REVIEW

Metabolomics as a diagnostic tool in gastroenterology

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Abstract

Metabolomics has increasingly been applied in addition to other "omic" approaches in the study of the pathophysiology of different gastrointestinal diseases. Metabolites represent molecular readouts of the cell status reflecting a physiological phenotype. In addition, changes in metabolite concentrations induced by exogenous factors such as environmental and dietary factors which do not affect the genome, are taken into account. Metabolic reactions initiated by the host or gut microbiota can lead to "marker" metabolites present in different biological fluids that allow differentiation between health and disease. Several lines of evidence implicated the involvement of intestinal microbiota in the pathogenesis of inflammatory bowel disease (IBD). Also in irritable bowel syndrome (IBS), a role of an abnormal microbiota composition, so-called dysbiosis, is supported by experimental data. These compositional alterations could play a role in the aetiology of both diseases by altering the metabolic activities of the gut bacteria. Several studies have applied a metabolomic approach to identify these metabolite signatures. However, before translating a potential metabolite biomarker into clinical use, additional validation studies are required. This review summarizes contributions that metabolomics has made in IBD and IBS and presents potential future directions within the field.

Core tip: Metabolic profiling is a powerful exploratory tool for understanding interactions between nutrients, the intestinal metabolism and the microbiota composition in health and disease and, to gain more insight in metabolic pathways. Metabolomics may advance our understanding, diagnosis and treatment of inflammatory bowel disease and irritable bowel syndrome. Metabolic reactions initiated by the host or gut microbiota can lead to "marker" metabolites present in different biological fluids that allow differentiation between health and disease. Disease-related mechanisms may be uncovered and verified, and candidate diagnostic biomarkers in biological samples are characterized.

INTRODUCTION

Different "Omic" approaches are currently applied to identify novel diagnostic targets and disease specific markers, and to characterize the link between gut microbiota or host metabolism and functional alterations in the pathophysiology of different diseases. Genomics, transcriptomics and proteomics provide extensive information regarding the genotype but convey limited information about the phenotype (Figure 1). Gene expression and protein data mainly indicate the potential for specific
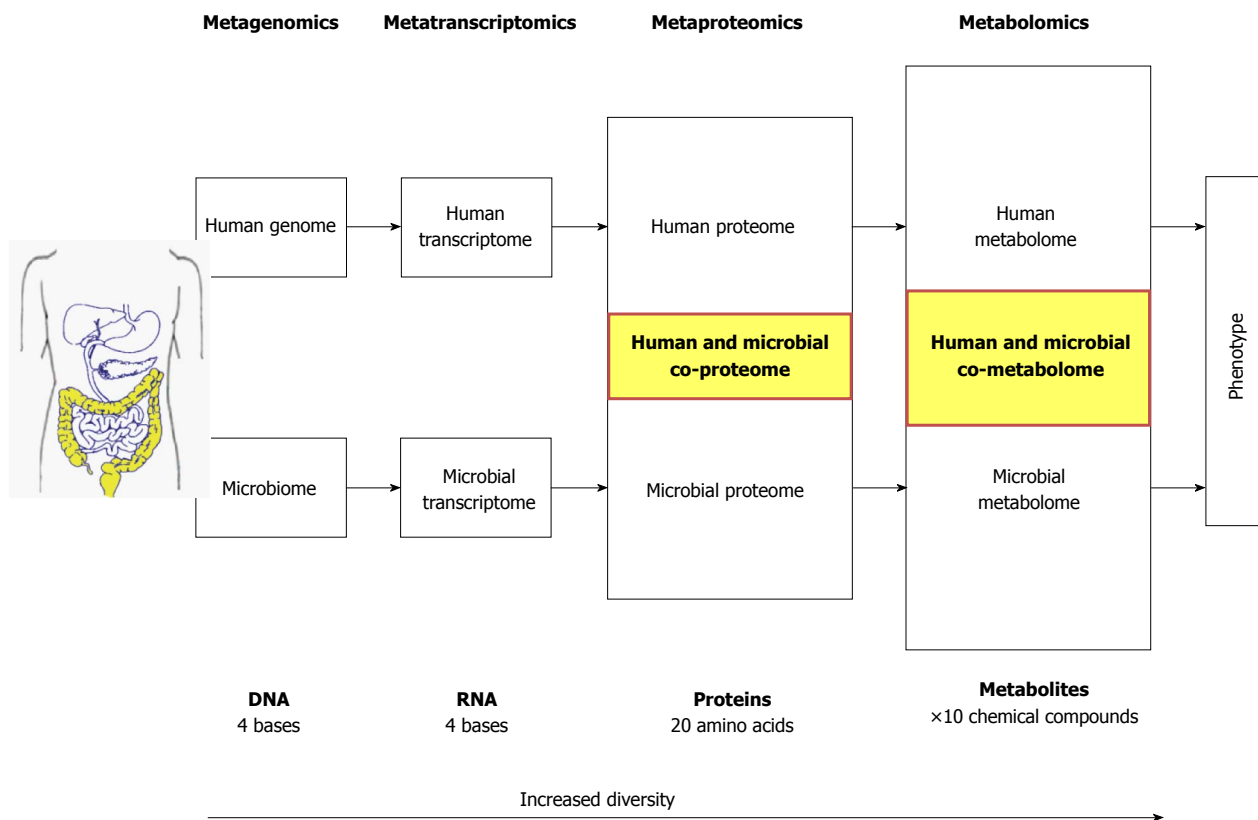
Figure 1 Overview of different "Omic" approaches (metagenomics, metatranscriptomics, metaproteomics and metabolomics) determining an individual's "Genotype to phenotype".

