PROBLEMS AND PROSPECTS OF METABOLOMIC STUDIES IN THE ALTERATION OF THE GUT MICROBIOME

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The review summarizes existing knowledge on the relationship between certain diseases and alteration (degeneration) of the intestinal microbiome. We consider major microbial metabolites firmly recognized as signaling molecules acting in communication between the microbiome and the host organism. These include short-chain fatty acids, bile acids, amines, amino acids, and their metabolites. Special attention is paid to metabolomic studies of the microbiome in chronic kidney diseases, in particular, immunoglobulin A nephropathy. The arguments supporting a concept of the microbiome of blood, previously considered an exclusively sterile environment in healthy humans, are considered. Metagenomic methods plays a key role in characterization of both the composition and potential physiological effects of microbial communities. The advantages and limitations of metabolomic analysis of blood serum/plasma and feces have been analyzed. Since the potential of clinical studies of the mutual impact of the microbiome-metabolome is limited by genetic and external factors, preclinical studies still employ both germ-free models and models based on the effects of antibiotics. The review considers the problems and prospects of metabolomics in studying the nature and mechanisms of the mutual impact of the microbiome and metabolome.

Keywords: metabolomics; microbiome; dysbiosis; biomarkers

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INTRODUCTION

Convincing evidence now exists that a significant proportion of severe diseases, including diseases leading to fatal outcomes, are associated with alteration (degeneration) of the gut microbiome. Their diagnostics, prognosis, and treatment would be much more successful provided that they are based on reliable metabolomic analysis data.

To date, signaling molecules that trigger pathological processes developing in the microbiome-metabolome system of connections have not been identified yet and this is a serious obstacle to the development of innovative drugs whose therapeutic effects are based on the correction and subsequent maintenance of a healthy microbiome. Identification of biomarkers of intestinal microbiome alterations for diagnosing the risks of diseases affecting the gut-brain, gut-kidney, and gut-heart axes thus represents an important task.

Various stress factors, including environmental ones, contribute to alterations in the intestinal microbiome, thus becoming a global problem. There is increasing evidence that environmentally conditioned diseases, in particular, decreased male fertility, are realized through intestinal microbiota dysbiosis [1]. It has been repeatedly confirmed that changes in the microbiome can affect cancer biology through various mechanisms, including the induction of chronic inflammation and modulation of the immune system [2].

The microbiome consists of trillions of bacteria, fungi, and viruses and contains an order of magnitude more cells than the human body [3]. Potentially affecting human health, microorganisms directly interact with host cells and affect metabolic processes [4]. Despite attempts to distinguish between the terms “microbiome” and “microbiota” [5], which define “microbiome” as a description of the genetic material encoding various populations of microorganisms, and “microbiota” as a community of microorganisms, these concepts are still more often used as synonyms. Jiang et al. [6] proposed to consider the microbiome as the combined potential of endogenous microorganisms. The general structure of the intestinal microbial community is the enterotype, which, even in the absence of a particular pathology, varies widely among different people depending on genetics, ethnicity and geographic location, body mass index, dietary patterns, and other environmental factors and lifestyle. The influence of the intestinal microbiome on drug metabolism, which largely determines interindividual variability in responses to therapy, requires special attention and thus remains beyond the scope of this review.

For diagnostic and therapeutic purposes, it is important to study the state of the microbiome that corresponds to the physiological norm and deviations from it, and ideally to differentiate microbiome alterations corresponding to a certain

disease. It is especially important to establish early predictors of diseases associated with the state of the microbiome, which can be registered before the appearance of specific clinical signs. The search for these early predictors or biomarkers can be carried out both by microbiological methods and on the basis of various omics technologies, with most studies in this area being carried out using the metabolomics platform. Metabolic profiles of the microbiome and specific microbial biomarkers are assessed for their ability to provide clinically significant information about disease states. Some researchers associate the prospects for diagnosing certain diseases with the study of specific signatures of the intestinal microbiome [7].

In this review, the microbiome is considered primarily in the context of its functions, or more precisely, the biomolecular mechanisms of its impact on physiological processes. The purpose of the review is to consider the complexity of the mutual influence of the microbiome-metabolome, focusing on identification of interactions between microbes and metabolites, as well as highlighting the problems and prospects of metabolomics in the diagnostics and therapy of diseases associated with the microbiome state.

1. THE STUDY OF THE RELATIONSHIP OF INTESTINAL MICROBIOME ALTERATION WITH DISEASE DEVELOPMENT

Over the last decade, “omics” technologies made a significant contribution to our understanding of the pathogenesis of many diseases. Genomics, epigenomics, transcriptomics, and proteomics have helped to better understand the origin and heterogeneity of various diseases. Risk factors for most autoimmune diseases are associated with the microbiome state. The most obvious connection seems to be between the alteration of the intestinal microbiome and inflammatory bowel diseases. These diseases are becoming global, with the number of registered cases increasing annually [8]. In 2021, a comprehensive systematic review [9] was published, where authors analyzed results of 143 studies aimed at elucidating microbial markers of inflammatory bowel diseases. General conclusions included a decrease in species diversity and the degeneration of the intestinal microbiome towards an increase in the number of pathogens in patients with inflammatory bowel diseases. It has been noted that only in the acute phase of intestinal inflammation the beneficial microflora significantly suppressed, while the proportion of pathogenic microflora increased markedly. If this is true, then this feature can be used to establish differences between the stages of remission and exacerbation of the disease. However, significant diversity in the methodology and design of experiments seriously complicated comparison of the results. Ning et al. [10] identified 36 candidate biomarkers of inflammatory bowel diseases. To establish the relationships between the gut microbiota composition and the metabolome, so-called multiomics biological correlation maps were constructed. The identified multiomics biomarkers can be considered as a valuable resource for further research, but they are too complex to be implemented into clinical practice in the recent future.

