Mucosa-Colonizing Microbiota Correlate With Host Autophagy Signaling in Patients With Inflammatory Bowel Disease

Wenxue Wang1,2*, Zhongjian Liu3, Wei Yue1†, Ling Zhu1, Huijie Zhong1,2, Chao Yang4, Tian He4, Ping Wan4* and Jiawei Geng1,2,5*

1Department of Infectious Disease and Hepatic Disease, First People’s Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, China; 2School of Medicine, Kunming University of Science and Technology, Kunming, China; 3Institute of Basic and Clinical Medicine, First People’s Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, China; 4Department of Gastroenterology, First People’s Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, China; 5Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, China

Both bacteria and autophagy are implicated in inflammatory bowel disease (IBD) pathogenesis. However, how bacteria crosstalk with autophagy signaling remains largely known, especially in intestinal mucosa. This study aimed to profile the internal complex autophagy signaling cascade and their external correlation with these bacteria, and consequently provide a systematic and precise target for future IBD diagnosis and therapy. We found the Ulcerative colitis (UC) patients exhibited more severe dysbiosis than the Crohn’s disease (CD) patients, as represented by alpha diversity, community phenotypes, and functional annotation compared with the control population. Meanwhile, CD patients showed greater transcriptional signaling activities of autophagy, endoplasmic reticulum (ER) stress, and bile acid production. Dominant bacteria (e.g., Rhodococcus, Escherichia, Shigella, and Enterococcus) were positively correlated and low-abundance bacteria (e.g., Bacillus, Acidovorax, Acinetobacter, and Stenotrophomonas) were negatively correlated with the autophagy signaling cascade (184 autophagy genes, 52 ER stress genes, and 22 bile acid production genes). Our observations suggested UC patients showed temporary and widespread microbiota turbulence and CD patients showed processive and local autophagy activity during IBD progression. Intestinal mucosa-colonizing bacteria were correlated with the bile/ER stress/autophagy signaling axis in IBD pathogenesis.

Keywords: autophagy, ER stress, bile, microbiome, transcriptome, inflammatory bowel disease

INTRODUCTION

Intestinal microbes and their metabolic products play key roles in human diseases, especially inflammatory bowel disease (IBD). Meanwhile, both adherent-invasive and diffusely adherent bacteria stick to the intestinal mucosa and induce host immune cell activity and are linked to Crohn’s disease (CD) and ulcerative colitis (UC), respectively (Mirsepasi-Lauridsen et al., 2019). Notably, mucosa samples can directly reflect bacteria-host interactions during IBD progression. However,
few multi-omics studies have investigated these interactions using mucosal biopsies. Furthermore, both the classification and mechanistic dissection of pathways involved in IBD remain challenging due to the complex correlative and interactive networks between host genetics and microbes (Plichta et al., 2019). Therefore, mucosal biopsy specimens are required to further explore these networks between host genetic factors and microbiota.

Genome-wide association studies have indicated that autophagy is an important mechanistic dissection of IBD pathogenesis, especially that of CD (Nguyen et al., 2013). Autophagy not only decreases intestinal epithelial permeability by inducing lysosomal degradation of tight junction proteins (Nighot et al., 2015), but also modulates programmed cell death in the intestinal epithelium (Matsuzawa-Ishimoto et al., 2017). In addition, autophagy is responsible for the elimination of intercellular bacteria from endoplasmic reticulum (ER) function defect-induced intestinal barrier leakage. For instance, autophagy can be induced by Autophagy related 16 like 1 (ATG16L1)-Transmembrane protein 59 (TMEM59) interactions in response to bacterial infection (Boada-Romero et al., 2016). Furthermore, activation of the bile acid receptor can strongly suppress the induction of autophagy (Lee et al., 2014). Thus, the profiles of mucosal bacteria-correlated autophagy gene networks and their interactions with ER stress and bile production signaling should be clarified in IBD patients.

Here, we collected intestinal mucosal biopsies from control population and IBD patients for dual-omics analysis of mucosal transcriptome and mucosa-colonizing bacterial diversity. Analysis focused on the autophagy signaling cascade and their complex correlations with intestinal mucosa-colonizing bacteria in IBD patients and control population. Combined with previous studies, our analysis suggested that mucosal bacteria regulated both ER stress and bile acid production and consequent autophagic activity, finally acting on host IBD progression. We systematically profiled the mucosal bacteria-autophagy correlation network in IBD patients, especially in those with CD, and provided a reliable analysis model of microbes-correlated intestinal diseases.

MATERIALS AND METHODS

Study Design
The control populations and IBD patients were recruited from the First People’s Hospital of Yunnan Province, China, from October 2017 to December 2019. Each participant provided information on age, occupation, and smoking and alcohol drinking history. The control population had no history of digestive tract disease or serious medical illnesses. Each IBD patient was diagnosed with UC or CD and had received no IBD therapy or antibiotics (within 3 months). All participants provided signed informed consents and completed a questionnaire regarding their age, sex, occupation, and antibiotic use, with family assistance if necessary.

Diagnosis of IBD
We used clinical feature inquiry, laboratory examination, and endoscopy and biopsy histopathological analysis to diagnose UC or CD according to clinical practice guidelines (Gomollón et al., 2017; Magro et al., 2017; Inflammatory Bowel Disease Group, Chinese Society of Gastroenterology, Chinese Medical Association, 2021). Firstly, outpatients with enteric symptoms of abdominal pain, diarrhea, blood in stools, and loose stools for more than 6 weeks, and/or parenteral manifestations, such as fatty liver and cholecystitis, were suggested for laboratory examination, which included a full blood count, electrolyte, liver function, and inflammatory marker [C-reactive protein (CRP)] tests. Secondly, when clinical features and laboratory tests suggested further investigation, an endoscopy (colon and/or intestinal endoscopy) was performed to obtain evidence and collect mucosal biopsies for histopathological analysis. Thirdly, histopathological investigations were performed to provide reliable information on mucosal architecture, lamina propria cellularity, neutrophil granulocyte infiltration, and epithelial abnormality. Finally, UC or CD was diagnosed when comprehensive analysis of clinical features, laboratory examinations, endoscopy, and histopathology gave positive responses.

