



Microbiome and metabolome analysis to clarify the interaction between the urine microbiota and serum metabolites in Chinese patients with immunoglobulin A nephropathy

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Background: The bacterial and metabolic networks in immunoglobulin A nephropathy (IgAN), the most common type of primary chronic glomerulonephritis worldwide, have not been extensively studied. To help develop better methods for the diagnosis, treatment, and prognosis of IgAN, we characterized the alterations of the urinary microbiome and serum metabolome in patients with IgAN.

Methods: We analyzed serum and urine samples from Chinese patients with IgAN and healthy controls (HCs) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and 16S ribosomal RNA gene sequencing.

Results: Patients with IgAN had a higher relative abundance of *Actinomyces* and a lower relative abundance of *Lactobacillus*. The elements of metabolism have been affected, including free amino acids, polyunsaturated fatty acids, and oligopeptides. We also identified the 9 metabolites that might be the core metabolites, including guanidinoacetic acid, apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)], and diethanolamine, which linked the metabolic networks between the urinary tract (UT) and blood. Other core metabolites, such as homocitrulline, apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)], butyrylcarnitine, formiminoglutamic acid (FIGLU), diethanolamine, and prolylhydroxyproline, were positively correlated with urinary mini-total protein (MTP). Conversely, *Lactobacillus* was negatively correlated with MTP.

Conclusions: We verified the connection between the disruption of the microbiota and serum metabolites, along with the clinical parameters, in patients with IgAN, which may help provide a tool for IgAN interventions.

Keywords: IgA nephropathy (IgAN); microbiota; metabolites; *Lactobacillus*

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Introduction

Immunoglobulin A nephropathy (IgAN) is recognized as the most common form of primary chronic glomerulonephritis worldwide. IgAN is responsible for approximately 45.26% of total primary glomerular disease cases in China. About 10–20 years after diagnosis, one-third of patients with IgAN progress to end-stage renal disease (ESRD). These patients require renal replacement therapies (including hemodialysis or peritoneal dialysis or transplantation) for survival (1-3). Currently, an accurate diagnosis of IgAN depends almost completely on percutaneous renal biopsies (4). Meanwhile, IgAN's etiology and pathogenesis remain unknown but it is generally accepted that the deposition of galactose-deficient IgA1 (Gd-IgA1) (5). A previous study has found that genes, environment, living habits and patterns play an important role in the pathogenesis of IgAN (6) and that systematic changes in cellular metabolism are caused by these intrinsic and extrinsic factors. In recent studies, some metabolites, such as urinary glycine, have been shown to be protective biomarkers for IgAN (7,8). Moreover, the alteration of fatty acids and amino acids has been shown to activate the unique metabolic pathway of IgAN (9). These metabolites were found to be changed in connection with changes in the microbiome and discovered because of the recent rise of gut/kidney axis research. Altered fecal microbiota has also been identified as a possible tool to distinguish between patients with IgAN and healthy controls (HCs) (10). An increasing

amount of research points to the close relationship between microbiota and metabolites as being critical in the occurrence and development of diseases. IgAN therapy currently depends on treatments supported by only low-level evidence, mainly relating to immunosuppressants and the renin-angiotensin system (RAS). Emerging research, however, has reported that probiotic supplements such as *Lactobacillus* (11) and polyunsaturated fatty acids are integral to both the prevention and treatment of IgAN.

While previously considered sterile, the urinary tract (UT) now hosts a range of bacteria in healthy individuals (12). At present, there are many studies related to gut microbiota, but we currently know little about the role of UT microbiota and its mechanism. Several studies have found specific bacterial communities in the healthy UT based on advances in molecular biology techniques (13,14), and we can observe changes in the UT microbiome in some urinary system diseases, such as chronic pelvic pain syndrome, urologic cancers, neurogenic bladder dysfunction and urinary incontinence (12,13). The network of the urinary microbiome and the serum metabolome has not thus far been analyzed, and yet the close relationship between microbiota and metabolites may likely have considerable relevance for therapy. This study thus aimed to clarify the network of different serum metabolites and urinary bacteria and to provide some means for clinical diagnosis and treatment. We present the following article in accordance with the MDAR checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5334/rc>).

