Metabolomics: is it useful for inflammatory bowel diseases?

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Purpose of review
The assessment of metabolite profiles in biofluids has become a powerful method for the detection of biomarker molecules and disease mechanisms. This review outlines the recent advances in the use of metabolomic techniques to study inflammatory bowel diseases (IBDs).

Recent findings
The last few years have seen an increase in the studies of experimental and human IBD focusing on the search for small metabolites, such as amino acids, bases, and tricarboxylic acid cycle intermediates. Experimental methods for the screening of metabolites in serum, urine, fecal extracts, and colon tissue include ^{1}H NMR spectroscopy, gas chromatography–mass spectrometry, and liquid chromatography methods. Several studies demonstrate that IBD patients and healthy individuals, as well as the IBD subtypes, can be distinguished using metabolic profiling. Metabolomic data of fecal extracts and urine have revealed disruptions in bacterial populations, findings that are indicative of a possible involvement of the microbiome in the development of IBDs.

Summary
Metabolites from biofluids can be detected in IBDs by different experimental methods that allow successful separation of IBD subtypes from healthy cohorts. Knowledge of a unique metabolomic fingerprint in IBDs could be useful for diagnosis, treatment, and detection of disease mechanisms.

Keywords
metabolic profile, metabolomics, metabonomics, multivariate analysis

INTRODUCTION

Several serologic markers for inflammatory bowel diseases (IBDs) are currently available. Although they are invaluable as an adjunct to endoscopic and radiologic findings, they are less helpful in differentiating between the two subtypes of IBDs, that is, Crohn's disease and ulcerative colitis, or in categorizing patients with indeterminate colitis. Thus, biomarkers that clearly discriminate between these two subtypes and between remission and flare-ups would be enormously useful for clinical practice. Additionally, changes in the concentrations of metabolites may reveal important clues about severity and activity of IBDs. Such information is important to support diagnosis and to provide an insight into the underlying pathophysiological mechanisms of IBDs, which are far from clear at present.

TECHNIQUES AND ANALYSIS OF METABOLOMICS

Techniques of metabolomic research have an advantage of being extremely sensitive and of allowing to perform experiments in a cost-effective high-throughput manner. Common technologies used for metabolomics include (with increasing sensitivity) ^{1}H NMR spectroscopy, gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS). Ion cyclotron resonance-Fourier transform mass spectrometry (ICR-FT/MS) with ultrahigh mass resolution has allowed for the separation of thousands of masses that correspond to small but complex metabolites [1], whereas another advanced

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KEY POINTS

- Metabolic profiling of biofluids combined with multivariate analysis is extremely useful for discriminating between IBD patients and healthy individuals.
- IBD subtypes can be discriminated by metabolic profiling of serum and plasma.
- Through metabolic profiling, we are able to identify biomarkers of IBDs as well as components of disease mechanisms.

high-resolution technique (ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight MS) has recently been employed in experimental IBDs [2▪].

Measuring metabolites by NMR and MS generates a large amount of data which needs to be organized by multivariate analysis methods in order to extract the important information. Thus, metabolomic data are usually interpreted by statistical methods such as principal component analysis (PCA) and orthogonal partial least squares (OPLS) projections [3]. OPLS is based on projection to latent structures (PLS), a pattern recognition method that reduces numerous collinear variables (metabolites) to a few subsets to demonstrate their interdependence. OPLS-discriminant analysis (DA) maximizes the independence of variables, resulting in better separation of data between cohorts and therefore in better discrimination of diseased individuals from healthy ones (Fig. 1).

METABOLOMICS AND MODELS OF INFLAMMATORY BOWEL DISEASES

Over the years, several mouse models of IBDs have been used to measure differences in the metabolic profiles of serum, urine, fecal extracts, and gut tissues between diseased and nondiseased animals. These models include the interleukin-$10^{-/-}$ knockout model [4–7], the $TNF^{\Delta ARE/WT}$ model that mimics Crohn's-disease-like ileitis with great similarity [8▪], and the dextran sulfate sodium (DSS)-induced experimental colitis model that largely resembles ulcerative colitis [9▪,10,11]. ^{1}H NMR spectroscopy [4,5,8▪,10,11], GC–MS [6,9▪], and LC–MS [8▪] were employed to profile metabolites in these models. Despite having lower sensitivity, ^{1}H NMR spectroscopy may be preferred to MS because of its higher reproducibility [12▪] and the application of 'targeted' metabolomics. 'Targeted' (quantitative) metabolic profiling in ^{1}H NMR spectroscopy, compared with the traditional spectral binning, significantly improves the chances for the discovery of disease-related biomarkers. The technique involves the analysis of a large group of compounds whose characteristics (e.g., individual NMR spectra) are established and stored in a database library. From the complex mixture of individual metabolite spectra in the biofluid, the spectra in the database can be used to identify and quantify the targeted metabolites [13]. Quantitative metabolic profiling has already been used in two different mouse models of colitis to discriminate between diseased vs. nondiseased states [4,10] and in serum, plasma, and urine of IBD patients [14▪▪].