Exclusion Criteria and Control Population Selection
To eliminate the possible effects of other intestinal diseases on UC and CD diagnosis, we carefully discriminated infectious enteritis, Clostridium difficile infection, intestinal tuberculosis, Behçet syndrome, amebiasis, schistosomiasis, and other intestinal diseases from IBD. Those participants without IBD or other digestive tract diseases and without serious diseases of other tissues and organs were chosen as the control population group. A total of 138 participants were initially recruited, 38 of which were excluded according to exclusion criteria (i.e., UC and/or CD and antibiotic use within the last 3 months). Finally, 25 control population, 26 CD patients, and 51 UC patients were enrolled in the study.

Sample Collection
The IBD patients and control population were first given an intravenous injection of propofol and etomidate, and then an enteroscopy was performed for mucosal sampling. All samples were collected from October 2017 to December 2019. Upon collection, the mucosal samples were immediately placed on ice and frozen at −80°C within 1h for microbiome and transcriptome analyses.

Bacterial DNA Isolation and 16S rRNA Gene Sequencing
Microbial community genomic DNA was extracted from intestinal mucosa using a QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, United States). The hypervariable V3–V4 region of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5’-ACTCTCCTACGG GAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCT CAA-3’) using an ABI GeneAmp® 9700 PCR thermocycler (ABI,
CA, United States). PCR amplification of the 16S rRNA gene was performed as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 45 s, single extension at 72°C for 10 min, and end at 4°C. The PCR mixture contained 5× TransStart FastPfu buffer 4 μl, 2.5 mM dNTPs 2 μl, forward primer (5 μM) 0.8 μl, reverse primer (5 μM) 0.8 μl, TransStart FastPfu DNA Polymerase 0.4 μl, template DNA 10 ng, and finally ddH2O up to 20 μl. PCR was performed in triplicate. The PCR products were extracted from 2% agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer’s instructions and quantified using a Quantus™ Fluorometer (Promega, United States).

Purified amplicons were pooled in equimolar concentrations and paired-end sequenced on the Illumina MiSeq PE300/NovaSeq PE250 platforms (Illumina, San Diego, United States) using standard protocols.

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered using fastp v0.20.0 (Chen et al., 2018), and merged using FLASH v1.2.7 (Magoc and Salzberg, 2011) with the following criteria: (i) 300-bp reads were truncated at any site receiving an average quality score of <20 over a 50-bp sliding window, with truncated reads shorter than 50 bp; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapping sequence. The maximum mismatch ratio of the overlapping region was 0.2. Reads that could not be assembled were discarded; and (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, two nucleotide mismatches in primer matching.

Operational taxonomic units (OTUs) with 97% similarity cutoff (Stackebrandt and Goebel, 1994; Edgar, 2013) were clustered using UPARSE v7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed using RDP Classifier v2.2 (Wang et al., 2007) against the 16S rRNA database (Silva v132) with a confidence threshold of 0.7. To remove possible contamination, we sequenced three samples of pure water as a negative control with the same procedures, including DNA extraction, PCR amplification, cDNA library construction, and final sequencing. Based on the negative control sequencing results, the same DNA sequence count detected in the negative control was extracted from all control and IBD samples. To confirm the reliability of 16S rRNA sequencing data, we also performed real-time PCR and found the data are replicable.

Bioinformatics Analysis of 16S rRNA Gene Sequencing Data

Community Hierarchical Clustering

Community clustering heatmap was conducted to show community variation at the genus level (top 47). Significant differences between control population and IBD patients were determined using the Kruskal-Wallis test at genus level. The FDR and Tukey–Kramer methods were used for multiple testing correction and post hoc tests, respectively.

Analysis of Abundance Differences

Significant differences between control population and IBD patients were determined using the Kruskal-Wallis H test at genus level. The FDR and Tukey–Kramer methods (CI = 0.95) were used for multiple testing correction and post hoc tests, respectively.

Functional Microbial Composition Analyses

The Functional Annotation of Prokaryotic Taxa (FAPROTAX) database extrapolates functions of cultured prokaryotes to estimate metabolic and other ecologically relevant functions (Louca et al., 2016). We used FAPROTAX to assess how the metabolic activities of intestinal mucosa-colonizing bacteria affect the host signaling network using the Tukey–Kramer method (CI = 0.95).

Phenotypic Prediction of Intestinal Mucosa-Colonizing Bacteria

Intestinal mucosa-colonizing bacteria phenotypes were predicted and compared using BugBase (Ward et al., 2017) and the Kruskal-Wallis H test. Briefly, BugBase uses an OTU table as an input file, which is normalized by the predicted 16S copy number. The preprocessed database and BugBase tool then automatically select thresholds to predict bacterial phenotypes.

Processing Library Preparation and Illumina Hiseq X Ten/NovaSeq 6000 Sequencing

The RNA-seq transcriptome library was prepared using a TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, CA, United States) with 1 μg of total RNA. In brief, messenger RNA (mRNA) was isolated according to the polyA selection method by oligo(dT) beads and then fragmented using fragmentation buffer. The double-stranded cDNA was synthesized using a SuperScript Double-Stranded cDNA Synthesis Kit.
(Invitrogen, CA, United States) with random hexamer primers (Illumina). The synthesized cDNA was then subjected to end repair, phosphorylation, and “A” base addition according to the library construction protocols of Illumina. Libraries were size-selected for 300-bp cDNA target fragments on 2% low-range ultra-agarose gel, followed by PCR amplification using Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantification by TBS380, the paired-end RNA-seq library was sequenced using an Illumina HiSeq X Ten/NovaSeq 6000 sequencer (2 × 150-bp read lengths).

**Read Mapping**

The raw paired-end reads were trimmed and quality controlled using SeqPrep\(^1\) and Sickle\(^2\) with default parameters. The clean reads were then separately aligned to the reference genome in orientation mode using HISAT2\(^3\) software (Kim et al., 2015). The mapped reads of each sample were assembled using StringTie\(^4\) with a reference-based approach (Pertea et al., 2015).