Certain attempts are undertaken to identify biomarkers of the gut microbiome in the context of not only inflammatory diseases, but also oncological, neurodegenerative, and other diseases. The involvement of microbiota in the development of colorectal cancer has been already recognized [11] and this stimulated numerous studies aimed at finding pathogenic microorganisms associated with this disease. Studying a relationship between the gut microbiome and chronic kidney disease, Pan et al. [26] found that the *Proteobacteria* phylum was an important factor in the pathogenesis of this pathology. High levels of bacterial liposaccharides in the blood, together with uremic toxins of intestinal origin and impaired immune regulation, played a decisive role in the development of both chronic kidney disease and associated complications. The gut-kidney relationship will be discussed in more detail below in Section 6.

Currently, the gut-brain axis is in the focus of researchers. The paper [12] presents results of a meta-analysis that identified 17 studies on the association of Alzheimer's disease (AD) with the microbiome state. The study included 438 AD patients and 672 healthy volunteers. Although the results were significantly various, the overall conclusion was that the dysbiosis observed in AD was directed towards a pro-inflammatory profile. Results of animal studies performed on mice indicate that the pathogenesis of Parkinson's disease (PD) is modulated, and possibly initiated, in the gastrointestinal tract [13, 14]. There is evidence that the gut microbiome composition affects neurological outcomes through several mechanisms, including production of metabolites. Cabral et al. [15] identified bacterial species that were increased or decreased in the colon of PD patients compared to a group of conditionally healthy controls. In the study [16], four types of bacteria were elucidated in the context of identification of PD patients. However, a later research, performed using metagenomic analysis [17], questioned the unambiguity of these conclusions. As experimental data accumulated, the task of establishing accurate diagnostic microbial biomarkers of PD began to look increasingly difficult. In a systematic review published in 2019 [18], the authors examined results of16 studies aimed at finding microbial PD markers. Although the association of microbiome alterations with PD was confirmed by almost all studies, the results of establishing specific microbial markers were most often not reproduced. The main reason, apparently, is the lack of fully standardizing the conditions

of clinical studies [18]. We believe that this may encourage a return to preclinical studies, in which it is much easier to eliminate confounding factors.

2. MODELLING THE MICROBIOME-METABOLOME INTERACTION IN PRECLINICAL STUDIES

There is a clear need in effective modeling to establish the microbiome-metabolome relationship. The development of small animal microbiota models helps to test microbiota subsets as causal and concomitant factors in diseases and to offer a system for finding therapeutic agents. Two main methods have been developed to study the influence of microbiota on physiology in health and disease: germ-free models and antibiotic treatment regimens. Both approaches have their strengths and weaknesses [19] and do not always show the same results. For example, using these two models, the authors [20] revealed different changes in neuropeptide signaling in mice.

The changes observed in germ-free mice were generally opposite to those found in mice treated with antibiotics. The antibiotic-based model is less expensive, does not require special conditions and equipment, and is applicable to any genotype, but it does not rule out the influence of survived bacteria, progressive fungal growth, and the effect of antibiotics on eukaryotic cells. The study [21] demonstrated significant changes in the relative abundance of various bacterial taxa at both the phylum and genus levels, thus emphasizing the profound effect of antibiotic therapy on intestinal microbial communities. A much more complex and expensive germ-free mouse model uses animals in which all organs and tissues are sterile and can be colonized by the microorganisms of interest. Using a germ-free mouse model, a link between obesity and the microbiome has been recognized [22]. It was demonstrated that microbiota transplantation could promote fat mass gain: when microbiota from low-weight mice was transplanted into sterile mice, the formerly sterile mice gained 27% of their body weight, while those that received microbiota from overweight mice gained 47% of their body weight over the same period. Using a germ-free mouse model, probiotics are selected and tested [23]. The use of the germ-free model helps to confirm the microbial origin of metabolites: their concentrations in germ-free animals are reduced compared to normal animals [24].

