Metabolomics facilitate the personalized management in inflammatory bowel disease

Rirong Chen*, Jieqi Zheng*, Li Li†, Chao Li, Kang Chao, Zhirong Zeng, Minhu Chen and Shenghong Zhang

Abstract: Inflammatory bowel disease (IBD) is a gastrointestinal disorder characterized by chronic relapsing inflammation and mucosal lesions. Reliable biomarkers for monitoring disease activity, predicting therapeutic response, and disease relapse are needed in the personalized management of IBD. Given the alterations in metabolomic profiles observed in patients with IBD, metabolomics, a new and developing technique for the qualitative and quantitative study of small metabolite molecules, offers another possibility for identifying candidate markers and promising predictive models. With increasing research on metabolomics, it is gradually considered that metabolomics will play a significant role in the management of IBD. In this review, we summarize the role of metabolomics in the assessment of disease activity, including endoscopic activity and histological activity, prediction of therapeutic response, prediction of relapse, and other aspects concerning disease management in IBD. Furthermore, we describe the limitations of metabolomics and highlight some solutions.

Keywords: disease activity, inflammatory bowel disease, metabolomics, relapse, therapeutic response

Received: 29 August 2021; revised manuscript accepted: 15 November 2021.

Introduction

Inflammatory bowel disease (IBD), a chronic recurrent inflammatory disease of the gastrointestinal tract, is composed of Crohn’s disease (CD) and ulcerative colitis (UC). These two disorders differ in many respects. CD is characterized by dispersed lesions and transmural inflammation that can occur anywhere in the gastrointestinal tract, with the most frequent symptoms of abdominal pain, diarrhea, fistulae, obstruction, and/or perianal lesions. In UC, the lesions are continuous and limited to the mucosal layer of the colon and rectum, which can most commonly lead to bloody diarrhea and rectal bleeding.

The prevalence of IBD is higher in developed countries than developing countries. Nevertheless, the prevalence and incidence of IBD are increasing globally, especially in newly industrialized countries. Therefore, the disease burden will become ever higher in both developed and developing countries. To reduce the disease burden, personalized management, including regular monitoring of disease activity, matching the patients with the proper treatment, and predicting prognosis, is important. Previous studies have referred to a variety of serological and fecal biomarkers, such as serum C-reactive protein (CRP), interleukin 6, fecal calprotectin, and lactoferrin, which serve as surrogate markers for the diagnosis and management of IBD. However, these available biomarkers could only assist with the activity monitoring and prognosis of IBD to some extent, instead of being considered as a gold standard in the personalized management of IBD. Additional studies or techniques are necessary to discover potential and effective biomarkers to guide the management of IBD.
Given that patients with IBD often exhibit specific metabolomic profiles, metabolomic analysis is a potential tool for identifying diagnostic and therapeutic markers of IBD. Metabolomic analysis is a technique with the advantages of high throughput, high sensitivity, and high accuracy for the qualitative and quantitative study of small metabolite molecules in biological samples, including feces, urine, serum, plasma, breath, and biopsy samples. Metabolomic analysis covers a wide range of metabolites with a molecular mass less than 1500 Da, including but not limited to sugars, lipids, amino acids, nucleic acids, organic acids, fatty acids, and some exogenous chemicals. Through metabolomics analysis, promising metabolites or models based on metabolites for facilitating accurate diagnosis and personalized management of IBD will be discovered. Although previous studies have summarized the role of metabolomics in the identification of diagnostic markers for IBD and their possible association with pathological mechanisms, few other clinical applications of metabolomics have been reviewed in detail. Therefore, in the present review, we highlight the possible value of metabolomics in evaluating disease activity for IBD, as well as its potential in predicting treatment response and disease relapse.

Metabolomics
Metabolomics is the qualitative and quantitative study of a suite of small metabolite molecules in biological samples, which symbolizes the metabolic phenotypes of a living system under specific conditions, or in other words, reflects the metabolic response to pathophysiological stimuli or even genetic modification at a certain time point. It includes both targeted and untargeted methods. Targeted metabolomics often focuses on specific known metabolic pathways and can be used for the quantification of metabolites. However, untargeted metabolomics tends to analyze a large number of metabolites in a sample without bias and can be used for the identification of unknown metabolites. In the existing literature, analytes in metabolomics of IBD involve an immense variety of metabolites participating in various metabolic pathways in organisms, such as the tricarboxylic acid cycle, urea cycle, or fat oxidation. These metabolites can be roughly classified as endogenous and exogenous substances. The former includes organic acids such as citrate and succinate, lipids such as sphingolipids and glycerolipids, amino acids such as histidine and cystine, short-chain fatty acids (SCFAs), glycosylation products, and other endogenous molecules. Exogenous substances including different kinds of xenobiotics have also been mentioned in some studies.

The primary analytical techniques for metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) coupled with gas or liquid chromatography. NMR spectroscopy distinguishes metabolites by the resonance frequency of nuclei in a magnetic field. Since the frequency and pattern of resonance vary with the chemical environment, each metabolite with a specific molecular structure generates a unique resonance, showing characteristic chemical shifts in the spectra. MS, however, ionizes and fragments the compounds into smaller molecules of different mass-to-charge ratios, which can be measured by a detector and then give rise to corresponding mass spectra. Ionization and fragmentation are to a large extent determined by the chemical structures of metabolites, which enables the identification of different compounds. Chromatography used prior to MS analysis physically separates the metabolites so that the detection of individual molecules can be enhanced. Both NMR spectroscopy and MS coupled with chromatography are high-throughput techniques and have their own advantages and limitations.

As compared to MS, NMR spectroscopy requires minimal sample preparation and has a higher quantitative ability and reproducibility without sample destruction. Nevertheless, signals of metabolites with low concentrations may be covered and cannot be identified due to overlapping signals, leading to a lower sensitivity and narrower detection range as compared to MS techniques. With the help of bioinformatic tools, data acquired from NMR or MS can be processed automatically. Multivariate statistical techniques, such as principal components analysis and partial least squares discriminant analysis, are widely applied in metabolomics studies to discover distinct metabolite profiles in different sample classes.

