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ORIGINAL ARTICLE

Basic Study

Combined metabolomic and metagenomic analysis reveals inflammatory bowel disease diversity in pediatric and adult patients

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Abstract

BACKGROUND

The gut microbiota displays pronounced compositional differences between pediatric and adult populations, both under normal conditions and during the development of inflammatory bowel disease (IBD). These structural variations are accompanied by substantial changes in microbial metabolic activity.

AIM

To identify novel early diagnostic biomarkers of IBD, we performed an integrated multi-omics analysis that included assessing microbial community structure and profiling microbial metabolic activity in pediatric and adult cohorts with ulcerative colitis (UC) and Crohn's disease (CD).

METHODS

The study cohort consisted of two distinct age groups with confirmed IBD diagnoses: Adult patients (aged 45 to 70) and pediatric patients (aged 5 to 15), each diagnosed with either CD or UC. 16S rRNA gene sequencing was performed using the MinION™ Mk1B platform, with data acquisition carried out *via* MinKNOW software version 22.12.7 (Oxford Nanopore Technologies). Stool samples were analyzed using a Shimadzu QP2010 Ultra GC/MS system equipped with a Shimadzu HS-20 headspace extractor.

RESULTS

Comparative analysis revealed significant age-related differences in the abundance of *Bacteroidota*, with pediatric IBD patients showing a lower prevalence compared to adults. Microbial profiling identified *Streptococcus salivarius* and *Escherichia coli* as potential biomarkers for assessing IBD risk in children. Furthermore, metagenomic analysis uncovered five microbial signatures with diagnostic potential for CD: *Ralstonia insidiosa*, *Stenotrophomonas maltophilia*, *Erysipelatoclostridium ramosum*, *Blautia spp.*, and *Coprococcus comes*. Using comprehensive metabolomic profiling, we developed and validated novel risk prediction algorithms for pediatric IBD. The CD risk stratification model identifies high-risk patients based on two key biomarkers: An elevated IBD risk coefficient score and reduced levels of 1H-indole-3-methyl. The UC risk prediction model incorporates three metabolic biomarkers indicative of increased disease risk: An elevated risk coefficient score, increased acetate levels, decreased pentanoic acid, and altered excretion of p-cresol (4-methylphenol).

CONCLUSION

Functional metabolomics holds transformative potential for IBD diagnostics across all age groups, with especially significant implications for pediatric patients. The distinct metabolic and metagenetic profiles observed in the pediatric cohort may represent primary alterations in IBD, providing valuable insights for exploring novel mechanisms underlying disease pathogenesis.

Key Words: Headspace-gas chromatography-mass spectrometry; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Metagenomics; Pediatric and adult patients

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Core Tip: Functional metabolomics combines the ability to simultaneously assess the diversity of the microbiome and evaluate its secretory activity in the development of pathologies. In this study, a diagnostic rule was developed for children with inflammatory bowel disease that enables calculation of the risk of disease development. This rule, based on a set of metabolic and metagenomic markers, also helps clarify the specific type of pathology. The combined use of metabolomic and metagenomic analysis will facilitate prompt assessment of the potential risks for developing inflammatory bowel disease in the future and support timely initiation of appropriate therapy.

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INTRODUCTION

Inflammatory bowel diseases (IBD) represent a major global gastroenterological challenge[1-4]. Within the IBD spectrum, two predominant forms account for most clinical presentations: Crohn's disease (CD) and ulcerative colitis (UC)[5-7]. As chronic, relapsing-remitting disorders, IBDs are fundamentally characterized by persistent mucosal inflammation[8]. IBD pathogenesis involves complex interactions between multiple biological systems that drive intestinal mucosal inflammation, including dysregulated immune responses and genetic predisposition[9-11]. Many theories have been developed about the trigger mechanism of IBD, but the main problem of untimely diagnosis still exists because, in most cases, the IBD can be asymptomatic for a long time[12].

While UC and CD share overlapping histopathological features, the age of onset represents a critical differentiating factor with distinct clinical implications. CD is typically developed in adolescence, although it also occurs in infants compared to the UC[13]. The CD symptoms, which are divided into intestinal and extraintestinal, depend on the severity and its specific location[14]. In children, CD can also cause delays in physical development and sexual maturation[15]. Early onset and lack of pronounced symptoms provoke long-term diagnosis and untimely treatment. In up to 10% of pediatric cases, the distinction between CD and UC is difficult, or the patient is being monitored for a long time with a diagnosis of IBD-unclassified[16]. As the disease progresses, some of these IBD unclassified cases later develop into either CD or UC. Thus, it is important to identify early markers of the development of IBD. On the other hand, it is necessary to investigate new molecular markers of the CD and UC in pediatric and adult patients for a more detailed understanding of the fundamental principles of IBD pathogenesis[17].

While genetic predisposition (*e.g.*, nucleotide-binding oligomerization domain 2, autophagy-related protein 16-like 1 variants) and autoimmune dysregulation (Th1/Th17 responses) contribute substantially to CD pathogenesis, the gut microbiota plays an equally critical and mechanistically distinct role[18]. The gut microbiota exhibits remarkable responsiveness to intestinal microenvironmental changes through dynamic adaptations and functional alterations[19]. Among the main components, which are actively produced by intestinal bacteria, it is possible to identify short-chain fatty acids (SCFAs), vitamins, essential amino acids, components of biotransformation and conjugation of bile acid salts, substances that promote intestinal motility and stimulate intestinal angiogenesis[20-23]. The gut microbial metabolome exhibits profound compositional shifts between homeostasis and IBD, yielding clinically actionable diagnostic signatures[24].

Contemporary multi-omics research has established distinct low-molecular signatures for IBD subtypes. Decreased fecal butyrate, elevated serum kynurenine/tryptophan ratio, and a unique bile acid profile have been observed for CD patients. Increased urinary N-methylhistamine, fecal prostaglandin E2, and plasma lysoPC (16:0) reduction (Area under the curve = 0.89) have been observed in UC patients' cohorts[25-27]. Strong evidence links IBD pathogenesis to SCFA depletion were also identified[28]. The altered secretory profile in IBD represents a functional consequence of microbial community restructuring.

Functional metabolomics provides a powerful integrative framework for IBD research by simultaneously characterizing microbial community dynamics and bacterial metabolic output profiling[29]. Volatile organic compound (VOC) profiling represents a transformative approach for evaluating microbial metabolic activity[30]. The gas chromatography-mass spectrometry method is used to analyze SCFA, medium-chain and long-chain fatty acids, acids with a phenyl radical, aldehydes, and heterocycles[31]. The vapor-phase extraction method realized by headspace-gas chromatography-mass spectrometry (HS-GC-MS) makes it possible to compare various biological samples, including feces, since the ratios of components in an equilibrium vapor do not depend on the amount of water contained in the samples[32,33]. In this study, we performed a detailed metabolomic analysis of IBD urine samples. We employed a headspace extractor in combination with a gas chromatograph-mass spectrometer. This approach enables the analysis of VOCs rather than the complete metabolite profile of urine. Therefore, investigating VOCs transferred into the vapor phase represents a promising method for studying low-molecular-weight compounds.

MATERIALS AND METHODS

Subjects

Pediatric patients (age range 5-15) with IBD were recruited from the Department of Propaedeutics of Children's Diseases of the N.F. Filatov Clinical Institute of Children's Health, I.M. Sechenov First Moscow State Medical University, Russia. Adult patients (age range 45 to 70) were recruited from the Department of Gastroenterology of the M.F. Vladimirovskiy Moscow Regional Research and Clinical Institute, Russia. All samples were collected between September 2024 to December 2024. Stool samples were collected from all pediatric and adult patients upon admission to the clinic during disease exacerbation. The diagnosis of CD and UC was based on conventional clinical, radiological, and endoscopic features and was finally confirmed by histological examination of intestinal biopsies[34]. The study included an adult IBD group ($n = 34$), a pediatric IBD group ($n = 66$), and a healthy control group comprising both adults and children ($n = 49$). The main criteria for inclusion in the healthy control group were no history of IBD, no recent antibacterial therapy, and no special dietary restrictions. Clinical characteristics of patients with IBD, including indices of disease activity and medication, are shown in [Supplementary Table 1](#).

Ethics statement

All experimental procedures were approved by the Local Ethical Committee of Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russian Federation, Russia. Informed consent was obtained from all subjects.

HS-GC-MS

50-100 mg stool samples plus 500 μ L water samples were placed into 10 mL screw-cap vials for a Shimadzu HS-20 headspace extractor. 0.2 g of a mixture of salts (ammonium sulphate and potassium dihydrogen phosphate in a ratio of 4:1) was added to increase the ionic strength of the solution. Headspace extractor settings used: Oven temperature 80 °C, sample line temperature 220 °C, transfer line temperature 220 °C, equilibrating time 15 minutes, pressurizing time 2 minutes, load time 0.5 minutes, injection time 1 minute, needle flush time 7 minutes. The vials were sealed and analysed on a Shimadzu QP2010 Ultra GC/MS with a Shimadzu HS-20 headspace extractor, a VF-WAXMS column with a length of 30 m, a diameter of 0.25 mm, and a phase thickness of 0.25 microns. Initial column temperature 80 °C, heating rate 20 °C/minute to 240 °C, exposure 20 minutes. Carrier gas - helium 99.9999, injection mode - splitless, flow rate 1 mL/minute. Ion source temperature -230 °C. Interface temperature 240 °C. The total ionic current monitoring mode was used. To analyse the obtained mass spectra, the National Institute of Standards and Technology 2014 mass spectra library with an automated mass spectral deconvolution and identification system [automated mass spectral deconvolution and identification system (AMDIS) version 2.72] was used. Details of the reagents are provided in [Supplementary Table 2-1](#).

Metabolome data processing

The HS-GC-MS data processing was carried out as follows: Peak areas for selected compounds, calculated using AMDIS, were converted into relative abundance values. The percentage of volatile compounds in each sample was estimated by summing the percentages of compounds confidently identified in the AMDIS database. These sums were then recalculated as a proportion of the total confidently identified compounds to minimize inaccuracies caused by noise and unreliable matrix signals. Subsequent data analysis was performed using MetaboAnalyst 5.0 (Available from: <http://www.metaboanalyst.ca>) and GraphPad Prism 8.0.1 software. Values obtained for each patient were treated as paired and consistent, with outliers identified using the ROUT test ($Q = 1\%$). Since normality could not be confirmed for all sample groups, the raw data were assumed to be non-normally distributed. Therefore, the non-parametric Mann-Whitney test was applied for initial group comparisons. After applying natural logarithm transformation and normalization, data were further examined using standard *t*-tests and analysis of variance (ANOVA). Statistical significance was defined as a two-tailed *P*-value below 0.05. To reduce dimensionality and further investigate the dataset, unsupervised principal component analysis was conducted following standard preprocessing steps. A χ^2 test guided the choice of imputation method for missing values, revealing that the data met the missing at random (MAR) assumption. Based on this, the Bayesian principal component analysis (BPCA) approach was selected as the optimal imputation technique. Data were then normalized and scaled accordingly. Finally, relationships between metabolites and the composition of gut microbiota were explored using Spearman's rank correlation coefficient, calculated with the R "psych" package.

DNA extraction

Fecal samples served as the source for total DNA extraction. Nucleic acids were isolated utilizing the MagicPure® Stool and Soil Genomic DNA Kit in conjunction with the Kingfisher Flex Purification System (Thermo Fisher Scientific, MA, United States), following the manufacturer's instructions. The extracted DNA was subsequently quantified using the Quant-iT dsDNA BR Assay Kit (Thermo Fisher Scientific, MA, United States) on a Qubit 4 fluorometer.

16S rRNA gene sequencing on the MinION™ platform: Between 1 and 5 ng of the extracted DNA was amplified using primers 27F (AGAGTTGATYMTGGCTAG) and 1492R (GGTTACCTTGTTAYGACTT) (Eurogen, Novosibirsk, Russia) with the Tersus Plus polymerase chain reaction (PCR) Kit (Eurogen, Novosibirsk, Russia) in a total reaction volume of 25 μ L. The PCR was carried out under the following conditions: An initial denaturation at 95 °C for 2 minutes; followed by 27 cycles of denaturation at 95 °C for 1 minute, annealing at 60 °C for 1 minute, and extension at 72 °C for 3 minutes; concluded with a final extension at 72 °C for 2 minutes and cooling at 4 °C. Amplification products were verified by electrophoresis on a 1.5% agarose gel. The resulting amplicons were purified using KAPA HyperPure Beads (Roche, Basel,

Switzerland) according to the manufacturer's instructions.