3. BLOOD MICROBIOME

Associations with the development of certain pathologies are traditionally studied in relation to the intestinal microbiome. Until recently the concept of microbiome in relation to blood, traditionally considered a sterile biological environment, was perceived as absurd. However, using modern molecular genetic methods, bacterial DNA was detected in the blood of basically healthy people with a negative blood culture [25]. The work [26] provides convincing evidence that presence of bacterial DNA in the blood is determined by the blood microbiota, which can persist for many years in the blood of healthy people. If we accept the fact of the existence of the blood microbiome as such, then we can consider blood dysbiosis and assume its significant impact on health parameters. Some recent findings, such as a positive correlation between the number of microbial DNA copies in the blood and important health factors such as glucose, insulin, free fatty acids and white blood cell count, have led to the hypothesis of blood dysbiosis as a potential prognostic biomarker for a number of human pathologies [27]. The bloodstream allows microbes to reach various parts of the body, but the low prevalence of microbes in the blood of healthy people highlights the transient and infrequent nature of this phenomenon [28]. The existence of microbial populations in “classically sterile” environments, including blood, is a rather new concept. Although the presence of bacteria-specific DNA in the blood was discovered relatively long ago, but its true origin still remained a matter of debate. The first review summarizing previous studies and substantiating the clinical significance of changes in the composition of the blood microbiome has been published only in 2023 [29]. It analyzes the relationship between blood-borne bacteria and some human diseases. Although potential biomarkers of blood microbiota for diagnostics of various diseases will probably be found in the future, certain evidence exists for the blood microbiome association with various pathological conditions, which include cardiovascular [30] or, to a greater extent, renal [31] diseases investigated in pilot studies. In the work [32], 20 blood samples from people with chronic kidney disease without signs of diabetes mellitus and 20 blood samples from healthy volunteers were analyzed. A significant decrease in bacterial diversity and an increase in the number of *Proteobacteria* (families *Enterobacteridaceae* and *Pseudomonadaceae*) in the blood in chronic kidney disease were found. Other studies confirmed blood dysbiosis, manifested in less bacterial heterogeneity and significant taxonomic variations, in chronic kidney disease [33]. Currently, the blood microbiome still receives much less attention than the intestinal microbiome.

4. MECHANISMS OF MUTUAL INFLUENCE OF INTESTINAL DYSBIOSIS AND DISEASE DEVELOPMENT

The mechanisms by which the microbiome mediates its impact on disease progression or protection still remain poorly understood. According to the “key pathogen” hypothesis proposed in [34],

certain low-abundance microbial pathogens can cause inflammatory diseases by transforming benign microbiota into dysbiotic ones. In this context, dysbiosis or dysbacteriosis refers to the composition of the intestinal microbiome that poses health risks, provoking the development of diseases that can accordingly be classified as "dysbiotic". Identification of key pathogens is important for clinical practice because it may facilitate the development of new treatments for complex dysbiotic diseases by focusing therapeutic strategies on only a limited number of bacterial targets that induce and stabilize dysbiosis. Moreover, new targeted diagnostic tools may be developed if a disease is shown to be caused by a key pathogen or a limited number of microorganisms acting in a similar manner.

A similar hypothesis, known as the "alpha-bug hypothesis", has been proposed in [35]. The hypothesis was based on the results punished in [36], where the ability of the enterotoxigenic human colon bacterium *Bacteroides fragilis* to cause colon tumors in mice with multiple intestinal neoplasia was demonstrated. Although the alpha-bug hypothesis was proposed in relation to colon carcinogenesis, it could be applicable not only to other types of cancer, but also to various immune chronic pathologies. New experimental preclinical models and, in the future, translational studies applied to humans will help to establish a potential critical set of key pathogens (alpha-bugs) and their bacterial collaborators acting as triggers in the community in the process leading to disease development. So far, the alpha-bug hypothesis has been most developed in relation to colorectal cancer, which is the third most commonly diagnosed form of cancer worldwide [37].

Proposed mechanisms linking microbiome alterations and carcinogenesis include both the alpha-bug hypothesis, which points to the potential of enterotoxigenic Bacteroides fragilis to promote cancer development in the colonic epithelium, and the "driver-passenger model" [38], which classifies microbes into indigenous ones that cause colorectal cancer, and opportunistic ones that proliferate and mediate colorectal oncogenesis. In a 2021 review, a group of French researchers focused on a specific potential microbial biomarker for colorectal cancer, a pathogenic strain of *Escherichia coli* that produces genotoxic colibactin [39].

There are a growing number of supporters of the opposite viewpoint; they propose to identify not specific pathogens, but the pathogenic potential of the human microbiota [40]. Even more radical is the proposal to consider a microbiome signature [41]. This hypothesis is based on the obvious fact that the microbiome is a dynamic symbiotic system [42]. Based on the results of studies aimed at establishing the relationship between the composition of the microbiome and various pathologies [38–42] some general conclusions can be drawn:

– although the microbiome consists of bacteria, fungi, and viruses, most studies of the human intestinal microbiome concerned only bacteria in the context of their relationship with the disease development;

– changes in the composition and abundance of intestinal microbiota apparently correlate with the onset of the disease and/or its pathogenesis and this gives hope that it will be possible to identify clinical microbial biomarkers (most likely indices of several types of intestinal microbes) to predict the risk of disease and choose a treatment regimen;

– some of the results of such studies could not be reproduced largely due to methodological and design differences; this is the main limiting factor on the way of obtaining clinically significant microbial biomarkers.

Successful identification of microbial biomarkers will require additional multicenter cohort studies involving patients who have not previously received treatment and who have achieved remission during treatment. To date, no recommendations have been proposed to take into account the impact of age, diet, lifestyle, ecology, antibiotic treatment regimen, and other factors on the microbiome. At present, the combination of these factors represents a significant obstacle to the development of universal microbial biomarkers of diseases.

