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Original Article

Mucosal and Plasma Metabolomes in New-onset Paediatric Inflammatory Bowel Disease: Correlations with Disease Characteristics and Plasma Inflammation Protein Markers

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Abstract

Background and Aims: To advance the understanding of inflammatory bowel disease [IBD] pathophysiology, we compared the mucosal and plasma metabolomes between new-onset paediatric IBD patients and symptomatic non-IBD controls, and correlated plasma inflammation markers and disease characteristics with the altered metabolites.

Methods: Paired colonic and ileal biopsies and plasma from 67 treatment-naïve children with incident Crohn's disease [CD; *n* = 47], ulcerative colitis [UC; *n* = 9], and non-IBD controls [*n* = 11] were analysed using ultra-performance liquid chromatography-mass spectrometry [UPLC-MS/MS]. Inflammatory plasma proteins [*n* = 92] were assessed.

Results: The metabolomes in inflamed mucosal biopsies differed between IBD patients and controls. In CD, mucosal levels of several lysophospholipids [lysophosphatidylcholines, lysophosphatidyletanolamines, lysophosphatidylinositols, and lysophosphatidylserines] were decreased, correlating with various plasma metabolites including amino acid analogues and *N*-acetylated compounds. In both CD and UC, mucosal sphingolipids, including ceramide [d18:2/24:1, d18:1/24:2], lactosyl-*N*-palmitoyl-sphingosine [d18:1/16:0], behenoyl sphingomyelin [d18:1/22:0], lignoceroyl sphingomyelin [d18:1/24:0], and/or sphingomyelin [d18:1/24:1, d18:2/24:0] were increased, correlating with sphingolipids, bile acids, and/or *N*-acetylated metabolites in plasma. Among proteins associated with CD, interleukin-24 correlated with plasma metabolites, including lactosyl-*N*-palmitoyl sphingosine [d18:1/16:0] and phosphatidyletanolamine [18:1/18:1], haemoglobin, and faecal calprotectin. In UC, interleukin-24, interleukin-17A, and C-C motif chemokine 11 correlated with several plasma metabolites, including *N*-acetyltryptophan, tryptophan, glycerate, and threonate, and with the Paediatric Ulcerative Colitis Activity Index, C-reactive protein, and faecal calprotectin.

Conclusions: Mucosal perturbations of lysophospholipids and sphingolipids characterised the metabolome in new-onset paediatric IBD and correlated with plasma metabolites. By integrating plasma metabolomics data with inflammatory proteins and clinical data, we identified clinical and inflammatory markers associated with metabolomic signatures for IBD.

Key Words: Inflammatory bowel disease; paediatric; metabolome

1. Introduction

Inflammatory bowel disease [IBD], comprising the predominant subtypes Crohn's disease [CD] and ulcerative colitis [UC], is a chronic inflammatory disease of the gastrointestinal tract. Up to 25% of IBD patients have a paediatric onset, often with a more severe disease course and specific paediatric complicating aspects.[1,2] The incidence of the disease has been rising globally for the past decades, particularly in recently industrialised regions.[3]

The precise aetiology of IBD is unknown, but according to a prevailing hypothesis, the inflammation arises due to an aberrant immune response to gut microbiota in genetically susceptible individuals. Exposure to environmental risk factors, including dietary items, seems to precede the loss of gut homeostasis and dysregulation of innate and adaptive immune pathways.[4,5] Ultimately, these initial triggers seem to cause intestinal gut barrier disruption and overt inflammation. Technical advancement in novel omics technologies may advance our understanding of the molecular changes in the pathophysiology of IBD and aid identification of potential diagnostic biomarkers.[6] Metabolomics, the measurement of small molecules [<2000 Da], is a powerful tool for characterising the interactions between nutrients, intestinal metabolism, gut microbiota, and host immune response.[7]

Untargeted metabolomic studies in diverse IBD populations have identified several molecular classes of potential biomarkers and of pathophysiological interest.[8–11] There are also numerous reports from targeted approaches, for example reduction of microbially-derived short-chain fatty acids in faecal samples from IBD patients.[8–11] Although children with treatment-naïve, new-onset IBD, without any comorbidity, have been described as an ideal population for metabolomic profiling, most studies have examined adult IBD patients with ongoing treatment.[9,11] Various biological samples have been analysed, most commonly blood and faeces,[11–18] and fewer studies have used mucosal biopsies.[11,19–22]

We hypothesised that by gaining insight into the diagnostic phase of paediatric IBD and characterising the metabolome in relation to clinical parameters and inflammatory markers, our knowledge about the IBD metabolome could be leveraged and key pathways of disease pathogenesis identified. Therefore, this study aimed to identify alterations in the plasma and mucosal [ileum and colon] metabolome of treatment-naïve, new-onset paediatric IBD patients compared with symptomatic non-IBD controls, ie, patients with gastrointestinal symptoms without objective findings of gastrointestinal inflammation. Furthermore, we aimed to identify correlations between disease characteristics, plasma inflammation markers, and plasma and mucosal metabolomic profiles.

2. Material and Methods

2.1. Study design

This was a prospective, single-centre, case-control study, where we analysed and compared the metabolomes of treatment-naïve paediatric patients with new-onset CD or UC with those of symptomatic paediatric patients without objective findings of IBD at inclusion or follow-up, ie, symptomatic non-IBD controls [Figure 1].

2.2. Patient cohort

Children aged <18 years with clinically suspected IBD, who were referred to the Uppsala University Children's Hospital, between 2009 and 2018, were invited to participate. The inclusion criterion was presence of gastrointestinal symptoms indicative of IBD. Exclusion criteria were a previous diagnosis of IBD, other chronic gastrointestinal diseases, and treatment with antibiotics within the preceding 3 months. After providing written informed consent, all children underwent a routine diagnostic workup for IBD in accordance with the European Society for Paediatric Gastroenterology, Hepatology and Nutrition/Porto criteria,[23] including gastroscopy and ileocolonoscopy with serial mucosal biopsies for histology. The study included 47 children with CD and nine with UC [Table 1]. The Paris classification was used to categorise patients by disease phenotype,[24] and the short Paediatric CD Activity Index [sPCDAI][25,26] and the Paediatric UC Activity Index [PUCAI][27,28] were used to assess disease activity. We used the Simple Endoscopic Score for CD [SES-CD] and the Mayo endoscopic score for UC to describe endoscopic activity.[29,30] In addition, we measured C-reactive protein [CRP] and haemoglobin to define the systemic inflammatory activity and faecal [f]-calprotectin as a proxy for gastrointestinal inflammation. Children who did not meet the diagnostic criteria for IBD were included as symptomatic non-IBD controls [$n = 11$]. One of these patients had a focal vascular malformation in the colonic mucosa, one had coeliac disease, and the remaining nine patients had normal endoscopy and histology on mucosal biopsies. In the long-term follow-up, all these nine patients were diagnosed with irritable bowel syndrome in accordance with the Rome IV criteria. The study was approved by the Uppsala University Ethics Committee [2008/395].

2.3. Sample collection and processing

Snap-frozen mucosal biopsies were collected from the ileum and transverse colon at ileocolonoscopy, except in one patient in whom biopsies were obtained from the descending colon instead of the transverse colon. Blood samples were collected at inclusion in immediate connection with the endoscopy [in 4-ml BD Vacutainer® CPTTM cell preparation tubes with sodium citrate] and processed to plasma within 4 h of sampling using centrifugation at 1800 *g* for 20 min. All samples were stored at – 80 °C.

2.4. Mass spectrometry analysis

Plasma and biopsy samples were extracted with methanol and subjected to non-targeted mass spectrometry [MS] analysis

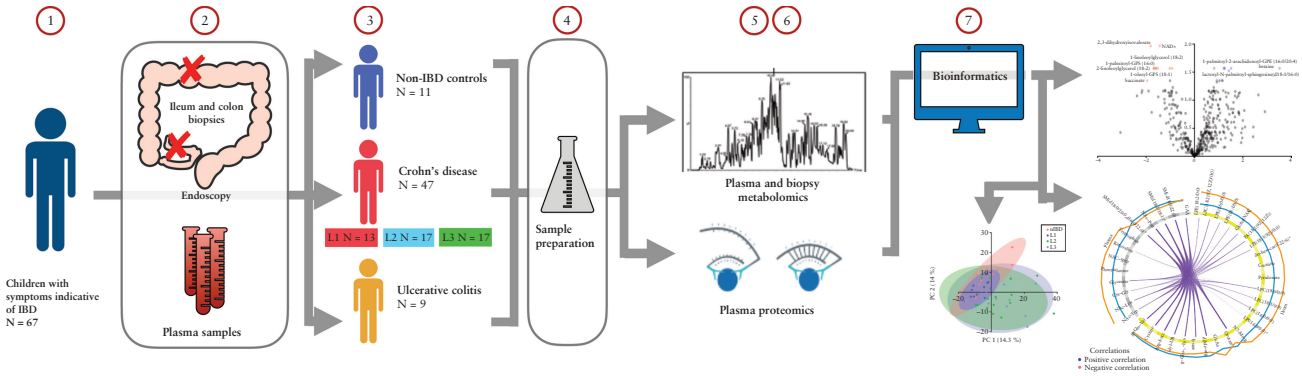

Figure 1. Study overview. 1. Children with symptoms indicative of inflammatory bowel disease [IBD]. 2. Endoscopy, collection of plasma samples and biopsies from mid-colon and ileum. 3. Children were separated into diagnostic groups. Collection of clinical data. 4. Sample preparation. 5. Ultra-performance liquid chromatography-tandem mass spectrometry on plasma samples and biopsies for untargeted metabolite separation, detection, and quantification. 6. Plasma proteomics with an OLINK Multiplex Inflammation 92 panel. 7. Bioinformatics, uni- and multivariable analyses.

Table 1. Demographics and clinical characteristics of treatment-naïve paediatric patients with new-onset inflammatory bowel disease [IBD; *N* = 56] and symptomatic non-inflammatory bowel disease [non-IBD] controls [*N* = 11].