metabolic functions and do not always reflect the effective physiological processes as several downstream regulatory mechanisms are involved. As compared to other "omics", metabolic profiling or metabolomics, integrates the effects of gene regulation, post-transcriptional regulation and pathway interactions. This downstream synthesis of diverse signals ultimately makes metabolites direct molecular readouts of cell status that reflect a meaningful physiological phenotype (Figure 1)[1,2]. In addition, changes in metabolite concentrations are also induced by exogenous factors such as environmental and dietary factors which do not affect the genome. Metabolomics is defined as "the non-biased identification and quantification of all metabolites in a biological system"[3]. For the quantitative analysis of metabolites in response to disease, Nicholson and colleagues introduced the term metabonomics or "the quantitative measurement of the multiparametric metabolic responses of a living system to pathophysiological stimuli or genetic modification"[4]. In practice, within human disease research, both terms are used indifferently.

Metabolic profiling is a powerful exploratory tool for understanding interactions between nutrients, the intestinal metabolism and the microbiota composition in health and disease and, to gain more insight in metabolic pathways. Metabolomic studies allow evaluation of metabolites by a top-down approach bypassing the need for an *a priori* hypothesis. Generally, metabolomic analysis has in view two major opportunities. First, untargeted analysis of a large number of metabolites enhances the chance to discover metabolites that are associated with the disease and might serve as biomarkers. In this respect, biomarker models are designed to discriminate with optimal sensitivity/specificity between groups, but do not presume biological understanding as an absolute prerequisite for biomarker development. However, understanding of the biological pathways can certainly support an assay[5]. Second, the profile of metabolites affected by the disease may provide new insights into the pathogenesis and eventually reveal new therapeutic targets.

Until now, genomic and proteomic methodologies have often been applied to uncover gastrointestinal related pathophysiological processes[6-11]. However, currently, metabolomics technologies are increasingly used for discovery of gastrointestinal disease signatures and have been applied for the screening of different pathological conditions that are linked with a metabolic imbalance. This review focuses on the contribution of metabolic profiling in advancing research in the field of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

THE MICROBIOTA AND ITS METABOLIC ACTIVITY

The microbiota residing in the human gastrointestinal tract, especially the large intestine, is recognized as one of the most metabolically active organs of the human

body. This microbial ecosystem is extremely complex and dynamic with high densities of living bacteria consisting of approximately 500-1000 different species[12,13]. In healthy adults, 80% of the identified fecal microbiota can be classified into three dominant phyla: *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, but there is substantial variation in the species composition between individuals[14]. A total of about 10^{14} bacterial cells are present in the adult intestine, which is ten times the number of cells in the human body[15]. This microbiome outnumbers the host's genetic potential by two orders of magnitude[16] and provides a diverse range of biochemical and metabolic activities to complement the host's physiology. The presence and metabolic activities of a specific bacterial community play an important role in maintaining the host's overall health and well-being, and has been shown to respond to metabolic challenges and dietary factors. This complex microbial system varies with the host's age, diet and health status[17].

Through the process of fermentation, colonic bacteria produce a wide range of compounds that may influence the physiological processes in the colon. The human microbiota is characterized by a significant degree of functional redundancy, meaning that different bacteria can perform similar functions and metabolize the same substrate, thereby producing similar metabolites[18]. Therefore, not only the composition but also the functional capacity of the intestinal microbiota is highly important regarding the clinical end points. Nevertheless, metabolic insights remain limited due to the inaccessibility of the intestinal habitat and the complexity of the microbiota composition[12]. A number of factors, such as nutrient availability, physicochemical nutrient properties, colonic transit time, and age of the host, influence the composition and the metabolic activity of the colonic microbiota. Nutrient availability is believed to be the most important regulator of bacterial metabolism. Especially the ratio of available carbohydrate to nitrogen determines the degree of saccharolytic *vs* proteolytic fermentation[19]. Colonic fermentation of carbohydrates results in the generation of short-chain fatty acids (SCFA) which are generally assumed to be beneficial for the host[20]. Protein fermentation gives rise to a variety of metabolites such as phenolic compounds, branched-chain fatty acids, S-containing compounds, amines and ammonia[21,22].