5. BIOMARKERS OF THE GUT MICROBIOME STATE

None of the above hypotheses contradicts the fact that people with similar species or profiles of microorganisms in their microbiomes can have completely different reactions to them. If we accept this paradigm, then, probably, diagnostics should be oriented primarily towards the search for signaling biomolecules that reflect the body's reactions, i.e., to study not the microbiome, but the metabolome. Since microbiomes are unique, there are many versions of a healthy microbiome. The concept of a healthy version of the metabolome seems to be not only better studied, but also more unambiguous. The established connection between the gut microbiome and the blood metabolome in 2009 was called amazing [43]. After 15 years, no one doubts that a significant part of the metabolites in the blood is of microbial origin. In order to establish which metabolites are associated with a particular disease, numerous studies have been undertaken, and their number is increasing every year. Chromatographic mass spectrometric technologies for identifying microbial metabolites in the human body have been improved [44]. However, conclusions about associations between the microbiome and diseases based on blood or fecal metabolome data require should be made with caution due to significant inconsistency of published results of epidemiological studies. The problem can be solved through complex metagenomic and metabolomic studies. Currently, the vast majority of metabolomic studies are conducted

using chromatographic-spectral methods [45]. The general scheme of an untargeted metabolomic study aimed at finding new candidate biomarkers using mass spectrometry is presented in Figure 1.

In practical terms, an important task is to establish the relationship between the intestinal microbiome and the circulating blood metabolome, which is to a certain degree genetically determined [46].

Among the microbial metabolites, which are involved in regulation of host physiology and characterized the microbiome quality, short-chain fatty acids (SCFA) [47, 48] and bile acids [49] have been studied to the greatest extent. SCFA containing from 1 to 6 carbon atoms in the chain, are formed as a result of intestinal anaerobic fermentation of polysaccharides and amino acids (Fig. 2).

Special attention is paid to deviations from normal amino acid metabolism during microbiome alterations [50]. Some researchers believe that certain pathologies correspond to the particular metabolism of certain amino acids, such as citrulline, tryptophan, leucine, valine, tyrosine, asparagine, and ornithine. Results of some studies have demonstrated an indicative potential in the differential diagnostics of some pathologies associated with microbiome alterations [51, 52]. High sensitivity and specificity in the diagnostics of pre-infarction conditions in patients with heart failure were demonstrated for eight amino acids (glutamic acid, taurine, aspartic acid, ornithine, ethanolamine, serine, sarcosine, and cysteine) [53]. Arginine and ornithine metabolism is associated with the preclinical stage of AD, while the glutamate degradation pathway was most clearly expressed in healthy volunteers [54]. In epidemiological studies, blood is widely used as a biological sample for metabolome profiling to facilitate the study of the association between gut microbiota-related metabolites and cardiometabolic diseases. For example, Nemet et al. [55] found that blood phenylacetylglutamine levels were positively correlated with incident cardiovascular disease and major adverse cardiovascular events. In [56], several gut microbiota-related blood metabolites including kynurenate, dimethylglycine, 2-hydroxyhippurate were found to be associated with the risk of type 2 diabetes mellitus. Circulating trimethylamine-N-oxide was associated with the risk of cardiovascular diseases.

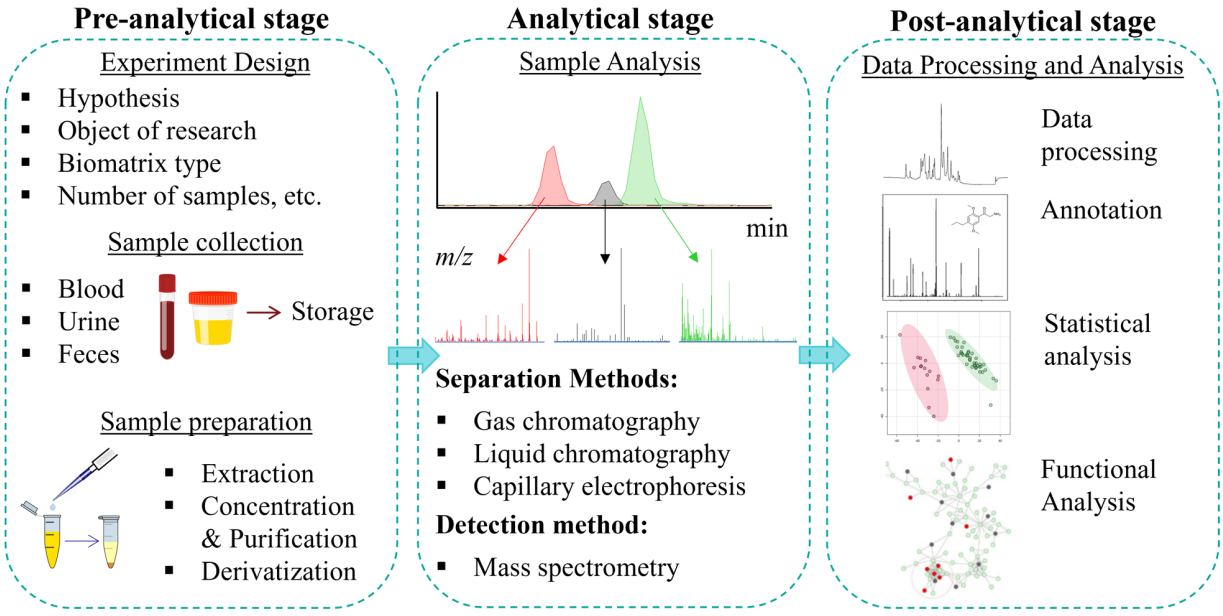

Figure 1. General scheme of the metabolomic study using mass spectrometry.