Libraries were prepared according to the manufacturer's protocol (ligation sequencing amplicons) with modifications. Amplicons were processed using the NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB). Barcodes (Native Barcoding Kit 96 (SQK-NBD109.96)) were ligated using Blunt/TA Ligase Master Mix (NEB). Barcoded libraries were purified using KAPA Pure Beads (Roche, Switzerland). Library concentrations were measured using the Quant-iT dsDNA Assay Kit, High Sensitivity (Thermo Fisher Scientific, MA, United States), and samples were mixed at equimolar concentrations. The final adapter [Adapter Mix II Expansion (Oxford Nanopore Technologies, Oxford, United Kingdom)] was ligated to the pooled library using the NEB Next Quick Ligation Module (NEB). The prepared DNA library (12 µL) was mixed with 37.5 µL sequencing buffer, 25.5 µL loading beads, and loaded onto the R10.4.1 flow cell (FLO-MIN114; Oxford Nanopore Technologies) and sequenced using the MinION™ Mk1B. MinKNOW software ver. 24.06.14 (Oxford Nanopore Technologies) was used for data acquisition.

Genome data processing

Technical sequences and bases with a quality lower than a Phred score of 9 were processed using Porechop and NanoFilt software[35,36]. The resulting data were evaluated using the Emu pipeline for taxonomic classification[37]. Alpha and beta diversity analyses were performed using the vegan package for GNU/R[38]. Alpha and beta diversity were assessed using the Bioconductor Microbiota Process package for GNU/R. Heatmap visualization was performed using the "pheatmap" package for GNU/R. Alpha diversity studies were performed using the Microbiota Process package[39]. To identify taxa with statistically significant differences in representation between comparison groups, the Maaslin2 v.1.22.0 {with the following thresholds: Minimum abundance = 0.01, minimum prevalence = 0.25, max significance [false discovery rate (FDR)] = 0.05} and LEfSe v.1.1.2 tools were utilized.

RESULTS

Metabolomic profiles of IBD in pediatric and adult patients

An HS GC-MS analysis of the volatile compounds was performed for all experimental groups. Raw metabolome data are available in [Supplementary Table 2-2](#). A minimum of 60% of the stable compounds identified were utilized for the metabolomic profile comparison. The most frequently observed compounds that met the screening criteria are presented in [Table 1](#). Among the identified compounds, SCFAs, medium- and long-chain fatty acids, and amino acid derivatives were observed. This investigation employed comprehensive volatile metabolomic profiling to characterize pediatric IBDs, with particular focus on CD. At the first stage, a verification metabolomic analysis was carried out, which allowed for identification of the spectrum of compounds in pediatric and adult IBD groups compared to the control. Based on the data obtained, the profile of all detectable ([Figure 1A and B](#)) and stably detectable ([Figure 1C and D](#)) metabolites revealed in the IBD group was significantly different from that of the control group. The same result was obtained in the IBD adult group, either for all ([Supplementary Figure 1A and B](#)) or for stable metabolites ([Supplementary Figure 1C and D](#)). The formation of metabolome profile diversity in the pediatric IBD group indicates significant disturbances in the intestinal microbiota of patients with UC and CD. Patients with IBD typically have reduced microbiota diversity, which should result in a reduction in the number of identified metabolites.

Contrary to initial expectations, comprehensive metabolomic profiling reveals paradoxical patterns in pediatric IBD. The control group was characterized by a smaller number of diversely represented volatile metabolites than the IBD group ([Figure 1E](#)) compared to the adult patients ([Supplementary Figure 1E](#)). A wide variety of components in patients with IBD compared to the control group may be associated with the therapy being taken, as well as the presence of excessive bacterial growth, which provokes the secretion of a number of volatile components. In contrast to pediatric patients, adults with chronic IBD demonstrate a more stable metabolomic profile, reflecting long-term adaptation to disease.

To delineate disease-specific metabolic signatures, we conducted a comprehensive comparative analysis of UC and CD groups. The principal component analysis and orthogonal projection to latent structures methods were used for metabolite profiles of the CD, UC, and control groups for comparison. As a result, we identified three separate profiles of volatile metabolites for all detected ([Figure 2A and B](#)) and stable detected metabolites ([Figure 2C and D](#)). As expected, the same result was obtained for adult patients for all detected ([Supplementary Figure 2A and B](#)) and for stable detected metabolites ([Supplementary Figure 2C and D](#)). The significant metabolomic differences between UC and CD strongly suggest corresponding variations in their underlying microbial ecosystems. Therefore, the heatmap data allow us to identify the entire spectrum of detectable metabolites, where pathologies are characterized by their own profile of volatile compounds for pediatric ([Figure 2E](#)) and for adult patients ([Supplementary Figure 2E](#)). Moreover, comparative analysis allows us to identify a decrease in the number of detectable metabolites in CD compared to the UC in children. Metabolomic profiling identified treatment-derived xenobiotic metabolites that significantly contribute to the observed metabolic signatures ([Figure 2E](#)). However, when assessing stably detectable metabolites, the UC and CD groups significantly differed from the control group.

Thus, assessing the overall spectrum of detectable metabolites in both groups, it is possible to assess the therapy being carried out and the characteristics of the patients' diet. Whereas the stably detected metabolites, which are the main metabolites secreted by the microbiota, undergo significant changes in the IBD group ([Figure 3A and B](#)). The heatmap indicates a significant spectrum of detectable metabolites in the pediatric group, which are not observed in the adult patients' group. Based on this comparison, it becomes obvious that pediatric IBD differs significantly from adult IBD ([Figure 3C](#)).

Table 1 Complete list of stably detected metabolites in four groups of stool samples. Control kids - control group, Crohn's disease, ulcerative colitis, all kids - inflammatory bowel disease group (Crohn's disease + ulcerative colitis)

Name	Control kids	CD kids	UC kids	All kids
Acetic acid	100.00	100.00	100.00	100.00
Propanoic acid, 2-methyl	100.00	100.00	100.00	100.00
Butanoic acid	100.00	100.00	100.00	100.00
Pentanoic acid	100.00	100.00	100.00	100.00
Propanoic acid	100.00	97.14	100.00	99.02
Hexanoic acid	96.97	100.00	100.00	99.02
Butanoic acid, 3-methyl	100.00	100.00	97.06	99.02
Benzeneacetic acid	100.00	100.00	91.18	97.06
n-Hexadecanoic acid	100.00	91.43	100.00	97.06
Phenol,2,4-bis(1,1-dimethylethyl)	81.82	94.29	97.06	91.18
Benzaldehyde	93.94	77.14	94.12	88.24
Indole	100.00	82.86	82.35	88.24
Butanoic acid, 2-methyl	96.97	88.57	79.41	88.24
Phenol, 4-methyl	100.00	74.29	73.53	82.35
Benzoic acid	90.91	71.43	82.35	81.37
Octanoic acid	72.73	71.43	76.47	73.53
Nonanoic acid	69.70	85.71	64.71	73.53
Tetradecanoic acid	96.97	48.57	64.71	69.61
Heptanoic acid	72.73	68.57	50.00	63.73
Benzenepropanoic acid	66.67	54.29	70.59	63.73

UC: Ulcerative colitis; CD: Crohn's disease.

In particular, compounds such as indole, pentanoic acid, phenol, 4-methyl, and 1H-indol-3-methyl characterize the pediatric IBD group (Figure 3D). Indole, pentanoic acid, phenol, 4-methyl, and acetic acid discriminate UC and control groups, while 1H-Indol, 3-methyl can be used for CD diagnostics in the pediatric group (Figure 3D). The main metabolome differences detected for the adult IBD and control group were the following: Phenol,2,4-bis(1,1-dimethylethyl), indole, pentanoic acid, N-hexadecanoic acid, and tetradecanoic acid (Supplementary Figure 3A and B). Metabolites such as hexadecane, indole, pentanoic acid, and phenol,2,4-bis(1,1-dimethylethyl), determined the difference between patients with UC and the control group. On the other hand, metabolites such as N-hexadecanoic acid and phenol determined the difference between patients with CD and the control group in adults (Supplementary Figure 3C and D).

CD and UC are long-term diseases, while the onset of CD is in childhood. However, long-term disease leaves an indelible mark on the microbiota diversity and its secretory activity. The overall spectrum of metabolites detected in pediatric and adult IBD groups was different, as expected (Figure 4A-C). The following metabolites were found to be different in two age variants of IBD: N-hexadecanoic acid and phenol,2,4-bis(1,1-dimethylethyl) (Figure 4D). The onset of CD occurs in childhood, so special attention was paid to comparing CD patients of different ages. Based on metabolomic profiling data, pediatric CD differs from adult CD and forms a separate metabolite profile (Figure 5A).

According to the data obtained, pediatric patients had a more consolidated metabolome spectrum than the adult group. Metabolomic profile is more homogeneous in pediatric patients compared to the adult group with CD. It may indicate that there is a special mechanism of CD development compared to the UC that determines its early onset. Whereas CD in adult patients may be caused not only by endogenous but also by exogenous factors, which dramatically change microbiota. In addition to the visual differences identified in the consolidation of the children's group with CD, it is also possible to identify the spectrum of metabolites that significantly distinguish the two age groups from each other (Figure 5B and C). The obtained difference in the metabolomic profiles of CD patients of different ages should be compared with metagenomic data to assess possible mechanisms of pathogenesis of CD. The metabolome difference between adults and children may be used for identifying familial cases.

In order to make a timely diagnosis, it is necessary to develop a universal method for assessing the risk of developing IBD in children based on metabolic profiling data. Calculating the risk coefficient for the IBD development in the pediatric group is one of the ways to translate relative metabolomic data to the clinical laboratory. Based on the data from the metabolomic profiling, a special algorithm was developed to calculate the risk coefficient for IBD in the pediatric group.

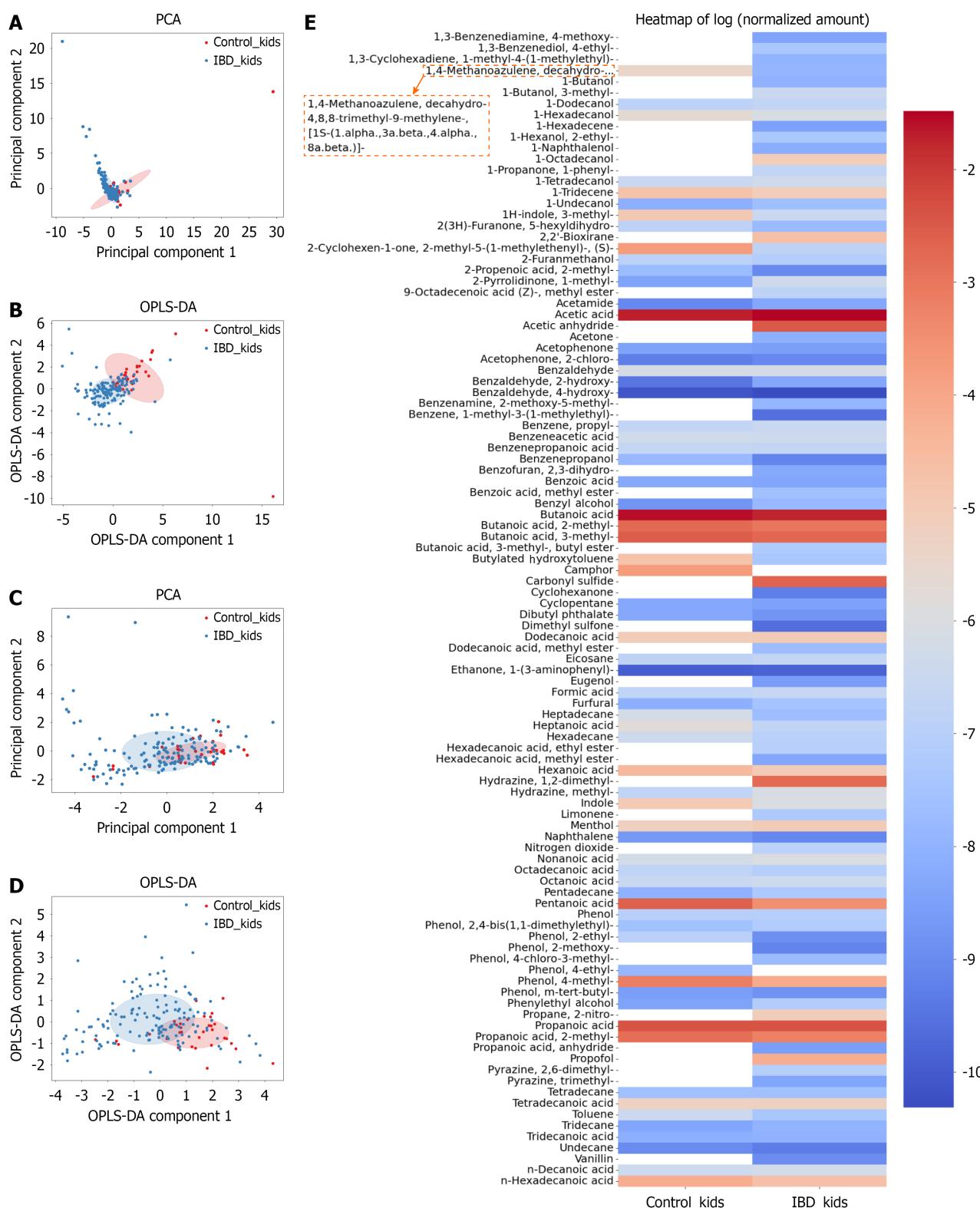


Figure 1 Total headspace-gas chromatography-mass spectrometry data obtained for inflammatory bowel disease and control groups of pediatric patients. A and B: Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis data represent two independent groups of samples [inflammatory bowel disease (IBD) kids and control kids] according to the relative concentration of all detected volatile compounds; C and D: Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis data represent two independent groups of samples (IBD kids and control kids) according to the relative concentration of stable detected volatile compounds; E: Comparison of volatile organic compound composition for two independent groups of samples (IBD kids and control kids). Relative concentrations in the vapor phase were used. PCA: Principal component analysis; IBD: Inflammatory bowel disease; OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis.