HOST AND INTESTINAL MICROBIOTA CO-METABOLOME

The intestinal microbiota produces a number of compounds during the metabolism of nutrients and xenobiotics (compounds of non-host origin that enter the gut with the diet or are produced by the microbiota). Some of these metabolites are excreted in feces whereas others are absorbed through the colonic mucosa and enter the systemic circulation where they can be further modified by human metabolism. For instance, p-cresol is a bacte-

rial fermentation product produced in the colon from tyrosine that is effectively absorbed. It is conjugated in the colon mucosa or liver to p-cresol sulfate or p-cresol glucuronide which improves the water solubility and facilitates its urinary excretion[23]. These metabolites are called human commensal co-metabolites. Also the opposite occurs. A number of metabolites that are derived from host metabolism are returned to the gut *via* biliary excretion where they can be further metabolized by the microbiota. For instance, bile acids that have escaped absorption in the terminal ileum can be deconjugated and converted to secondary bile acids by microbial metabolism[24].

These host-microbiota metabolic interactions complicate the interpretation of metabolite profiles. In addition, this co-metabolism explains that the outcome of metabolome analyses clearly depends on the biomatrix chosen. The contribution of the microbial metabolism is more likely reflected in the fecal metabolome than in urinary, serum of breath profiles. Urinary profiles contain human and human-microbial co-metabolites whereas serum profiles seem less influenced by bacterial metabolism.

METABOLOMICS-BASED METHODS

Analytical strategies

Fiehn *et al*[1] defined metabolomic analysis as "a comprehensive and quantitative analysis of the metabolome" with the metabolome defined as the whole of metabolites produced by an organism. However, due to the chemical diversity and different physicochemical properties of the metabolites and the large dynamic range of metabolite concentrations in different biological samples, it is virtually impossible to measure the complete metabolome. By selecting a specific analytical platform and a biofluid in which metabolites will be measured, the metabolome will be reduced to those specific conditions. Serum, plasma, urine, feces and tissue are the most studied biological matrices[25]. An overview of the different steps involved in the analytical process is shown in Figure 2.

Multiple analytical techniques have been used for the analysis of the metabolome. Gas chromatography (GC), liquid chromatography (LC) and high/ultra performance liquid chromatography (H/UPLC) coupled to mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR) enable detection, identification and quantification of metabolites[26,27]. Other analytical options consist of Fourier transform infrared spectroscopy (FTIS) or capillary electrophoresis (CE) coupled to MS[28,29].