**Differential Expression Analysis and Functional Enrichment**

To identify differentially expressed genes (DEGs) between two samples, the expression level of each transcript was calculated according to the transcripts per million reads (TPM) method. RSEM\(^5\) was used to quantify gene abundances (Li and Dewey, 2011). Essentially, differential expression analysis was performed using DESeq2 (Love et al., 2014)/EdgeR (Robinson et al., 2010) with Q value ≤ 0.05 and DEGs with |log2FC| > 1 and Q value ≤ 0.05 (DESeq2 or EdgeR)/Q value ≤ 0.001 (DEGseq) deemed significant. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses were performed to identify DEGs significantly enriched in GO terms and metabolic pathways (Bonferroni-corrected value of \(p \leq 0.05\)) compared to the whole transcriptome background. GO functional enrichment and KEGG pathway analysis were carried out using GOATools\(^6\) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do; Xie et al., 2011).

**Alternative Splicing Event Identification**

All alternative splicing events that occurred in the samples were identified using the recently released program rMATS (http://rnaseq-mats.sourceforge.net/index.html; Shen et al., 2014). Only isoforms similar with the reference or comprising novel splice junctions were considered; and splicing differences were detected as exon inclusion and exclusion, alternative 5′ and 3′, and intron retention events.

---

\(^1\)https://github.com/jstjohn/SeqPrep

\(^2\)https://github.com/najoshi/sickle

\(^3\)http://ccb.jhu.edu/software/hisat2/index.shtml

\(^4\)https://ccb.jhu.edu/software/stringtie/index.shtml?t=example

\(^5\)http://deweylab.biostat.wisc.edu/edgeR

\(^6\)https://github.com/tanghaibao/Goatools

**Transcriptome Analysis**

**Time Series Expression Trend Analysis**

STEM is used for clustering, comparing, and visualizing time series gene expression data (Ernst and Bar-Joseph, 2006). The STEM clustering algorithm is a supervised algorithm, that is, clustering is classified into artificially set trends. First, the software simulates \(n\) of the most representative possible trends according to the preset, and then calculates the correlation coefficient between each gene and the preset trends. Finally, each gene is classified into the trend to which it is most similar. We used STEM (v1.3.11) to explore the gene expression pattern of the healthy controls and CD and UC patients. Analysis explored functional enrichment of genes with a certain expression pattern and predicted the genetic regulatory network of intestinal mucosa.

**Gene Expression Correlation Analysis**

Genes obtained from expression trend analysis and/or related to autophagy, ER stress, and bile acid production were used to calculate Spearman correlation coefficients based on the correlation of gene expression. In the correlation networks, the larger the node, the greater the number of correlations between the expression of the gene and other genes.

**Protein–Protein Interaction Analysis**

We used the STRING database\(^7\) to perform Protein–Protein Interaction (PPI) network analysis of genes of interest. The interactions corresponding to genes of interest were directly extracted from the database to construct the network. NetworkX (Python) was used to visualize the network of the genes of interest.

**KEGG Functional Enrichment Analysis**

We used R script and Fisher’s precision probability test to perform KEGG pathway enrichment analysis. To control the false positives of enrichment analysis, multiple testing corrections (Benjamini-Hochberg, BH) were carried out. KEGG pathways reaching a corrected \(p\) value of 0.05 were defined as significantly enriched.

**Ethics Approval**

All study protocols and procedures were approved by the Medical Ethics Board of the First People’s Hospital of Yunnan Province (GXBSC-2021001, 2021 updated), China, and were carried out in accordance with all relevant provincial, national, and international guidelines, including the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

**Statistical Analysis**

GraphPad Prism v9.0.0 (San Diego, CA, United States) and the R stats package were used to analyze all data (R Core Team, 2013). The Kruskal-Wallis H test with FDR correction

---

\(^7\)http://string-db.org/
(CI = 0.95) for multiple comparisons was conducted to compare abundant bacterial taxa. Spearman rank-order correlation was used to evaluate associations between genus-level bacterial relative abundances and autophagy-related gene expression levels. In all analyses, \( p < 0.05 \) was considered statistically significant.

**RESULTS**

**Characteristics of IBD Patients and Controls**

We initially recruited 138 participants, 38 of which were excluded due to antibiotic use within the last 3 months and/or suffering from other serious illnesses. In total, 23 healthy control population, 26 CD patients, and 51 UC patients were enrolled (Table 1). Most IBD patients were male (68 out of 77 subjects) and had no history of smoking. The control population was recruited from outpatients suffering abdominal discomfort, but with no gastrointestinal or other serious illness. For transcriptome analysis, an additional biopsy sample was collected from six control population, five CD patients, and 12 UC patients (Supplementary Table S1) with informed consent.

**IBD Patients, Especially UC Patients, Show Intestinal Mucosa-Colonizing Microbial Dysbiosis and Corresponding Dysfunction**

The intestinal microbiota play a key role in IBD progression. IBD patients exhibit clear dysbiosis, mainly represented by a decrease in bacterial diversity (Ott et al., 2004; Manichanh et al., 2006). Mucosal biopsies are advantageous as they can be used to explore the real interactions between dysbiosis and host responses. Here, we collected intestinal mucosal biopsies from control population and IBD patients to investigate the compositional movement and functional variation potential of intestinal mucosa-colonizing bacteria. Consistent with previous study (Ott et al., 2004), we found that alpha diversity (Sobs and Shannon indices) of the intestinal mucosal bacterial community was significantly decreased in IBD patients compared with the control population. Furthermore, UC patients showed poorer alpha diversity than CD patients (Figures 1A,B). The decrease in the Sobs index mainly originated from the reduced abundances of Proteobacteria and Bacteroidetes (Supplementary Figure S1). Correspondingly, FAPROTAX analysis indicated that the metabolic activities of nitrite, nitrate, and fumarate were significantly decreased in IBD patients, especially UC patients (Figure 1D; Supplementary Figure S3). Notably, these metabolic activities had brought focuses because of their key roles in IBD incidence, prevention, and therapy (Dykhuizen et al., 1996; Saijo et al., 2010; Jädert et al., 2014; Casili et al., 2016). Redox of these nitrogenous salts is also associated with IBD pathology (Bourgonje et al., 2020). Based on community phenotype analysis, we also found that IBD patients showed a decrease in oxidative stress tolerance, primarily due to the lower abundances of *Escherichia*, *Shigella*, and *Pseudomonas* (Figure 1E; Supplementary Figures S4A,B).