Metabolomics for assessing disease activity in IBD
The assessment of disease activity in IBD is an essential step in judging the stage of the disease, selecting an appropriate treatment plan, monitoring the progress of the disease, and evaluating the...
treatment effect. Clinical remission and endoscopic healing in IBD are recommended as intermediate and long-term therapeutic targets, respectively. Histological healing also assists in assessing the depth of treatment response in UC. Therefore, a precise and convenient assessment of disease activity is necessary for the management of IBD. Studies have found that changes in the levels of some metabolites in patients with IBD are strongly associated with the severity of disease activity, and models based on metabolomic findings are capable of accurately distinguishing patients with active disease from those in remission. Since researches that focus on metabolomics and clinical disease activity contribute to revealing disease characteristics rather than patient management, in this section, we just review studies aimed at assessing the endoscopic or histological activity of IBD through metabolomics (Table 1).

**Metabolomic analysis and endoscopic activity**

Several studies have revealed that a variety of metabolites from different samples are related to endoscopic activity in patients with IBD. Bodelier et al. conducted a prospective cohort study of 725 exhaled air samples from 191 patients with CD to explore potential volatile organic compounds (VOCs) to discriminate the active and remission patients. They found that remission patients were characterized with elevated decanal level and reduced concentration of other nine VOCs. Moreover, in breath samples, ethane was mentioned to be positively correlated with the endoscopic score of UC in another study. Through metabolomics analysis, several metabolites in urine sample, including ethylmalonate, l-3,4-dihydroxy-phenylalanine, levoglucosan, and propylene glycol, were demonstrated to associate with endoscopic recurrence in CD patients with ileocolonic resection. In addition, urine metabolites associated with endoscopic recurrence differed between patients with and without the use of biological agents. These results suggest the impact of biologics therapy in metabolites should be considered in future metabolomic researches. Furthermore, the association between fecal metabolites and mucosal healing (using calprotectin levels as a surrogate) in pediatric CD was explored in a recent study. As a principal discriminator, fecal pentanoate was detected at higher concentrations in the low calprotectin group. A similar result was also reported in another study. The study discovered that fecal allantoin and gamma-glutamylcysteine in pediatric patients with CD, and fecal aminoadipic acid, carnosine, and ribose-5-phosphate in pediatric patients with UC exhibited a high correlation with fecal calprotectin concentration, which is a sensitive biomarker to detecting endoscopic activity in both CD and UC. In mucosal biopsies, the levels of several lipid metabolites were reported to be discrepant in patients with active UC and remission UC.

Besides single metabolites, previous studies have reported statistical models based on metabolomics for distinguishing different severities of endoscopic inflammation in patients with IBD. Predictive models based on mucosal biopsies were confirmed to exhibit good performance in predicting endoscopic inflammation in UC. Probert et al. found that the plasma metabolite profiles were able to discriminate endoscopic remission from endoscopic activity with a sensitivity, specificity, and accuracy of 82%, 82%, and 77%, respectively. In patients with CD, an established statistical model based on the breathograms of 10 discriminatory VOCs from breath samples also had a high sensitivity and specificity for the identification of remission and active patients. Moreover, the model combined with urine ethylmalonate, l-3,4-dihydroxy-phenylalanine, levoglucosan, and propylene glycol was accurate to distinguish endoscopic remission from endoscopic recurrence after ileocolonic resection in patients with CD. However, Di Giovanni et al. established a model with the serum metabolome from patients with CD and found that it failed to completely separate the high, low, and quiescent endoscopic activity groups. Since these studies used different samples, metabolomic techniques, and definitions of remission, further investigations are needed to determine whether the metabolomic model can identify endoscopic remission in CD.

**Metabolomic analysis and histological activity**

Histological activity can persist in patients with IBD despite endoscopic remission and is now considered to be a significant indicator in predicting disease progression in patients with UC. However, only one study has investigated the ability of metabolomics to assess histological activity. Probert et al. performed plasma NMR
**Table 1.** Metabolomic analysis and endoscopic disease activity in IBD.

| Patients | Activity index | Technique | Sample | Metabolic index/model | Performance | Reference |
|----------|----------------|-----------|--------|-----------------------|-------------|-----------|
| 38 CD    | Rutgeerts score| NMR       | Urine  | Levoglucosan          | Correlated with Rutgeerts score \(r = 0.33\) | Keshteli et al.²⁴ |
| 38 CD    | Rutgeerts score| NMR       | Urine  | Propylene glycol      | Correlated with Rutgeerts score \(r = -0.31\) | Keshteli et al.²⁴ |
| 69 Pediatric IBD | Fecal calprotectin (surrogate marker of intestinal inflammation) | UPLC-MS | Feces | Carnosine | Correlated with fecal calprotectin \(r = 0.887\) | Kolho et al.²⁵ |
| 17 UC    | Unclear        | GC        | Breath | Ethane                | Correlated with endoscopic score \(r = 0.45\) and symptom score \(r = 0.34\) | Sedghi et al.²⁴ |
| 25 Pediatric CD | Fecal calprotectin | NMR       | Feces  | Pentanoate concentration | 1.35-times greater in low calprotectin group compared to high calprotectin group | Taylor et al.²⁷ |
| 25 Pediatric CD | Fecal calprotectin | NMR       | Feces  | Lysine concentration  | 1.54-times greater in high calprotectin group compared to low calprotectin group | Taylor et al.²⁷ |
| 20 UC    | UCDAl and Mayo score | UHPLC-MS/MS | Colon biopsies | Oxylipins, endocannabinoids | Significant variance between treatment-naive and deep remission groups | Diab et al.²⁸ |
| 38 CD    | Rutgeerts score| NMR       | Urine  | 4 Metabolites         | Detecting recurrence \(AUC = 0.91; 100\% sensitivity; 84.6\% specificity\) | Keshteli et al.²⁴ |
| 33 UC    | UCDAl and Mayo score | UPLC-MS/MS | Mucosa biopsies | 67 Lipids | Relative concentration altered between treatment-naive and deep remission groups | Diab et al.²⁹ |
| 43 UC    | Mayo score     | NMR       | Colon biopsies | Metabolomics data sets | AUC >0.95 for activity prediction | Bjerrum et al.³⁰ |
| 40 UC    | UCEIS and Nancy Index | NMR       | Plasma | Metabolite profiles   | Identifying low and high UCEIS (accuracy of 77\%); Identifying low and high Nancy score (accuracy of 65\%) | Probert et al.³¹ |
| 191 CD   | Harvey–Bradshaw Index along with C-reactive protein and fecal calprotectin | GC-TOF-MS | breath | Model based on 10 volatile organic compounds | Predicting activity with sensitivity 0.81, specificity 0.80, and AUC 0.88 | Bodelier et al.³² |
| 28 UC    | Mayo score     | GC-TOF-MS; UHPLC-MS | Mucosa biopsies | PCA plot | Separation between the inflamed and noninflamed mucosa | Diab et al.³³ |
| 35 CD    | Crohn’s disease endoscopic index score | GC-TOF-MS | Serum | Three-dimensional PCA plot and PLS-DA plot | Incompletely separated between the three CD groups | Di Giovanni et al.³⁴ |
| 191 CD   | Harvey–Bradshaw Index along with C-reactive protein and fecal calprotectin | GC-TOF-MS | Breath | Activity classifying score based on 10 volatile organic compounds | 22% of samples could not be classified (superior to clinical parameters) | Bodelier et al.³² |