The applicability of these analytical techniques differs. GC-MS provides an extraordinary resolution, permitting the separation of structurally similar compounds. However, this technique requires the compounds to be volatile and thermally stable. A chemical derivatization step can be applied prior to the chromatographic separation to render polar metabolites more volatile. Purge-
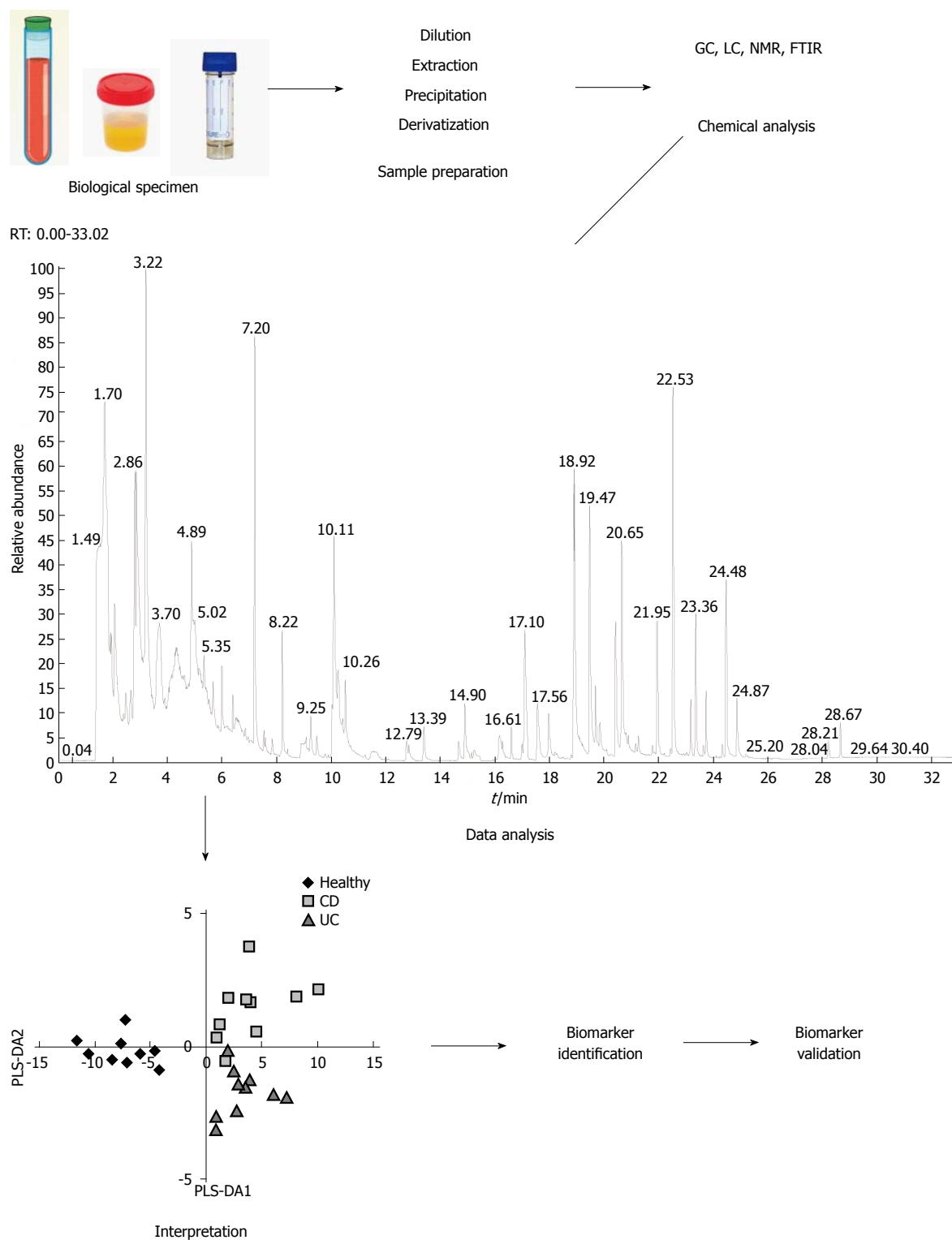
Figure 2 **Overview of the different steps involved in the analytical process of metabolomics.** GC: Gas chromatography; LC: liquid chromatography; NMR: Nuclear magnetic resonance; FTIR: Fourier Transform infrared spectroscopy; PLS-DA: Partial least squares discriminant analysis; CD: Crohn's disease; UC: Ulcerative colitis.

and-trap and solid phase micro-extraction are sample preparation techniques often used in combination with GC. For metabolites that are not volatile and which cannot be derivatized, LC-MS is applied. LC-MS can detect a relatively broad spectrum of (polar and non-polar) metabolites with ample selectivity and sensitivity[30]. CE-MS is a rather new technique in metabolomics that is more sensitive and detects a wider spectrum of (polar) compounds than LC-MS[31,32]. ^{1}H-NMR is a non-destructive technique that does not require prior separation of the compounds in the biofluid. It provides detailed information on molecular structure and requires only minimal

sample preparation, but has a lower sensitivity than the MS based techniques[33]. Often, a combination of different techniques is applied as none of the individual methods will cover the full metabolome[27]. Several on-line databases for identifying metabolites from experimental NMR and/or MS data are available, as summarized in Table 1. These databases contain chemical, spectral, clinical and molecular data about metabolites found in different human biofluids.

Depending on the technique, detection limits vary: detection limits for NMR and FTIS (mM sensitivity) are much higher as compared to GC-MS (< mM sensitivity) and LC-MS (nM sensitivity). As a consequence, MS-based techniques are preferably applied for quantification of specific metabolites.

Data handling

The analysis and interpretation of complex metabolomics data is facilitated by the application of chemometric and bio-statistical tools. Commonly used tests in metabolomic studies include principal component analysis (PCA) and partial-least squares discriminant analysis (PLS-DA). PCA is an unsupervised classification method, since the variation in the data is analyzed without a priori designation of samples into their classes. In contrast, PLS-DA is considered a supervised classification method, as the samples are designated into their classes for comparison.