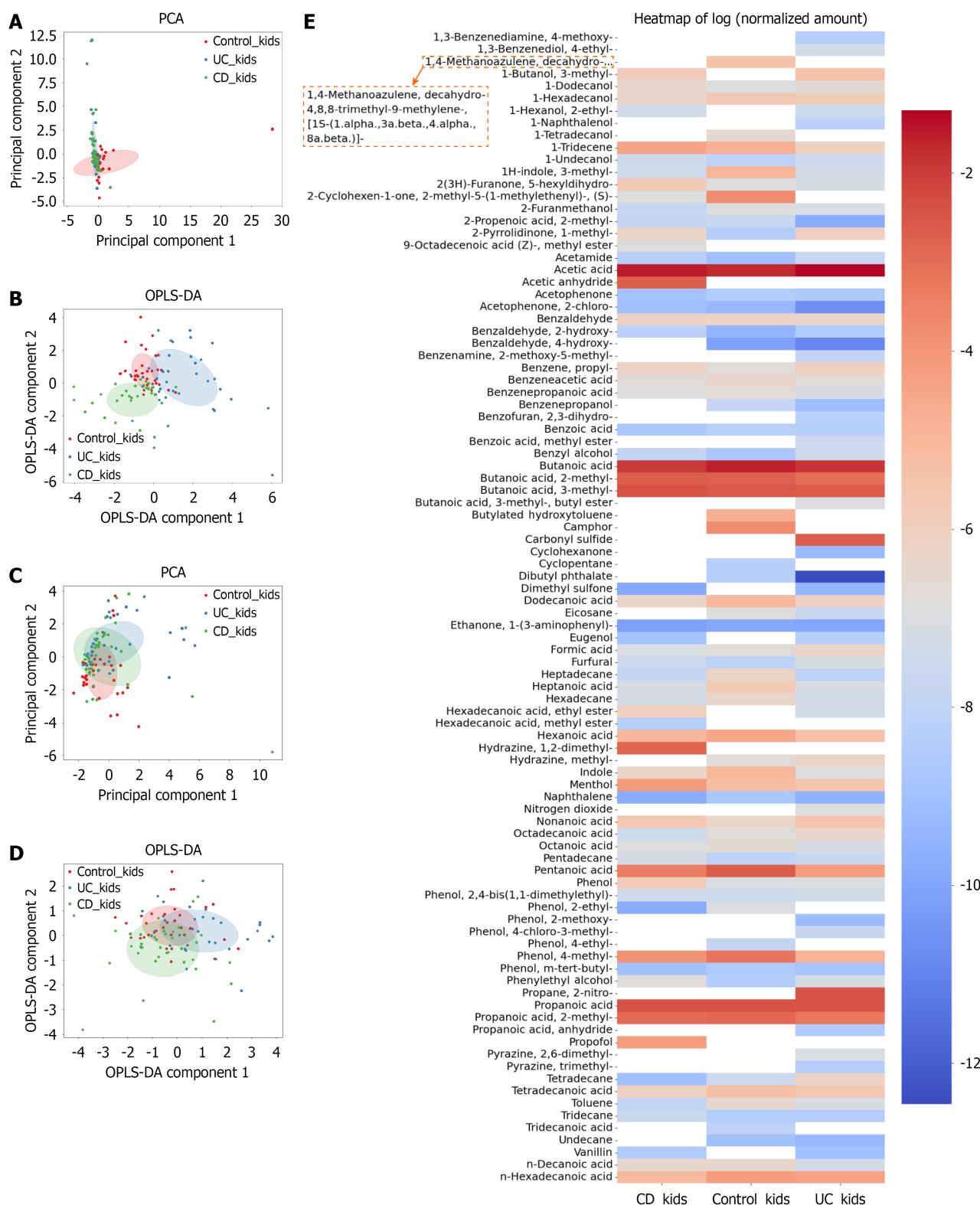


Figure 2 Total headspace-gas chromatography-mass spectrometry data obtained for ulcerative colitis and Crohn's disease and control groups of pediatric patients. A and B: Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis data represent three independent groups of samples [ulcerative colitis (UC) kids, Crohn's disease (CD)-kids, and control kids] according to the relative concentration of all detected volatile compounds; C and D: Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis data represent three independent groups of samples (UC kids, CD-kids, and control kids) according to the relative concentration of stable detected volatile compounds; E: Comparison of volatile organic compound composition for three independent groups of samples (UC kids, CD-kids, and control kids). Relative concentrations in the vapor phase were used. UC: Ulcerative colitis; CD: Crohn's disease; PCA: Principal component analysis; IBD: Inflammatory bowel disease; OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis.

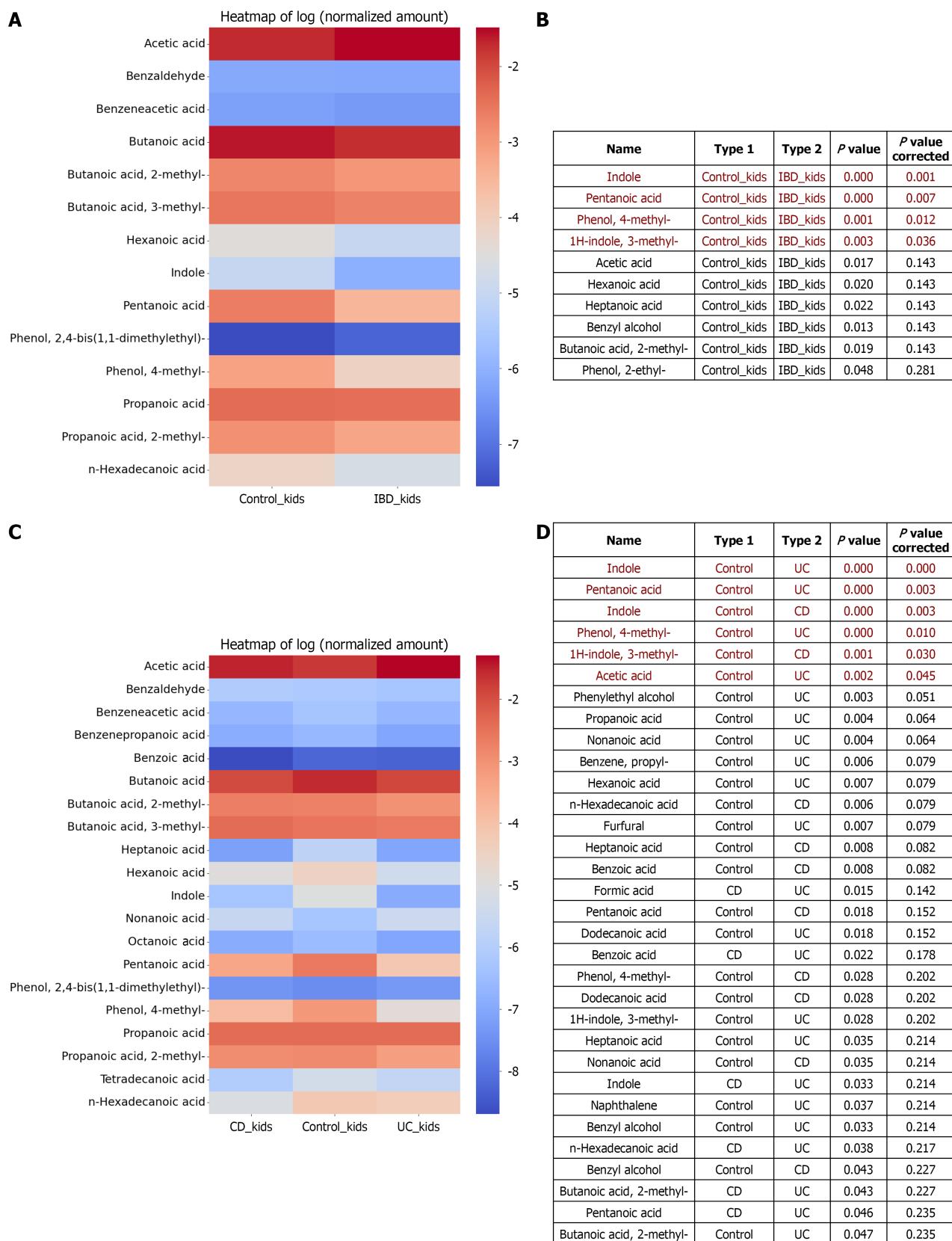


Figure 3 Total headspace-gas chromatography-mass spectrometry data obtained for ulcerative colitis and Crohn's disease and control groups of pediatric patients. A: Comparison of the volatile organic compound composition of stable detected metabolites for two independent groups of samples (inflammatory bowel disease kids and control kids). Relative concentrations in the vapor phase were used; B: The nonparametric Mann-Whitney test was used for the primary comparisons between groups, inflammatory bowel disease kids, and Control kids. Statistical significance was determined by a two-sided P-value of less than 0.05. False discovery rate correction was also applied; C: Comparison of the volatile organic compound composition of stable detected metabolites for three independent groups of samples (ulcerative colitis kids, Crohn's disease kids, and control kids). Relative concentrations in the vapor phase were used; D: The nonparametric Mann-Whitney test was used for the primary comparisons between groups: Ulcerative colitis kids, Crohn's disease kids, and control kids. Statistical

significance was determined by a two-sided *P*-value of less than 0.05. False discovery rate correction was also applied. UC: Ulcerative colitis; CD: Crohn's disease; IBD: Inflammatory bowel disease.

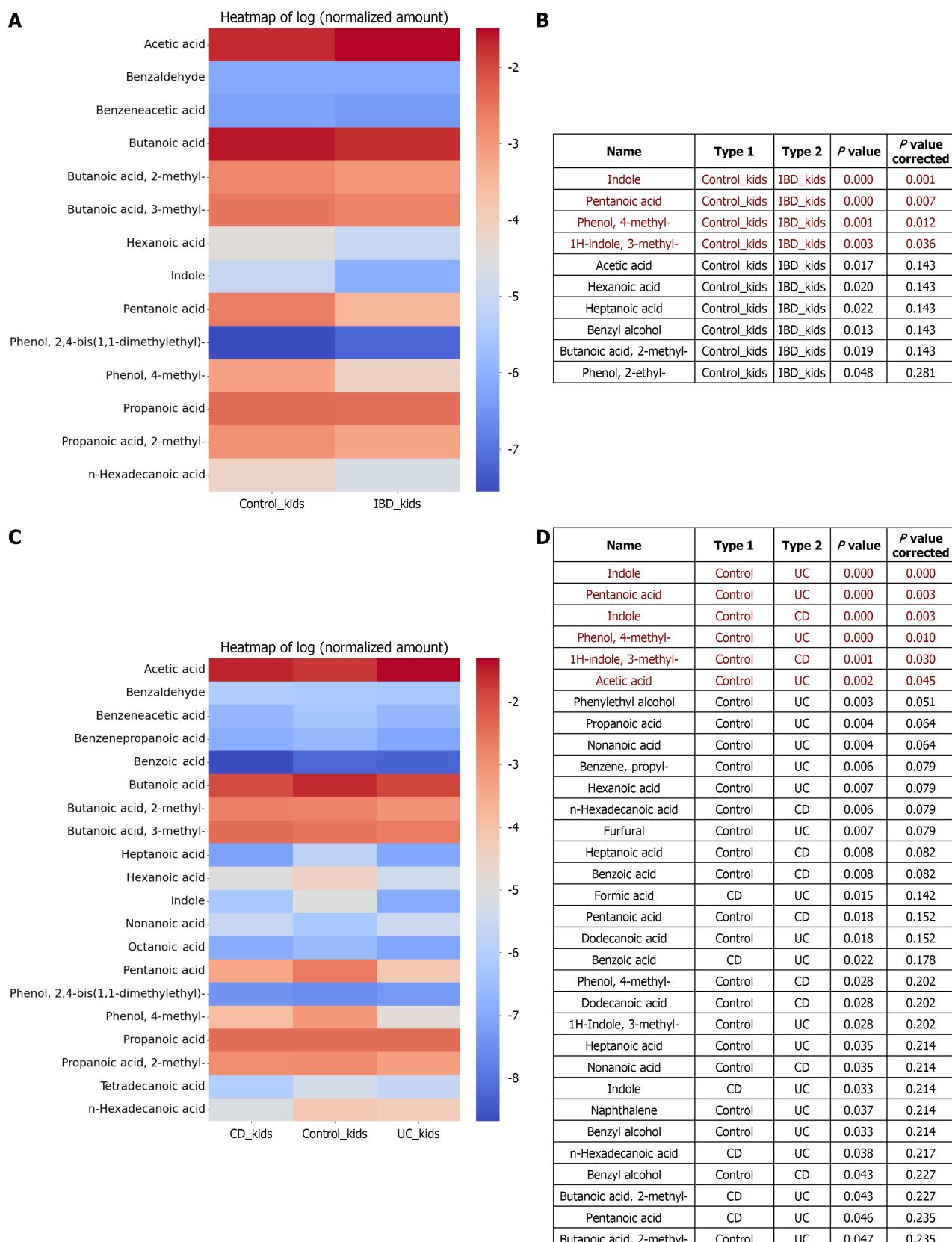


Figure 4 Total headspace-gas chromatography-mass spectrometry data obtained for the inflammatory bowel disease group of pediatric