	CD N = 47	UC N = 9	Non-IBD N = 11
Sex, male, *N* [%]	28 [60]	7 [78]	5 [45]
Median [IQR] age, years	15 [12–16]	15 [13–16]	14 [9–16.5]
Plasma samples, *N*	46	9	11
Ileal biopsies, *N*	35	8	10
Inflamed ileal biopsies, *N*	19	0	0
Colonic biopsies, *N*	45	8	10
Inflamed colonic biopsies, *N*	27	6	0
CD location			
L1 Ileal, *N* [%]	13 [28]		
L2 Colonic, *N* [%]	17 [36]		
L3 Ileocolonic, *N* [%]	17 [36]		
L4a Upper gastrointestinal disease, *N* [%]	12 [26]		
CD behaviour			
B1 Non-stricturing, non-penetrating, *N* [%]	44 [94]		
B2 Stricturing, *N* [%]	3 [6]		
B3 Penetrating, *N* [%]	0 [0]		
sPCDAI 0-90, median [IQR]	40 [25–57]		
SES-CD 0-60, median [IQR]	12 [6–20]		
UC extent			
E1 Proctitis, *N* [%]		2 [22]	
E2 Left-sided colitis, *N* [%]		1 [11]	
E3 Extensive colitis, *N* [%]		5 [56]	
E4 Total colitis, *N* [%]		1 [11]	
PUCAI 0–85, median [IQR]		45 [30–50]	
Mayo endoscopic score		2 [2–2]	
Non-IBD diagnoses			
Irritable bowel syndrome, *N*			9
Coeliac disease, *N*			1
Focal vascular malformation, *N*			1
Median [IQR] faecal calprotectin, µg/g[a]	1381 [737–2146]	1380 [808–1999]	59 [36–64]

IQR, interquartile range; CD, Crohn's disease; UC, ulcerative colitis; sPCDAI, Short Paediatric Crohn's Disease Activity Index; SES-CD, Simple Endoscopic Score-Crohn's Disease; PUCAI, paediatric ulcerative colitis activity index.
[a]Information on faecal calprotectin was missing in four children.

using ultra-performance liquid chromatography [UPLC-MS/MS]. All methods used a Waters ACQUITY UPLC and a Thermo Fisher Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionisation source and Orbitrap mass analyser operated at 35 000 mass resolution. Sample extracts were dried and then reconstituted in solvents compatible with one of four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure consistency in injection and chromatography. One aliquot was analysed in acidic positive ion conditions, chromatographically optimised for more hydrophilic compounds. In this method, the extract was gradient-eluted from a C18 column [Waters UPLC BEH C18-2.1 × 100 mm, 1.7 µm] using water and methanol, containing 0.05% perfluoropentanoic acid and 0.1% formic acid. Another aliquot was analysed in acidic positive ion conditions, chromatographically optimised for more hydrophobic compounds. In this method, the extract was gradient-eluted from the aforementioned C18 column using methanol, acetonitrile, water, 0.05% perfluoropentanoic acid, and 0.01% formic acid, at an overall higher organic content level. The third aliquot was analysed in basic negative ion optimized conditions with a separate dedicated C18 column. The basic extracts were gradient-eluted from the column using methanol and water with 6.5 mM ammonium bicarbonate, at pH 8. The fourth aliquot was analysed via negative ionisation following elution from a hydrophilic interaction chromatography column [Waters UPLC BEH Amide 2.1 × 150 mm, 1.7 µm] using a gradient consisting of water and acetonitrile with 10 mM ammonium formate, at pH 10.8. The MS analysis alternated between MS and data-dependent tandem MS scans using dynamic exclusion. The scan range varied slightly between methods, but covered 70–1000 m/z.

2.5. Identification of metabolites

Metabolites were identified through automated comparison of the ion features in the experimental samples with a reference library of chemical standard entries that included retention time, molecular weight [m/z], preferred adducts, in-source

fragments, and associated MS spectra. They were curated with visual inspection as quality control using software developed at Metabolon. Identification of known chemical entities was based on comparison with metabolomic library entries of purified standards. Commercially available purified standard compounds were acquired for determination of their detectable characteristics. Additional mass spectral entries were created for structurally unnamed biochemicals, which were identified by virtue of their recurrent nature [both chromatographic and mass spectral]. Peaks were quantified using the area under the curve. The raw area counts for each metabolite in each sample were normalised to correct for variation resulting from instrument inter-day tuning differences, using the median value for each run day. Therefore, the medians were set to 1.0 for each run. Missing values were imputed using the observed minimum after normalisation.

2.6. Classification of metabolites

Quality-controlled data were automatically organised into curated metabolic classes and pathways and piped into a secure, cloud-based digital environment for further analysis using custom-built bioinformatics tools, providing detailed pathway annotation based on the integration of literature and institutional knowledge. For quality analyses, pooled quality-controlled plasma replicates from study samples were used to determine endogenous biochemical variability, with a representative relative standard deviation of 10% across all biochemicals. Experimental samples were randomised across the platform run to avoid batch effects.

2.7. Measurement of inflammatory proteins

Inflammatory proteins in plasma were measured using the commercially available Multiplex Inflammation 96 panel [OLINK Proteomics, Uppsala, Sweden]. The methodology enables analysis of 92 inflammation-related protein biomarkers across 96 samples simultaneously, using proximity extension assay technology, and has been described in detail elsewhere. In short, pairs of antibodies against each target antigen are used. When both antibodies bind to the same antigen in close proximity, attached oligonucleotides hybridise. The oligonucleotide templates are extended and amplified using polymerase chain reaction [96.96, Dynamic Array IFC, Fluidigm Biomark] on a Biomark HD Instrument. For each panel, 92 oligonucleotide-labelled antibody probe pairs are allowed to bind to their respective target present in the sample. Olink Proteomics, Uppsala, Sweden, analysed all samples. Data were pre-processed with the Olink Wizard for GenEx [Multid Analyses, Sweden] to generate normalised $\log_2$-values corresponding to relative protein quantities. Proteins were excluded if signals were below the limit of detection in >75% of the samples in both cases and controls.

2.8. Statistical analyses

Continuous variables, representing clinical characteristics, are presented as medians and interquartile ranges, and categorical data as frequencies. Metabolites were considered in the analysis if at least half of the mass signals were detected in any of the investigated groups. Missing values were imputed at one-fifth of the lowest observed value for each compound. Prior to the multivariable statistical analysis, each metabolite intensity was auto-scaled. Univariable analyses were performed using Welch's two-sample t test. A false-discovery rate [FDR] approach was used to account for multiple comparisons and adjusted *p*-values are reported. Multivariable analyses comprised both principal component analysis [PCA], partial least squares discriminant analysis [PLS-DA], and hierarchical cluster analysis. To assess the validity of the PLS-DA model, we performed permutation analyses and R^2 and Q^2 analyses. We also produced variable importance in projection plots to identify major metabolites that could be used to distinguish between different groups of patients. For the multivariable analyses, patients with CD were stratified based on presence of inflammation in the ileum and transverse colon. Univariable and multivariable analyses of metabolomics data were performed with MetaboAnalyst v. 5.0.[31]

For integration analyses, we used DIABLO [Data Integration Analysis for Biomarker discovery using Latent cOmponents],[32,33] which is part of the mixOmics R package version 6.15.33 [2021-01-27]. A design matrix was chosen based on the correlation between the variables of each dataset. For this, correlation values of component 1 of a PLS regression analysis between the datasets were calculated. Using the 'perf' function, the number of components and the type of distance to be included in the tuning of each model were selected based on the lowest balanced error rate achieved. Next, a grid of variables for each dataset was created and tested in tune.block.splsda, using leave-one-out cross-validation to create the final models. In cases where the resulting models included a large number of variables, the maximum was set to 25 for each dataset. Correlation coefficients between important variables in each dataset were calculated in accordance with the method of Ignacio González *et al*.[34] and positive and negative correlations were visualised in Circos plots.

3. Results

Mass spectrometry-based metabolomics analysis using UPLC-MS/MS was performed on 182 biological samples collected at the diagnostic ileocolonoscopy from 56 treatment-naïve paediatric patients with incident IBD [CD, *n* = 47, or UC, *n* = 9] and 11 symptomatic non-IBD controls [Table 1, Figure 2A]. One of the symptomatic controls had a focal vascular malformation in the colonic mucosa, one had coeliac disease, and the remaining nine patients had normal endoscopy and histology on mucosal biopsies. During a median [interquartile range] follow-up period of 6.5 [4.6–7.5] years, all these nine patients were diagnosed with irritable bowel syndrome in accordance with the Rome IV criteria. The analysis was performed on biopsies from ileum [CD; *n* = 35, UC; *n* = 8, and non-IBD symptomatic controls; *n* = 10] and colon [CD; *n* = 45, UC; *n* = 8, and non-IBD symptomatic controls, *n* = 10]. Matched biopsies from both the ileum and the colon were available in 35 patients with CD, eight with UC, and 10 symptomatic controls. Plasma samples were analysed from all individuals, except one patient with CD.