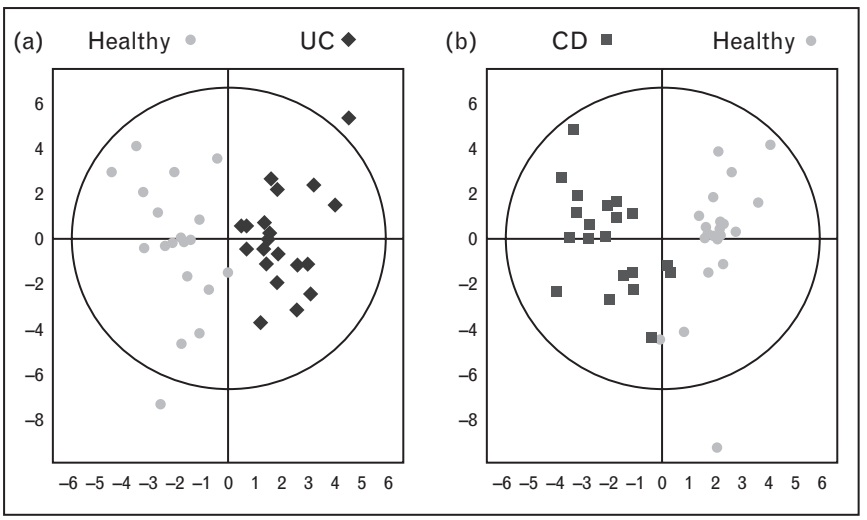

FIGURE 1. Score plots of OPLS-DA coefficients from plasma metabolites measured by ^{1}H NMR spectroscopy in patients with (a) ulcerative colitis (UC) and (b) Crohn's disease (CD) demonstrating good separation of metabolites that could help to diagnose IBD subtypes in patients. IBD, inflammatory bowel disease; OPLS-DA, orthogonal partial least squares-discriminant analysis.

Changes in metabolic profiles: a possible clue to pathogenesis?

Changes in the metabolism of cholesterol, triglycerides, and phospholipids that were demonstrated in a mouse model of Crohn's disease [8▪], highlight the importance of inflammatory lipid mediators as potential biomarkers of IBDs. *Helicobacter hepaticus* $Rag2^{-/-}$ mice, another model of IBD, revealed significant differences between diseased and nondiseased animals in compounds involved in tryptophan, fatty acid, and purine metabolism, as well as in methionine–homocysteine and tricarboxylic acid (TCA) cycles [15▪]. Some of these changes, such as those in tryptophan metabolism and TCA cycle, were also observed in other studies of experimental IBD [5,10]. In IL-$10^{-/-}$ knockout mice, a model in which mice develop a spontaneous colitis, NMR-based metabolic profiling was studied over time and revealed many changes in molecules related to energy household including lactate, pyruvate, and citrate [4,5]. In a DSS colitis model, metabolic profiles were measured by GC–MS to differentiate between acute colitis and the resolution phase, suggesting that metabolomics could be useful for determining disease progression [9▪]. NMR has also been successfully used to monitor the effects of antibiotics on the urine and fecal metabolic profiles of mice [16].

METABOLOMICS AND HUMAN INFLAMMATORY BOWEL DISEASE

The use of metabolomic analysis in human IBD arose from the need to improve diagnosis and differentiation of IBD subtypes and to classify indeterminate colitis. Simultaneously, the method offered a new approach to screen for small molecules that might play a role in disease mechanisms. A noninvasive approach of diagnosing IBDs may also be preferable over endoscopy which is not without risk, although serious complications are reportedly low [17]. Noninvasive fecal and serologic markers with variable specificity and sensitivity for IBDs are already in use. Among them, lactoferrin, calprotectin, PMN-elastase, and CRP are used to differentiate active from inactive IBD as well as from irritable bowel syndrome (IBS) with quite good diagnostic accuracy (e.g. 83% for lactoferrin in ulcerative colitis and 81.4% for calprotectin in Crohn's disease [18]). Other serologic markers such as anti-*Saccharomyces cerevisiae* (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (pANCAs) or the more experimental anti-CBir1 and anti-OmpC are less sensitive for IBDs [19], although a combination of these markers has recently shown high predictive value in distinguishing IBD subtypes [20▪].