and adult patients. A and B: Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis data represent two independent groups of samples [inflammatory bowel disease (IBD)-kids, IBD-adults] according to the relative concentration of all detected volatile compounds; C: Comparison of volatile organic compound composition for two independent groups of samples (IBD-kids, IBD-adults). Relative concentrations in the vapor phase were used; D: The nonparametric Mann-Whitney test was used for the primary comparisons between groups. Statistical significance was determined by a two-sided *P*-value of less than 0.05. False discovery rate correction was also applied. UC: Ulcerative colitis; CD: Crohn's disease; IBD: Inflammatory bowel disease.

The formula for the IBD risk included metabolites that, in combination, are significantly different in groups of patients with IBD *vs* controls. The main advantage of this testing is the possibility of non-invasive analysis of the secretory activity of the microbiota and frequent monitoring of the intestinal state as much as is required for timely diagnosis of IBD (**Figure 5D**). For statistically significant metabolites, biserial correlation was calculated with subsequent FDR correction; metabolites with an absolute correlation coefficient $|r| > 0.3$ were included in the formula: Positively correlated metabolites were placed in the numerator, negatively correlated ones in the denominator. The receiver operating characteristic analysis was then performed to determine risk coefficient thresholds: Values below the 5th percentile indicated absence of risk, while values above the 25th percentile indicated high risk. The upper cutoff threshold for outliers was determined using the interquartile range.

Metagenomic profiles of IBD in pediatric and adult patients

The metagenome study was performed for all experimental and control groups. Sequencing identified 554 microbial species belonging to 224 total genera in 71 experimental fecal samples of pediatric patients and 654 microbial species belonging to 267 total genera in 36 experimental fecal samples of adult patients. Sample metadata is presented in **Supplementary Table 3**. Raw data and sequencing statistics after quality control are represented in **Supplementary Table 4**. Relative species abundances and taxonomic tables are presented in additional materials (**Supplementary Table 5**). The distribution across microbial phyla is presented in **Figure 6**.

Microbiome analysis revealed significant α -diversity reductions in IBD patients *vs* healthy controls. Quantitative microbiome analysis demonstrated consistent Firmicutes depletion across all IBD groups (**Figure 6A** and **Supplementary Figure 4A**). The taxonomic composition of the children's gut microbiome shows significant differences between healthy and diseased children, based on alpha diversity expressed as Shannon coefficient and Bray-Curtis dissimilarity, indicating that clinical status determines and influences the overall community structure of the gut microbiome in children (**Figure 6B** and **C**). The same results were obtained for adult patients (**Supplementary Figure 4B** and **C**). Comparative microbiome analysis revealed significantly greater microbial diversity in pediatric *vs* adult IBD patients across multiple taxonomic levels (**Figure 6D** and **Supplementary Figure 4D**).

Metagenomic analysis of pediatric IBD patients revealed several key patterns. Metagenomic analysis revealed distinct phylum-level alterations in pediatric IBD subtypes, including Marked Proteobacteria expansion in the CD group and Significant Bacteroidetes depletion in the UC group. A significant predominance of *Bifidobacterium* was observed in both patients with UC and CD, which clearly distinguishes both pathologies from the control group. The *Escherichia* genus was also increased in the CD group compared to the controls and patients with UC. Multiple lines of evidence establish specific *Escherichia coli* strains as significant CD risk factors[40]. Significant enrichment of *Enterococcus* and *Lactobacillus* representatives was observed in the UC group (**Figure 6E** and **Supplementary Figure 4E**). It is possible that *Enterococcus* and *Lactobacillus* increased as a result of taking a probiotic. Another important feature is a significant decrease in representatives of the genus *Akkermansia* in patients with UC and CD. It is known that *Akkermansia spp.* have the ability to restrain autoimmune reactions and reduce proinflammatory cytokines[41].

By analyzing the quantitative representation of individual bacterial species, several metagenomic markers characteristic of CD and UC in children can be identified. Notably, there was a significant increase in the abundance of species such as *Ralstonia insidiosa*, *Stenotrophomonas maltophilia*, and *Erysipelatoclostridium ramosum*, while a decrease was observed in species like *Blautia* sp. and *Coprococcus comes* within the CD group among pediatric patients (**Figure 6F**). While *Ralstonia insidiosa* is known as an opportunistic pathogen - particularly in hospital settings - and has been associated with various infections, including those in immunocompromised patients, its direct role in IBD remains unclear[42]. Although IBD is linked to gut microbiome dysbiosis, *Ralstonia* species, including *Ralstonia insidiosa*, are not typically among the most studied or implicated bacteria in IBD pathogenesis. There is no direct evidence that *Ralstonia insidiosa* increases susceptibility to CD; however, both *Ralstonia insidiosa* and CD patients share a vulnerability to opportunistic infections due to compromised immune systems or disruptions of the body's natural barriers. *Stenotrophomonas maltophilia* is also considered an opportunistic pathogen, more likely to cause infections in individuals with weakened immune defenses or other health issues. It is known for its resistance to many commonly used antibiotics, which complicates treatment. Colonization with *Stenotrophomonas maltophilia* can worsen symptoms in IBD patients, potentially leading to increased inflammation, malabsorption, and other complications[43].

Eubacterium ramosum has been detected in both fecal and biopsy samples from IBD patients, particularly those with UC. It has also been identified as a cause of invasive infections in various tissues, especially in immunocompromised individuals, highlighting its potential to cause complications beyond the gut. This bacterium produces an enzyme capable of degrading immunoglobulin A, a crucial antibody in the mucosal lining of the gut. This activity may impair the immune system's ability to combat other harmful bacteria or pathogens, potentially worsening inflammation in IBD[44]. *Blautia* is a genus of bacteria commonly found in the human gut, and some species within this genus have been associated with IBD. While several studies report a decrease in *Blautia* abundance in IBD patients - including those with CD and UC - suggesting a potential protective role, other research indicates a more complex relationship. Some *Blautia* species may

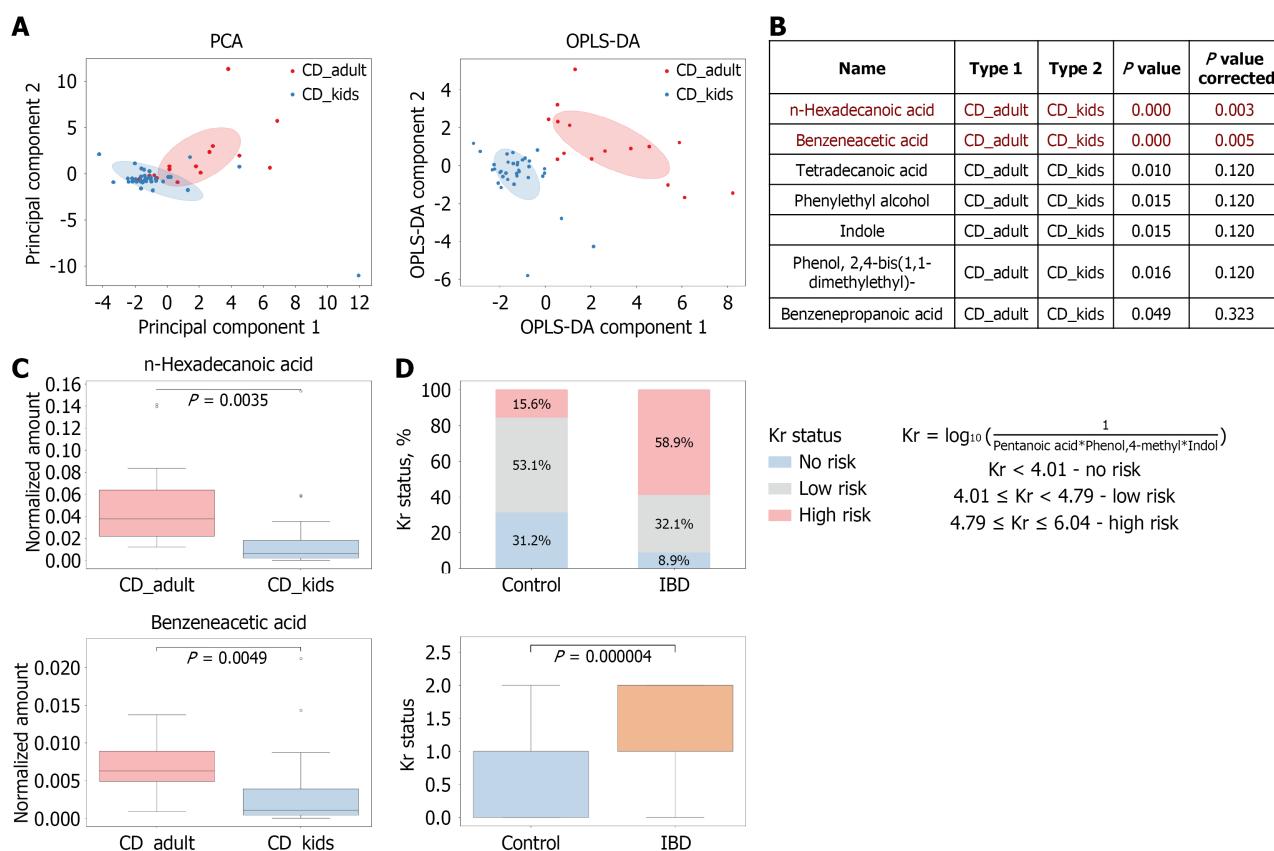
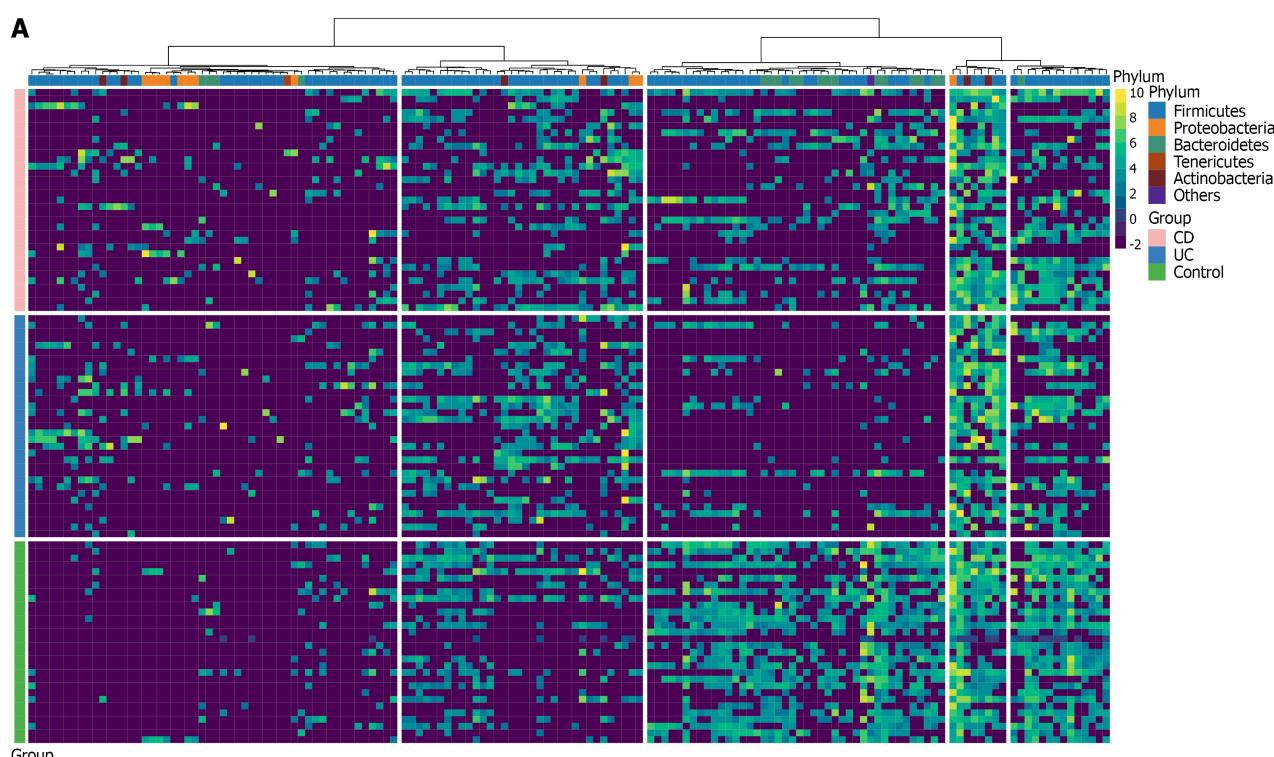


Figure 5 Total headspace-gas chromatography-mass spectrometry data obtained for Crohn's disease group of pediatric and adult patients. A: Principal component analysis and Orthogonal Partial Least Squares Discriminant Analysis data represent two independent groups of samples [Crohn's disease (CD)-kids, CD-adults]. According to the relative concentration of all detected volatile compounds; B: The nonparametric Mann-Whitney test was used for the primary comparisons between groups. Statistical significance was determined by a two-sided P-value of less than 0.05. False discovery rate correction was also applied; C: Box plots the quantitative differences in the relative contents of metabolites detected in the analyzed groups (CD-kids, CD-adults); D: Risk coefficient calculation. UC: Ulcerative colitis; CD: Crohn's disease; PCA: Principal component analysis; IBD: Inflammatory bowel disease; OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis.