3.1. Metabolomic profile by diagnosis

To examine the paediatric IBD metabolome, we first performed unsupervised multivariable analyses and compared treatment-naïve paediatric patients with incident CD and UC with symptomatic non-IBD controls, stratified by the three biological specimens, ie, plasma, ileal, and colonic biopsies. PCA plots were generated to identify similarities and differences across the sample groups for each investigated sample type. Analyses of ileal samples demonstrated a discrete separation between CD patients with ileal involvement [L1/

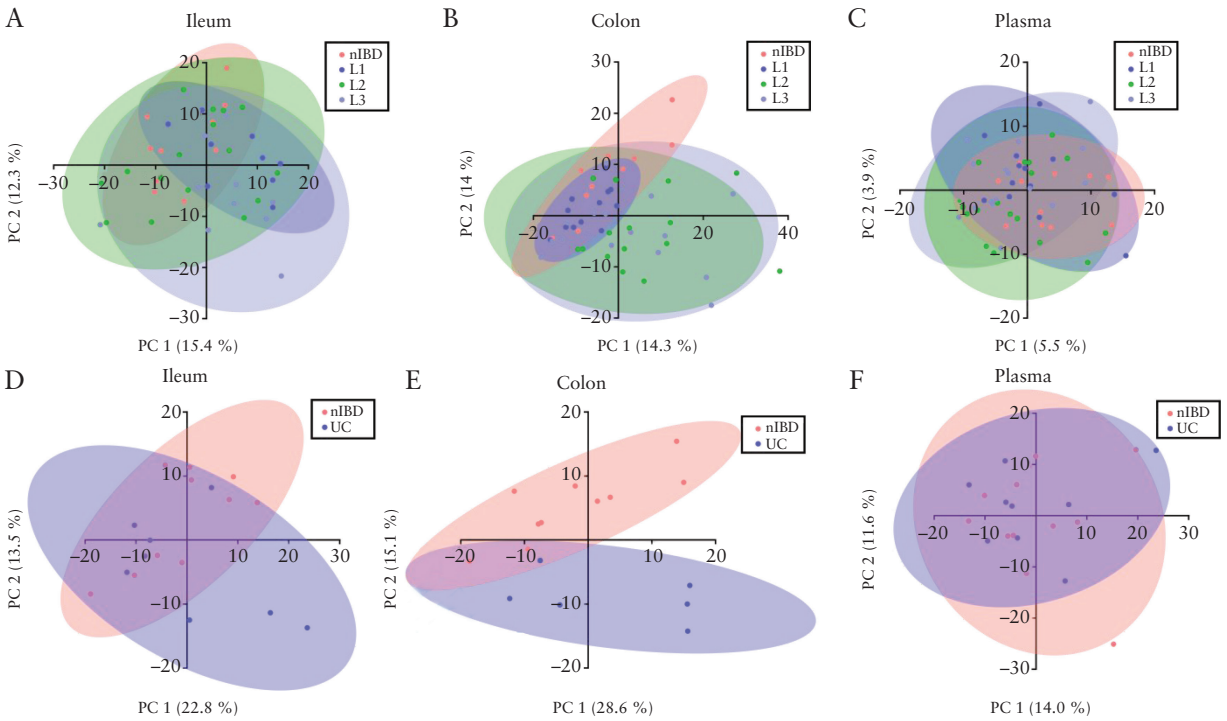

Figure 2. Principal component analysis [PCA] for the metabolomics data showing the degree of discrimination between children with Crohn's disease [phenotypes L1–L3] and symptomatic non-inflammatory bowel disease [nIBD] controls in: A] ileal biopsies; B] colonic biopsies; and C] plasma samples. The lower row shows the corresponding PCA for children with ulcerative colitis [UC] versus symptomatic nIBD controls in: D] ileal biopsies; E] colonic biopsies; and F] plasma samples.

L3] and non-IBD controls [Figure 2A]. When colonic biopsies were examined, the CD patients with inflamed colonic mucosa [L2/L3] and non-IBD controls were partly separated [Figure 2B], whereas CD patients with ileal involvement [L1] were highly similar to the non-IBD controls. Plasma analyses did not result in any clear pattern or separation between the investigated groups [Figure 2C].

A comparison of inflamed colonic biopsies from UC patients and biopsies from non-IBD controls revealed clear separation [Figure 2E], whereas no separation was observed in plasma or ileal samples for UC patients [Figure 2D, F]. No clear pattern or separation was observed when comparing inflamed colonic biopsies and plasma samples from patients with CD [L2, L3] and UC [Supplementary Figure 4A–D], or when comparing inflamed ileal [L1, L3] and inflamed colonic biopsies [L2, L3] from patients with CD [Supplementary Figure 5A–C].

Collectively, these unsupervised analyses of the metabolome signatures showed some disease-associated profiles in inflamed mucosa of CD and UC patients, but not in non-inflamed tissue or plasma.

3.2. Individual metabolites by diagnosis

Since most global metabolomic profiles of children with CD and UC did not clearly differ from those of non-IBD controls, we examined levels of individual metabolites stratified by type of biological material.

3.2.1. Crohn's disease patients versus symptomatic non-IBD controls

Univariable results of stratified analyses by biological material are shown in Table 2 and Figure 3A–F. Several dysregulated metabolites were identified in inflamed ileal and colonic biopsies from CD patients as compared with biopsies from symptomatic non-IBD controls [Table 2, Figure 3A and B], with a fold change of at least 2 and FDR-adjusted p-values <0.05. In contrast, no metabolites were found to be significantly altered in the plasma samples [Figure 3C].

A total of 20 metabolites were upregulated and 25 downregulated in inflamed ileal biopsies from CD patients compared with non-IBD controls [Table 2]. The major altered metabolite class was lipids [10 upregulated and 18 downregulated]. The subclasses of upregulated lipids were mainly sphingolipids, plasmalogens, and glycerophospholipids, whereas most of the downregulated lipids were lysophospholipids.

In inflamed CD colonic biopsies, 12 metabolites were upregulated and 11 downregulated compared with colonic biopsies from non-IBD controls [Table 2]. The main altered metabolite class was lipids [seven upregulated and eight downregulated]. Upregulated lipids were either glycerophospholipids or sphingolipids, whereas most downregulated lipids belonged to either the lysophospholipid [n = 2] or monoacylglycerol [n = 3] subclasses. Some amino acids or their modifications were also upregulated in these colonic samples. The top four differentially regulated metabolites in colonic and ileal tissue, as well as examples of plasma metabolites are shown in Figure 3D–F.

3.2.2. Ulcerative colitis patients versus symptomatic non-IBD controls

In UC patients, metabolic differences compared with non-IBD controls were observed in inflamed colonic biopsies, but not in ileal or plasma samples [Table 2, Figure 4A–F]. These

Table 2. Univariable analyses of single metabolites in inflamed biopsies from ileum and colon in Crohn's disease patients and from colon in ulcerative colitis patients, showing bold p-values for statistically significantly altered metabolites and their compound classes when compared with data from symptomatic non-inflammatory bowel disease [non-IBD] controls

General class	Compound class	Metabolite	CD Ileum	CD Colon	UC Colon
Amino acid	Creatine metabolism	Creatine	**0.03**		
Amino acid	Glutamate metabolism	Carboxyethyl-gaba	**0.02**		
Amino acid	Glutamate metabolism	Glutamine	**0.01**		
Amino acid	Glycine, Serine, and Threonine metabolism	N-acetylserine	**0.01**	**0.03**	**<0.01**
Amino acid	Glycine, serine, and threonine metabolism	N-acetylthreonine	**0.03**	**<0.05**	
Amino acid	Glycine, serine, and threonine metabolism	Betaine		**0.03**	
Amino acid	Leucine, isoleucine, and valine metabolism	Methylsuccinate	**<0.05**		
Amino acid	Lysine metabolism	Hydroxy-N6,N6,N6-trimethyllysine	**<0.05**		
Amino acid	Methionine, cysteine, s-adenosylmethionine, and taurine metabolism	Methionine sulfoxide	**<0.05**		
Amino acid	Tryptophan metabolism	C-glycosyltryptophan	**<0.05**		
Peptide	Gamma-glutamyl amino acid	Gamma-glutamyl-epsilon-lysine			**0.04**
Carbohydrate	Aminosugar metabolism	N-acetylglucosaminitol	**0.01**		
Carbohydrate	Aminosugar metabolism	N-acetylneuraminate		**0.03**	
Carbohydrate	Pentose phosphate pathway	Sedoheptulose-7-phosphate	**0.04**		
Cofactors and vitamins	Nicotinate and Nicotinamide metabolism	Nicotinamide adenine dinucleotide	**0.02**	**0.01**	
Cofactors and vitamins	Nicotinate and Nicotinamide metabolism	Nicotinamide	**<0.05**		
Cofactors and vitamins	Nicotinate and Nicotinamide metabolism	1-methylnicotinamide	**0.03**		
Cofactors and vitamins	Vitamin B6 metabolism	Pyridoxate	**0.03**		**0.04**
Energy	Tricarboxylic acid cycle	Succinate		**0.03**	
Nucleotide	Purine metabolism, guanine-containing	Guanine			**0.04**
Nucleotide	Pyrimidine metabolism, uracil-containing	Beta-alanine	**<0.05**		
Nucleotide	Pyrimidine metabolism, uracil-containing	N-acetyl-beta-alanine	**0.01**		
Xenobiotics	Food component/plant	2,3-dihydroxyisovalerate		**0.01**	
Lipid	Fatty acid metabolism [acyl carnitine, long-chain saturated]	Margaroylcarnitine [C17]	**<0.05**		
Lipid	Fatty acid metabolism [acyl carnitine, polyunsaturated]	Linoleoylcarnitine [C18:2]	**<0.05**		
Lipid	Fatty acid metabolism [acyl carnitine, polyunsaturated]	Docosahexaenoylcarnitine [C22:6]	**0.02**		
Lipid	Fatty acid metabolism [acyl choline]	Oleoylcholine	**<0.05**		
Lipid	Long-chain polyunsaturated fatty acid [n3 and n6]	Linoleate [18:2n6]	**0.01**		
Lipid	Monoacylglycerol	1-myristoylglycerol [14:0]		**<0.05**	
Lipid	Monoacylglycerol	1-linoleoylglycerol [18:2]		**0.03**	
Lipid	Monoacylglycerol	2-linoleoylglycerol [18:2]		**0.03**	
Lipid	Phospholipid metabolism	Glycerophosphorylcholine [GPC]		**0.03**	
Lipid	Phospholipid metabolism	Glycerophosphoserine		**0.03**	
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-myristoyl-2-palmitoyl-GPC [14:0/16:0]	**<0.05**	**0.04**	**0.04**
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1,2-dipalmitoyl-GPC [16:0/16:0]	**0.01**		**0.04**
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-palmitoyl-2-stearoyl-GPC [16:0/18:0]	**0.03**		