To date, ^{1}H NMR spectroscopy has been employed most widely to characterize activity and severity of human IBDs. Studies have been performed on biofluids such as serum, plasma, urine, and fecal extracts as well as on biopsies, isolated colonocytes, and lymphocytes of IBD patients and were compared to matched healthy persons. These studies have primarily focused on small and noncomplex molecules, such as amino acids and related metabolites, on TCA cycle intermediates, and on metabolites involved in fatty acid and purine metabolism. Differences in metabolic profiles were reported to exist between IBD patients and healthy controls [14▪▪,21▪,22,23▪,24,25] as well as between the IBD subtypes [14▪▪,21▪,25]. Although multivariate statistical models are the best choice to discriminate patients from healthy individuals, Balasubramanian *et al.* [26] were able to detect significant shifts using univariate statistical methods in a variety of metabolites of the colonic mucosa in patients with Crohn's disease and ulcerative colitis in comparison to healthy persons or IBD patients in remission.

With regard to confounding factors, medication does not seem to influence the metabolomic data very much [14▪▪,24,25], although in one study IBD patients who were on TNF-α antibodies had different urinary metabolic profiles than those without TNF-α antibody treatment [22]. Inclusion of IBD patients with bowel resections may also confound statistical outcome [22].

URINARY METABOLOMICS

In human IBDs, the type of biofluid investigated may have a significant impact on the metabolic profile. For instance, intersubject variability is reported to be higher in urine than in serum [27]. Several studies have demonstrated that urinary metabolites are more influenced by environmental cues (diet, diurnal rhythms, age, sex, and cultural influences) than serum or plasma metabolites [28–30]. Despite these variations, urinary metabolomic data may provide an important insight into changes within the bacterial population that colonize the gut, information that may be of high relevance. Metabolites of gut bacteria can usually be detected in urine [31]. In fact, a lot has already been learned about the role of the gut microbiome through various metabolomic studies [32▪▪].

The gut microbiome is intricately involved in the pathogenesis of IBDs [33] and dysbiosis may occur either as a cause or as a consequence of the disease [34]. An altered population in the microbiome may thus lead to an altered urinary metabolic profile. Williams *et al.* [25] investigated

the metabolites in longitudinal samples of urine from Crohn's disease and ulcerative colitis patients by ^{1}H NMR spectroscopy using multivariate analysis and PLS-DA. Their model of evaluation distinguished well between IBD patients and healthy individuals, and also between ulcerative colitis and Crohn's disease patients. Significant decreases in hippurate, a metabolite that derives from microbiota, were found in IBD patients. Similarly, good separation of patients with IBD from healthy individuals by ^{1}H NMR spectroscopy and multivariate analysis were reported by two other groups [14▪▪,22]. They also noticed low hippurate levels in urine of IBD patients, suggesting that hippurate may be a biomarker candidate. The findings are of high interest as hippurate levels were recently shown to correlate with the presence of *Clostridia* in the gut [35] and *Clostridia* bacteria have been found widely in Crohn's disease patients [34]. Nevertheless, more data are necessary concerning the specificity of such markers as NMR can, for instance, reveal changes in hippurate, citrate, 2-oxoglutarate and creatinine already after slight changes in weight, suggesting that these compounds may represent general stress markers rather than specific biomarkers [36]. Other studies [14▪▪,22,24] failed to distinguish between ulcerative colitis and Crohn's disease through urinary metabolic profiling, emphasizing the influence of environmental variation in urinary metabolomics and the multifactorial nature of IBDs. In fact, IBD shows high variations in phenotype and severity, and ulcerative colitis is easily confounded with colonic Crohn's disease and indeterminate colitis [37].