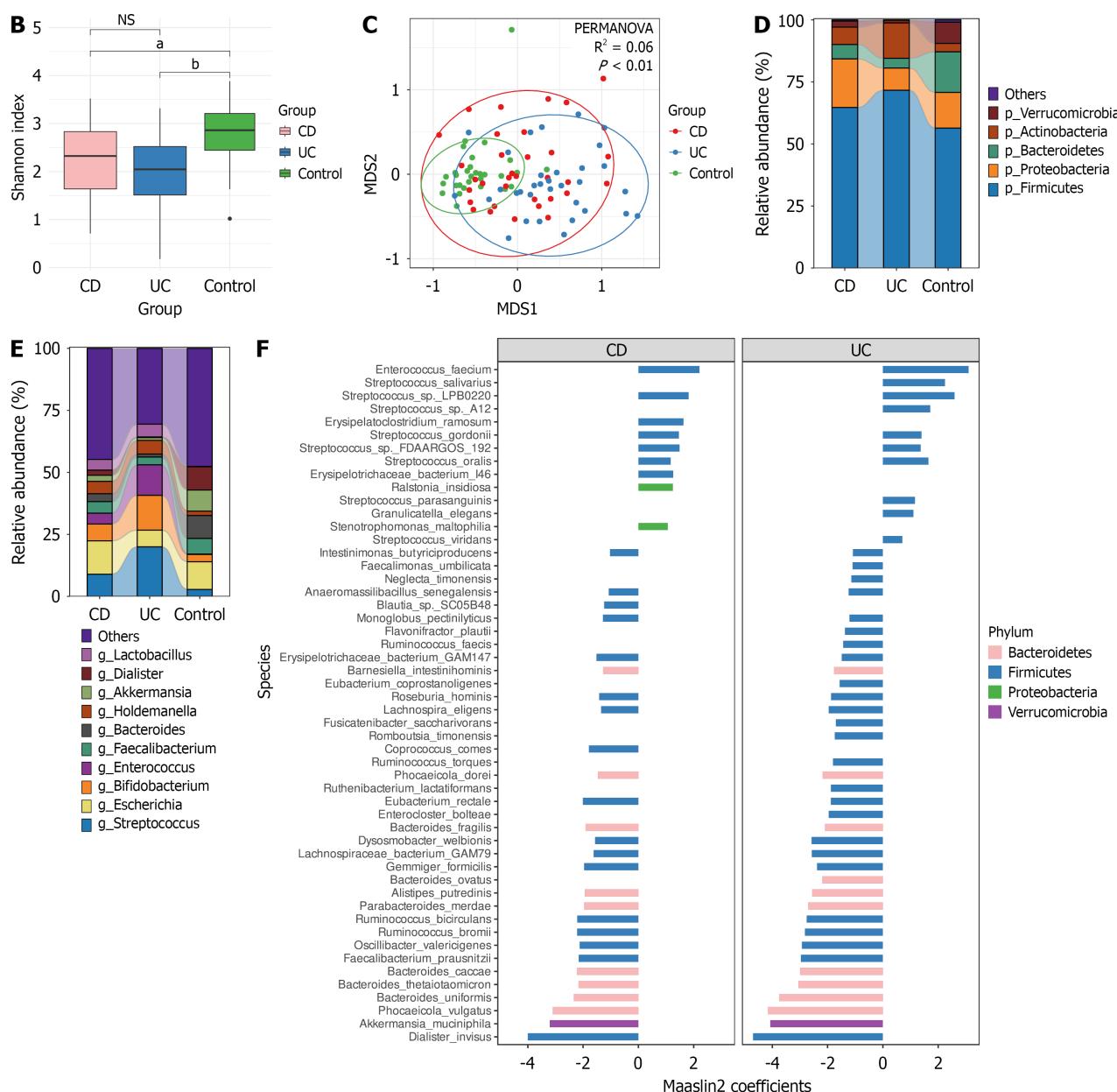


Figure 6 Results of exploratory analysis of gut microbiota diversity in experimental groups (ulcerative colitis and Crohn's disease, and control). A: The most abundant genera in the gut microbiota of patients are presented as a heatmap. Colors denote the relative abundance of species obtained by the emu pipeline after log transformation using pseudo counts. The figure shows the top 90 by relative abundance species. The rows correspond to the samples/patients; the phylum-level taxonomic ranks are denoted with a right color bar. Hierarchical clustering was performed using the Euclidean distance and complete linkage; B: Shannon index distribution of stool samples across experimental groups. Different colors denote different experimental groups. Horizontal brackets show the P-value obtained by comparing corresponding groups using the Wilcoxon rank-sum test; C: Non-metric multidimensional scaling biplot obtained using taxonomic profiles at the species level of patients' and healthy controls' stool samples. Different groups are shown by different color dots. Permutational multivariate analysis of variance results are presented in the lower left corner; D: Bar plots denote the distribution of phylum abundance across experimental groups. The X-axis showed experimental groups, Y-axis showed relative abundance. Different colors show different phyla; E: Bar plots denoted the distribution of genus abundance across experimental groups. The X-axis showed experimental groups, Y-axis showed relative abundance. Different colors show different genera. F: Results of identifying differentially abundant species between ulcerative colitis and coronavirus disease 2019 patient groups using Maaslin2 with the following thresholds: Minimum abundance = 0.01, minimum prevalence = 0.25, maximum significance = 0.05. The Y-axis shows microbial species, the X-axis - the Maaslin2 regression coefficient. Color indicates taxonomic affiliation at the order level for each identified species. ^aP > 0.05, obtained by comparison corresponded groups using the Wilcoxon rank-sum test. ^bP > 0.01, obtained by comparison corresponded groups using the Wilcoxon rank-sum test. UC: Ulcerative colitis; CD: Crohn's disease; PERMANOVA: Permutational Multivariate Analysis of Variance; MDS: Multidimensional scaling ordinate; NS: Not significant.

even have beneficial effects; for instance, certain strains produce SCFAs, which are beneficial for gut health, and can modulate immune responses. Experimental studies have shown that specific *Blautia* strains can alleviate colitis in animal models by reducing inflammation and enhancing gut barrier integrity[45].

Coprococcus, particularly the species *Coprococcus comes*, has been associated with both the presence and alleviation of IBD[46]. While some studies suggest a potential link between certain *Coprococcus* species and IBD, others indicate that *Coprococcus eutactus*, a related species, may exert a protective effect against the disease. It appears to promote anti-inflam-

matory responses and help restore gut barrier integrity by enhancing goblet cell maturation - cells responsible for mucus production - and by increasing the expression of tight junction proteins. *Coprococcus comes* is a producer of SCFAs, primarily acetate, which is known for its anti-inflammatory properties[47]. Conversely, antibodies against *Coprococcus comes* have been found at higher concentrations in individuals with CD compared to healthy controls[48]. Further research is necessary to fully understand the roles of different *Coprococcus* species in IBD and to evaluate their potential as therapeutic targets.

Quantitative assessment of microbiota species variability in pediatric patients with UC helps identify specific bacterial species that characterize this condition and distinguish it from CD. Notably, patients with UC often exhibit a significant increase in certain *Streptococcus* species, such as *Streptococcus salivarius*, *Streptococcus viridans*, and *Streptococcus sanguinis*. *Streptococcus salivarius* is a common bacterium found in the human oral cavity and gut; some studies suggest it may play a role in IBD, although its exact function remains under investigation. Evidence indicates that *Streptococcus salivarius* can possess anti-inflammatory properties and modulate immune responses. For example, it has been shown to inhibit the nuclear factor-kappaB pathway in human intestinal epithelial cells *in vitro*, suggesting potential anti-inflammatory effects [49]. Certain strains, like JIM8772, have demonstrated protective effects in mouse models of colitis, further supporting their possible therapeutic role[50]. Additionally, *Streptococcus salivarius* may influence immune responses by affecting cytokine production and regulating proliferator-activated receptor gamma transcription activity in intestinal epithelial cells[50]. Increased levels of *Streptococcus salivarius* have also been observed in the saliva of individuals with IBD, especially those with oral manifestations such as orofacial granulomatosis or oral CD. Conversely, children with colitis show increased abundance of bacteria such as *Ruminococcus faecis* and *Bacteroides ovatus*. *Ruminococcus faecis* is part of the human gut microbiome; although less studied than other *Ruminococcus* species, some research suggests a potential link between *Ruminococcus* spp., including *Ruminococcus faecis*, and IBD. Studies have shown that species like *Ruminococcus gnavus* and *Ruminococcus torques* are more prevalent in individuals with IBD and can produce molecules that may contribute to gut inflammation[51]. *Bacteroides ovatus* is a common gut bacterium that can have both beneficial and potentially harmful effects in IBD. While generally considered a commensal microbe, some studies indicate it can trigger antibody responses in IBD patients. Conversely, other research suggests that *Bacteroides ovatus* may reduce colitis severity and promote epithelial healing in animal models of IBD[52].

Comparison of the IBD metagenomes occurring in childhood *vs* adulthood reveals significant differences that characterize the distinct features of disease progression across age groups. Primarily, it is important to note that the intestinal microbiota of children and adults differ markedly, which is entirely normal (Figure 7A and B). Consequently, making direct comparisons between the microbiota of CD -CD-associated colitis in children and adults can be challenging. Nonetheless, certain features related to bacterial diversity involved in the development of IBD in children and adults can still be identified.

In particular, Shannon's alpha diversity index is significantly lower in children compared to adults (Figure 7C). Reduced bacterial diversity was observed in children across both the control group and the groups with colitis and CD. It is important to note that, during disease development, the microbiomes of children and adults undergo similar changes (Figure 7D). However, significant differences in bacterial species composition are evident between pediatric and adult patients when comparing groups with CD and UC (Figure 7D). Analyzing the species diversity of UC and CD in both children and adult groups reveals that potential biological markers not only distinguish between the disease types but also show an association with age (Figure 8).

According to the data obtained, *Streptococcus salivarius* appears to be a universal metagenomic marker characterizing the development of IBD in the pediatric group ($\log_{2}\text{FoldChange} = 3.31$, $\text{FDR} < 0.0001$). Similarly, *Escherichia coli* can also serve as a universal marker for IBD in children, as a significant increase in this bacterial species was observed in both UC ($\log_{2}\text{FoldChange} = 2.45$, $\text{FDR} = 0.049$) and CD groups ($\log_{2}\text{FoldChange} = 3.45$, $\text{FDR} = 0.007$). However, the expected increase in *Escherichia coli* was not observed in adult patients with CD. Nonetheless, distinct metagenomic profiles are formed in both CD and UC across adult and pediatric groups, which can be utilized for diagnosis and differential diagnosis (Figure 9).