Table 2. Continued

General class	Compound class	Metabolite	CD Ileum	CD Colon	UC Colon
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-palmitoyl-2-linoleoyl-GPC [16:0/18:2]	0.01		
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-palmitoyl-2-arachidonoyl-GPC [16:0/20:4n6]		<0.05	0.04
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-palmitoyl-2-docosahexaenoyl-GPC [16:0/22:6]		<0.05	
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-oleoyl-2-linoleoyl-GPC [18:1/18:2]			0.04
Lipid	Glycerophospholipid, phosphatidylcholine [PC]	1-linoleoyl-2-linolenoyl-GPC [18:2/18:3]	0.01		0.04
Lipid	Glycerophospholipid, phosphatidylethanolamine [PE]	1-palmitoyl-2-arachidonoyl-GPE [16:0/20:4]		0.03	
Lipid	Glycerophospholipid, plasmalogen	1-[1-enyl-palmitoyl]-2-oleoyl-GPC [P-16:0/18:1]	0.01		
Lipid	Glycerophospholipid, plasmalogen	1-[1-enyl-palmitoyl]-2-arachidonoyl-GPC [P-16:0/20:4]	0.01		
Lipid	Glycerophospholipid, plasmalogen	1-[1-enyl-palmitoyl]-2-arachidonoyl-GPE [P-16:0/20:4]	0.02	0.03	
Lipid	Lysophospholipid, lysophosphatidylcoline [LPC]	1-palmitoyl-GPC [16:0]	<0.05		
Lipid	Lysophospholipid, lysophosphatidylcoline [LPC]	1-stearoyl-GPC [18:0]	<0.05		
Lipid	Lysophospholipid, lysophosphatidylcoline [LPC]	1-oleoyl-GPC [18:1]	<0.05		
Lipid	Lysophospholipid, lysophosphatidylcoline [LPC]	1-linoleoyl-GPC [18:2]	0.01		
Lipid	Lysophospholipid, lysophosphatidylethanolamine [LPE]	1-palmitoyl-GPE [16:0]	0.01		
Lipid	Lysophospholipid, lysophosphatidylethanolamine [LPE]	1-oleoyl-GPE [18:1]	0.01		
Lipid	Lysophospholipid, lysophosphatidylethanolamine [LPE]	1-linoleoyl-GPE [18:2]	<0.01		
Lipid	Lysophospholipid, lysophosphatidylinositol [LPI]	1-stearoyl-GPI [18:0]	0.03		
Lipid	Lysophospholipid, lysophosphatidylinositol [LPI]	1-oleoyl-GPI [18:1]	0.02		
Lipid	Lysophospholipid, lysophosphatidylinositol [LPI]	1-linoleoyl-GPI* [18:2]	0.01		
Lipid	Lysophospholipid, lysophosphatidylserine [LPSe]	1-palmitoyl-GPSe [16:0]		0.03	
Lipid	Lysophospholipid, lysophosphatidylserine [LPSe]	1-oleoyl-GPSe [18:1]		0.03	
Lipid	Lysoplasmalogen	1-[1-enyl-stearoyl]-GPE [P-18:0]		<0.05	
Lipid	Sphingolipid, ceramide	Ceramide [d18:2/24:1, d18:1/24:2]		0.03	0.04
Lipid	Sphingolipid, ceramide	N-palmitoyl-phytosphingosine [t18:0/16:0]			<0.01
Lipid	Sphingolipid, lactosylceramide	Lactosyl-n-palmitoyl-sphingosine [d18:1/16:0]	0.04	0.03	<0.01
Lipid	Sphingolipid, sphingomyelin	Behenoyl sphingomyelin [d18:1/22:0]	0.03		
Lipid	Sphingolipid, sphingomyelin	Lignoceroyl sphingomyelin [d18:1/24:0]	0.03		
Lipid	Sphingolipid, sphingomyelin	Sphingomyelin [d18:1/24:1, d18:2/24:0]	0.02		
Lipid	Sphingolipid synthesis	Phytosphingosine	0.03		

Red indicates decreased levels and blue increased levels compared with non-IBD controls. In the univariable analyses, there were no significantly altered metabolites in ileal biopsies in UC patients or in plasma samples in either CD or UC patients. The data are false-discovery rate-adjusted *p*-values for each metabolite.
CD, Crohn's disease; UC, ulcerative colitis.

findings were in line with observed differences in the PCA [Figure 2D–F]. In total, 12 colonic metabolites were significantly altered in UC patients compared with non-IBD controls [Table 2, Figure 4B]. Most metabolites were connected to lipid metabolism, with eight increased and four decreased in UC patients. The four most altered metabolites based on fold change for the three biological samples studied are shown in Figure 4D–F [two upregulated and two downregulated were selected].

To further address the metabolomic profiles of paediatric IBD patients, we performed supervised analyses [PLS-DA] and examined levels of individual metabolites, stratified by subtype of IBD and biological material. Information on the results of the PLS-DA is provided in Supplementary Figures 1 and 2.

To examine the diagnostic performance of the most differentially regulated metabolites within the dataset, we performed a hierarchical cluster analysis of the top 50 metabolites, stratified by biological material. The majority of ileal biopsies from CD patients with ileal involvement [L1, L3] were found in a single cluster, and this cluster did not include any non-IBD controls [Supplementary Figure 3A]. In line with this, the majority of the colonic biopsies from CD patients with colonic involvement [L2, L3] clustered separately from non-IBD controls [Supplementary Figure 3B]. In general, phospholipids were upregulated and lysophospholipids were downregulated in CD-derived biopsies compared with those in biopsies from non-IBD controls. In plasma samples, the separation between groups was less evident, although one cluster was represented by samples from CD patients only [Supplementary Figure 3C].

Also, in inflamed colonic metabolites of UC patients, a clear clustering from non-IBD controls was observed [Supplementary Figure 3E], suggesting that these metabolites had a discriminative capacity within the dataset. A similar separation was also seen in ileal biopsies and plasma samples [Supplementary Figure 3D and F].

3.2.3. Crohn's disease versus ulcerative colitis patients and analyses by Crohn's disease location

No dysregulated metabolites were identified in univariable analyses of inflamed colonic biopsies from CD patients compared with UC patients, whereas one single metabolite, eicosenamide [20:1], was downregulated in plasma samples from CD patients [Supplementary Figure 4A–D].

Univariable analyses of inflamed ileal biopsies [L1, L3], compared with inflamed colonic biopsies [L2, L3] in patients with CD, demonstrated downregulations of two single metabolites, beta-hydroxyisovalerate and adenosine, in ileal biopsies [Supplementary Figure 5A–C].

3.3. Correlation between mucosal metabolites and plasma metabolites

Next, we aimed at identifying disease-associated metabolites at the site of inflammation, ie, colonic or ileal mucosa, which correlated with plasma metabolites. Using DIABLO, we performed extensive model building, testing, and evaluation to compare metabolites of CD and UC patients with those of non-IBD controls. In CD patients versus non-IBD controls, our final model with integration of metabolites from inflamed ileum and plasma had an error rate of 15%, with 16 and eight ileal metabolites and eight and five plasma metabolites in components 1 and 2, respectively [Figure 5, Supplementary Tables 2 and 3]. The corresponding fine-tuned model of inflamed colonic and plasma metabolites comprised two components with 10 and five colonic metabolites and five and five plasma metabolites, respectively [Supplementary Tables 2 and 3]. The error rate of this model was 4%.

For UC, the final model, integrating colon and plasma metabolites, included two components with 12 colon metabolites and 18 plasma metabolites and had an error rate of 13%. The error rate for the model with ileum and plasma metabolites in UC was 16% and it included 30 ileal and 14 plasma metabolites [Supplementary Tables 2 and 3].

3.4. Correlation between plasma inflammation markers, disease characteristics, and individual metabolites

To examine the relationships between inflammation, disease characteristics, and plasma metabolites, we investigated correlations between plasma inflammation markers, clinical parameters [age, haemoglobin levels, disease activity], clinically established markers of inflammation [CRP and f-calprotectin], and plasma metabolites, stratified by subtype of IBD. Interleukin [IL]-24 and IL-17A were among the top contributing plasma inflammation markers in the models for CD and UC [Figure 6]. In CD, IL-24 correlated with disease characteristics [haemoglobin], clinically established inflammatory markers [faecal calprotectin] and several plasma metabolites, including some glycerophospholipids. Matrix metalloproteinase [MMP]-1 was important and correlated with the same plasma metabolites as IL-24 and negatively with haemoglobin, whereas IL-17A did not show any correlations with clinical parameters or plasma metabolites. In UC, six plasma inflammation markers correlated with disease characteristics, and some also with plasma metabolites. Here IL-24, IL-17A, and CCL11 correlated with both clinically established inflammatory markers [CRP and f-calprotectin] and the disease activity score [PUCAI]. In addition, these elevated plasma inflammation markers correlated negatively with several plasma metabolites, including *N*-acetyltryptophan, tryptophan, glycerate, and threonate.

4. Discussion

This study demonstrates specific patterns of metabolomic alterations in the inflamed mucosa of treatment-naïve children with new-onset IBD compared with symptomatic non-IBD controls. These findings suggest pervasive mucosal alterations in several metabolic pathways, related to lipid, amino acid, and carbohydrate metabolism, already at IBD diagnosis. In contrast, we did not observe any significant metabolomic alterations in biopsies from non-affected gastrointestinal segments or plasma. By performing integrated correlation analyses, we showed that several of the mucosal metabolic alterations were associated with plasma metabolic alterations. These findings are in line with the general view of IBD as a systemic disease. The associations between key inflammatory markers in plasma and well-established clinical IBD parameters and metabolites further supported that the observed metabolomic alterations are of pathophysiological importance.