Unexpectedly, pathogen causing diarrhea and gastroenteritis potentials also decreased significantly in IBD patients. Potential pathogens, such as *Rhodococcus*, *Streptococcus*, *Enterococcus*, *Veillonella*, *Ruminococcus torques*, and *Ruminococcus gravis*, were enriched in the intestinal mucosa of IBD patients (Figure 1C; Supplementary Figure S2), which contributed to high Gram-positive performance, another community phenotype (Supplementary Figures S4C,D).

**Active Intestinal Mucosa Signaling in CD Patients Exceeds That in UC Patients**

Functional genomics network analysis is a powerful tool for identifying the key regulatory networks involved in IBD progression (Peters et al., 2017). Here, gene expression clustering analysis revealed that the intestinal mucosa signaling network was comprised of eight clusters based on differential expression in control population and UC and CD patients (Figure 2A). Three clusters, i.e., cluster 4 (3,339 genes, \( p = 0.0000012 \)), cluster 6 (6,355 genes, \( p = 1.2e–156 \)), and cluster 7 (9,773 genes, \( p = 0 \)), were significant according to the differential transcription levels in the control population and UC and CD patients. Cluster 6 genes were highly expressed in the intestinal mucosa of both UC and CD patients, whereas cluster 7 and 4 genes showed a gradual decrease in expression in UC patients (Figures 2B–D). Thus, these findings indicate that intestinal mucosa signaling activity was higher in CD patients than in UC patients.

**Environmental Factor Correlation Analysis Reveals Bacterial Community-Matched Autophagy Signaling in Intestinal Mucosa of IBD Patients**

Increasing studies emphasize that crosstalk between the mucosal microbiome and host signaling network greatly affects IBD progression and clinical outcome (Morgan et al., 2015; Ryan et al., 2020). Recent review work provides new insights of the interplay between autophagy and intestinal bacteria and suggested that...
IBD-associated autophagy alleles and their interactions with environmental triggers, such as resident microbiota, are crucial for developing new therapeutic strategies for IBD treatment (Larabi et al., 2020). Thus, we performed correlation analysis between intestinal mucosa-colonizing bacteria and autophagy gene expression levels in clusters 6 and 7, which covered most KEGG autophagy-related genes (Supplementary Figure S7). In this analysis, 144 autophagy genes (58 in cluster 6 and 86 in cluster 7) were significantly correlated with intestinal mucosa-colonizing bacteria (Figures 3A,D). Gene expression heatmap analysis showed that these bacteria-correlated autophagy genes were more highly expressed in CD patients than in UC patients (Supplementary Figure S8). Furthermore, these autophagy genes not only formed a correlation network, but also a PPI network. For example, bacteria-correlated autophagy genes Autophagy related 4A cysteine peptidase (ATG4A), Autophagy related 5 (ATG5), ATG16L1, Autophagy related 9A (ATG9A), Signal transducer and activator of transcription 3 (STAT3), NRAS proto-oncogene, GTPase (NRAS), and Heat shock protein 90 alpha family class A member 1 (HSP90AA1) in cluster 6 (Figures 3B,C) and Beclin 1 (BCN1), Mitogen-activated protein kinase 1 (MAPK1), Phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3), Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), Charged multivesicular body protein 2A (CHMP2A), Charged multivesicular body protein 4A (CHMP4A), Charged multivesicular body protein 3 (CHMP3), Charged multivesicular body protein 4A (CHMP4A), Vacuolar protein sorting 4 homolog A (VPS4A), Vacuolar protein sorting 25 homolog (VPS25), Translocase Of outer mitochondrial membrane 20 (TOMM20), and AKT serine/threonine kinase 2 (AKT2) in cluster 7 (Figures 3E,F) were core members of both the correlation and interaction networks.

The above bacteria could also be clustered into two groups, i.e., those showing positive correlations with autophagy genes in...
both cluster 6 and cluster 7 (e.g., *Haemophilus*, *Ruminococcus torques*, and *Rhodococcus*) and those showing negative correlations (e.g., *Bacillus*, *Acidovorax*, *Acinetobacter*, and *Stenotrophomonas*; Figures 3A,D). It is worth noting that most of the intestinal mucosa-colonizing bacteria showing positive correlation with the above autophagy genes have been reported in previous IBD studies (Goyal et al., 2018; Soltys et al., 2020). For those bacteria negatively correlated with the autophagy genes, *Bacillus* is recognized as a probiotic bacterium in IBD prevention and therapy (Ghavami et al., 2020; Shinde et al., 2020; Zhang et al., 2020; Liu et al., 2021), but the remaining bacteria (e.g., *Acidovorax*, *Acinetobacter*, and *Stenotrophomonas*) were first reported to involve in IBD progression. Interestingly, the co-occurrence network indicated that low-abundance intestinal mucosa-colonizing bacteria were negatively and densely associated with autophagy genes, whereas high-abundance bacteria were positively and sparsely associated with autophagy genes (Supplementary Figure S6).

The autophagy genes in cluster 4, which were highly expressed in CD patients but not in UC patients compared with control population, showed more positive correlations and fewer negative correlations with the intestinal mucosa-colonizing bacteria than found in clusters 6 and 7. The positive and negative correlations were represented by *Haemophilus*, *Ruminococcus torques*, and *Rhodococcus*, and *Bacillus*, *Acidovorax*, *Acinetobacter*, and *Stenotrophomonas*, respectively. These cluster 4 bacteria-correlated autophagy genes also showed correlation and interaction networks, both of which dominated by Tumor protein P53 (*TP53*), Stimulator of interferon response CGAMP interactor...
These results are highly consistent with previous study suggesting that STING senses bacterial viability to orchestrate autophagy (Moretti et al., 2017). The cluster 4 co-occurrence network also showed more positive correlations between autophagy genes and intestinal mucosa-colonizing bacteria (e.g., *Haemophilus, Ruminococcus torques, Enterococcus, Veillonella, Escherichia,* and *Shigella*), but strong negative correlations between *Bacillus* and *Streptococcus* and autophagy genes (*e.g.,* Ring finger protein 41 (*RNF41*), *BNIP3P5*, and Interleukin 10 (*IL10*); Supplementary Figure S5). However, this correlation only existed in CD patients. Although similar correlations between mucosa-colonizing bacteria and autophagy activity have been reported (Nakagawa et al., 2004; Sudhakar et al., 2019; Wu et al., 2019), we used clinical biopsies to explore real crosstalk between mucosal bacteria and autophagy genes during IBD progression and to clarify the complex signaling pathways connecting intestinal microbes and host disease pathogenesis.