Highlight box

Key findings

- We verified the connection between the disruption of the microbiota and serum metabolites, along with the clinical parameters, in patients with IgAN.

What is known and what is new?

- A relational network between gut microbiota and serum metabolites in IgAN was found. Altered fecal microbiota has also been identified as a possible tool to distinguish between patients with IgAN and HCs.
- While previously considered sterile, the UT now hosts a range of bacteria in healthy individuals. We observed a shift in the abundance of bacteria and metabolites in patients with IgAN. Our study clarified the relationship between serum metabolites, urine microbiota, and disease in those with IgAN.

What is the implication, and what should change now?

- This will help to provide further research and develop new tools for preventing and delaying the occurrence of the disease.

Methods

Patient information and sample collection

A total of 14 patients diagnosed with IgAN according to renal biopsies and 15 healthy people were recruited at the First Affiliated Hospital of Jinan University. These participants were informed of the study and signed an informed consent form. The basic clinical information of the participants, including gender, age, body mass index (BMI), fasting blood glucose, hypertension, liver and renal functions, and pathological data of IgAN, were recorded using the Oxford classification system. Patients were excluded if they had malignant tumors, infectious diseases, diabetes mellitus, severe liver dysfunction, cardiac disease, other immune diseases, a history of excessive alcohol intake, or received any anti-inflammatory or probiotics treatment within the past 3 months (15). Participants in

the HCs group were also excluded if they had a history of autoimmune disease.

Serum and urine samples from all volunteers were collected on the morning before a recent renal biopsy. Before collection, the volunteers had to thoroughly wash their hands and clean their external genital area with water and soap. Women washed the area around the external urethral orifice and vaginal introitus, then dipped 4 cotton swabs in iodophor, and moved them back and forth to wipe the genitals. The men were asked to disinfect the area around the urethral orifice after the foreskin had been withdrawn and the glans of the penis exposed. Without interrupting the micturition, the volunteers urinated into the toilet and then into a sterile container. The self-collected midcourse clean urine specimens were quickly transferred to the biological sample laboratory. These samples were stored in sterile Eppendorf (EP) tubes at -80°C . Blood samples were taken in the morning after volunteers had fasted all night. Volunteers with hemolysis present in their serum samples were excluded from the study. The collected supernatants, which were centrifuged at 3,000 rpm at 4°C for 10 minutes, were then transferred to a -80°C refrigerated cabinet for long-term storage. This study was authorized and reviewed by the institutional review board of the First affiliated Hospital of Jinan University (No. KY-2020-034) and conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Metabolite extraction and liquid chromatography-tandem mass spectrometry analysis

A total of 400 μL of extract solution (acetonitrile: methanol =1:1; containing isotopic labeled internal standard mixture) was added to the 100 μL sample in an EP tube. After the samples were rotated for 30 s, we sonicated them in an ice-water bath for 10 min and cultured them at -40°C for 1 h to precipitate. The samples were then centrifuged at 12,000 rpm at 4°C for 15 min. We collected the resulting supernatant in a clean bottle for analysis. We then mixed the aliquots from all samples and analyzed the samples with liquid chromatography-tandem mass spectrometry (LC-MS/MS; UHPLC system with UPLC BEH amide column and Q Exactive HFX mass spectrometer, Thermo Fisher Scientia) and subjected them to multivariate analysis. The detailed experimental methods we used are outlined in previous papers published by our research group (9).

Metabolomics data preprocessing and annotation

The original data was converted to the mzXML format using ProteoWizard software. The peak extraction, calibration, and integration based on XCMS were performed using an internal program written in R. A further processing of the data matrix was carried out by removing more than 50% of the peaks of the missing values in the samples using half of the minimum of the simulation method as a simulation and filling in some gaps in the data. The new data matrices were standardized by internal standards, and 3 databases were applied to metabolite annotation: an in-house MS2 database (BiotreeDB), the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (www.genome.jp/kegg), and the Human Metabolome Database (HMDB) online database (www.hmdb.ca). The cutoff value for comments was set as 0.3. We used a multivariate statistical analysis that included a principal component analysis and an orthogonal projection to latent structures discriminant analysis (OPLS-DA) to compare HCs and IgAN metabolomic characteristics.