METABOLOMICS OF SERUM AND PLASMA

Schicho *et al.* [14▪▪] showed that quantitative metabolic profiling of serum and plasma by ^{1}H NMR spectroscopy and multivariate analysis discriminated between ulcerative colitis and Crohn's disease patients, albeit with a weaker predictability than between healthy and ulcerative colitis/Crohn's disease patients. These observations were recently confirmed in serum in a comparable study [21▪]. In a mouse model of colitis, serum metabolomics measured by the same method were clearly different between diseased and nondiseased animals [10]. Another group that analyzed the plasma of patients with IBD with the aim to investigate differences in metabolites demonstrated that amino acid profiles evaluated by a novel statistical data modeling (aminogram) discriminated between IBD and healthy individuals, and between ulcerative colitis and Crohn's disease [38▪▪]. Statistical indices also correlated well with disease activity, indicating that metabolic profiles of amino acids could be useful in monitoring disease activity during progression of IBD [38▪▪]. A further report using GC/MS showed that IBD patients with either ulcerative colitis or Crohn's disease could be readily distinguished from each other on the basis of amino acids and TCA cycle-related molecules [39]. Altogether, these studies demonstrate that metabolic serum and plasma profiles can separate between patients with IBD and healthy controls as well as between the IBD subtypes irrespective of the technique, favoring the use of serum and plasma for the reliable measurement of metabolic profiles. However, analysis of serum may not provide us with information on important changes in the population of the gut microbiota.

METABOLOMICS OF FECAL EXTRACTS

The possible involvement of microbiota in the pathogenesis of IBD has also led to an interest in the use of metabolic profiling of fecal extracts for diagnosis and biomarker detection [40]. Work by Marchesi *et al.* [41] has already highlighted that NMR-based metabolic profiles in human fecal extracts of IBD patients show decreased levels of short-chain fatty acids and that profiles are significantly distinct from healthy individuals. Recently, another study using ^{1}H NMR spectroscopy of fecal samples showed clear separation of ulcerative colitis patients from control cohorts, whereas the model was less successful in distinguishing between IBS patients and healthy controls [23▪]. NMR spectra in this study correlated well between gut microbiota profiles as well as fecal metabolite composition, indicating that metabolomics of fecal samples not only discriminates between diseased and healthy individual, but reveals valuable insight into disturbances of gut bacteria [23▪]. Fecal extracts may therefore provide important clues on how the microbiome contributes to the pathology of IBD. Jansson *et al.* [1] used ICR-FT/MS with an ultrahigh mass resolution in fecal extracts of Crohn's disease patients. They were able to measure thousands of masses that differentiated between healthy and diseased individuals. Compounds of amino acid pathways and bile acid metabolism, saturated and unsaturated fatty acids, and arachidonic acid were among the discriminating metabolites. This points again to the important role of inflammatory lipid mediators in IBD and to their use in metabolic profiles for classifying IBD patients and healthy individuals.

METABOLOMICS OF COLON TISSUE

Biopsies from colon tissue represent another source material to study metabolomics in IBD. Sharma *et al.*

[42] assessed macroscopically involved and uninvolved mucosa of the colon from IBD patients by ^{1}H NMR spectroscopy and detected similar metabolic profiles in these biopsies, whereas another study reported differences between biopsies from IBD patients and healthy individuals with regard to amino acids, membrane components, and lactate [26]. Using multivariate analysis, Bjerrum *et al.* [24] demonstrated that active ulcerative colitis can be separated from inactive ulcerative colitis by metabolic profiles of biopsies and isolated colonocytes. Interestingly, these workers showed that 20% of inactive ulcerative colitis patients had metabolic profiles similar to those with active ulcerative colitis. The authors suggested that the ulcerative-colitis-like profiles in these patients could have indicated pathogenic changes that occurred prior to or immediately after flare-ups instead of representing subclinical inflammation [43].

CONCLUSION

Metabolic profiling of biofluids from IBD patients represents a powerful approach for the detection of biomarkers and underlying mechanisms of IBD. In addition, the biofluids investigated can hold different information on IBD, that is, whereas metabolic profiles of serum and plasma allow reproducible separation of patients with IBD from healthy controls and additionally allow separation of IBD subtypes, urinary metabolic profiles are harder to interpret because of a higher susceptibility of urine composition to environmental variation. However, as gut microbiota are regarded as possible important players in IBD pathogenesis, urinary and fecal metabolic profiles may provide important information on disease development, perpetuation, and progression. A recent study shows that fecal transplants significantly improved recurrent *Clostridium difficile* infection [44], suggesting such treatment might also be useful in alleviating or curing IBD, or maintaining remission. It would be very interesting to follow these patients in future studies following such treatment in order to determine whether their serum, urine, and fecal extract profiles returned to normal and to explore the prognostic value of metabolomic analysis in this setting. Whatever technique and biofluid are going to be used for metabolic profiling of IBD patients in the future, metabolomics certainly will become an important adjunct in the clinical assessment and management of IBDs.

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Conflicts of interest

Disclosures: The authors have no conflicts of interest to disclose.

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