AUC, area under receiver operator characteristic curve; CD, Crohn’s disease; GC, gas chromatography; IBD, inflammatory bowel disease; MS, mass spectrometer; NMR, nuclear magnetic resonance; PCA, principal component analysis; PLS-DA, partial least squares discriminate analysis; TOF-MS, time-off-flight mass spectrometer; UC, ulcerative colitis; UCDAl, ulcerative colitis disease activity index; UCEIS, ulcerative colitis endoscopic index of severity; UHPLC, ultra-high-pressure liquid chromatography; UPLC, ultra performance liquid chromatography.
spectroscopy to study the discrepant metabolite profile between patients with UC showing histological remission and moderate to severe histological activity. The study found that plasma concentrations of five lipoproteins were elevated in patients with histological activity, while concentrations of valine, glucose, and myo-inositol declined. Furthermore, the researchers built a model based on these metabolites to identify histological remission with an accuracy of 65%. In the future, additional studies are needed to explore the abilities of different methods of metabolomics or differently sourced samples for assessing histological activity in IBD.

**Metabolomics for predicting the therapeutic response in IBD**

Because of the heterogeneity of IBD, there is not one specific therapy that can satisfy all the patients. Thus, the prediction of therapeutic response and matching the right patients with the right treatment are essential in clinical practice. In this section, distinctive metabolomic signatures that might be useful for identifying different responses of patients with IBD after certain therapies are summarized (Table 2).

**Metabolomic profiles and therapeutic response of biologics**

In a recent guideline, anti-tumor necrosis factor (TNF) agents, such as infliximab, adalimumab, and certolizumab pegol, have been recommended to be used in patients with moderate-to-severe CD who are refractory to corticosteroids or immunomodulators. It is also strongly recommended that vedolizumab, tofacitinib, or anti-TNF therapy including adalimumab, infliximab, or golimumab should be used for induction and maintenance of remission in moderately to severely active UC. Nevertheless, around 30–50% of patients show primary or secondary nonresponse to biologics therapy. Thus, prediction of the therapeutic response to these biologic agents is important for optimizing disease management. Through metabolomic analysis, several studies have uncovered that metabolite concentration could reflect and even predict the therapeutic response of these biological agents in patients with IBD. Bjerrum et al. conducted a retrospective cohort study including 87 patients with IBD and 37 healthy controls to investigate the variation of serum lipid metabolomic profiles after 14-week anti-TNF therapy. They demonstrated that the lipid profiles of patients with IBD were clear discriminated from the healthy controls before anti-TNF therapy. However, only the lipid profiles of patients with remission UC, instead of nonresponse UC or remission CD, became inseparable from the healthy controls after 14-week anti-TNF therapy. These results indicated that patients with UC who responded to anti-TNF treatment had a remarkable serum metabolomic change, which may help for discovering potential prognostic metabolites. In addition to different types of IBD, different biological agents also had distinct impacts in serum metabolites. A sustained increase in serum tryptophan (TRP) levels was observed in patients with IBD who responded to infliximab therapy, while patients nonresponding to infliximab or receiving vedolizumab therapy did not have a significant change in TRP levels.

Furthermore, metabolomics was capable of exploring promising metabolites or models to predict therapeutic response to biologics in IBD. Ding et al. performed a prospective, longitudinal cohort study of 76 patients with CD and found that many bile acids from feces, serum, and urine manifested differentiated signatures between responders and nonresponders. By combining changes in the levels of these representative markers in each sample type, especially in fecal samples, the anti-TNF response could be accurately predicted. Moreover, several lipid markers in serum and feces were reported to exhibit altered concentrations in responders compared to nonresponders, and the fecal lipid profile had a higher predictive accuracy than the serum lipid profile. Excitingly, another study found that response to infliximab in pediatric patients with CD could be predicted at baseline through fecal metabolomic approach. Patients responding to infliximab showed relatively high levels of glycine, linoleic acid, and l-lactic acid and low levels of N-acetylserotonin, methylglutaric acid, adipic acid, 4-aminohippuric acid, citramalic acid, isovaleric acid, and nicotinic acid before receiving infliximab. Thus, the baseline metabolomic profile could separate the responders from nonresponders clearly. In line with this, specific fecal lipid metabolites of patients with IBD were also mentioned in a prospective study of two cohorts, of which reduced 3-methyl-thiopropionic acid and methyl 2-(methylthio) acetate were the representative biomarkers detected in remission.

journals.sagepub.com/home/tag
Table 2. Metabolomic changes can reflect therapeutic response in IBD.