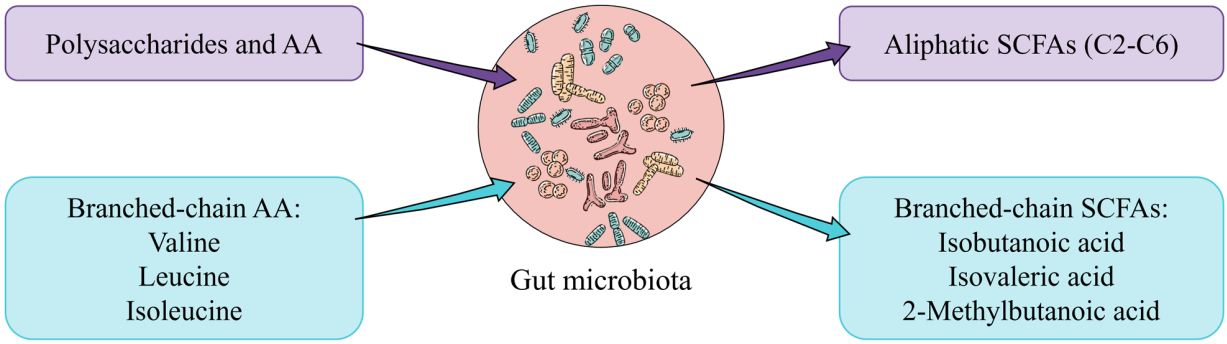

Figure 2. Biosynthesis of short-chain fatty acids (SCFAs) and amino acids (AAs) by the gut microbiota.

Dysbiotic gut microbiome is associated with increased production of uremic toxins [57], which include a wide range of substances that accumulate in the blood during kidney dysfunction. The focus of this review is on microbial-derived uremic toxins. The bioprecursors of microbial uremic toxins are aromatic amino acids, choline, carnitine, and betaine (Fig. 3).

In recent years, the greatest attention has been paid to tryptophan metabolism pathways [58, 59], which can be significantly altered in dysbiosis. For example, lactic acid of microbial origin activates tryptophan conversion to 5-hydroxytryptamine in anxiety syndrome [60]. The microbiological origin of uremic toxins associated with terminal renal failure was studied in [61]. Significant changes in the intestinal microbiota in patients with renal failure may be associated with accelerated biosynthesis of numerous toxic compounds, subsequent increase in the concentration of uremic toxins in the blood plasma and progression of renal failure [61]. A method for the combined determination of nine uremic toxins and choline in blood serum was proposed in [62]. The uremic toxins determined in blood serum are metabolites of aromatic amino acids and choline.

6. ADVANCES AND PITFALLS OF METABOLOMIC RESEARCH OF THE MICROBIOME USING IMMUNOGLOBULIN A NEPHROPATHY AS AN EXAMPLE

Immunoglobulin A nephropathy (IgAN) is the most common form of glomerulonephritis and the leading cause of fatal kidney disease worldwide. Non-targeted metabolomics was used in a preclinical study to identify IgAN-related serum metabolic profiles and then evaluate the effect of anti-inflammatory treatment [63]. High-performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS) was used as an analytical method. The identified serum metabolites were related to dysbiosis, mainly affecting such processes as lipid metabolism, signal transduction, carbohydrate metabolism, amino acid metabolism, etc. These changes were obviously realized through 784 compounds identified in the positive ion detection mode and 647 in the negative ion detection mode [63]. One of the new biomarkers of cardiovascular complications in renal failure is the monocyte to high-density lipoprotein ratio (MHR). A significant association between indoleacetic acid and MHR was shown in [64]. Prospective studies are needed to assess whether decreasing indoleacetic acid concentrations can reduce MHR levels, prevent cardiovascular events and improve clinical outcomes in IgAN patients. Higher MHR is mainly associated with multivessel coronary artery disease [65]. Changes in the blood amino acid profile at the early stage of IgAN compared to healthy volunteers were investigated in [66] within the framework of untargeted metabolomics. A number of dysregulations of amino acid metabolism were revealed in IgAN patients compared to healthy individuals. These included the increase in tryptophan, kynurenine, and ornithine associated with IgAN. The results of the metabolomic study also showed impairments of tryptophan metabolism in IgAN patients, with a significant increase in the levels of 5-hydroxyindoleacetate and kynurenine. Thus, dysregulation of amino acid metabolism occurred in IgAN patients and influenced the disease process. Among the amino acids

