mitigating intestinal inflammation. This hypothesis needs further validation with a larger sample size.

In Fuchs syndrome patients, certain microbes are primarily involved in the occurrence of Fuchs syndrome by influencing linoleic acid metabolism. LA is an n-6 polyunsaturated fatty acid, and its downstream product is arachidonic acid. The latter is converted into metabolites of the arachidonic acid cascade, including prostaglandin E2, thromboxane A2, and leukotriene B4 [42]. LA levels were markedly elevated in the faeces of patients with acute anterior uveitis and the serum of BU patients [43,44]. In vivo, diet components rich in LA, such as soybean oil, could impair intestinal barrier function and increase the susceptibility to colitis in wild-type mice [45]. Furthermore, in this study, LA levels were positively linked to the abundance of *Moraxella* and *Rhodococcus*, both of which demonstrated a correlation with zonulin levels. However, further experiments are required to explore the roles of polyunsaturated fatty acids and related microbes in the onset of Fuchs syndrome.

In our study, we found serum zonulin levels were markedly greater in BU and Fuchs syndrome patients compared to controls. However, none of the patients enrolled in this study had gastrointestinal symptoms. This discrepancy may be due to changes in the intestinal mucosa that are subclinical and not sufficient to cause gastrointestinal symptoms. Probiotic supplementation can regulate gut flora, restore barrier function, and modulate immune responses, thereby providing potential therapeutic benefits for various inflammatory diseases, including uveitis [46,47]. Preclinical studies have shown that oral administration of IRT-5 probiotics [48] or *Escherichia coli* Nissle 1917 [49] during EAU induction can ameliorate uveitis by affecting initial antigen presentation and T-cell activation. Two case reports demonstrated that probiotics also had a relieving effect as an adjunctive therapy [50,51]. Patients with BU and Fuchs had reduced Lactobacillus compared to controls in this study. Lactobacillus acidophilus can produce antibacterial substances such as lactic acid and hydrogen peroxide [52]. Although probiotics have shown promising potential in improving uveitis, further studies are needed to determine the optimal strain, dose, and treatment regimen.

The limitations of this study include the following three points. First, the study was based on a small cohort of single-center patients. Additionally, biological factors (genetics, environment, BMI, and diet) that influence the gut microbiota and metabolites were not included, and could be addressed through stratified analysis. Furthermore, a longitudinal comparison between the active and remission phases is needed to investigate the relationship between disease progression and dynamic changes in the gut microbiota.

In conclusion, our study identified and explored the variations in the intestinal microbiota and metabolites in BU and Fuchs syndrome patients. Certain fatty acids and vitamins were identified as relevant factors in the pathogenesis of uveitis. Intestinal microbes may initiate uveitis by disrupting the intestinal barrier. However, further investigations in animal disease models are needed to confirm the impacts of specific microbes and metabolites on the intestinal barrier and clinical manifestations in uveitis.

CRediT authorship contribution statement

Mingzhu Liu: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Mengyao Li:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Siyan Jin:** Writing – review & editing, Validation, Supervision, Project administration, Investigation. **Xia Wang:** Writing – review & editing, Validation, Supervision. **Jiawei Geng:** Writing – review & editing, Visualization, Validation. **Xiaoli Liu:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Ethics statement

All procedures in this study received approval from the Medical Ethics Committee of the Second Hospital of Jilin University [approval No. 2024(142)]. Written informed consent forms have been obtained from all adult participants at the initiation of the study. For participants under the age of 18, written informed consent forms were obtained from their parents or legal guardians, in addition to assent from the participants themselves.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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