| Patients | Therapy | Technique | Sample | Sampling time | Metabolic index/model | Performance | Reference |
|----------|---------|-----------|--------|---------------|-----------------------|-------------|-----------|
| 76 CD    | Anti-TNF | UPLC-MS   | Urine  | Baseline; 3 months after therapy | Cysteine | Predicting response (AUC = 0.70) | Ding et al. | 37 |
| 3 CD/6 UC | Anti-TNF or vedolizumab | HILIC-LC-MS/MS | Feces | Baseline; weeks 2, 6, and 14 after induction | Butyric acid | Increased uniquely in anti-TNF remission patients | Aden et al. | 38 |
| 3 CD/6 UC | Anti-TNF or vedolizumab | HILIC-LC-MS/MS | Feces | Baseline; weeks 2, 6, and 14 after induction | Ethanol or acetaldehyde | Significantly associated with remission following anti-TNF therapy | Aden et al. | 38 |
| 81 CD/67 UC | Infliximab or vedolizumab | UPLC-MS | Serum | Baseline; weeks 2, 6, and 14; 6 months | Tryptophan | Sustained increase of levels in Res but not in non-Res (Infliximab therapy); no significant effect (Vedolizumab therapy) | Nikolaus et al. | 39 |
| 15 Pediatric CD | EEN | A modified spectrophotometric method | Feces | Baseline; 15, 30, and 60 days on EEN | Change magnitude of butyric acid and total sulfide levels | Increased when non-Rem were excluded | Gerasimidis et al. | 40 |
| 26 Pediatric IBD | EEN or corticosteroid | Multisegment injection-capillary electrophoresis-MS | Urine | Baseline; over 8 weeks | Octanoyl glucuronide, pantothenic acid, and pyridoxic acid | Specific dietary biomarkers of EEN for clinical remission | Yamamoto et al. | 41 |
| 7 Pediatric UC | FMT | GC-TOF-MS | Feces | Before and after FMT | Short-chain fatty acids | Changed in Res after FMT | Nusbaum et al. | 42 |
| 73 UC | FMT | High-performance GC | Feces | Baseline; week 8; 12 months | Short-chain fatty acids | No correlation with FMT effect | Costello et al. | 43 |
| 81 UC | FMT | UPLC-MS/MS | Feces | Baseline; week 8 of FMT | Short-chain fatty acid biosynthesis and secondary bile acids | Increased in Rem after FMT | Paramsothy et al. | 44 |
| 81 UC | FMT | UPLC-MS/MS | Feces | Baseline; week 8 of FMT | 15 Metabolites | Independent predictors of response at baseline | Paramsothy et al. | 44 |
| 43 Pediatric CD | EEN | NMR | Feces | Baseline; during EEN; at end of EEN; at follow-up | Individual metabolites | Normalized in Res but not non-Res at follow-up | Diederen et al. | 45 |
| 69 CD | FMT | NMR | Urine | Pre-first FMT; pre-second FMT | 7 Metabolites | Elevated right before the second FMT | Li et al. | 46 |
| 76 CD | Anti-TNF | UPLC-MS | Feces | Baseline; 3 months after therapy | Models of lipids | Predicting response (AUC = 0.94) | Ding et al. | 37 |
| 76 CD | Anti-TNF | UPLC-MS | Feces | Baseline; 3 months after therapy | Models of bile acids | Predicting response (AUC = 0.81) | Ding et al. | 37 |
| 76 CD | Anti-TNF | UPLC-MS | Serum | Baseline; 3 months after therapy | Models of bile acids | Predicting response (AUC = 0.74) | Ding et al. | 37 |
| 43 Pediatric CD | EEN | NMR | Feces | Baseline; during EEN; at end of EEN; at follow-up | Metabolic profiles | Predicting response at baseline (AUC = 0.8) | Diederen et al. | 45 |

(Continued)
patients treated with anti-TNF agents. In this study, the authors also developed a silico model based on fecal metabolomic profiles to identify patients who would achieve clinical remission after anti-TNF treatment.

**Metabolomic profiles and therapeutic response of fecal microbiota transplantation**

Fecal microbiota transplantation (FMT) is an effective method of directly changing the gut microbiota of recipients to normalize the composition, thereby achieving therapeutic benefits. As previous studies have shown that FMT is proposed as a therapeutic approach to induce remission in patients with CD and UC. Several studies have found that changes in metabolomics can reflect disease remission after FMT treatment. One prospective study proved that significant alterations in urinary metabolic profiles of patients with CD who experienced clinical improvement or remission were achieved at the pre-second FMT compared with those at pre-first FMT. In addition, Paramsothy et al. found that the primary outcome of FMT treatment could be predicted at baseline according to the fecal metabolomic profiles. The increased levels of SCFA biosynthesis and secondary bile acids, as well as the decreased levels of heme and lipopolysaccharide biosynthesis, were considered as markers that enabled patients in remission at week 8 to be distinguished from those who did not achieve remission. Similarly, another study of fecal metabolomes of pediatric patients with UC analyzed fecal metabolites and found that at subsequent follow-up points during EEN, there was a decrease in the

---

**Table 2. (Continued)**

| Patients | Therapy | Technique | Sample | Sampling time | Metabolic index/model | Performance | Reference |
|----------|---------|-----------|--------|---------------|-----------------------|-------------|-----------|
| 29 Pediatric CD | Infliximab | LC–tandem MS | Feces | Before and after treatment | Metabolomic profiles | Distinct patterns between responders and nonresponders before and after treatment | Wang et al. |
| 87 IBD | Infliximab | NMR | Serum | Baseline; during a 14-week treatment | Metabolomic profiles | Potentially distinct in non-Res versus other groups | Bjerrum et al. |
| 69 CD | FMT | NMR | Urine | Pre-first FMT; pre-second FMT | Metabolomic profiles | Significant difference | Li et al. |

Anti-TNF, anti-tumor necrosis factor; AUC, area under receiver operator characteristic curve; CD, Crohn’s disease; EEN, exclusive enteral nutrition; FMT, fecal microbiota transplantation; GC, gas chromatography; HILIC-LC-MS/MS, hydrophilic interaction liquid chromatography coupled to mass spectrometry; IBD, inflammatory bowel disease; MS, mass spectrometry; NMR, nuclear magnetic resonance; Rem, remission; Res, responders; TOF-MS, time-of-flight mass spectrometer; UC, ulcerative colitis; UPLC-MS, ultra performance liquid chromatography mass spectrometry.
levels of butyric acid and an increase in the concentrations of total sulfide. The magnitude of these changes became larger when excluding the patients who did not experience clinical remission from the analysis, implying different metabolomic characteristics in patients who achieved remission after EEN treatment and those who did not. Metabolic trajectories of urinary metabolites over 8 weeks were also monitored in a pediatric retrospective cohort study of 26 patients with IBD who received corticosteroid or EEN therapy.\textsuperscript{41} Urinary octanoyl glucuronide, pyridoxic acid, and pantothenic acid were shown as specific dietary biomarkers of clinical remission after EEN treatment in pediatric patients with IBD. These findings offer support for the utility of urinary metabolomics in the early therapeutic monitoring of pediatric patients.