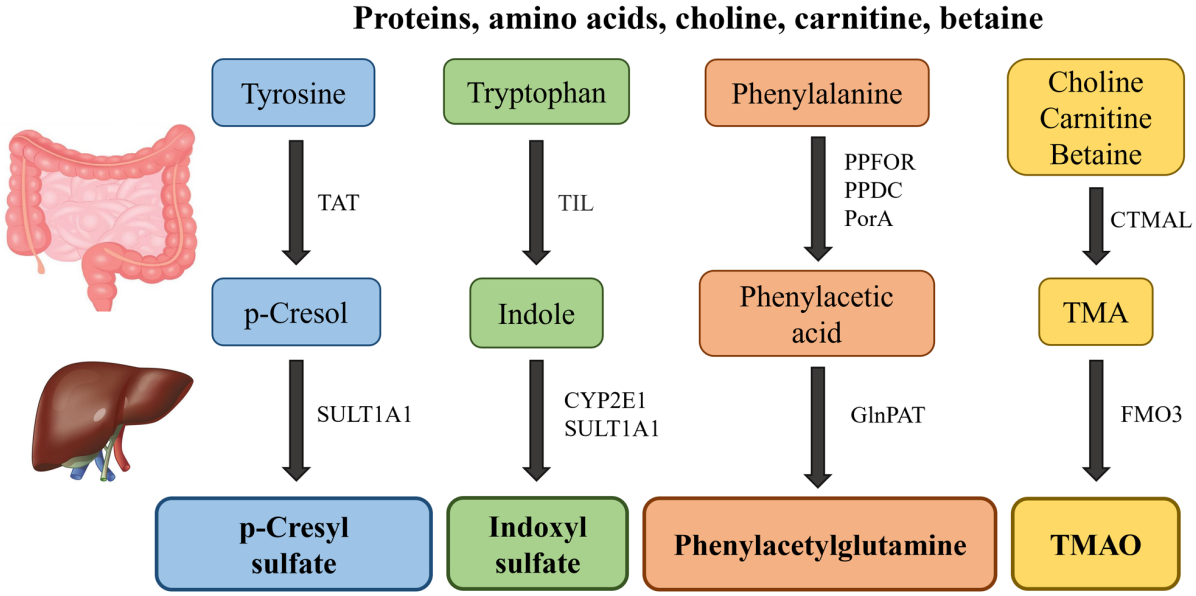

Figure 3. Formation of uremic toxins. TAT – tyrosine aminotransferase; TIL – tryptophan indole lyase; PPFOR – phenylpyruvate ferredoxin oxidoreductase; PPDC – phenylpyruvate decarboxylase; PorA – porphyrinase A; CYP2E1 – cytochrome P450 2E1; SULT1A1 – sulfotransferase 1A1; GlnPAT – glutamine phenylacetyl transferase; CTMAL – carnitine TMA-lyase; FMO3 – flavin-containing monooxygenase 3.

associated with the progressive stage of IgAN, leucine, valine, and glutamine were identified in urine, and threonine in blood [67]. The study [68] revealed significant differences in the content of phenyl sulfate, indoleacetic acid, proline, tyrosine and tryptophan in serum samples of IgAN patients and healthy volunteers. Urinary glycine as a predictor of IgAN was identified in the work [69].

In 2024, the results of a large-scale study, aimed at searching for biomarkers in blood plasma for the early diagnosis of IgAN were published [70]. The study was performed on a group of patients with an established diagnosis of IgAN, who did not have concomitant diseases and did not receive treatment before blood sampling. This study resulted in isolation of six metabolites, which had diagnostic value: indole 3-lactic acid, indole 3-carboxylic acid, kynurenic acid, tryptophan, kynurenine, indole 3-carboxaldehyde. It should be noted that the listed biomarkers are not specific for the IgAN diagnostics and, as follows from the previous sections of this review, are also characteristic of other diseases. Does this mean that intestinal dysbiosis poses a threat with consequences, which depend on the risks caused by genetic and external factors, or does it play the role of a trigger in the development of a particular pathology? This question may not find an unambiguous answer even in the long term perspective.

Thus, convincing evidence now exists that changes in the gut microbiota contribute to the development of a number of diseases, while the links between the gut microbiota and the composition of circulating blood metabolites still remain largely uncertain and are the subject of further research.

7. METAGENOMIC AND METABOLOMIC STUDIES OF FACES AND BLOOD

Historically, classical microbiological methods based on the isolation and cultivation of individual species of bacteria associated with the gut have been used to study the microbial colonization of higher organisms. Relatively recently, metagenomic methods have been used to characterize both the composition and potential physiological effects of entire microbial communities without the need to cultivate individual community members. Metagenomic DNA sequencing technology is considered as the “gold standard” for analyzing the microbiota composition. The 16S rRNA gene, a unique and highly conserved region of the nucleotide chain of all bacteria, by which they are usually identified, is isolated from the samples. In the 2000s, genes for specific metabolic pathways such as amino acid and glycan metabolism were found to be overrepresented in the distal gut microbiome [71], thus supporting the notion that human metabolism is not only directly linked to but is also integrated with the metabolism of its coexisting microbiota. Since genetic testing becomes more and more popular among consumers and the link between gut microbial composition and disease becomes clearer, an industry has grown to offer consumers personalized healthcare advice based on metagenomic analysis of their microbiomes.

Feces and blood are widely used biological matrices for investigation of the links between gut microbiome alterations and disease. Numerous studies have shown that gut microbiota significantly explains the variance in blood metabolome [72, 73], while other studies suggest that gut microbiota composition is significantly correlated with fecal metabolome [74, 75]. In addition, as already noted, diet and genetics are also important factors forming the blood and fecal metabolome.