In metabolomics, typically the number of variables or metabolites largely exceeds the number of samples measured. This can lead to the discovery of a number of variables that randomly, *i.e.,* by chance, correlate to the outcome variable and in this way give the impression of a good predictive ability. However, if such a set of variables is chosen and the model is applied to new samples, the predictive ability might be very poor. This is known as over-fitting or fitting to the noise and can be avoided by careful cross-validation of the model. Cross-validation implicates that the data set is split in a training set and a test set. The biomarker model is discovered using the training set and the performance of the model is evaluated using the test set. In case of a relatively low sample number (< 100), multiple rounds of cross validation are performed using different partitions of the data in training set/test set and the performance results are averaged[5].

METABOLIC SIGNATURE AND GASTRO-INTESTINAL PHENOTYPES

Dysbiosis in gastrointestinal disorders

In gastrointestinal diseases such as IBD and IBS, there is an emerging consensus hypothesis that a dysbiosis of the microbiota is involved in initiating the disease or maintaining it. Several studies identified a disproportion of the predominant bacteria in fecal samples of IBD patients[18,42] and IBS patients[43]. For example, a reduction in the abundance and diversity of Firmicutes is fre-

quently associated with IBD and IBS. At present, studies comparing the metabolic activity of the microbiota of IBD and IBS patients as compared to normal individuals are emerging thereby investigating whether eventual differences could be related to the pathogenesis of the disease[44,45] or whether they could be used as a classification tool in clinical diagnosis.

Metabolomics in IBD

Inflammatory bowel diseases comprise Crohn's disease and ulcerative colitis as the two major phenotypes. Although both phenotypes share similar pathophysiological and clinical features, they require different therapeutic management and display different prognosis. Both manifestations are influenced by genetic predispositions as well as microbial and environmental factors. At present, the diagnosis of IBD mainly relies on clinical, endoscopic, radiologic and histologic examination which implicates that diagnosis is only possible at a relatively advanced stage of the disease. Less invasive methods such as analysis of biomarkers from urine, serum, or feces, however, would be of significant advantage and useful for primary diagnosis, surveillance, and early detection of relapses.

Several biomarkers or sets of biomarkers have been tested in clinical trials including acute phase proteins such as C-reactive protein, fecal markers (lactoferrin, calprotectin, and PMN-elastase) and serological markers (antibodies against luminal antigens and anti-glycan antibodies)[46]. Recently, the exploration of metabolomics in IBD rose from the need to improve diagnosis and to allow better stratification of patients into IBD subtypes.

Several studies have applied a metabolomic approach to discriminate IBD patients from healthy controls, to discriminate CD from UC and patients with active disease from patients in remission. An overview of the studies in humans is presented in Table 2.

The growing acceptance of the involvement of the gut microbiota in the chronic mucosal immune activation underlying the pathogenesis of IBD has led to an interest in the use of fecal extracts or fecal samples as biofluids to apply metabolite profiling.

Marchesi et al[47] was the first to differentiate IBD patients from healthy controls based on ^{1}H-NMR analysis of fecal water extracts and to discriminate CD patients from UC patients. Fecal water extracts from IBD patients were characterized by a depletion of bacterial degradation products such as SCFA, dimethylamine and trimethylamine suggesting a disruption of the normal bacterial ecology, called dysbiosis, either as the cause or consequence of the disease. In a study in identical twins including healthy twins and twins with inactive CD, either concordant or discordant, fecal extracts were analyzed using ICR-FT/MS with ultrahigh mass resolution. Healthy subjects could nicely be discriminated from CD patients. In addition, it was possible to separate patients with predominantly ileal involvement from patients with predominantly colonic involvement of the disease. Inter-

Table 1 Overview of web-based databases for metabolite identification		
Database	URL or web address	Extra information
Human Metabolome Database (HMDB)	http://www.hmdb.ca/	Wishart *et al*[34]
Madison Metabolomics Consortium (MMC) Database	http://mmcd.nmrfam.wisc.edu/	Cui *et al*[35]
Biological Magnetic Resonance Data Bank (BMRB)	http://www.bmrb.wisc.edu/	Ulrich *et al*[36]
Golm Metabolome Database	http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html	Kopka *et al*[37]
BiGG (a knowledgebase of Biochemically, Genetically and Genomically structured genome-scale metabolic network reconstructions)	http://bigg.ucsd.edu/	Schellenberger *et al*[38]
SetupX and BinBase	http://fiehnlab.ucdavis.edu/	Skogerson *et al*[39]
MassBank	http://www.massbank.jp/	Horai *et al*[40]
METLIN	http://metlin.scripps.edu/	Smith *et al*[41]

estingly, the separation of the groups was higher for the metabolite profiles than for the microbial community profiles, analyzed on the same samples with T-RFLP fingerprints generated using general bacterial and *Bacteroides* group-specific primers[48]. The higher discrimination of the metabolite data was attributed to a direct link of the metabolites to function.