| Patients | Definition of recurrence | Technique | Sample | Metabolic index/model | Performance | Reference |
|----------|--------------------------|-----------|--------|-----------------------|-------------|-----------|
| 355 UC | CAI ≥5 or necessity for additional treatment | High-performance LC-electrospray ionization MS | Plasma | Histidine | HR (1st versus 4th quartile) = 2.55 | Hisamatsu et al.\textsuperscript{60} |
| 108 CD/56 UC | Symptomatic worsening | LC-MS | Serum | Propionyl-l-carnitine | Associated with relapse (β = −1.24) | Borren et al.\textsuperscript{61} |
| 108 CD/56 UC | Symptomatic worsening | LC-MS | Serum | Sarcosine | Associated with relapse (β = −0.92) | Borren et al.\textsuperscript{61} |
| 108 CD/56 UC | Symptomatic worsening | LC-MS | Serum | Carnitine | Associated with relapse (β = −0.95) | Borren et al.\textsuperscript{61} |
| 108 CD/56 UC | Symptomatic worsening | LC-MS | Serum | Sorbitol | Associated with relapse (β = 1.06) | Borren et al.\textsuperscript{61} |
| 108 CD/56 UC | Symptomatic worsening | LC-MS | Serum | Risk score based on four metabolites | Predicting relapse (AUC = 0.70; OR = 5.79) | Borren et al.\textsuperscript{61} |
| 40 UC | Increase of UCEIS ≥1 | NMR | Plasma | Baseline metabolomic profiles | Predicting postoperative improvement or worsening [74% accuracy; 81% specificity; 81% sensitivity] | Probert et al.\textsuperscript{31} |
| 20 UC | Partial Mayo score ≥3 | Direct infusion/LC-MS/MS; NMR | Serum and urine | Baseline metabolomic profiles | Predicting relapse within 12 months | Keshteli et al.\textsuperscript{62} |

AUC, area under receiver operator characteristic curve; CAI, Lichtiger Clinical Activity Index; CD, Crohn’s disease; HR, hazard ratio; IBD, inflammatory bowel disease; LC, liquid chromatography; MS, mass spectrometry; NMR, nuclear magnetic; OR, odds ratio; UC, ulcerative colitis; UCEIS, ulcerative colitis endoscopic index of severity.

### Metabolomics for predicting disease relapse

IBD is a disease characteristic of a relapsing–remitting course. Approximately 10–30% of patients tend to experience a disease relapse annually despite achieving remission.\textsuperscript{58,59} Therefore, it is critical to pre-identify patients with a high risk of relapse so as to prospectively modify the strategies of disease management, which make it possible to conduct effective interventions for relapse prevention in patients. Several articles that elaborate the potential metabolic markers for the prediction of relapse are presented in this section (Table 3).

A prospective cohort study of 40 patients with UC showed that a baseline plasma metabolite profile performed well in predicting worsening of postoperative endoscopic activity with an
Two studies have investigated the relationship between the probability of clinical recurrence of UC and the metabolome. A total of 355 patients with remission UC were observed prospectively in one of the studies,\textsuperscript{60} and it was shown that a decreased concentration of plasma histidine was associated with an increased risk of relapse. In another study,\textsuperscript{62} patients with clinical relapse were demonstrated to have significantly higher levels of serum 3-hydroxybutyrate, acetoacetate, acetone, and urinary \textit{trans}-aconitate, while urinary acetamide and cystine levels decreased. In addition, a prospective cohort study of 164 patients with IBD\textsuperscript{61} identified four serum metabolomic markers, sarcosine, carnitine, propionyl-l-carnitine, and sorbitol, which were associated with clinical relapse in IBD within 2 years. A metabolomics risk score developed based on these metabolites was confirmed to have a good performance for the prediction of relapse, with an area under the receiver operating characteristic curve of 0.70.

**Limitations of metabolomics and corresponding solutions**

There remain some limitations of metabolomics that, to some extent, hamper the transition between laboratory research and the clinical application of metabolomics in IBD.

Above all, previous studies have demonstrated that age, gender, and environmental factors such as diet, lifestyle, toxins, and cultural trends can influence metabolic phenotypes.\textsuperscript{70–73} Therefore, both clinical research and applications of metabolomics should take the influence of these confounding factors into account, especially urinary metabolomics, which are thought to be more susceptible to environmental factors than metabolomics of other sample types.\textsuperscript{70,72} Otherwise, biased results and even erroneous conclusion may be drawn. As noted in a prospective study for relapse prediction of UC, concentrations of plasma histidine and glutamate are affected by age and gender in patients with UC.\textsuperscript{60} After gender and age adjustment, the association between histidine and the risk of relapse was more obvious, while the opposite was true for glutamate. Moreover, disease severity, concomitant therapy, and genetic variants in patients with IBD are also non-negligible confounders in prognostic studies with metabolomics.\textsuperscript{13,74} For example, the metabolism and drug toxicity of thiopurine, a widely used drug in IBD, is strongly influenced by the genetic variants of NUDT15, TPMT, and HLA.\textsuperscript{75–77} If these genetic biomarkers are not considered in metabolomics research concerning the therapeutic efficacy of thiopurine, biased results will be generated. Thus, it is important to have an appropriate study design and perform robust statistical adjustments for potential confounders to make metabolomic profiles reliable for application in clinical practice.

In addition, some contradictory results have been reported in previous studies, which may result from the heterogeneity of cohorts, sampling techniques, or analytical methods. Previous studies have revealed that the quality of acquired metabolomic data can be strongly influenced by sample...
preparation and the analytic approaches undertaken, which is likely to lead to significant variability in the final metabolomic profiles. Since the metabolome of an organism is highly complex, there is no single and uniform strategy to analyze all metabolites, resulting in distinct analytic methods and instruments used for different sample types and research purposes, which makes it difficult to compare and integrate the results from different studies. Therefore, it is necessary to further verify the role of metabolites that have been identified as predictive biomarkers in the disease management and prognosis of IBD following the standardized procedures from sample preparation to analysis, and to determine the most appropriate sampling and analytical methods according to the properties of the target metabolites for clinical application.

Another limitation of metabolomics is that metabolomic profile alterations in patients with different disease activity or prognosis are hard to interpret since the mechanism behind these changes remains unknown. Fortunately, the integration of metabolomics and other omics, especially the microbiome, can provide more comprehensive and profound insights into metabolic characterization. Metabolites are downstream of the genome–transcriptome–proteome and are affected by the metabolism of various microbiota in vivo, so that multiomics studies create an opportunity to explore the interactions of genes, proteins, metabolites, and microbiota and to uncover the pathophysiological mechanisms behind the characteristics of metabolomics. This enables to explain the causes of metabolic changes in patients with IBD. In addition, the integration of multiomics may enable the identification of an optimal set of biomarkers for the management of IBD. For instance, a multivariable model combining metabolomic and proteomic risk scores to predict clinical relapse in IBD exhibited a superior performance to a metabolomic or proteomic model alone in a prospective cohort study. Similarly, another study revealed that a model of baseline clinical, proteomic, metabolomic, and metagenomic markers showed a higher predictive value in predicting the biologic therapeutic response than a model of microbial taxa alone. Therefore, metabolomics is still a promising and reliable technology to facilitate the personalized managements in IBD when addressing these limitations.