**Autophagy-Induced ER Stress Is Correlated With Intestinal Mucosa-Colonizing Bacteria in IBD Patients**

Moretti et al. (2017) found that STING initiates autophagy after bacterial infection and that this autophagy is mediated by ER stress. ER stress also regulates autophagy processes during IBD progression (Kaser and Blumberg, 2009; Kaser and Blumberg, 2011; Grootjans et al., 2019). According to our analysis, 15 ER stress-related genes in cluster 6 were
ER Stress-Related Bile Acid Production Signaling Is Correlated With Mucosa-Colonizing Bacteria in IBD Patients

Increasing evidence indicates that intestinal dysbiosis induces bile acid dysmetabolism and consequent IBD progression (Duboc et al., 2013; Lloyd-Price et al., 2019; Quinn et al., 2020; Sinha et al., 2020). Bacterial-driven bile acid metabolites have also been investigated to describe the pathophysiological basis of bacteria-bile acid associations (Long et al., 2017; Lavelle and Sokol, 2020). Here, we expanded the association between bile acid production-related genes and intestinal mucosa-colonizing bacteria. Eleven genes [e.g., Leptin (LEP), cytochrome P450 family 46 subfamily A member 1 (CYP46A1), KIT proto-oncogene, receptor tyrosine kinase (KIT), Phospholipase A2 group IB (PLA2G1B), and Retinoid X receptor alpha (RXRA)] in cluster 6 and eight genes [e.g., Sulfotransferase family 7 member 1 (SULT2A1), Fibroblast growth factor receptor 4 (FGFR4), and fatty acid binding protein 1 (FABP1)] in cluster 7 were significantly correlated with the intestinal mucosa-colonizing bacteria (Figures 5A, D). Similar to autophagy and ER stress signaling, genes in both cluster 6 and cluster 7 formed correlation and interaction networks, dominated by RXRA (Supplementary Figures S14B, C) and FABP1 (Supplementary Figures S14D, E), respectively. Notably, KIT and PLA2G1B in cluster 6 (Supplementary Figure S14A) and SULT2A1 in cluster 7 (Supplementary Figure S14D) showed strong correlations with the intestinal mucosa-colonizing bacteria. Bile-regulated lipid metabolism can induce ER stress in intestinal epithelial cells and intestinal bacteria modulate this metabolic process (Ko et al., 2020). Here, we identified bile acid-related genes in cluster 6 (RXRA, Aldo-keto reductase family 1 member C2 (AKR1C2), and Oxysterol binding protein like 7 (OSBPL7)) and cluster 7 [FGFR4, Nuclear receptor coactivator 2 (NCOA2), and FABP1], which were all significantly correlated with intestinal mucosa-colonizing bacteria and formed both correlation and interaction networks with ER stress signaling (Figures 5B, C, E, F). KEGG functional analysis also revealed the upregulation of genes related to bile biosynthesis (Supplementary Figure S15) and secretion (Supplementary Figure S16). Furthermore, the expression differentiation of bile acid-producing gene between UC and CD patients increased in cluster 7 than in cluster 6 (Supplementary Figure S17).

In the other hand, the bacteria that correlated with intestinal mucosa bile acid signaling, mainly comprised of Proteobacteria. The correlations between Stenotrophomonas, Acinetobacter, Bacillus, and Corynebacterium and cluster 6 genes (Figure 5A; Supplementary Figure S14A) and Rhodococcus and Acidovorax and cluster 7 genes (Figure 5B; Supplementary Figure S14B), exhibited the most significant correlations with host bile acid signaling. In addition, FGF19 in cluster 4, which is reported to modulate the connection between intestinal microbiota and host inflammation (Gadaleta et al., 2020), was correlated with multiple intestinal bacteria, including Staphylococcus, Peptomonas, Haemophilus, Ruminococcus torques, and Bacteroides (Supplementary Figure S13).
DISCUSSION

Increasing studies focus on the mucosal microbiome and local host immune activities in IBD patients (Howell et al., 2018; Lloyd-Price et al., 2019; Ryan et al., 2020) and the role of autophagy in IBD progression, especially in CD patients (Khor et al., 2011; Larabi et al., 2020). To date, however, no studies have reported on how mucosal bacteria interact with host autophagy activity based on biopsy samples even that obviously hold a great interest of precise diagnosis and therapy of IBD. Here, we used intestinal mucosal biopsies to elucidate the interaction network between mucosal bacteria and host autophagy signaling, as this network can reveal the bacterial infection-related pathogenesis of IBD and highlight potential and precise therapeutic targets for IBD.

Our results showed that active mucosal signaling, including autophagy, could be divided into cluster 4, 6, and 7 based on signaling extensity in UC patients. In general, all genes in the three clusters were highly activated in CD patients, but only genes in cluster 6 were highly expressed in UC patients. In addition, genes in cluster 7 showed increased expression in UC patients compared with that in the control population. The genes in cluster 4 also showed the same activities in UC patients and control population. These results are consistent with previous study showing that autophagy genes are pivotal for intestinal homeostasis and antimicrobial function in IBD, especially CD (Larabi et al., 2020).