Functional metabolomics results

Correlation analysis enables the evaluation of certain aspects of functional metabolomics, which can be applied to identify differential markers distinguishing CD and UC in children and adults. Notably, pediatric patients with CD are characterized by an increased level of tetradecanoic acid and quantitative changes in several microorganisms, including *Bacteroides eggerthii*, *Bacteroides cellulolyticus*, *Alistipes putredinis*, *Barnesiella intestihominis*, and *Bacteroides thetaiotaomicron* (Figure 10A). Another marker associated with co-directional correlation is butyrate and propionate derivatives, which increase in tandem with changes in bacteria such as *Prevotella copri*, *Bifidobacterium adolescentis*, and others. *Escherichia coli*, a well-known risk factor associated with CD, shows an inverse correlation with benzoic acid levels. In the pediatric group with UC, a high abundance of *Streptococcus salivarius* was observed. Interestingly, the content of *Streptococcus salivarius* is inversely proportional to the level of heptanoic acid (Figure 10B). Most members of the genus *Streptococcus* exhibit an inverse relationship with heptanoic acid levels. Notably, heptanoic acid has been found to be reduced in patients with IBD.

Studies suggest that alterations in gut microbiota composition and decreased fermentation of dietary fibers in IBD can lead to changes in SCFAs, including heptanoic acid. Although the precise mechanisms are still under investigation, SCFAs are known to play crucial roles in regulating immune responses and promoting tissue repair within the gut. Significant amounts of *Blautia* bacteria (*Blautia faecis*, *Blautia glucurasea*, *Blautia obeum*, *Blautia luti*) positively correlate with levels of various propionate derivatives, which can also serve as markers for differential diagnosis between UC and CD. Additionally, the correlation analysis reveals notable differences in the microbiota structure of children and adults with CD and UC. Specifically, when comparing pediatric and adult CD groups, opposite trends are observed in several compounds and bacterial species. For example, propionate and acetate do not show significant correlation with bacterial

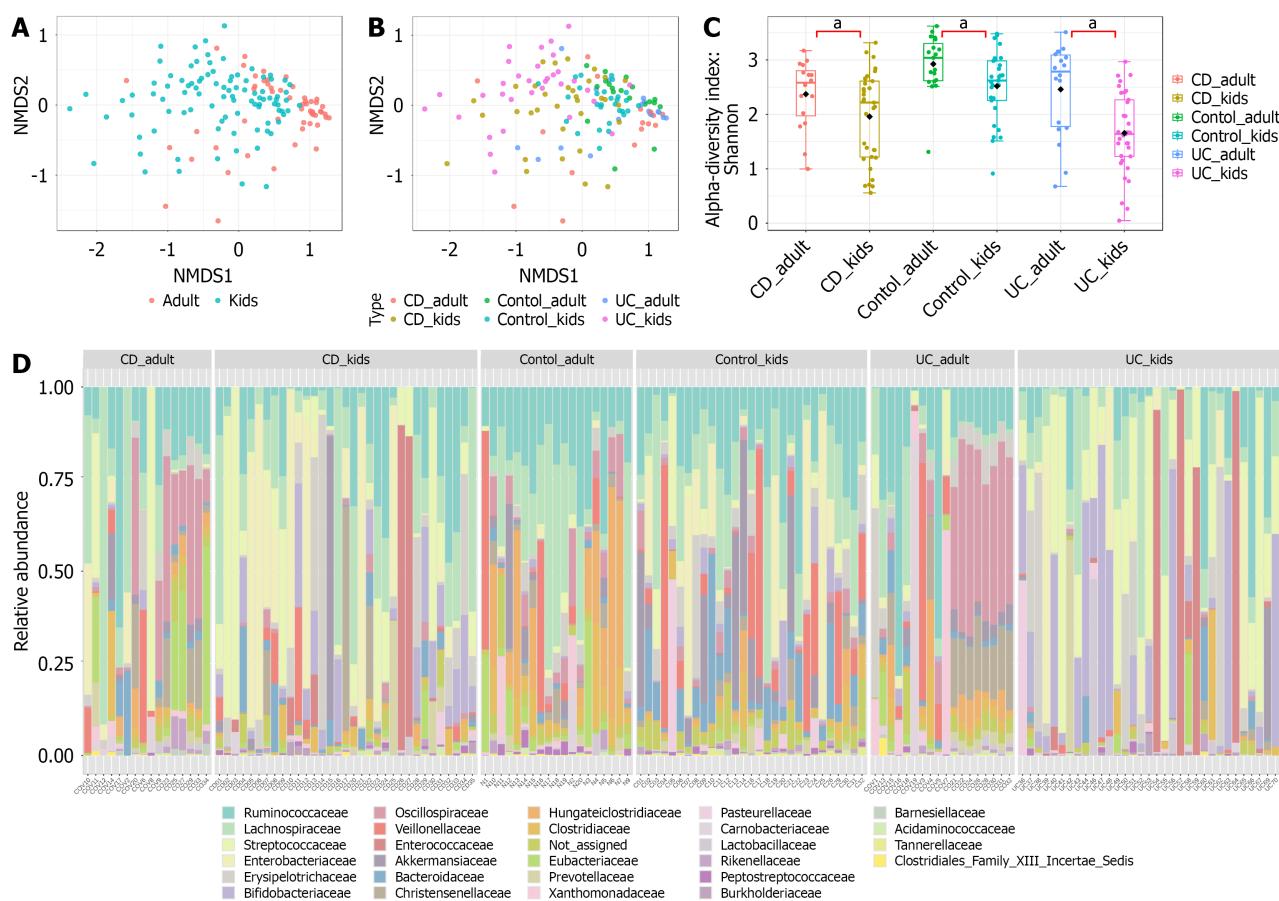


Figure 7 Gut microbiome analysis of pediatric and adult patients. A and B: Nonmetric multidimensional scaling based on Bray-Curtis dissimilarity; C: Boxplots representing Shannon's alpha diversity index; D: Bar plots of bacterial family relative abundance. ^aP < 0.05, assessed by Kruskal-Wallis test. UC: Ulcerative colitis; CD: Crohn's disease; NMDS2: Non-metric multidimensional scaling ordinate 2.

species numbers in the pediatric CD group; however, in adults, correlations are evident, indicating a decline in the activity of bacteria responsible for producing SCFAs.

Summarizing the correlation data, it is evident that there is considerable variability in the Shannon diversity index for SCFAs in UC compared to CD (Figure 10C). This finding is particularly intriguing when considering the potential mechanisms underlying the pathogenesis of these two IBDs. The extensive microbiota alterations observed at early stages of UC may suggest a broader range of pathogenic factors influencing its onset and progression. In contrast, CD - both in childhood and adulthood - is characterized by a more limited spectrum of metabolic and metagenomic changes, which may enhance the prospects for identifying specific pathognomonic factors responsible for its development.

DISCUSSION

IBDs are polyetiological conditions that develop as a result of the influence of numerous exogenous and endogenous factors[53]. Among these, the most common are UC and CD. Although both conditions can manifest at different ages, CD is more frequently diagnosed during childhood and adolescence[54]. Nonetheless, both pathologies are extremely challenging to diagnose accurately and differentiate from each other[55]. Despite the extensive data currently available on genetic predispositions and independent markers associated with disease activity, there remains a need to identify new determinants that could enable timely targeted interventions. Laboratory tests today can help assess the risk of developing IBD[56], but they are typically used only when specific symptoms appear. Common clinical manifestations include diarrhea, weight loss, nausea, and blood in the stool, among others[57]. Importantly, long before these symptoms become evident, the intestinal microbiota - the body's primary sensor - reacts to changes within the gut environment. Microorganisms adapt to inflammatory conditions in CD and UC by altering their diversity and metabolic activity[58]. These microbial adaptations involve shifts in bacterial community composition and changes in the secretion levels of certain metabolites[59]. Therefore, analyzing microbial presence and their secretory activity offers a promising approach for monitoring disease progression and improving early diagnosis and differential diagnosis of IBD. It is important to recognize that both conditions have a chronic course, often beginning early in life and persisting into adulthood with alternating periods of exacerbation and remission[60]. Studying microbiome diversity in adult patients reflects the established equilibrium of bacterial communities resulting from long-standing inflammation[61]. Consequently, investigating microbiome dynamics in young patients is especially crucial for understanding disease pathogenesis and iden-