**Conclusion**
Owing to the advantages of satisfactory sensitivity and precision, high throughput, convenient sampling, and noninvasive characteristics, metabolomics has attracted increasing attention in IBD research in recent years. The potential of metabolomic profiling in the management of IBD is gradually being recognized. Many metabolites from different types of samples were found to be candidate predictors of disease activity, therapeutic response, or relapse. Several statistical models based on some of the representative metabolites were considered to have good performance in distinguishing patients with active IBD from those in remission or in identifying responders from non-responders during treatment. Much of this evidence shows that high-throughput and high-sensitivity metabolomics technology not only allows for the quantification of small changes in a single metabolite but can also integrate the changes of multiple metabolites with the help of multivariate analysis to obtain a comprehensive judgment of metabolic characteristics. This assists in monitoring the progress of the disease and in evaluating the therapeutic effect, which has broad prospects in the clinical management of IBD.

Although previous studies showed the promising role of metabolomics in the personalized management of IBD, more efforts are warranted for adopting metabolomics in real-world medicine. On one hand, the predictive ability and generalization of established metabolomic profiles need further validation in research with large sample sizes because of the numerous variables in the metabolomic profiles and the heterogeneity of IBD. On the other hand, the mechanisms of metabolite alteration in IBD patients with different disease activity or prognosis should be elucidated through basic research or multiomics studies, so that clinicians can perform a more targeted management with a profound interpretation of metabolomic profiles. Fortunately, the fact that metabolomics can be applied in clinical practice has been supported by the routine employment of the targeted metabolomic profiling of some metabolites, such as steroids, organic acids, amino acids, biogenic amines, and acylcarnitines, in newborn screening, especially in detecting inborn metabolism errors. Furthermore, metabolomics also exerts an important role in personalized medicine with respect to diabetes and cancer. All of these applications in other fields
provide references for the clinical establishment of metabolomics in IBD. It is believed that the adoption of metabolomics in practical medicine of IBD will be witnessed in the near future.

Author contributions
SZ is the guarantor of the article. SZ, MC, and LL designed the study and revised the review. RC and JZ wrote and revised the manuscript. SZ, CL, KC, and ZZ revised the contents of the manuscript. All authors approved the final manuscript and agreed to be responsible for this review.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by grants from the National Natural Science Foundation of China (Nos 81630018, 82070538, 81870374, 82000520), Guangzhou Science and Technology Department (No. 202002030041), and Guangdong Science and Technology Department (Nos 2017A030306021, 2020A1515010249)

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iDs
Li Li https://orcid.org/0000-0002-3203-901X
Shenghong Zhang https://orcid.org/0000-0002-9088-8781

References
1. de Souza HS and Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol 2016; 13: 13–27.
2. Torres J, Mehandru S, Colombel J-F, et al. Crohn’s disease. Lancet 2017; 389: 1741–1755.
3. Ungaro R, Mehandru S, Allen PB, et al. Ulcerative colitis. Lancet 2017; 389: 1756–1770.
4. Abraham C and Cho JH. Inflammatory bowel disease. N Engl J Med 2009; 361: 2066–2078.
5. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 2017; 390: 2769–2778.
6. Kaplan GG and Ng SC. Understanding and preventing the global increase of inflammatory bowel disease. Gastroenterology 2017; 152: 313–321.e2.
7. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015; 12: 720–727.
8. Sands BE. Biomarkers of inflammation in inflammatory bowel disease. Gastroenterology 2015; 149: 1275–1285.e2.
9. Storr M, Vogel HJ and Schicho R. Metabolomics: is it useful for inflammatory bowel diseases? Curr Opin Gastroenterol 2013; 29: 378–383.
10. Wishart DS. Metabolomics for investigating physiological and pathophysiological processes. Physiol Rev 2019; 99: 1819–1875.
11. Bauset C, Gisbert-Ferrandiz L and Cosin-Roger J. Metabolomics as a promising resource identifying potential biomarkers for inflammatory bowel disease. J Clin Med 2021; 10: 622.
12. Gallagher K, Catesson A, Griffin JL, et al. Metabolomic analysis in inflammatory bowel disease: a systematic review. J Crohns Colitis 2021; 15: 813–826.
13. Lin HM, Helsby NA, Rowan DD, et al. Using metabolomic analysis to understand inflammatory bowel diseases. Inflamm Bowel Dis 2011; 17: 1021–1029.
14. Patti GJ, Yanes O and Siuzdak G. Innovation: metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 2012; 13: 263–269.
15. Dudney E, Yousef M, Wang Y, et al. Targeted metabolomics and mass spectrometry. Adv Protein Chem Struct Biol 2010; 80: 45–83.
16. Dunn WB, Bailey NJC and Johnson HE. Measuring the metabolome: current analytical technologies. Analyst 2005; 130: 606–625.
17. Beckonert O, Keun HC, Ebbels TMD, et al. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2007; 2: 2692–2703.
18. Dettmer K, Aronov PA and Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev 2007; 26: 51–78.
19. Pan Z and Raftery D. Comparing and combining NMR spectroscopy and mass spectrometry in metabolomics. Anal Bioanal Chem 2007; 387: 525–527.
20. Lindon JC and Nicholson JK. Analytical technologies for metabolomics and metabolomics, and multi-omic information recovery. *TrAC: Trend Anal Chem* 2008; 27: 194–204.

21. Harbord M, Eliakim R, Bettenworth D, *et al.* Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 2: current management. *J Crohns Colitis* 2017; 11: 769–784.

22. Gomollón F, Dignass A, Annese V, *et al.* 3rd European evidence-based consensus on diagnosis and management of Crohn’s disease 2016: part 1: diagnosis and medical management. *J Crohns Colitis* 2017; 11: 3–25.

23. Turner D, Ricciuto A, Lewis A, *et al.* STRIDE-II: an update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology* 2021; 160: 1570–1583.

24. Keshteli AH, Tso R, Dieleman LA, *et al.* A distinctive urinary metabolomic fingerprint is linked with endoscopic postoperative disease recurrence in Crohn’s disease patients. *Inflamm Bowel Dis* 2018; 24: 861–870.