In another study, ^{1}H-NMR profiling of fecal extract allowed to discriminate patients with UC from healthy controls. Elevated levels of taurine and cadaverine in UC patients were the major discriminative findings. Samples were also analyzed for microbiota composition using DGGE. Canonical correlation analysis between NMR and DGGE data sets, based on PC scores accounting for 90% of the original variance, revealed a good correlation ($r = 0.85$, $P < 0.002$) between gut microbiota profile and metabolite composition suggesting a direct link between both parameters[49].

A recent study analyzed metabolite profiles in feces of chronic gastrointestinal disorders using GC-MS with headspace sample preparation. Samples of CD patients showed significant higher levels of ester and alcohol derivatives of SCFA and indole as compared to healthy controls, UC and IBS patients. Following treatment, the metabolite profile was altered to more closely resemble that of healthy volunteers[50]. As many microbial metabolites are absorbed and excreted in urine, either as such or after further metabolism by human enzymes, metabolite profiles of urine samples may also reflect the impact of the microbiota composition[51]. Several studies were able to discriminate IBD patients from healthy controls based on metabolite profiling of urine samples[52-54]. In all these studies, hippurate levels were lower in IBD patients as compared to controls suggesting hippurate as a biomarker of IBD. Hippurate or N-benzoylglycine is a mammalian-microbial co-metabolite that originates from bacterial fermentation of dietary aromatic compounds (polyphenols, purines or aromatic amino acids) to benzoic acid which is further conjugated to glycine in the liver[55]. Remarkably, urinary metabolite profiling allowed to differentiate between UC and CD in only one study[51], whereas two other studies failed to do so[53,54]. This discrepancy may highlight the fact that IBD is a multifactorial disease with a high variety in phenotypes and severity[56]. Indeed, the notion that IBD is actually a syndrome comprising several disease subtypes, is gaining

more and more acceptance[46].

Metabolite profiles in serum or plasma or in colonic mucosal biopsies rather reflect changes in the host's metabolism and provide less information on the impact of the gut microbiota composition and/or activity. As compared to urinary and fecal profiles, metabolite profiles in serum or plasma may be less affected by environmental factors and are subject to less inter-individual variation[57].

Results from studies that analyzed serum/plasma or colonic mucosa cells indicate that both CD and UC have an impact on the amino acid metabolism[53,58-63]. Several amino acids occurred in lower levels in colonic mucosal cells from IBD patients as compared to controls. As higher amino acid levels were found in fecal extracts[47,64], this may be the result of malabsorption due to inflammation. An alternative explanation is that inflammatory conditions induce large energy requirements to repair the damaged mucosa leading to enhanced protein catabolism. Specifically, the role of decreased levels of glutamine in the pathogenesis of IBD has been studied. Besides butyrate, glutamine is an important energy source for the colonocytes and accounts for about 30% of their energy needs. In a mouse model of DSS-induced colitis, similar reductions in glutamine levels in serum and colonic tissue were observed and supplementation with glutamine attenuated the DSS-induced colitis[65].

In a recent study, Hisamatsu *et al*[66] calculated an AminoIndex based on multivariate analysis of amino acid profiles in serum of IBD patients which allowed to distinguish between CD and UC and also reflected disease activity.

Zhang *et al*[67] specifically recruited patients with active UC and short disease duration (2.7 years) and evaluated the efficacy of ^{1}H-NMR metabolic profiling of serum for early stage diagnosis of UC. Although active UC patients could be discriminated from healthy controls in a multivariate OPLS-DA model, the number of altered metabolites in this study was rather limited.

Metabolomics in IBS

Irritable bowel syndrome is a multifactorial functional disorder of the gastrointestinal tract that affects about 10%-15% of the adult population. IBS patients have symptoms of pain and bowel dysfunction. A subset of patients that developed IBS after an infection, so-called

Table 2 Overview of the studies that applied metabolomics to discriminate inflammatory bowel diseases and/or irritable bowel syndrome patients from controls