Studying associations between gut microbiota and fecal and blood metabolomes, it is important to collect fecal and serum (plasma) samples at the same time points and to follow carefully all preanalytical requirements. These requirements were emphasized in a study [76], in which several fecal and blood metabolites that were well predicted by gut microbiota were identified in 1007 middle-aged and older Chinese adults. Phenotypic and genetic correlations between paired fecal and blood samples were confidently established for 30 metabolites, which were ranked by phenotypic correlations. Phenotypic correlations between metabolites in paired fecal and blood samples were assessed using partial Spearman correlation analysis adjusted for age, sex, and body mass index. Dong et al. [77] investigated the associations of blood metabolome disturbances with changes in the gut microbiota composition in gestational diabetes mellitus. A multi-omics study of blood metabolome-gut microbiome associations was conducted based on metagenomic sequencing of fecal microbiota and proton NMR profiling of blood plasma metabolome. The experiment involved 40 pregnant women: 20 women with diagnosed gestational diabetes and 20 women with a reliable absence of this disease. The results have shown that the fecal microbiota and plasma metabolome of pregnant women can be divided according to the vector of hyperglycemia development. Evaluation of the correlation between the fecal microbiota and blood plasma metabolome in gestational diabetes revealed five plasma metabolites (glycerol, lactic acid, proline, galactitol, and methylmalonic acid) and 98 bacteria from the fecal microbiota, which provided the microbiome/metabolome link. Further Spearman rank correlation analysis showed that four of the five identified plasma metabolites (except galactitol) correlated with hyperglycemia. Coexistence network analysis showed that 15 of the 98 fecal microbiota members were positively correlated with each other, forming a community mainly consisting of representatives of the *Firmicutes* phylum. The fact that galactitol failed to pass the retest for a connection with glycemia was not interpreted

by the authors of the study. Despite the diversity of microbial metabolites relevant for disease diagnostics, they can be systematized within several main groups (Table 1).

A great advantage of feces is the simplicity and non-invasiveness of sampling, which can be carried out independently outside a medical institution. Among the most significant fecal metabolomics studies, it is worth mentioning the work [78], in which fecal metabolomics is presented as an additional approach to studying the metabolism of intestinal microbial communities and their interaction with genetics and host diet. During the metabolic profiling of fecal samples, 1116 metabolites were identified, including 647 metabolites, which were unique to feces. Thus, the results of fecal metabolomics significantly complement the results of blood metabolomics.

The task of studying the fecal metabolome appears to be much more complex not only due to the significant variability of the composition, but also due to problems with providing stability during storage and standardization of fecal samples. A special device containing a stabilizing solution optimized for preserving the fecal metabolome was developed and patented [79]. According to HPLC-MS/MS analysis, the metabolic profiles of infant fecal samples, collected using this device, were comparable to those of snap-frozen samples [80]. More recently, it was found that stabilization of feces with both a patented solution and 95% ethanol provided the suitability of the biospecimen for metabolomic and metagenomic analysis [81].

The metabolic profiles of six fecal samples from different donors after their storage under different conditions were studied using GC-MS/MS [82]. The study has shown importance of following a standard protocol for collecting and storing fecal samples prior to analysis and it is especially important to standardize samples in order to obtain comparable results in different laboratories. Thus, the problem of discrepancies in metabolomic analysis results due to use of different research protocols appears to be much more acute for feces than for serum/plasma.

The growing number of publications reporting the identification of new microbial metabolites in the human body prompts us to consider these metabolites as an open system with a significant degree of uncertainty rather than an understudied set of substances. The paper [83] presents the concept of the microbial exposome, i.e. the entire community of microbial metabolites in the host organism. Based on the literature data, one of the first versions of the Exposome-Explorer microbial metabolite database has been created. The main difficulty with forming the microbial exposome consists in differentiation of metabolites obtained exclusively or partially as a result of microbial metabolism from metabolites produced by the host or ingested with food.

The authors [83] propose three types of evidence for the microbial origin of metabolites:

(1) metabolites are produced *in vitro* by human fecal bacteria;

(2) concentrations of metabolites decrease in humans or experimental animals exposed to antibiotics;

(3) metabolites exhibit reduced concentrations in germ-free animals compared to conventional animals.

Table 1. Known microbial metabolites involved in mediating microbiome-metabolome communication

Group of metabolites	Some metabolites	Biological medium	Comments	References
Short chain fatty acids (SCFA)	Acetic, propionic, butyric, isobutyric, valerianic, isovaleric, hexanic acids	Blood serum/plasma, feces	SCFA production decreases in intestinal microbiome alterations	[47, 48, 54]
Amino acids	Tyrosine, tryptophan, asparagine, citrulline, threonine, leucine, isoleucine, valine, ornithine	Blood serum/plasma, urine	Information on increased/decreased concentrations with microbiome alterations is contradictory	[50, 52–54, 63, 67, 68, 70, 77]
Metabolites of aromatic amino acids	*p*-cresol, *p*-cresyl sulfate, *p*-cresyl glucuronide, indole derivatives: indoleacetic, indolelactic, indolepropionic, indoleacrylic acids	Blood serum/plasma, urine	They are considered as “uremic toxins” and their concentrations are significantly increased in serum/plasma of kidney disease patients	[26, 55–59, 62–64, 66, 68, 70]
Amines	Carnitine, betaines, trimethylamine, trimethylaminoxy methyl and dimethylamines	Blood serum/plasma	Substrates and metabolites of intestinal bacteria	[56, 57, 62, 63, 70]
Bile acids (BA)	Cholic, deoxycholic, taurocholic, glycocholic, and other acids	Feces, blood serum/plasma	The relationship between BA content in feces, the composition of the intestinal microbiome and liver function has been recognized	[49, 54, 56, 63, 70]