25. Kolho KL, Pessia A, Jaakkola T, *et al.* Faecal and serum metabolomics in paediatric inflammatory bowel disease. *J Crohns Colitis* 2017; 11: 321–334.

26. Sedghi S, Keshavarzian A, Klamut M, *et al.* Elevated breath ethane levels in active ulcerative colitis: evidence for excessive lipid peroxidation. *Am J Gastroenterol* 1994; 89: 2217–2221.

27. Taylor H, Serrano-Contreras JI, McDonald JAK, *et al.* Multiomic features associated with mucosal healing and inflammation in paediatric Crohn’s disease. *Aliment Pharmacol Ther* 2020; 52: 1491–1502.

28. Diab J, Al-Mahdi R, Gouveia-Figueira S, *et al.* A quantitative analysis of colonic mucosal oxylipins and endocannabinoids in treatment-naive and deep remission ulcerative colitis patients and the potential link with cytokine gene expression. *Inflamm Bowel Dis* 2019; 25: 490–497.

29. Diab J, Hansen T, Goll R, *et al.* Lipidomics in ulcerative colitis reveal alteration in mucosal lipid composition associated with the disease state. *Inflamm Bowel Dis* 2019; 25: 1780–1787.

30. Bjerrum JT, Rantalainen M, Wang Y, *et al.* Integration of transcriptomics and metabolomics: improving diagnostics, biomarker identification and phenotyping in ulcerative colitis. *Metabolomics* 2014; 10: 280–290.

31. Probert F, Walsh A, Jagielowicz M, *et al.* Plasma nuclear magnetic resonance metabolomics discriminates between high and low endoscopic activity and predicts progression in a prospective cohort of patients with ulcerative colitis. *J Crohns Colitis* 2018; 12: 1326–1337.

32. Bodelier AG, Smolinska A, Baranska A, *et al.* Volatile organic compounds in exhaled air as novel marker for disease activity in Crohn’s disease: a metabolomic approach. *Inflamm Bowel Dis* 2015; 21: 1776–1785.

33. 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.

34. Di Giovanni N, Meuwis MA, Louis E, *et al.* Untargeted serum metabolic profiling by comprehensive two-dimensional gas chromatography-high-resolution time-of-flight mass spectrometry. *J Proteome Res* 2020; 19: 1013–1028.

35. Mosli MH, Zou G, Garg SK, *et al.* C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol* 2015; 110: 802–819; quiz 820.

36. Gupta A, Yu A, Peyrin-Biroulet L, *et al.* Treat to target: the role of histologic healing in inflammatory bowel diseases: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2021; 19: 1800–1813.e4.

37. Ding NS, McDonald JAK, Perdones-Montero A, *et al.* Metabolomics and the gut microbiome associated with primary response to anti-TNF therapy in Crohn’s disease. *J Crohns Colitis* 2020; 14: 1090–1102.

38. Aden K, Rehman A, Waschima S, *et al.* Metabolic functions of gut microbes associate with efficacy of tumor necrosis factor antagonists in patients with inflammatory bowel diseases. *Gastroenterology* 2019; 157: 1279–1292.e11.

39. Nikolaus S, Schulte B, Al-Massad N, *et al.* Increased tryptophan metabolism is associated with activity of inflammatory bowel diseases. *Gastroenterology* 2017; 153: 1504–1516.e2.

40. Gerasimidis K, Bertz M, Hanske L, *et al.* Decline in presumptively protective gut bacterial species and metabolites are paradoxically associated with disease improvement in pediatric Crohn’s disease.
52. Singh S, Fumery M, Sandborn WJ, et al. Systematic review and network meta-analysis: first- and second-line biologic therapies for moderate-severe Crohn’s disease. *Aliment Pharmacol Ther* 2018; 48: 394–409.

53. Wang J-W, Kuo C-H, Kuo F-C, et al. Fecal microbiota transplantation: review and update. *J Formos Med Assoc* 2019; 118(Suppl. 1): S23–S31.

54. Colman RJ and Rubin DT. Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. *J Crohns Colitis* 2014; 8: 1569–1581.

55. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 2015; 149: 102–109.

56. Heuschkel RB, Menache CC, Megerian JT, et al. Enteral nutrition and corticosteroids in the treatment of acute Crohn’s disease in children. *J Pediatr Gastroenterol Nutr* 2000; 31: 8–15.

57. Borrelli O, Cordischi L, Cirulli M, et al. Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn’s disease: a randomized controlled open-label trial. *Clin Gastroenterol Hepatol* 2006; 4: 744–753.

58. Fukuda T, Naganuma M, Sugimoto S, et al. The risk factor of clinical relapse in ulcerative colitis patients with low dose 5-aminosalicylic acid as maintenance therapy: a report from the IBD registry. *PLoS ONE* 2017; 12: e0187737.

59. Yamamoto T, Shimoyama T, Umegae S, et al. Endoscopic score vs. fecal biomarkers for predicting relapse in patients with ulcerative colitis after clinical remission and mucosal healing. *Clin Transl Gastroenterol* 2018; 9: 136.

60. Hisamatsu T, Ono N, Imaizumi A, et al. Decreased plasma histidine level predicts risk of relapse in ulcerative colitis patients with low dose 5-aminosalicylic acid as maintenance therapy. *PLoS ONE* 2015; 10: e0107416.

61. Borrelli NZ, Plichta D, Joshi AD, et al. Multi-“omics” profiling in patients with quiescent inflammatory bowel disease identifies biomarkers predicting relapse. *Inflamm Bowel Dis* 2020; 26: 1524–1532.

62. Keshteli AH, van den Brand FF, Madsen KL, et al. Dietary and metabolomic determinants of relapse in ulcerative colitis patients: a pilot prospective cohort study. *World J Gastroenterol* 2017; 23: 3890–3899.

63. Clerc F, Novokmet M, Dotz V, et al. Plasma N-glycan signatures are associated with features during enteral nutrition. *Inflamm Bowel Dis* 2014; 20: 861–871.

41. Yamamoto M, Shanmuganathan M, Hart L, et al. Urinary metabolites enable differential diagnosis and therapeutic monitoring of pediatric inflammatory bowel disease. *Metabolites* 2021; 11: 245.

42. Nusbaum DJ, Sun F, Ren J, et al. Gut microbial and metabolomic profiles after fecal microbiota transplantation in pediatric ulcerative colitis patients. *FEBS Microbiol Ecol* 2018; 94: fiy133.