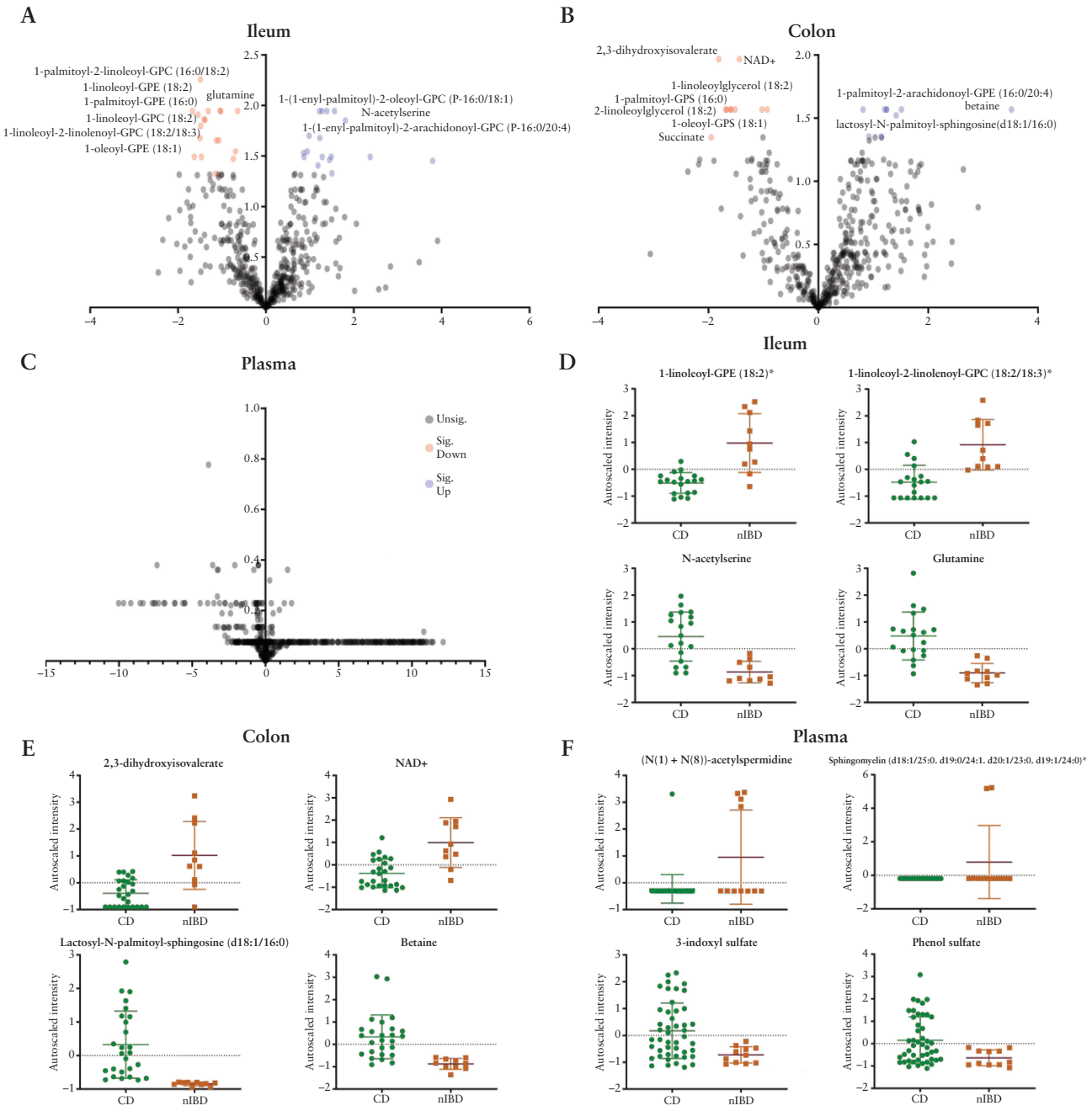

Figure 3. Volcano plots of metabolites from: A] ileal biopsies; B] colonic biopsies; and C] plasma samples in Crohn's disease patients compared with symptomatic non-inflammatory bowel disease [nIBD] controls. Differentially regulated metabolites are annotated and were selected based on $\log_2$-fold change [≥2] and significance [false-discovery rate-adjusted p <0.05]. The last three panels show mean [+/- three standard deviations] levels of the top four altered metabolites for each comparison for: D] ileum; E] colon; and F] plasma. Levels of the top four mucosal metabolites were significantly different between Crohn's disease patients and non-inflammatory bowel disease controls.

Metabolomics is increasingly explored to leverage knowledge about disease mechanisms in IBD, and several studies have reported metabolomic alterations in blood, urine, and faeces.[11,19–22] However, most studies have examined adult populations with long-standing disease, and fewer studies have addressed the intestinal epithelium where the primary inflammation occurs.[11,19–22] To our knowledge, this is the first untargeted exploration of the mucosal metabolome in biopsies from treatment-naïve children with new-onset IBD.

One of the most prominent IBD-related metabolomic patterns was a decrease in several lysophospholipids in inflamed biopsies of children with CD. Lysophospholipids [LP] are bioactive lipids with different immune regulatory and signalling functions, categorised based on their polar head group (choline: lysophosphatidylcholine [LPC], ethanolamine: lysophosphatidylethanolamine [LPE], inositol: lysophosphatidylinositol [LPI], and serine: lysophosphatidylserine [LPSe]).[35,36] Alterations in various LPC, LPE, and also other LP families have been linked to IBD[12,20,36,37] and to inflammation in murine models of IBD.[36,38–43] Diab *et al.* identified an imbalance between pro- and anti-inflammatory bioactive mucosal lipids, including LPC, as a key feature in adult patients with new-onset UC.[20] Recently, decreased serum levels of a wide range of LPC

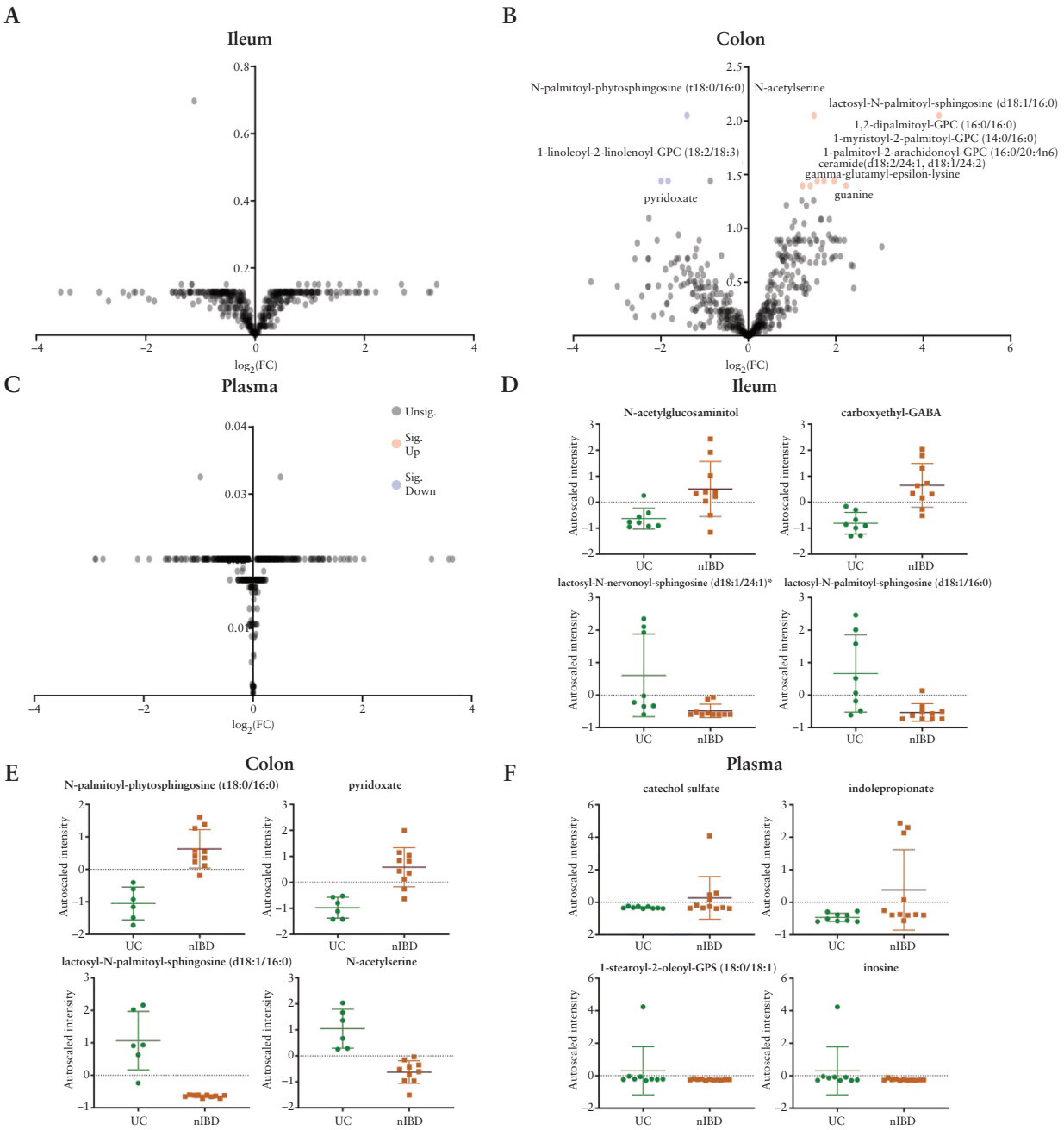

Figure 4. Volcano plots of metabolites from: A] ileal biopsies; B] colonic biopsies; and C] plasma samples in ulcerative colitis patients compared with symptomatic non-inflammatory bowel disease controls. Altered metabolites are annotated and were selected based on $\log_2$-fold change [≥2] and significance [false-discovery rate-adjusted p <0.05]. The last three panels show mean [+/- three standard deviations] levels of the top four altered metabolites for each comparison for; D] ileum; E] colon; and F] plasma.

were reported in treatment-naïve children with new-onset IBD.[12] Interestingly, a disturbed colonic wound healing process has also been associated with mucosal lipidomic alterations, including decreased levels of LP and other bioactive phospholipids, in patients with quiescent UC.[44] In a study of stool samples from adult UC patients, Zou *et al.* demonstrated an association between low levels of LPE [16:0 and 18:1] and inflammation.[39] The authors also associated depletion of mucosal LPE 18:1 in mice with epithelial barrier disruption and colonic inflammation, and found that oral LPE 18:1 supplementation reversed the barrier dysfunction.[39] In line with these previous findings, we observed reduced mucosal levels of several metabolites of the LPC, LPE, LPI, and LPSe families in children with CD. Also, in CD patients, ileal LPC, LPE, and LPI levels correlated positively with plasma levels of *N*-acetyltryptophan, *N* – acetylleucine, cysteinylglycine, and glycerate. Furthermore, in CD patients, colonic LPC and LPI levels correlated positively with plasma levels of amino acid analogues, trimethylamine *N*-oxide, and hexadecadienoate [16:2n6]. These findings together indicate that LP dysregulation in inflamed mucosa is associated with systemic metabolic alterations in children with new-onset CD. In contrast, no statistically significant LP alterations were identified in children with UC.