Reference	Analytical platform	Biofluid	Samples	Observations
Marchesi *et al*[47]	^{1}HNMR	Faecal extracts	CD (*n* = 10), UC (*n* = 10), HC (*n* = 13)	Depletion of SCFA and methylamine and trimethylamine in CD patients
				Higher amounts of amino acids in UC and CD compared to healthy controls
Jansson *et al*[64]	ICR-FT/MS	Faecal water	10 twin pairs with CD, 7 healthy twin pairs	Discrimination based on disease location (ileal or colonic CD) significant differences in the types and number of metabolites within specific pathways, including tyrosine and phenyl-alanine metabolism and bile acid and fatty acid biosynthesis
Le Gall *et al*[49]	^{1}HNMR	Faecal water	UC (*n* = 13; 31 samples), IBS (*n* = 10; 21 samples), HC (*n* = 22; 72 samples)	Discrimination between UC and HC; no classification of IBS
				Increased taurine and cadaverine in UC
Walton *et al*[50]	GC-MS	Faeces	UC (*n* = 20), CD (*n* = 22), IBS (*n* = 26), HC (*n* = 19)	Increased concentrations of ester and alcohol derivates of short-chain fatty acids and indole in CD
				After treatment, metabolite patterns are more similar to those of HC
Williams *et al*[52]	^{1}HNMR	Urine	CD (*n* = 86), UC (*n* = 60), HC (*n* = 60)	Discrimination between CD, UC and HC
				Significantly different metabolites include hippurate, p-cresol sulfate and formate
				Clustering independent of diet and medication
Schicho *et al*[53]	^{1}HNMR	Urine, serum, plasma	CD (*n* = 20), UC (*n* = 20), HC (*n* = 40)	IBD patients could be discriminated from HC, differences between CD and UC less pronounced
				Discriminating metabolites include amino acids, creatine, creatinine, metabolites of urea cycle, monosaccharides, hippurate (urine)
Stephens *et al*[54]	^{1}HNMR	Urine	CD (*n* = 30), UC (*n* = 30), HC (*n* = 60)	Metabolites for distinguishing IBD from HC: TCA cycle intermediates, amino acids metabolites derived from gut microflora (methanol, formate, hippurate, acetate, and methylamine); as well as the other metabolites trigonelline, creatine, urea, and taurine
				No discrimination between UC and CD after removal of patients with surgical intervention confounder
Ooi *et al*[58]	GC-MS	Colonic biopsies, serum	Colonic biopsies: UC (*n* = 22), serum: UC (*n* = 13), CD (*n* = 21), HC (*n* = 17)	Reduced levels of amino acids resulting in reduced levels of TCA cycle related downstream molecules in colonic tissue of UC
				Serum amino acid profiling enabled discrimination between UC and CD
Bjerrum *et al*[60]	^{1}HNMR	Colonic biopsies, colonocytes, lymphocytes, urine	Active UC (*n* = 35), quiescent UC (*n* = 33), HC (*n* = 25)	No discrimination between active UC, inactive UC and HC based on urine or lymphocyte profiles
				Inactive UC could not be differentiated from HC
				Active UC characterized by higher antioxidants and amino acids and lower levels of lipid, myo-inositol, betaine and glycerophosphoglycine
				20% of inactive UC had similar profile as active UC
Bezabeh *et al*[63]	^{1}HNMR	Colonic biopsies	UC (*n* = 26; 45 samples), CD (*n* = 21; 31 samples), controls (38 non-inflamed IBD, 25 cancer patients)	Accurate classification of UC *vs* CD
				Some non-inflamed tissues from IBD had abnormal NMR-spectra
Balasubramanian *et al*[61]	^{1}HNMR	Colonic biopsies	Active UC (*n* = 20), Inactive UC (*n* = 11), Active CD (*n* = 20), Inactive CD (*n* = 6), HC (*n* = 26)	Higher α-glucose and lower amino acids, membrane components, lactate and succinate in active UC and CD compared to HC
				Lower lactate, glycerophosphorylcholine and myo-inositol in inactive UC and lower lactate in inactive CD compared to HC
				Lower formate in active UC *vs* active CD
Sharma *et al*[62]	^{1}HNMR	Colonic biopsies (inflamed and non-inflamed)	UC (*n* = 12), CD (*n* = 9), controls (*n* = 25)	No differentiation between inflamed and non-inflamed samples
				Lower levels of amino acids, membrane components, lactate and formate in IBD *vs* controls and higher levels of glucose
Hisamatsu *et al*[66]	AA analyzer	plasma	CD (*n* = 165), UC (*n* = 222), HC (*n* = 210)	Multivariate indexes established from plasma aminograms distinguish CD or UC from HC
				Other indexes distinguish active UC and CD from each remission patients and correlate with disease activity indices
Zhang *et al*[67]	^{1}HNMR	Serum	Active UC (*n* = 20), HC (*n* = 19)	Active UC displayed increased 3-hydroxybutyrate, β-glucose, α-glucose and phenylalanine and decreased lipid compared to healthy controls
Ponnusamy *et al*[71]	GC-MS	Faeces	IBS (*n* = 11) *vs* non-IBS (n = 8)	Elevated levels of amino acids and phenolic compounds that were highly correlated with abundance of lactobacilli and Clostridium