43. Costello SP, Hughes PA, Waters O, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA* 2019; 321: 156–164.

44. Paramsothy S, Nielsen S, Kamm MA, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology* 2019; 156: 1440–1454.e2.

45. 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.

46. Li P, Zhang T, Xiao Y, et al. Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn’s disease. *Appl Microbiol Biotechnol* 2019; 103: 349–360.

47. Wang Y, Gao X, Zhang X, et al. Microbial and metabolic features associated with outcome of infliximab therapy in pediatric Crohn’s disease. *Gut Microbes* 2021; 13: 1–18.

48. 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.

49. Lichtenstein GR, Loftus EV, Isaacs KL, et al. ACG clinical guideline: management of Crohn’s disease in adults. *Am J Gastroenterol* 2018; 113: 481–517.

50. Rubin DT, Ananthakrishnan AN, Siegel CA, et al. ACG clinical guideline: ulcerative colitis in adults. *Am J Gastroenterol* 2019; 114: 384–413.

51. Singh S, Murad MH, Fumery M, et al. First- and second-line pharmacotherapies for patients with moderate to severely active ulcerative colitis: an updated network meta-analysis. *Clin Gastroenterol Hepatol* 2020; 18: 2179–2191.
of inflammatory bowel diseases. *Gastroenterology* 2018; 155: 829–843.

64. Mackner LM, Hatzakis E, Allen JM, et al. Fecal microbiota and metabolites are distinct in a pilot study of pediatric Crohn’s disease patients with higher levels of perceived stress. *Psychoneuroendocrinology* 2020; 111: 104469.

65. Mardini HE, Kip KE and Wilson JW. Crohn’s disease: a two-year prospective study of the association between psychological distress and disease activity. *Dig Dis Sci* 2004; 49: 492–497.

66. Langhorst J, Hofstetter A, Wolfe F, et al. Short-term stress, but not mucosal healing nor depression was predictive for the risk of relapse in patients with ulcerative colitis: a prospective 12-month follow-up study. *Inflamm Bowel Dis* 2013; 19: 2380–2386.

67. Borren NZ, van der Woude CJ and Ananthakrishnan AN. Fatigue in IBD: epidemiology, pathophysiology and management. *Nat Rev Gastroenterol Hepatol* 2019; 16: 247–259.

68. Borren NZ, Plichta D, Joshi AD, et al. Alterations in fecal microbiomes and serum metabolomes of fatigued patients with quiescent inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2021; 19: 519–527.e5.

69. Bushman FD, Conrad M, Ren Y, et al. Multi-omic analysis of the interaction between *Clostridioides* difficile infection and pediatric inflammatory bowel disease. *Cell Host Microbe* 2020; 28: 422–433.e7.

70. Slupsky CM, Rankin KN, Wagner J, et al. Investigations of the effects of gender, diurnal variation, and age in human urinary metabolomic profiles. *Anal Chem* 2007; 79: 6995–7004.

71. Stella C, Beckwith-Hall B, Cloarec O, et al. Susceptibility of human metabolic phenotypes to dietary modulation. *J Proteome Res* 2006; 5: 2780–2788.

72. Lenz EM, Bright J, Wilson ID, et al. Metabonomics, dietary influences and cultural differences: a 1H NMR-based study of urine samples obtained from healthy British and Swedish subjects. *J Pharm Biomed Anal* 2004; 36: 841–849.

73. Patterson AD, Gonzalez FJ and Idle JR. Xenobiotic metabolism: a view through the metabolometer. *Chem Res Toxicol* 2010; 23: 851–860.

74. Stephens NS, Siffledeen J, Su X, et al. Urinary NMR metabolomic profiles discriminate inflammatory bowel disease from healthy. *J Crohns Colitis* 2013; 7: e42–e48.

75. Moon W and Loftus EV Jr. Review article: recent advances in pharmacogenetics and pharmacokinetics for safe and effective thiopurine therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2016; 43: 863–883.

76. Heap GA, Weendon MN, Bewshea CM, et al. HLA-DQA1-HLA-DRB1 variants confer susceptibility to pancreatitis induced by thiopurine immunosuppressants. *Nat Genet* 2014; 46: 1131–1134.

77. Moriyma T, Nishii R, Perez-Andreu V, et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nat Genet* 2016; 48: 367–373.

78. Jacob M, Lopata AL, Dasouki M, et al. Metabolomics toward personalized medicine. *Mass Spectrom Rev* 2019; 38: 221–238.

79. Gu Q, David F, Lynen F, et al. Evaluation of automated sample preparation, retention time locked gas chromatography-mass spectrometry and data analysis methods for the metabolomic study of Arabidopsis species. *J Chromatogr A* 2011; 1218: 3247–3254.

80. Beale DJ, Pinu FR, Kouromenos KA, et al. Review of recent developments in GC-MS approaches to metabolomics-based research. *Metabolomics* 2018; 14: 152.

81. Dunn WB and Hankemeier T. Mass spectrometry and metabolomics: past, present and future. *Metabolomics* 2013; 9(Suppl. 1): 1–3.

82. Loscalzo J, Kohane I and Barabasi A-L. Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. *Mol Syst Biol* 2007; 3: 124.

83. Ussher JR, Elmariah S, Gerszten RE, et al. The emerging role of metabolomics in the diagnosis and prognosis of cardiovascular disease. *J Am Coll Cardiol* 2016; 68: 2850–2870.

84. Lee JWJ, Plichta D, Hogstrom L, et al. Multi-omics reveal microbial determinants impacting responses to biologic therapies in inflammatory bowel disease. *Cell Host Microbe* 2021; 29: 1294–1304.e4.

85. Al-Dirbashi OY, Jacob M, Al-Hassnan Z, et al. Diagnosis of methylmalonic acidemia from dried blood spots by HPLC and intramolecular-excimer fluorescence derivatization. *Clin Chem* 2005; 51: 235–237.
86. Al-Dirbashi OY, Santa T, Rashed MS, et al. Rapid UPLC-MS/MS method for routine analysis of plasma pristanic, phytanic, and very long chain fatty acid markers of peroxisomal disorders. *J Lipid Res* 2008; 49: 1855–1862.

87. Rashed MS, Bucknall MP, Little D, et al. Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. *Clin Chem* 1997; 43: 1129–1141.

88. Chace DH, Kalas TA and Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. *Clin Chem* 2003; 49: 1797–1817.