LPs are derived from mucosal cell membrane phospholipids by phospholipases A1 and A2 [PLA1 and PLA2].[36] The fact that we observed decreased ileal levels of sn-1 LPs and decreased colonic levels of polyunsaturated fatty acid linoleate [18:2n6] in children with CD indicates that the disease onset is associated with reduced PLA2 enzymatic activity, since PLA2 deacetylate membrane-bound phospholipids to free sn-1 LPs and free polyunsaturated fatty acids.[36] The observed increase of some phospholipid precursors (phosphatidylcholine [PC] 16:0/16:0, PC 16:0/18:0, PC 16:0/20:4n6, phosphatidylethanolamine 16:0/20:4) and the absence of reduced levels of such LP precursors further support this hypothesis. In contrast to these findings, some previous studies have reported increased PLA2 activity in both colon mucosa and serum of patients with UC and CD.[45,46] These differences in outcome may reflect differences between study populations, particularly given that the previous studies examined adults with long-standing disease and ongoing medication, whereas we studied paediatric patients with incident IBD.

Another prominent IBD-related metabolomic pattern in this study was an increase in several mucosal sphingolipids in both CD and UC. As regards LP, mucosal dysregulation of sphingolipids was associated with the presence of inflammation. Bioactive sphingolipids are increasingly recognised as important regulators of inflammation, and mucosal alterations in various sphingolipids, including ceramides and sphingomyelins, have been reported in adult UC patients.[20] The recent approval of a sphingosine-1-phosphate receptor

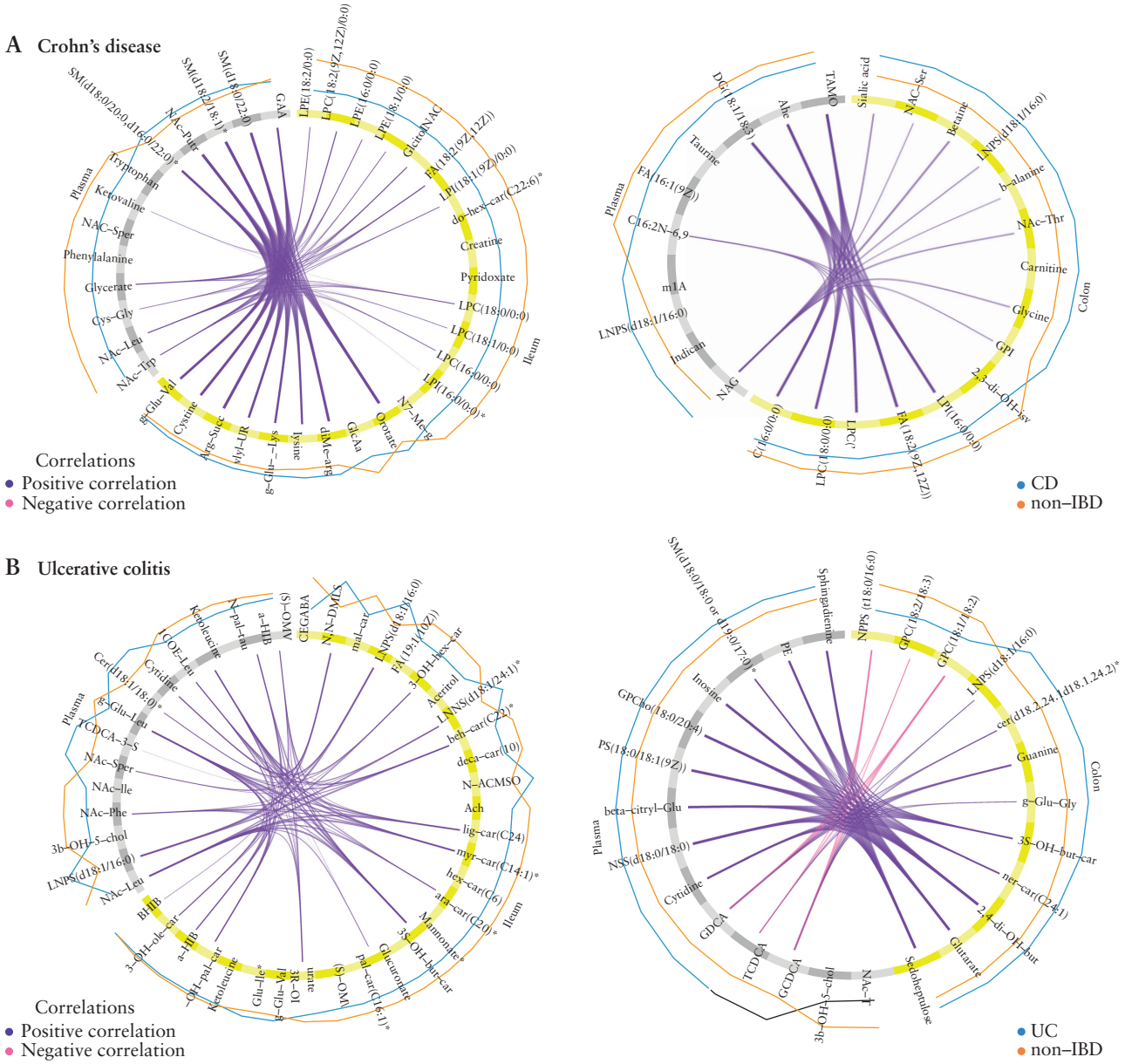

Figure 5. Circos plots depicting correlations between metabolites in biopsies and plasma for Crohn's disease [CD] patients [A] and ulcerative colitis [UC] patients [B], based on integrative partial least squares discriminant analysis. Positive and negative correlations with a value exceeding 0.7 are shown as purple and pink lines, respectively. The weight of a line increases with correlation strength. Lines outside the plots represent overall expression of the variables for children with CD and UC in blue, and symptomatic non-inflammatory bowel disease [non-IBD] controls in orange. Metabolites with grey background are from plasma, and yellow indicates biopsy metabolites from ileum [left panel] and colon [right panel], respectively. Abbreviations for metabolites are explained in Supplementary Table 2.

modulator [ozanimod] for the treatment of moderately to severely active UC in adults underscores a role of sphingolipids in IBD.[47] In line with recent reports from paediatric and adult IBD populations,[14,15,22] we observed an increase in lactosylceramide lactosyl-*N*-palmitoyl-sphingosine [LCER d18:1/16:0] in inflamed mucosa of children with CD and UC. In UC, the correlation analysis also identified positive correlations of mucosal LCER d18:1/16:0 with its plasma levels and with three plasma bile acids. Lactosylceramide seems to support plasma membrane stability, activate specific receptor molecules, and bind to specific bacteria.[48] We also observed a decrease of *N*-palmitoyl-phytosphingosine [t18:0/16:0] in inflamed mucosa of children with UC and CD compared with those in non-IBD controls.

These associations were supported by our PLS-DA, where levels of LCER d18:1/16:0 and *N*-palmitoyl-phytosphingosine [t18:0/16:0] in colonic biopsies were identified as the most important variables for distinguishing children with UC from non-IBD controls. In UC, mucosal levels of *N*-palmitoyl-phytosphingosine [t18:0/16:0] negatively correlated with three plasma bile acids. In line with these results, Montenegro-Burke *et al.* recently reported decreased levels of phytosphingosine in mice with 2,4,6-trinitrobenzene sulphonic acid-induced colitis and demonstrated its anti-inflammatory effect upon oral administration.[49] Thus, phytosphingosine or its *N*-palmitoyl analogue may potentially be used to alleviate inflammation in IBD. A pathophysiological role of sphingolipids in IBD was further supported by the results of the multivariable analyses, showing correlations between CD mucosal sphingolipid levels and plasma levels of various metabolites including sphingolipids, bile acids, and *N*-acetylglucosamine/*N*-acetylgalactosamine. Impaired *N*-acetylation of glucosamine and metabolic perturbations of other *N*-acetylated metabolites have previously been linked to IBD.[50,51] Dawiskiba *et al.* described an altered overall acetylation pattern by comparing serum samples from IBD patients with those from healthy controls.[51] In line with this observation, we identified correlations between plasma levels of several *N*-acetylated metabolites, including *N*-acetylleucine, and metabolomic alterations of both lysophospholipids and sphingolipids in ileal mucosa of patients with CD.

Bile acids and tryptophan are other metabolites that have been implicated in the pathogenesis of IBD.[8] In the present study, plasma levels of two primary bile acids (glycochenodeoxycholate [glyco-CDCA] and taurochenodeoxycholate [tauro-CDCA]) and a secondary bile acid (glycodeoxycholate [glyco-DCA]) were negatively correlated with mucosal phospholipid levels in colonic biopsies from UC patients. Glyco-CDCA and tauro-CDCA are released into the intestine, where specific gut microbiome bacteria deconjugate the glycine or taurine moieties to yield CDCA,[52] whereas glyco-DCA is a product of the human host-microbiome co-metabolism. Thus, all three bile acids are involved in microbiome metabolic transformation, as either substrates or products, and may have additional metabolic and immune effects through binding to different receptors.[53,54] In line with the previously reported negative association between disease activity and tryptophan levels in a large cohort of IBD patients,[55] we observed a negative correlation between inflammation markers and plasma levels of tryptophan in patients with UC. The observed increase of *C*-glycosyltryptophan in inflamed ileal mucosa of CD patients further supports an increased tryptophan metabolism in active IBD.