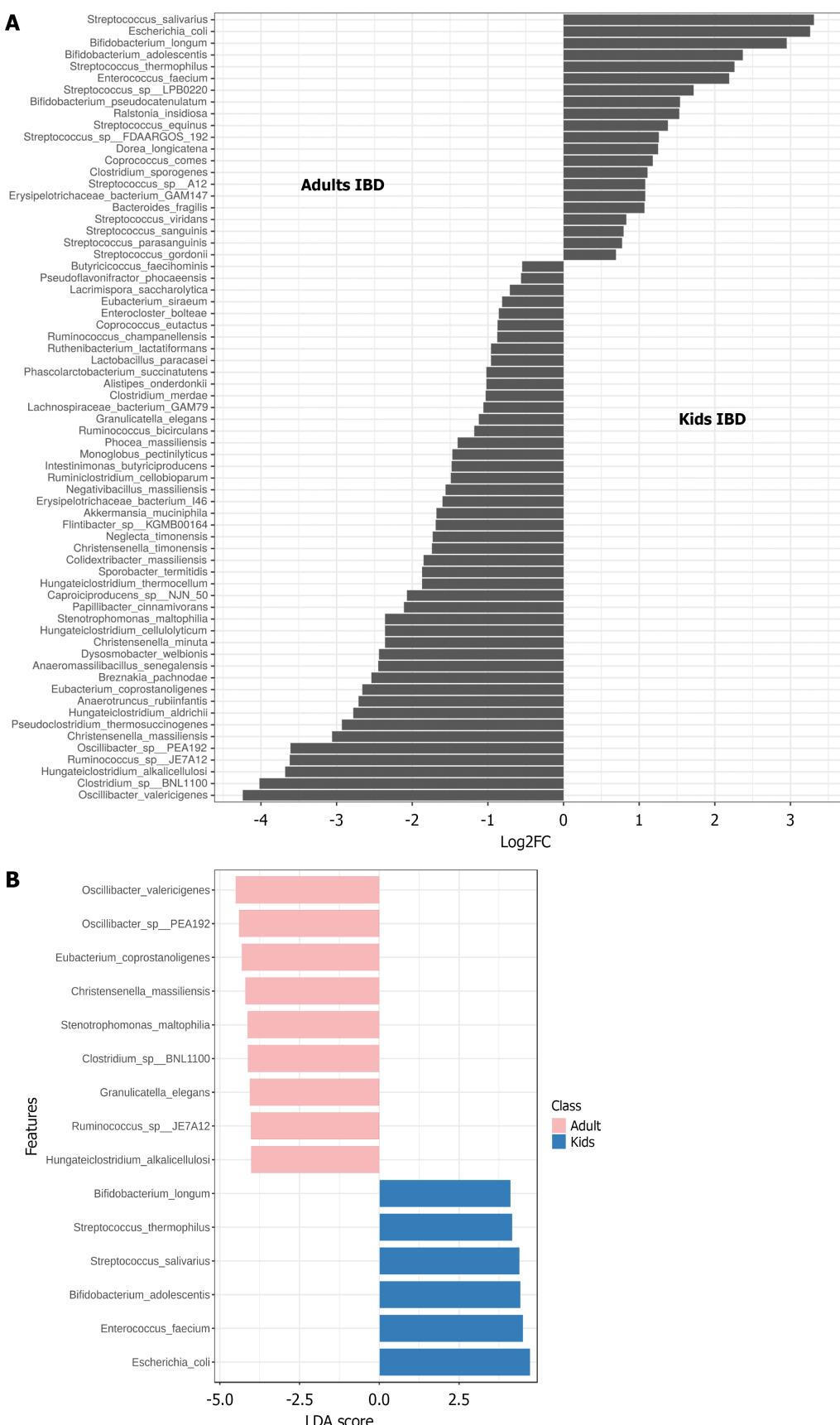
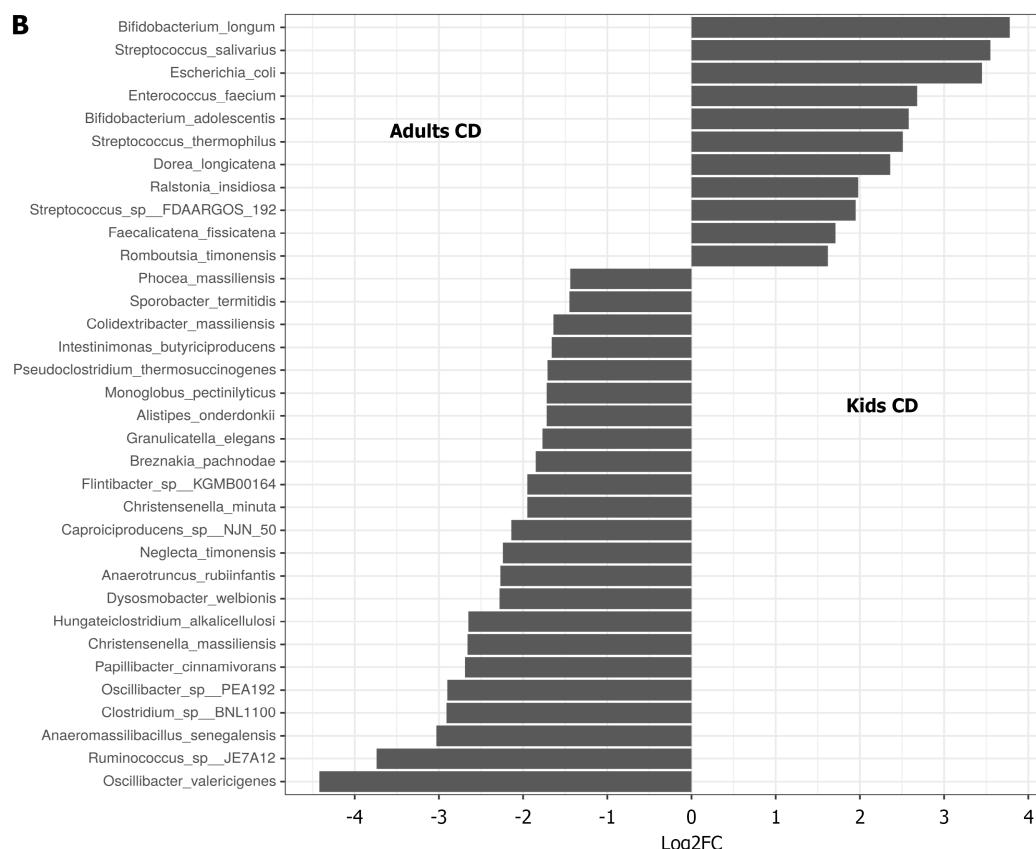
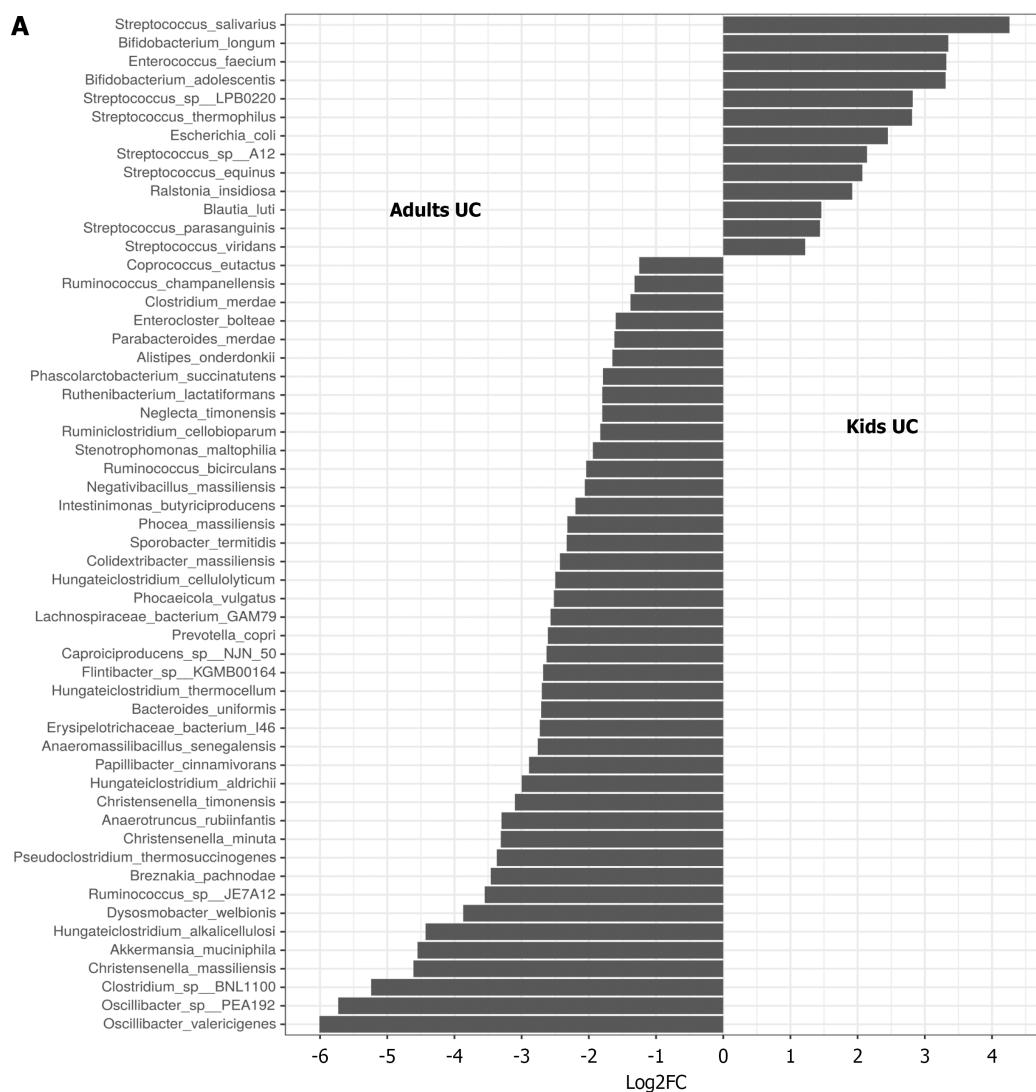


Figure 8 Difference between the taxonomic composition of the gut microbiome of pediatric and adult patients with inflammatory bowel disease. A: Results of identifying differentially abundant species between pediatric and adult inflammatory bowel disease patient groups using Maalsin2 with the following thresholds: Minimum abundance = 0.01, minimum prevalence = 0.25, maximum significance = 0.05; B: Results of identifying biomarker species of pediatric and adult inflammatory bowel disease patient groups using linear discriminant analysis effect size. The colors indicate biomarker species for the studied groups. IBD: Inflammatory bowel disease; LDA: Linear discriminant analysis.



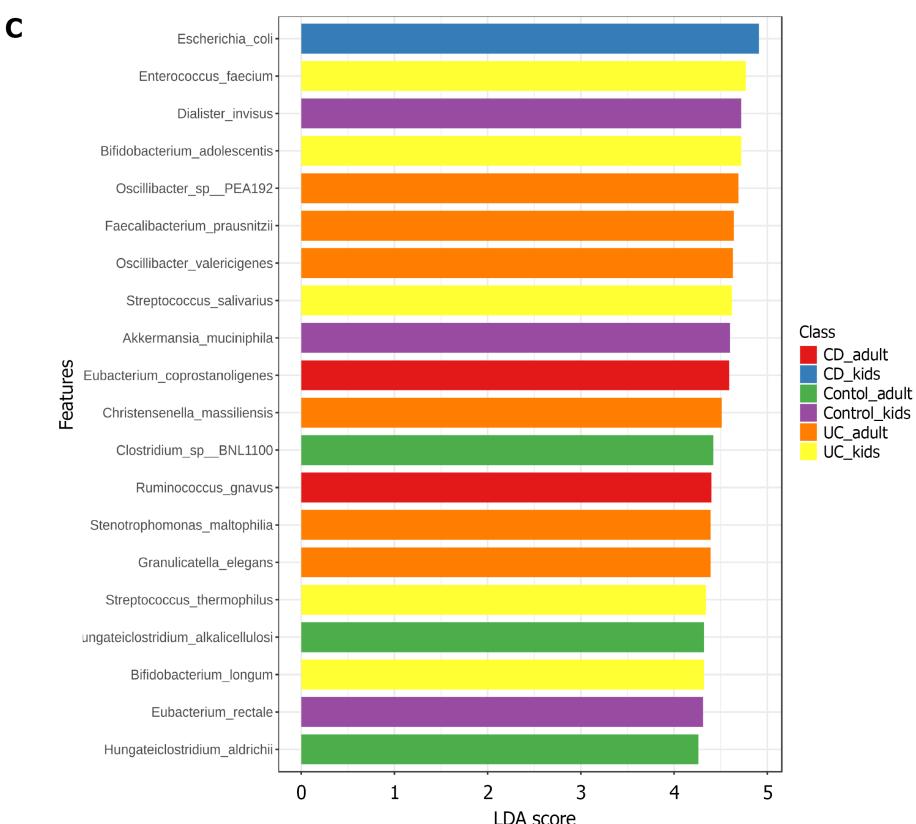


Figure 9 Difference between taxonomic composition of gut microbiome of paediatric and adult patients with ulcerative colitis and Crohn's disease. A: Results of identifying differentially abundant species between pediatric and adult ulcerative colitis patient's groups using Maalsin2 with following thresholds minimum abundance = 0.01, minimum prevalence = 0.25, max significance = 0.05; B: Results of identifying differentially abundant species between pediatric and adult Crohn's disease patient's groups using Maalsin2 with following thresholds minimum abundance = 0.01, minimum prevalence = 0.25, maximum significance = 0.05; C: Results of identifying biomarker species of pediatric and adult controls, ulcerative colitis and Crohn's disease patient's groups using linear discriminant analysis effect size. The colors indicate biomarker species for the studied groups. UC: Ulcerative colitis; CD: Crohn's disease; LDA: Linear discriminant analysis.

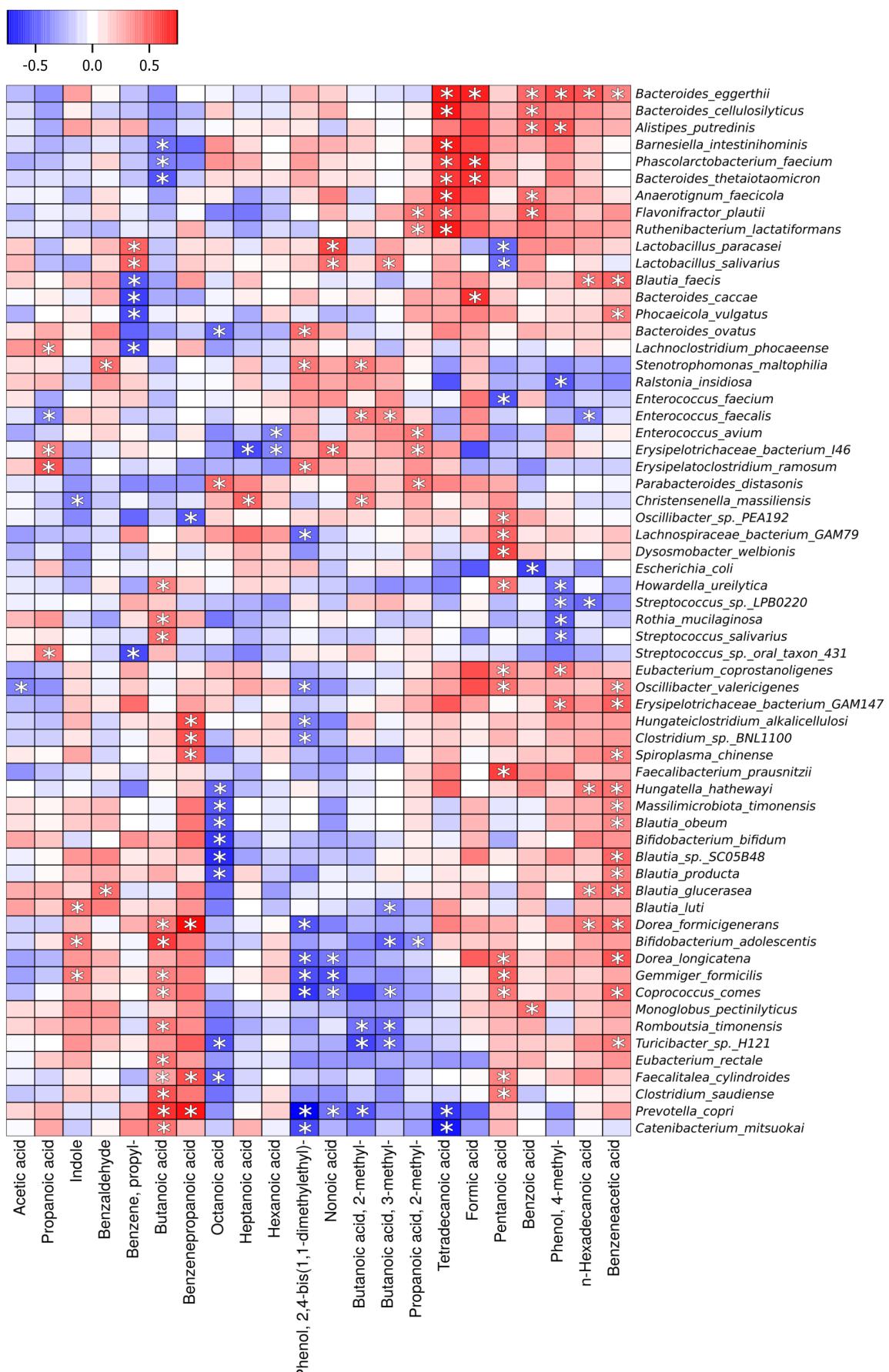
tifying effective diagnostic markers.