In our study, UC patients exhibited greater disturbance in mucosal microbial diversity, community phenotype, and underlying functional spectrum, whereas CD patients exhibited greater autophagy and associated signaling cascades. These
differences may have originated from the different pathological durations of UC and CD in patients. The long disease course in CD patients can strongly induce autophagy signaling to fight pathogenic microbial invasion. In another hand, pathogenic microbial invasion can induce CD progression, whereas diffusion behavior tends to increase UC progression (Mirsepasi-Lauridsen et al., 2019). Certainly, mucosal biopsies are ideal samples to determine bacterial diffusion behavior. Previous studies using fecal samples found that CD patients show more extensive microbial community disturbance than UC patients (Halfvarson et al., 2017; Pascal et al., 2017). Based on our findings, we suggest that CD patients continuously repair their gut defenses and establish new gut immune hemostasis during the long-term pathological progression of the disease, which is different from normal states and UC UC.

We also found that most dominant bacteria were positively correlated with autophagy genes and related signaling cascades, whereas less abundant bacteria showed negative correlations. Dominant bacteria may form stronger community structures that can withstand serious environmental shifts or may change into opportunistic pathogens for survival. In contrast, low-abundance bacteria communities may be more sensitive to environmental shift, and therefore tightly related to host metabolism and healthy conditions and finally process a high sensitivity and great value for intestinal disease diagnosis and therapy, including IBD. However, the low-abundance bacteria also brought some uncertainty. For example, Acidovorax (<0.2% of total abundance) was negatively correlated with most autophagy genes and related signaling, even these genes or signaling play an adversarial role in autophagy. In addition, we observed several bacterial species that were not

**FIGURE 5 |** Correlation pattern of bile acid production with intestinal mucosa-colonizing bacteria community and ER stress in IBD patients. Spearman correlation heatmap shows 11 bile-related genes in cluster 6 (A) and eight bile-related genes in cluster 7 (D) were significantly correlated with intestinal mucosa-colonizing bacteria (top 47). All bacteria were named to genus level unless otherwise noted in brackets. * 0.01 < p ≤ 0.05, ** 0.001 < p ≤ 0.01, and *** p ≤ 0.001. Correlation network of both ER stress- and bile acid production-related genes in cluster 6 (B) and cluster 7 (E). ER stress- and bile acid production-related genes are labeled in red and green, respectively. Circle area is positively correlated with number of connected genes. Correlation network was constructed based on Spearman rank correlation coefficients (δ Spearman |Coef| ≥ 0.8, p < 0.05). Multiple testing correction: BH. Interaction network of ER stress- and bile acid production-related genes in cluster 6 (C) and cluster 7 (F). Circle or equilateral triangle areas are positively correlated with number of connected genes. Interaction between circle-labeled genes with others has been reported. Interaction between equilateral triangle-labeled genes with others was predicted based on primary structure of gene-coding proteins. Control population: n = 6; UC patient: n = 12; and CD patient: n = 5.
significantly correlated with any host genes (data not shown); it was difficult to explain how they survive under severe immune stress from host and fierce competition from other microbes if they do not clearly show cooperative (or confrontational) properties.

Our study has several limitations. Both the recruited patients (average age > 45) and control population (average age > 35) were older than participants in other studies, which is likely due to the consciousness deficiency of IBD risk in the local young population. In this study, the microbiota-correlated autophagy genes concurrently showed a strong correlation with ER stress. Integrating previous study (Kökten et al., 2018), the autophagy in our study could be either macroautophagy or chaperone-mediated autophagy, but the specific subtype was not clear and needs to be further explored. The relationship between mucosal bacteria and host autophagy activity needs more mucosa biopsies to conform their correlation profiles, but our matched analysis of microbiome and transcriptome from same subjects and location still drew an interactive map of bacteria-autophagy-IBD progression and could provide more valuable diagnostic and therapeutic targets based on autophagy mechanism.

CONCLUSION

In conclusion, we found that UC patients exhibited more severe dysbiosis and the functional phenotype of intestinal mucosa-colonizing bacteria, whereas CD patients exhibited more active autophagic signaling compared to the controls. Dominant bacteria and low-abundance bacteria showed positive and negative correlations with host autophagy genes, respectively. In addition, correlation and interaction networks were found between bile acid production and ER stress, with the latter showing an interesting interaction with autophagy activity. Thus, the present study elucidated how the intestinal mucosa-colonizing bacterial community interacts with host bile/ER stress/autophagy signaling cascades during IBD progression, which could aid in disease diagnosis and autophagy-targeted therapy.

DATA_AVAILABILITY_STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA797846.

REFERENCES

Boada-Romero, E., Serramito-Gómez, I., Sacristán, M. P., Boone, D. L., Xavier, R. J., Pimentel-Muiños, F. X., et al. (2016). The T300A Crohn’s disease risk polymorphism impairs function of the WD40 domain of ATG16L1. Nat. Commun. 7:11821. doi: 10.1038/ncomms11821

Bourgonje, A. R., Feelsch, M., Faber, K. N., Pasch, A., Dijkstra, G., and van Goor, H. (2020). Oxidative stress and redox-modulating therapeutics in inflammatory bowel disease. Trends Mol. Med. 26, 1034–1046. doi: 10.1016/j.molmed.2020.06.006

Casili, G., Cordaro, M., Impellizzeri, D., Bruschetta, G., Paterniti, L., Cuzzocrea, S., et al. (2016). Dimethyl fumarate reduces inflammatory responses in experimental colitis. J. Crohns Colitis 10, 472–483. doi: 10.1093/ecco-jcc/jjv231

Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884–i890. doi: 10.1093/bioinformatics/bty560

Coope, A., Pascoal, L. B., Botezelli, J. D., da Silva, F. A. R., Ayrizono, M. D. L. S., Rodrigues, B. L., et al. (2019). ER stress activation in the intestinal mucosa but not in mesenteric adipose tissue is associated with inflammation in Crohn’s disease patients. PLoS One 14:e0223105. doi: 10.1371/journal.pone.0223105

Duboc, H., Rajca, S., Raineteau, D., Benarous, D., Maubert, M.-A., Quervain, E., et al. (2013). Connecting dysbiosis, bile-acid dysmetabolism and gut

ETHICS_STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Board of the First People's Hospital of Yunnan Province (GXBSFC-2021001, 2021 updated). The patients/participants provided their written informed consent to participate in this study.