This study has several strengths, but also some weaknesses. The examination of a paediatric inception IBD cohort,

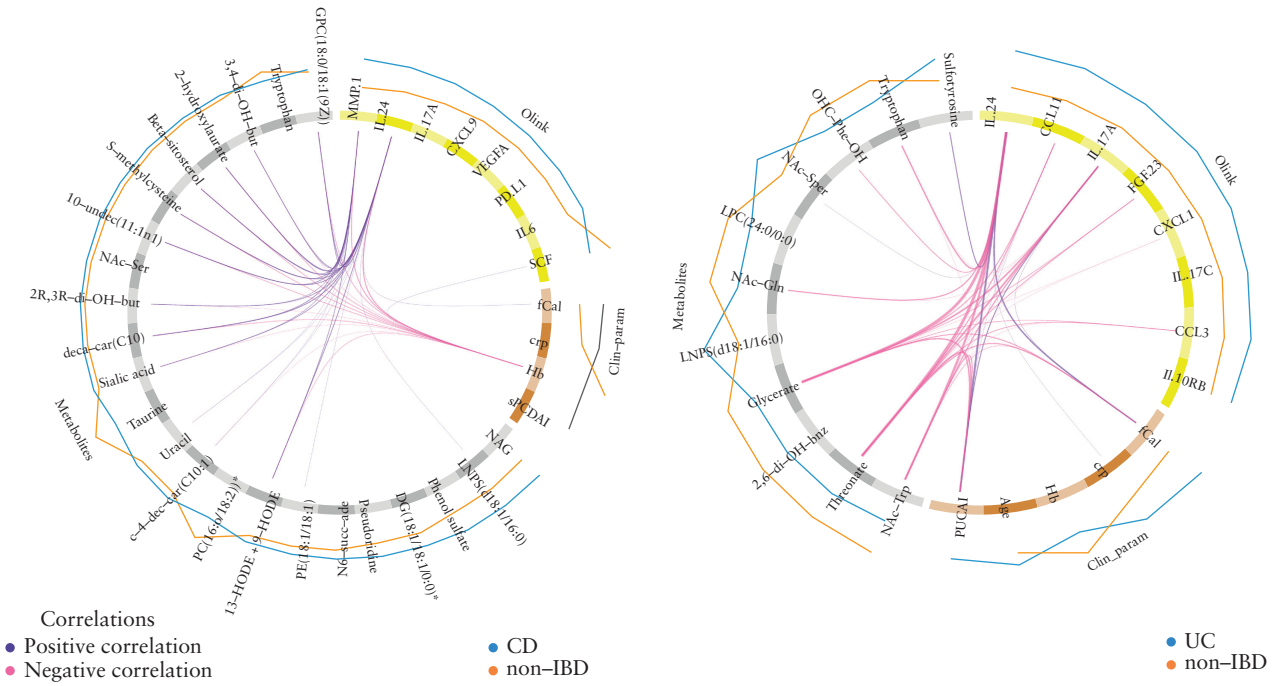

Figure 6. Circos plots depicting correlations between plasma metabolites [grey], Olink inflammation markers [yellow] and selected disease characteristics [light brown] for Crohn's disease [CD, left] and ulcerative colitis [UC, right] based on integrative partial least squares discriminant analyses. Positive and negative correlations with a value exceeding 0.6 are shown as purple and pink lines, respectively. The weight of a line increases with correlation strength. Lines outside the plots represent overall expression of the variables for children with CD and UC in blue, and symptomatic non-inflammatory bowel disease [non-IBD] controls in orange. Abbreviations for metabolites are explained in Supplementary Table 2.

where samples were obtained before the initiation of treatment, is a major strength. The use of children with symptoms mimicking IBD as controls shows the relevance of the identified compounds as targets in future diagnostic biomarker discovery efforts in IBD. By analysing matched ileal and colonic biopsies and plasma samples, we explored the extent to which the mucosal and plasma IBD metabolomes reflect each other. Furthermore, by integrating a large set of inflammatory proteins, we were able to correlate inflammatory pathways with metabolic signatures and clinical measures of disease activity. Several of these inflammatory markers, including IL-17A, IL-24, MMP-1, and CCL-11, have previously been identified as key inflammatory markers in CD and UC.[56–59] The use of the Metabolon platform allowed us to assess semi-quantitative differences in a large set of metabolites across various compound classes and provided a comprehensive metabolic pathway coverage. The number of total metabolites examined in this study [n = 1300] exceeds the number of metabolites obtained by several other analytical techniques, including nuclear magnetic resonance spectroscopy.

However, the UC and non-IBD control groups were small, and non-significant findings should be interpreted with caution, as we probably lacked statistical power to fully identify possible differences between these groups. The small sample size has probably reduced our possibilities in detecting a true difference in our comparisons of patients with CD and UC, as well as when we stratified patients with CD by location of inflammation. Nevertheless, results of previous metabolomic studies comparing subtypes of IBD or different phenotypes of CD are inconsistent. In accordance with our findings, several studies have failed to demonstrate metabolomic profiles in plasma, serum, or mucosal samples which clearly discriminate between CD and UC.[16,60,61] The number of patients in each disease category was based on the inclusion rate, ie, number of patients referred to Uppsala University Children's Hospital with suspected IBD and available biological material during the study period, and was not predefined by a formal estimation of sample size and power analysis. The use of children with gastrointestinal symptoms instead of healthy children as controls may have hampered our possibilities of identifying metabolomic alterations in non-inflamed tissue and plasma, since children with gastrointestinal symptoms probably represent a heterogeneous population. In contrast to our findings of dysregulated metabolites only in inflamed tissue, others have reported metabolomic changes in serum and non-inflamed biopsies of both children and adult patients with IBD.[12,50] Even though we integrated inflammatory proteins in the analyses, we acknowledge that the absence of data on the gut microbiome represents a limitation. Future studies would benefit from adding shotgun metagenomics and integrated analyses of mucosa-associated microbiota composition and function. The absence of a validation cohort is another major limitation that questions the possibility to generalise our findings to other children with IBD. Consequently, findings from our study need to be reproduced and diagnostic algorithms from metabolites should be identified and validated in future studies. The fact that we were not able to match controls by sex and age increases the impact of bias due to potential differences in demographics. Among inflammatory markers used in clinical practice, we analysed CRP and haemoglobin and did not include other markers in blood, such as erythrocyte sedimentation rate or platelet count. Last, we did not adjust our analyses for possible differences in body mass index or lifestyle factors, such as smoking and dietary habits.

In conclusion, we identified specific metabolomic alterations in the inflamed mucosa of treatment-naïve children with new-onset IBD compared with symptomatic non-IBD controls. Perturbations of mucosal levels of bioactive lysophospholipids and sphingolipids which correlated with plasma metabolites were the most prominent IBD-associated patterns. By integrating metabolomic findings with inflammatory protein markers in plasma and clinical parameters, we also identified underlying inflammatory and clinical markers associated with the altered mucosal metabolomic signatures. Our novel exploratory findings demonstrate that the study of mucosal metabolic patterns provides an important foundation for improving the understanding of IBD pathogenesis and for future diagnostic biomarker discoveries in paediatric IBD.

Data Availability Statement

The data underlying this article will be shared upon reasonable request to the corresponding author.

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Conflict of Interest

JH has served as speaker, consultant and/or advisory board member for AbbVie, Aqilion, BMS, Celgene, Celltrion, Ferring, Galapagos, Gilead, Hospira, Janssen, MEDA, Medivir, MSD, Olink Proteomics, Novartis, Pfizer, Prometheus Laboratories, Sandoz, Shire, Takeda, Thermo Fisher Scientific, Tillotts Pharma, Vifor Pharma, and UCB, outside of the submitted work. JH also has received grant support from Janssen, MSD, and Takeda, also outside of the submitted work. NN has served as speaker for Abigo, Baxter, Ferring, Fresenius-Kabi, Mylan/Meda, Nutricia, Shire, Takeda, Thermo Fisher Scientific, Tillotts Pharma, and as consultant and advisory board member for Takeda, outside the submitted work. All other authors declare no conflicts of interest.

Author Contributions

Study concept and design: all authors. Patient recruitment and sample processing: NN, ISK, LE. Data analysis: all authors Drafting the manuscript: NN, SPN, JH. Study coordination: ISK, JH. All authors were involved in critical review, editing, revision, and approval of the final manuscript.

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Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