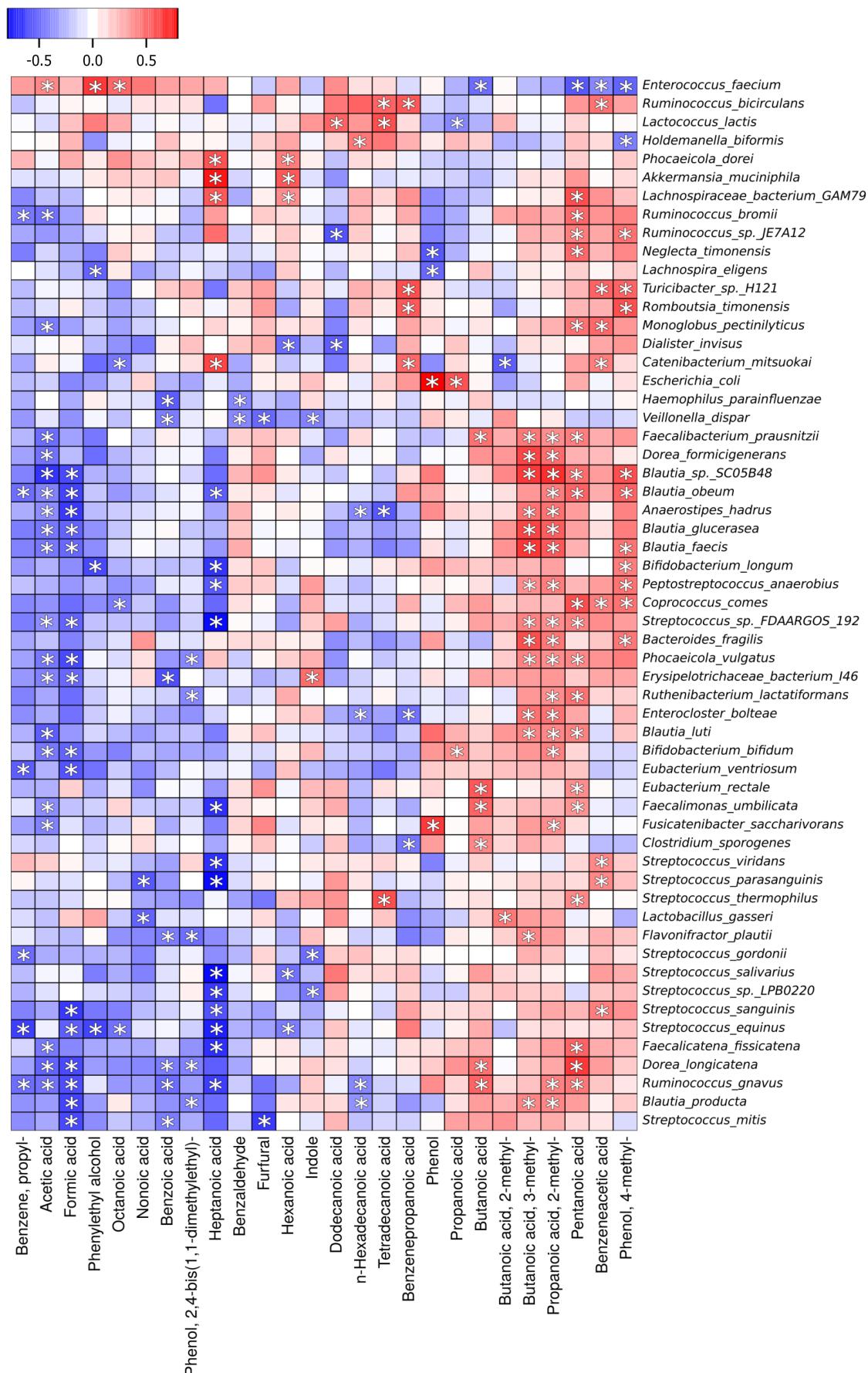
It is well established that the microbiota of children and adults differ significantly[62]. Therefore, the response to inflammatory processes in children will directly influence the composition of their microbiota and the secretory activity of bacteria. The objectives of this study were to comprehensively assess the metagenomic and metabolomic diversity of molecular markers characteristic of early CD and UC, as well as their later stages. Stool samples were collected from patients, as this is the most convenient option not only for initial diagnosis but also for subsequent monitoring of the inflammatory process.

Based on the obtained metabolomic data, it is possible to identify distinct profiles of inflammatory diseases when compared to controls, in both children and adults. Thus, in both age groups, the secretory activity of bacteria and overall enzymatic processes in the human body undergo significant changes during the development of inflammatory diseases. When comparing pediatric patients with IBD to healthy controls, several metabolites were found to be significantly altered: Indole, pentanoic acid, phenol, 4-methyl, and 1H-indole-3-methyl. Notably, when assessing differential differences between disease groups, the indole derivative 1H-indole-3-methyl showed significant variation between CD and UC. Importantly, indole levels were significantly reduced across the entire IBD group and within individual analyses of CD and UC patients, a finding supported by several studies[63]. This consistent decrease indicates a diminished presence of bacteria actively producing and secreting indole, reflecting microbiota alterations associated with these conditions.

It is well established that the gut microbiome plays a crucial role in tryptophan metabolism, producing various indole derivatives that can influence the host's immune response and intestinal health[64]. It has been previously demonstrated that indole metabolites produced by gut microbiota play a crucial role in maintaining intestinal health and modulating immune responses, with significant implications for IBD, including CD, in both adults and children[65]. A retrospective study of pediatric patients diagnosed with CD further confirms the high diagnostic value of volatile metabolite analysis, including indole. This approach proves useful for assessing the extent of intestinal mucosal changes and disease activity, especially when combined with other metagenomic indicators[66].

1H-indole-3-methyl is a naturally occurring compound found in the gut that results from tryptophan metabolism. While it is not directly implicated as a causative factor for CD, it is known that skatole (3-methylindole) can enhance promoter activity of cytokines such as IL-6 and TNF- α , increasing IL-6 mRNA expression and protein secretion. The ability of skatole to elevate IL-6 and TNF- α levels may significantly influence the development and progression of IBD[67].

A Spearman's correlation coefficient

B Spearman's correlation coefficient

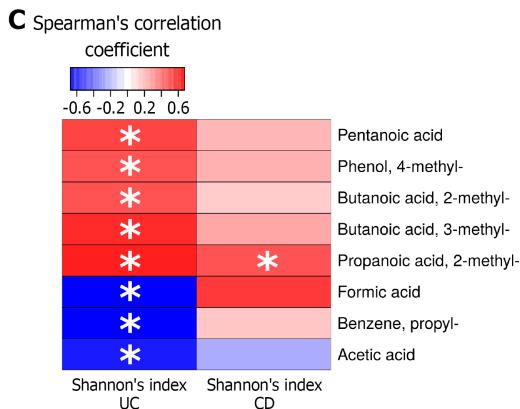


Figure 10 Spearman correlation between metabolite levels and relative abundance of microbial species. $P < 0.05$. A: Crohn's disease; B: Ulcerative colitis; C: Shannon's index diversity of metabolites in ulcerative colitis and Crohn's disease groups. UC: Ulcerative colitis; CD: Crohn's disease.

Unlike CD, UC also exhibits characteristic changes in the levels of pentanoic acid, 4-methylphenol (p-cresol), and acetate. Gut bacteria produce SCFAs like pentanoic acid as byproducts of digesting dietary fiber. These changes in SCFA profiles are considered potential indicators of dysbiosis and may reflect early microbial and metabolic disturbances linked to disease onset and progression. In UC, alterations in gut microbiota composition can affect SCFA production. Similar alterations in the composition of SCFAs have been observed in numerous studies focused on identifying molecular markers associated with the risk of developing UC[68]. Research suggests that pentanoic acid, along with other SCFAs, may play a role in regulating intestinal inflammation and potentially influence UC severity[68]. Studies have shown that increased levels of pentanoic acid and other SCFAs are associated with a reduction in UC symptoms[23]. Therefore, similar to indole, the concentration of pentanoic acid decreases with the development of IBDs. As demonstrated in this study, this decrease appears to be more characteristic of UC than CD.

Although metabolomic analysis can identify individual disease markers in clinical practice, applying these results is often challenging. For this reason, it is optimal to develop an assessment metric that allows evaluation of IBD risk in children, considering that IBD can manifest at this age. A formula incorporating phenol, 4-methylpentanoic acid, and indole can be used to calculate a risk coefficient for developing IBD. A coefficient value less than or equal to 4.01 indicates no IBD risk. Values between 4.01 and 4.79 suggest a low risk; however, additional clinical markers should be considered to clarify the diagnosis. A coefficient above 4.79 indicates a high risk of IBD and strongly recommends further examination, which can also detect asymptomatic cases. Moreover, in cases with a high-risk coefficient, 1H-indole-3-methyl can serve as a marker for differential diagnosis and assessing the risk of CD. Conversely, levels of pentanoic acid, phenol 4-methyl, and acetate can be used for differential diagnosis to assess the risk of developing UC.

Metagenomic data, especially when combined with metabolomic data, can significantly enhance the accuracy of diagnostics. However, as demonstrated, there is no need to sequence the entire metagenome for routine IBD diagnosis. Instead, focus can be placed on developing PCR-based tests to quantify specific microorganisms that characterize IBDs in general and enable differential diagnosis between CD and UC. Studies have indicated that *Streptococcus salivarius* and *Escherichia coli* may serve as potential microbial markers for assessing the risk of developing IBD in children, a finding that is supported by several research investigations[69]. These bacteria are often associated with dysbiosis observed in pediatric IBD and could potentially aid in early diagnosis or risk stratification. Importantly, this study conducted metagenomic analyses on older patients, thereby identifying bacterial differences that characterize pathologies developing during childhood. Specifically, in pediatric CD patients, a significant increase was observed in bacteria such as *Ralstonia insidiosa*, *Stenotrophomonas maltophilia*, and *Erysipelatoclostridium ramosum*, while a decrease was noted in species like *Blautia spp.* and *Coprococcus comes*. Functional metabolomics testing can also be useful for diagnosing IBD in adults, particularly when the disease is initially identified. However, it is crucial to recognize that the spectrum of metabolic and metagenomic differences at disease onset may differ substantially from those observed during later stages or established pathology.

CONCLUSION

Functional metabolomics combines the ability to simultaneously assess the diversity of the microbiome and evaluate its secretory activity in the development of pathologies. When evaluating IBDs in children and adults, it is important to consider differences in bacterial community composition and, consequently, their secretory activity, which are reflected in the overall profile of potential diagnostic markers. In this study, a diagnostic rule was developed for children with IBD that enables calculation of the risk of disease development. This rule, based on a set of metabolic and metagenomic markers, also helps clarify the specific type of pathology. The combined use of metabolomic and metagenomic analysis will facilitate prompt assessment of the potential risks for developing IBD in the future and support timely initiation of appropriate therapy.

FOOTNOTES

Author contributions: Zakharzhevskaya NB, Erdes SI, Belousova ES, Lomakina EY, Kardonsky DA, Kalachnuk TN, and Efimov BA contributed to designing experiments; Samolygo IS, Manina MM, Kondrashova PV, Shagaleeva OY, Silantiev AS, Kazakova VD, Kashatnikova DA, Chaplin AV, Veselovsky VA, Morozov DM, Zoruk PY, Boldyreva DI, Vorobyeva EA, Markelova MI, Grigoryeva TV, Kolesnikova IV, Olekhnovich EI, and Vanyushkina AA contributed to performed the experiments; Vorobyeva EA, Markelova MI, Grigoryeva TV, Kolesnikova IV, Olekhnovich EI, and Vanyushkina AA contributed to analyzed the data; Zakharzhevskaya NB, Erdes SI, Belousova ES, Samolygo IS, and Kardonsky DA contributed to wrote the paper; Zakharzhevskaya NB, Belousova ES, and Efimov BA contributed to supervised the project.

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Institutional review board statement: All experimental procedures were approved by the local ethical committee of Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russian Federation, Russia. Informed consent was obtained from all subjects, No. 1-IBD/ONCO-2.09.2024.

Institutional animal care and use committee statement: The local ethical committee of Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine, Moscow, Russian Federation, hereby confirms that no animal experiments were conducted as part of the scientific and clinical study titled "Development of a Comprehensive Panel of Diagnostic Markers for Verification of Inflammatory and Oncological Bowel Diseases", No. 2-IBD/ONCO-2.09.2024.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The genome data supporting the findings of this study are openly available in the NCBI BioProject under accession ID: PRJNA1315228. The metabolome data supporting the findings of this study are openly available in the Mendeley database (Mendeley Data, V1, Available from: <https://data.mendeley.com/datasets/f6vtf4gpcc/1>) [DOI: 10.17632/f6vtf4gpcc.1].

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