8. MODERN PLATFORMS FOR INTEGRATION OF THE METABOLOME-MICROBIOME SYSTEM

A microbial community maintains its dynamics through metabolite cross-talk. Methods using metagenomic sequencing data can predict the metabolic potential of a community, i.e. its ability to produce or utilize certain metabolites. These, in turn, can potentially serve as markers of biochemical pathways that are associated with different microbial communities. Gautam et al. [84] developed the Microbiome Metabolome Integration Platform (MMIP), an analytical and predictive web-based tool that can be used to compare the taxonomic content, diversity, and metabolic potential of two microbial communities; it is based on targeted amplicon sequencing data. Based on MMIP, the authors plan to create a user-friendly online web server for analysis of microbiomes linked to targeted amplicon sequencing data, prediction of metabolite signatures, and using learning-based linkage analysis, without the need for an initial metabolomic analysis.

In parallel, an opposite approach is being developed; it is aimed at identifying all components of the biological environment (e.g., blood serum) using high-resolution chromatography-mass spectrometry (MS) and subsequent processing of raw MS data using software that filters only microbial metabolites [85]. The microbeMASST search tool aims to solve the problem of annotating microbial metabolites in untargeted metabolomics. Using a database containing more than 60,000 microbial monocultures, users can extract known and unknown MS/MS spectra from high-resolution tandem mass spectrometry (MS/MS) data and assign them to the corresponding microbial producers. Coordinated chromatography-MS and microbiological studies seem to be the most productive in the search for microbiome-metabolome associations, which is facilitated by the rapid development of omics technologies. Although most omics studies describe changes in the immune system, more results are still needed in the context of the constantly developing multicenter models of autoimmune disease pathogenesis. To study the relationship between the microbiome and metabolome, Shtossel et al. [86] developed the LOCATE system, a machine learning tool for predicting metabolite concentrations based on microbiome composition. According to the authors, the accuracy of metabolome prediction using LOCATE is higher than that of all existing predictors. It is important to note that the proposed system metabolite concentrations but not sets of identification points or metabolic pathways. To test such predictions, it is necessary to have reference samples and validated methods for determining metabolites that have previously been detected and reliably identified as biomarkers of the corresponding microbes.

CONCLUSIONS

A number of factors influence the human metabolome: genetics, diet, environment, lifestyle, and microbiome. Among these factors, the microbiome has only recently been considered as one of the leading factors. Understanding the nature and mechanisms of the mutual influence of the microbiome and metabolome can be the key to the diagnostics and treatment of many diseases. In the systems “gut-kidney”, “gut-brain”, “gut-liver”, and others, metabolites are the main means of communication. Signaling molecules enter the bloodstream and affect the functions of organs and systems of the body. Metabolomics is designed to identify and measure concentrations of these signaling molecules that communicate between the microbiome and the host, as well as to elucidate the systemic impact on the body in health and in the development of pathology.

As the medical community indicates, the available information on the mechanisms by which the intestinal microbiota is involved in the pathogenesis of a number of diseases is still insufficient for their use in clinical practice [87, 88].

Comparing both microbiomes and metabolomes of patients with an established diagnosis and healthy volunteers, it is not yet possible to determine whether the recognized differences are the cause of the disease or its consequence. This circumstance seems to be the main limitation of the use of even reliably established differences in both microbiomes and metabolomes for early diagnostics of diseases. At the same time, the hope remains that specific changes in the microbiome and/or metabolome can be identified before the appearance of the first clinical signs of the disease or at least clearly expressed functional disorders of organs and systems.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ПРОБЛЕМЫ И ПЕРСПЕКТИВЫ МЕТАБОЛОМНЫХ ИССЛЕДОВАНИЙ ПРИ АЛЬТЕРАЦИИ КИШЕЧНОГО МИКРОБИОМА

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Суммированы аргументы, обосновывающие связь ряда заболеваний с альтерацией (перерождением) кишечного микробиома. Приведены основные установленные метаболиты микробного происхождения, которые являются средством коммуникации между микробиомом и организмом хозяина: короткоцепочечные жирные кислоты, желчные кислоты, амины, аминокислоты и их метаболиты. Особое внимание уделено метаболомным исследованиям микробиома при хронических болезнях почек, в частности, иммуноглобулин А-нефропатии. Обоснована правомерность концепции микробиома крови, ранее считавшейся исключительно стерильной средой у здорового человека. Отмечена ключевая роль метагеномных методов для характеристики как состава, так и потенциальных физиологических эффектов микробных сообществ. Проанализированы преимущества и ограничения метаболомного анализа сыворотки/плазмы крови и фекалий. Ввиду того, что потенциал клинических исследований взаимного влияния микробиом–метаболом ограничен генетическими и внешними факторами, сохраняют актуальность доклинические исследования с использованием как безмикробных моделей, так и моделей, основанных на воздействии антибиотиков. В обзоре рассмотрены проблемы и перспективы метаболомики при исследовании характера и механизмов взаимного влияния микробиома и метаболома.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: метаболомика; микробиом; дисбиоз; биомаркеры

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