AUTHOR_CONTRIBUTIONS

WW and JG designed the project and reviewed and revised the final version of the manuscript. WW and ZL analyzed the 16S rRNA sequencing data. LZ, WY, HZ, CY, TH, PW, and JG collected mucosal biopsies and clinical documents. WW, ZL, and JG finished the draft. WW, LZ, PW, and JG supervised the study and rendered foundation supports. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (81860437 and 82160514), the Yunnan Province Innovation Team of Intestinal Microecology-Related Disease Research and Technological Transformation (202005AE160010), Eminent Doctors Program of Yunnan Province (YNWR-MY-2019-072), Yunnan Digestive Endoscopy Clinical Medical Center Foundation [2X2019-01-02]- (2019LCZXF-XH05, 2020LCZXF-XH01, 2021LCZXF-XH01, and 2021LCZXF-XH15), and Fundamental Research Projects of Yunnan Province (202101AT070275, 202101AY070001-236, and 2018FE001-130).

ACKNOWLEDGMENTS

We thank WW for help with identification of the UC and CD patients.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.875238/full#supplementary-material
inflammation in inflammatory bowel diseases. Gut 62, 531–539. doi: 10.1136/gutjnl-2012-302578

Dykhuizen, R. S., Masson, J., Mc Knight, G., Mowat, A. N., Smith, C. C., Smith, L. M., et al. (1996). Plasma nitrate concentration in infective gastrointestinal and inflammatory bowel disease. Gut 39, 393–395. doi: 10.1136/gut.39.3.393

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998. doi: 10.1038/nmeth.2604

Ernst, J., and Bar-Joseph, Z. (2006). STEM: a tool for the analysis of short time series gene expression data. BMC Bioinformatics 7:191. doi: 10.1186/1471-2105-7-191

Franké, L., el Bannoudi, H., Jansen, D. T. S. L., Kok, K., Trynka, G., Diogo, D., et al. (2016). Association analysis of copy numbers of FC gamma receptor genes for rheumatoid arthritis and other immune-mediated phenotypes. Eur. J. Hum. Genet. 24, 263–270. doi: 10.1038/ejhg.2015.95

Gadala, R. M., Garcia-Irigoyen, O., Cariello, M., Scialpi, N., Perez, C., Vetrano, S., et al. (2020). Fibroblast growth factor 19 modulates intestinal microbiota and inflammation in presence of Farnesoid X receptor. ElBioMedicine 54:102719. doi: 10.1016/j.ebiom.2020.102719

Ghavami, S. B., Yadegar, A., Aghdaei, H. A., Sorrentino, D., Farmani, M., Mir, A. S., et al. (2020). Immunomodulation and generation of tolerogenic dendritic cells by probiotic bacteria in patients with inflammatory bowel disease. Int. J. Mol. Sci. 21:6266. doi: 10.3390/ijms2117266

Gomollón, F., Dignass, A., Annese, V., Tálg, H., Van Assche, G., Lindsay, J. O., et al. (2017). 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: part 1: diagnosis and medical management. J. Crohns Colitis 11, 3–25. doi: 10.1093/ecco-jcc/jw168

Goyal, A., Yeh, A., Bush, B. R., Firek, B. A., Siebold, L. M., Rogers, M. B., et al. (2018). Safety, clinical response, and microbiome findings following fecal microbiota transplant in children with inflammatory bowel disease. Inflammm. Bowel Dis. 24, 410–421. doi: 10.1093/ibd/ixz035

Grootjans, J., Krupka, H., Hosomi, S., Matute, J. D., Hanley, T., Savejova, S., et al. (2019). Epithelial endoplasmic reticulum stress orchestrates a protective Igα response. Science 363, 993–998. doi: 10.1126/science.aat7186

Hartvarson, J., Brislawn, C. J., Lamendella, R., Vázquez-Baeza, Y., Hanley, T., Saveljeva, S., et al. (2016). Long-term degradation. Nature 54:102719.

Howell, K. J., Kraizcy, J., Nakay, K. M., Gasparett, M., Ross, A., Lee, C., et al. (2018). DNA methylation and transcription patterns in intestinal epithelial cells from pediatric patients with inflammatory bowel diseases differentiate disease subtypes and associate with outcome. Gastroenterology 154, 585–598. doi: 10.1053/j.gastro.2017.10.007

Inflammatory Bowel Disease Group, Chinese Society of Gastroenterology, Chinese Medical Association (2021). Chinese consensus on diagnosis and treatment in inflammatory bowel disease (2018, Beijing). J. Dig. Dis. 22, 299–317. doi: 10.1111/1751-2980.12994

Jadert, C., Pullipson, M., Holm, L., Lundberg, J. O., and Borniquel, S. (2014). Interactions between gut bacteria and bile in health and disease. Mol. Asp. Med. 56, 54–65. doi: 10.1016/j.mam.2017.06.002

Liu, Y., Yin, F., Huang, L., Teng, H., Shen, T., and Qin, H. (2021). Long-term and continuous administration of during remission effectively maintains the remission of inflammatory bowel disease by protecting intestinal integrity, regulating epithelial proliferation, and reshaping microbial function. Food Funct. 12, 2201–2210. doi: 10.1039/d0fo02786c

Lloyd-Price, J., Arze, C., Ananthakrishnan, A. N., Schirmer, M., Avila-Pacheco, J., Poon, T. W., et al. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature 569, 655–662. doi: 10.1038/s41586-019-1237-9

Long, S. L., Gahan, C. G. M., and Joyce, S. A. (2017). Interactions between gut bacteria and bile in health and disease. Mol. Asp. Med. 56, 54–65. doi: 10.1016/j.mam.2017.06.002

Louca, S., Parfrey, L. W., and Doeble, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. Science 353, 1272–1277. doi: 10.1126/science.aaf4507

Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550. doi: 10.1186/s13059-014-0550-8

Magoč, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. doi: 10.1093/bioinformatics/btr507