References

1. Ludvigsson JF, Büsch K, Olén O, *et al*. Prevalence of paediatric inflammatory bowel disease in Sweden: a nationwide population-based register study. *BMC Gastroenterol* 2017;**17**:23.
2. Kuenzig ME, Fung SG, Marderfeld L, *et al*. Twenty-first century trends in the global epidemiology of pediatric-onset inflammatory bowel disease: systematic review. *Gastroenterology* 2022;**162**:1147–59.e4.
3. Kaplan GG, Windsor JW. The four epidemiological stages in the global evolution of inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 2021;**18**:56–66.
4. Graham DB, Xavier RJ. Pathway paradigms revealed from the genetics of inflammatory bowel disease. *Nature* 2020;**578**:527–39.
5. Gubatan J, Boye TL, Temby M, *et al*. Gut microbiome in inflammatory bowel disease: role in pathogenesis, dietary modulation, and colitis-associated colon cancer. *Microorganisms* 2022;**10**:1371.
6. Lloyd-Price J, Arze C, Ananthakrishnan AN, *et al*. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019;**569**:655–62.
7. Ursell LK, Haiser HJ, Van Treuren W, *et al*. The intestinal metabolome: an intersection between microbiota and host. *Gastroenterology* 2014;**146**:1470–6.
8. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 2020;**17**:223–37.
9. Bauset C, Gisbert-Ferrándiz L, Cosín-Roger J. Metabolomics as a promising resource identifying potential biomarkers for inflammatory bowel disease. *J Clin Med* 2021;**10**:622.
10. Longo S, Chieppa M, Cossa LG, *et al*. New insights into inflammatory bowel diseases from proteomic and lipidomic studies. *Proteomes* 2020;**8**:18.
11. Gallagher K, Catesson A, Griffin JL, Holmes E, Williams HRT. Metabolomic analysis in inflammatory bowel disease: a systematic review. *J Crohns Colitis* 2021;**15**:813–26.
12. Daniluk U, Daniluk J, Kucharski R, *et al*. Untargeted metabolomics and inflammatory markers profiling in children with Crohn's disease and ulcerative colitis: a preliminary study. *Inflamm Bowel Dis* 2019;**25**:1120–8.
13. Diederen K, Li JV, Donachie GE, *et al*. Exclusive enteral nutrition mediates gut microbial and metabolic changes that are associated with remission in children with Crohn's disease. *Sci Rep* 2020;**10**:18879.
14. Filimoniuk A, Blachnio-Zabielska A, Imierska M, Lebensztejn DM, Daniluk U. Sphingolipid analysis indicates lactosylceramide as a potential biomarker of inflammatory bowel disease in children. *Biomolecules* 2020;**10**:1083.
15. Filimoniuk A, Daniluk U, Samczuk P, *et al*. Metabolomic profiling in children with inflammatory bowel disease. *Adv Med Sci* 2020;**65**:65–70.
16. Kolho KL, Pessia A, Jaakkola T, de Vos WM, Velagapudi V. Faecal and serum metabolomics in paediatric inflammatory bowel disease. *J Crohns Colitis* 2017;**11**:321–34.
17. Marques JG, Shokry E, Frivolt K, *et al*. Metabolomic signatures in pediatric Crohn's disease patients with mild or quiescent disease treated with partial enteral nutrition: a feasibility study. *SLAS Technol* 2021;**26**:165–77.
18. Scoville EA, Allaman MM, Brown CT, *et al*. Alterations in lipid, amino acid, and energy metabolism distinguish Crohn's disease from ulcerative colitis and control subjects by serum metabolomic profiling. *Metabolomics* 2018;**14**:17.
19. Balasubramanian K, Kumar S, Singh RR, *et al*. Metabolism of the colonic mucosa in patients with inflammatory bowel diseases: an in vitro proton magnetic resonance spectroscopy study. *Magn Reson Imaging* 2009;**27**:79–86.
20. Diab J, Hansen T, Goll R, *et al*. Mucosal metabolomic profiling and pathway analysis reveal the metabolic signature of ulcerative colitis. *Metabolites* 2019;**9**:291.
21. Masoodi M, Pearl DS, Eiden M, *et al*. Altered colonic mucosal polyunsaturated fatty acid [pufa] derived lipid mediators in ulcerative colitis: new insight into relationship with disease activity and pathophysiology. *PLoS One* 2013;**8**:e76532.
22. Bazarganipour S, Hausmann J, Oertel S, *et al*. The lipid status in patients with ulcerative colitis: sphingolipids are disease-dependent regulated. *J Clin Med* 2019;**8**:971.
23. Levine A, Koletzko S, Turner D, *et al*. ESPGHAN revised Porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014;**58**:795–806.
24. Levine A, Griffiths A, Markowitz J, *et al*. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011;**17**:1314–21.
25. Hyams JS, Ferry GD, Mandel FS, *et al*. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr* 1991;**12**:439–47.
26. Kappelman MD, Crandall WV, Colletti RB, *et al*. Short pediatric Crohn's disease activity index for quality improvement and observational research. *Inflamm Bowel Dis* 2011;**17**:112–7.
27. Turner D, Otley AR, Mack D, *et al*. Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. *Gastroenterology* 2007;**133**:423–32.
28. Turner D, Hyams J, Markowitz J, *et al*. Appraisal of the pediatric ulcerative colitis activity index [PUCAI]. *Inflamm Bowel Dis* 2009;**15**:1218–23.
29. Daperno M, D'Haens G, Van Assche G, *et al*. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004;**60**:505–12.
30. Samuel S, Bruining DH, LoftusThia EVKT, *et al*. Validation of the ulcerative colitis colonoscopic index of severity and its correlation with disease activity measures. *Clin Gastroenterol Hepatol* 2013;**11**:49–54.e1.
31. Pang Z, Chong J, Zhou G, *et al*. Metaboanalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 2021;**49**:W388–96.
32. Singh A, Shannon CP, Gautier B, *et al*. Diablo: an integrative approach for identifying key molecular drivers from multi-omics assays. *Bioinformatics* 2019;**35**:3055–62.
33. Tenenhaus A, Philippe C, Guillemot V, Le Cao K-A, Grill J, Frouin V. Variable selection for generalized canonical correlation analysis. *Biostatistics* 2014;**15**:569–83.
34. González I, Cao KA, Davis MJ, Déjean S. Visualising associations between paired 'omics' data sets. *BioData Min* 2012;**5**:19.
35. Leuti A, Fazio D, Fava M, Piccoli A, Oddi S, Maccarrone M. Bioactive lipids, inflammation and chronic diseases. *Adv Drug Deliv Rev* 2020;**159**:133–69.
36. Alhouayek M, Ameraoui H, Muccioli GG. Bioactive lipids in inflammatory bowel diseases - from pathophysiological alterations to therapeutic opportunities. *Biochim Biophys Acta Mol Cell Biol Lipids* 2021;**1866**:158854.
37. Braun A, Treede I, Gotthardt D, *et al*. Alterations of phospholipid concentration and species composition of the intestinal mucus barrier in ulcerative colitis: a clue to pathogenesis. *Inflamm Bowel Dis* 2009;**15**:1705–20.
38. Bauer J, Liebisch G, Hofmann C, *et al*. Lipid alterations in experimental murine colitis: role of ceramide and imipramine for matrix metalloproteinase-1 expression. *PLoS One* 2009;**4**:e7197.
39. Zou D, Pei J, Lan J, *et al*. A SNP of bacterial BLC disturbs gut lysophospholipid homeostasis and induces inflammation through epithelial barrier disruption. *EBioMedicine* 2020;**52**:102652.
40. Sturm A, Zeeh J, Sudermann T, Rath H, Gerken G, Dignass AU. Lisofylline and lysophospholipids ameliorate experimental colitis in rats. *Digestion* 2002;**66**:23–9.
41. Frasch SC, Bratton DL. Emerging roles for lysophosphatidylserine in resolution of inflammation. *Prog Lipid Res* 2012;**51**:199–207.

42. Frasch SC, McNamee EN, Kominsky D, *et al*. G2a signaling dampens colitic inflammation via production of IFN-γ. *J Immunol* 2016;**197**:1425–34.
43. Masquelier J, Alhouayek M, Terrasi R, Bottemanne P, Paquot A, Muccioli GG. Lysophosphatidylinositols in inflammation and macrophage activation: altered levels and anti-inflammatory effects. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018;**1863**:1458–68.
44. Bjerrum JT, Wang Y, Zhang J, Riis LB, Nielsen OH, Seidelin JB. Lipidomic trajectories characterize delayed mucosal wound healing in quiescent ulcerative colitis and identify potential novel therapeutic targets. *Int J Biol Sci* 2022;**18**:1813–28.
45. Almer S, Franzén L, Olaison G, Smedh K, Ström M. Phospholipase a2 activity of colonic mucosa in patients with ulcerative colitis. *Digestion* 1991;**50**:135–41.
46. Minami T, Tojo H, Shinomura Y, Matsuzawa Y, Okamoto M. Increased group ii phospholipase a2 in colonic mucosa of patients with Crohn's disease and ulcerative colitis. *Gut* 1994;**35**:1593–8.
47. Sandborn WJ, Feagan BG, D'Haens G, *et al*. Ozanimod as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2021;**385**:1280–91.
48. Wishart DS, Guo A, Oler E, *et al*. Hmdb 5.0: the human metabolome database for 2022. *Nucleic Acids Res* 2022;**50**:D622–d31.
49. Montenegro-Burke JR, Kok BP, Guijas C, *et al*. Metabolomics activity screening of T cell-induced colitis reveals anti-inflammatory metabolites. *Sci Signal* 2021;**14**:eabf6584.
50. Williams HR, Willsmore JD, Cox IJ, *et al*. Serum metabolic profiling in inflammatory bowel disease. *Dig Dis Sci* 2012;**57**:2157–65.
51. Dawiskiba T, Deja S, Mulak A, *et al*. Serum and urine metabolomic fingerprinting in diagnostics of inflammatory bowel diseases. *World J Gastroenterol* 2014;**20**:163–74.
52. Heinken A, Ravcheev DA, Baldini F, *et al*. Systematic assessment of secondary bile acid metabolism in gut microbes reveals distinct metabolic capabilities in inflammatory bowel disease. *Microbiome* 2019;**7**:75.
53. Kim I, Ahn SH, Inagaki T, *et al*. Differential regulation of bile acid homeostasis by the farnesoid x receptor in liver and intestine. *J Lipid Res* 2007;**48**:2664–72.
54. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* 2014;**11**:55–67.
55. Nikolaus S, Schulte B, Al-Massad N, *et al*. Increased tryptophan metabolism is associated with activity of inflammatory bowel diseases. *Gastroenterology* 2017;**153**:1504–16.e2.
56. Andersson E, Bergemalm D, Kruse R, *et al*. Subphenotypes of inflammatory bowel disease are characterized by specific serum protein profiles. *PLoS One* 2017;**12**:e0186142.
57. Ónody A, Veres-Székely A, Pap D, *et al*. Interleukin-24 regulates mucosal remodeling in inflammatory bowel diseases. *J Transl Med* 2021;**19**:237.
58. O'Sullivan S, Gilmer JF, Medina C. Matrix metalloproteinases in inflammatory bowel disease: an update. *Mediators Inflamm* 2015;**2015**:964131.
59. Bergemalm D, Andersson E, Hultdin J, *et al*. Systemic inflammation in preclinical ulcerative colitis. *Gastroenterology* 2021;**161**:1526–39.e9.
60. Schicho R, Shaykhutdinov R, Ngo J, *et al*. Quantitative metabolomic profiling of serum, plasma, and urine by [1]h nmr spectroscopy discriminates between patients with inflammatory bowel disease and healthy individuals. *J Proteome Res* 2012;**11**:3344–57.
61. Bjerrum JT, Steenholdt C, Ainsworth M, *et al*. Metabonomics uncovers a reversible proatherogenic lipid profile during infliximab therapy of inflammatory bowel disease. *BMC Med* 2017;**15**:184.