Ohman et al[69]	GC-MS	Faeces	IBS-D (n = 30), CD (n = 62), UC (n = 48), HC (n = 109)	Significantly more esters in IBS-D, association of aldehydes with IBD Accurate separation of IBS-D from active CD, UC and HC

SCFA: Short-chain fatty acids; IBD: Inflammatory bowel diseases; IBS: Irritable bowel syndrome; TCA: Tricarboxylic acid; HNMR: Nuclear magnetic resonance; ICR-FT/MS: Fourier transform ion cyclotron resonance mass spectrometry; GC-MS: Gas chromatography-mass spectrometry.

post-infectious IBS, may have microscopic inflammation but with normal mucosal appearance on endoscopy. Recently, a new IBS entity, IBD-IBS, has been described: these patients have pain and diarrhea similar to IBS in association with minimal or no evident intestinal inflammation[68]. The exact etiology has not been identified. Yet, environmental, psycho-social, physiological and genetic factors are believed to play a role. Also the role of an abnormal microbiota composition is supported by clinical and experimental data[69]. Disruption of the balance between the host and the intestinal microbiota results in changes in the mucosal immune system that range from overt inflammation, as seen in Crohn's disease, to low-grade inflammation without tissue injury, as seen in a subset of IBS patients[70].

Surprisingly, very little work has been conducted on metabolite profiling of IBS patients. ^{1}H-NMR metabolite profiling of fecal extracts allowed to separate IBS patients from healthy controls with moderate success (sensitivity = 57%, specificity = 76%)[49]. Two other studies applied GC/MS to analyze fecal samples although with different sample preparation. Ahmed *et al*[71] analyzed the volatile organic compounds (VOC) in the headspace of the fecal samples, *i.e.*, compounds with a vapour pressure that is sufficiently high to enable them to move from the solid or liquid phase into the gaseous phase. Those VOC comprise hydrocarbons, alcohols, aldehydes, ketones, esters and organic acids[72]. In contrast, the volatility of polar compounds in the fecal samples was increased by trimethylsilyl derivatization in the study by Ponnusamy *et al*[73]. Although both studies were able to discriminate IBS patients from healthy controls, the metabolites responsible for discrimination were different which is inherent to the different sample preparation. Ahmed *et al*[71] found increased abundance of esters in diarrhea predominant IBS-patients compared with healthy controls. Of the 28 VOCs positively associated with IBS, 22 belonged to the class of esters. Ponnusamy *et al*[73] highlighted higher levels of specific amino acids (alanine and pyroglutamate) and phenolic compounds (hydroxyphenyl acetate and hydroxyphenyl propionate) and associated these metabolic changes to alterations of specific gut microbial populations including lactobacilli and *Clostridium*.

Translating metabolites in gastrointestinal biomarkers
Metabolites are promising biomarkers because they can be easily measured from non-invasive breath, urine, feces or blood samples. Several studies have identified such metabolite signatures that should allow classification of samples as healthy or diseased. To translate these models into the clinic, additional validation studies are required. First of all, experiments to validate the biomarker model

need to be performed. This level of validation includes (1) lab repeatability studies where the same samples are analyzed in the same laboratory by the same observer; (2) lab replication studies, where independent samples are analyzed in the same lab by the same observer; (3) inter-lab repeatability studies, were the original samples are analyzed in a different laboratory by a different observer; and (4) inter-lab replication studies, were independent samples are analyzed in a different laboratory.

Secondly, most studies mentioned above have compared metabolite profiles obtained from patients with a distinct diagnosis of either IBD or IBS to healthy subjects. To ensure clinical utility, it might be necessary to include additional control groups including patients with other gastrointestinal or inflammatory disorders like patients with infectious GI disease or neoplastic disease.

Thirdly, the influence of potential confounders on the performance of the models needs to be established. Confounding factors might be related to the subject (gender, age, disease location, comorbidities) or might be of environmental origin (diet, diurnal variation, medication, smoking).

CONCLUSION
Metabolomics may advance our understanding, diagnosis and treatment of inflammatory bowel disease and irritable bowel syndrome. Using this approach, disease-related mechanisms may be uncovered and verified, and candidate diagnostic biomarkers in biological samples are characterized. Before usage as clinical diagnostics, metabolites must be verified and validated in large clinical trials. To translate metabolomics data into a more profound biological understanding of the disease, more knowledge on the relevance of a decrease or increase in certain metabolites is warranted. Metabolomic profiling mainly detects associations between profiles and specific phenotypes which may not always be meaningful. In addition, it often remains unknown whether changes in metabolites are the cause or a consequence of the disease. Integration of other "Omics" with metabolomic may enable a further understanding of gastrointestinal related pathophysiological processes.

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