Mastrobuono-Ishimoto, Y., Shono, Y., Gomez, L. E., Hubbard-Lucey, V. M., Cammer, M., Neil, J., et al. (2017). Autophagy protein ATG16L1 prevents necroptosis in the intestinal epithelium. J. Exp. Med. 214, 3687–3705. doi: 10.1084/jem.20170558

Mirsepasi-Lauridsen, H. C., Vallance, B. A., Krogfelt, K. A., and Petersen, A. M. (2019). Pathobiotics associated with inflammatory bowel disease. Clin. Microbiol. Rev. 32, e00606–e00618. doi: 10.1128/CMR.00606-18

Moretti, J., Roy, S., Bozec, D., Martínez, J., Chapman, J. R., Ueberheide, B., et al. (2017). STING senses microbial viability to orchestrate stress-mediated autophagy of the endoplasmic reticulum. Cell 171, 809–823.e13. doi: 10.1016/j.cell.2017.09.034

Morgan, X. C., Kabakchiev, B., Waldron, L., Tyler, A. D., Frangeul, L., et al. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 55, 205–211. doi: 10.1136/gut.2005.073817

Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Nakamura, K., et al. (2004). Autophagy defends cells against invading group A Streptococcus. Nat. Rev. Immunol. 10.1038/s41575-019-0258-z

Nguyen, H. T. T., Lapasquete, P., Bringer, M.-A., and Darfeuille-Michaud, A. (2013). Autophagy and Crohn's disease. J. Innate Immun. 5, 434–443. doi: 10.1159/000345129

Nighot, P. K., Hu, C.-A. A., and Ma, T. Y. (2015). Autophagy enhances intestinal epithelial tight junction barrier function by targeting claudin-2 protein degradation. J. Biol. Chem. 290, 7234–7246. doi: 10.1074/jbc.M114.597492
## GLOSSARY

| Gene         | Description                                      |
|--------------|--------------------------------------------------|
| AKR1C2       | Aldo-keto reductase family 1 member C2           |
| AKT2         | AKT serine/threonine kinase 2                   |
| AT6          | Activating transcription factor 6               |
| ATG16L1      | Autophagy related 16 like 1                     |
| ATG4A        | Autophagy related 4A cysteine peptidase         |
| ATG5         | Autophagy related 5                             |
| ATG9A        | Autophagy related 9A                            |
| BAG6         | BAG cochaperone 6                               |
| BBC3         | BCL2 binding component 3                        |
| BCL2L11      | BCL2 like 11                                    |
| Becn1        | Beclin 1                                         |
| BNIP3P       | BCL2 interacting protein 3 pseudogene 1         |
| Casp17P      | Caspase 17, pseudogene                          |
| CD           | Crohn's disease                                 |
| CHAC1        | ChAc glutathione specific gamma-glutamycyclotransferase 1 |
| CHMP2A       | Charged multivesicular body protein 2A          |
| CHMP3        | Charged multivesicular body protein 3           |
| CHMP4A       | Charged multivesicular body protein 4A          |
| CYP4A1       | Cytochrome P450 family 46 subfamily A member 1 |
| DNAJB9       | DnaJ heat shock protein family (hsp40) member B9 |
| EIF2S1       | Eukaryotic translation initiation factor 2 subunit alpha |
| ER           | Endoplasmic reticulum                           |
| ERp27        | Endoplasmic reticulum protein 27                |
| FBXO15       | Fatty acid binding protein 1                    |
| FAPROTAX     | Functional annotation of prokaryotic taxa       |
| FCGR2B       | Fc gamma receptor IIIb                          |
| FGFR4        | Fibroblast growth factor receptor 4            |
| HERPUD1      | Homocysteine inducible ER protein with ubiquitin like domain 1 |
| HSPA1A       | Heat shock protein 90 alpha family class A member 1 |
| HSPA1B       | Heat shock protein 90 beta family member 2      |
| HSPA5        | Heat shock protein family A (hsp70) member 1A    |
| IB1          | Heat shock protein family A (hsp70) member 5     |
| IBD          | Inflammatory bowel disease                      |
| IFI16        | Interferon gamma inducible protein 16           |
| IL10         | Interleukin 10                                  |
| KEGG         | Kyoto encyclopedia of genes and genomes         |
| KIT          | KIT proto-oncogene, receptor tyrosine kinase    |
| LEP          | Leptin                                           |
| MANF         | Mesencephalic astrocyte derived neurotrophic factor |
| MAP3K5       | Mitogen-activated protein kinase kinase kinase 5 |
| MAPK1        | Mitogen-activated protein kinase kinase 1       |
| MBTPS1       | Membrane bound transcription factor peptidase, site 1 |
| NCOA2        | Nuclear receptor coactivator 2                  |
| NHLRC1       | NHL repeat containing E3 ubiquitin protein ligase 1 |
| NRAS         | NRAS proto-oncogene, GTPase                     |
| OSBPL7       | Oxysterol binding protein like 7                |
| PDIASP1      | Protein disulfide isomerase family A member 3 pseudogene 1 |
| PIK3C3       | Phosphatidylinositol-3-kinase catalytic subunit type 3 |
| PIK3CA       | Phosphatidylinositol-4,5-bisphosphate 3-catalytic subunit alpha |
| PLA2G1B      | Phospholipase A2 group IB                       |
| PMAIP1       | Phorbol-12-myristate-13-acetate-induced protein 1 |
| RNF41        | Ring finger protein 41                          |
| RXRA         | Retinoid X receptor alpha                       |
| STAT3        | Signal transducer and activator of transcription 3 |
| STC2         | Stanniocalcin 2                                 |
| STING        | Stimulator of interferon response CGAMP interactor 1 |
| SULT2A1      | Sulfotransferase family 2A member 1             |
| TMEM59       | Transmembrane protein 59                        |
| TOMM20       | Translocase Of outer mitochondrial membrane 20   |
| TP53         | Tumor protein P53                                |
| TXNDC12      | Thioredoxin domain containing 12                |
| UC           | Ulcerative colitis                              |
| VCP          | Valosin containing protein                      |
| VPS25        | Vacular protein sorting 25 homolog             |
| VPS4A        | Vacular protein sorting 4 homolog A            |
| WFS1         | Wolframin ER transmembrane glycoprotein        |