Urine DNA extraction and 16S sequencing

The Mobio Powersoil DNA Isolation Kit (Qiagen) was used to extract genomic DNA from urine samples according to the manufacturer's instructions. The V3 and V4 regions of the 16S ribosomal RNA (rRNA) genes were amplified with the following primers: F-primer: 5'-ACTCCTACGGGAGGCAGCA-3'; and R-primer: 5'-GGACTACHVGGGTWTCTAAT-3'. With the Illumina HiSeq, we analyzed the samples using a paired-end sequencing strategy after purifying the polymerase chain reaction products using ampoule XP magnetic beads (Beckman Coulter, UK).

Sequencing data analysis

Filtered paired-end reads were modified using Trimmomatic v. 0.33 software (Illumina, USA) (16). Using Cutadapt (version 1.9.1), the merging primer sequences were identified and removed. The reads were next combined using FLASH 1.2.11 software (17), and the chimeric sequences were removed using UCHIME 8.1 (18). The high-quality sequences that were obtained were then used for subsequent analysis. USEARCH software version 10.0 classified sequences with a similarity level of 97% as operational taxonomic units (OTUs) (19). We then annotated the

taxonomic information based on the Silva database (20), and different phylogenetic levels (phylum, class, order, family, genus, and species) were assigned to the OTUs. We used QIIME2 (<https://qiime2.org/>) to analyze alpha diversity and the Wilcoxon rank sum test to compare the amount of data and statistical difference diversity. In order to identify the bacterial taxa whose sequences were differentially abundant between the IgAN and control groups, we used linear discriminant analysis (LDA) and effect size (LEfSe). All sequencing data can be found in the National Center for Biotechnology Information (NCBI) database (accession No. SUB11247081).

Statistical analysis

We used the Student's *t*-test and the Wilcoxon rank sum test to conduct the differential analysis of the measured data. We assessed the correlations between UT bacteria and serum metabolites using Spearman correlation analysis and found a correlation between the microbiome and metabolome and clinical indicators. Statistical calculations were performed with the SPSSAU project, an online software application (<https://www.spssau.com>). Metabolites were identified that had a variable importance in the project (VIP) >1 and a P value <0.05 that indicated a significant difference. The plot was optimized using GraphPad Prism 9 (GraphPad Software) and the OmicStudio tools (<https://www.omicstudio.cn/tool>). We calculated the specificity and sensitivity of the core metabolites, microbiome, and clinical symptoms using the receiver operating characteristic (ROC) curve.

Results

Summary of clinical characteristics

A total of 29 participants, including 14 patients with IgAN (mean age 36.50±13.83 years) and 15 HCs (mean age 31.00±12.14 years), were enrolled in the study at the First Affiliated Hospital of Jinan University. We used independent sample *t*-tests to compare the two groups. There was a statistical difference in the serum albumin, 24-h proteinuria, full blood count, urea nitrogen, creatine, and uric acid (P<0.05) between the two groups. However, no significant differences were found in age, BMI, alanine aminotransferase (ALT), or aspartate transaminase (AST). Although the systolic blood pressure of those with IgAN

was in the normal range (90–130 mmHg) according to the guidelines of the American College of Cardiology (ACC) and the American Heart Association (AHA), it was significantly higher than that of the HCs group. Detailed descriptions of the IgAN patients and HCs can be found in [Tables S1,S2](#).

OTUs and diversity analyses in bacteria

The urinary bacteria of 14 patients with IgAN and 15 HCs were analyzed using 16S rRNA gene sequencing. In this study, we obtained an average of 74,399 valid tags (average length: 418 bp) and 2,157,579 total reads. We then identified 1,464 OTUs in the HC group and 1,466 OTUs in the IgAN group, including 1,463 shared OTUs, indicating a 97% similarity between the two groups ([Figure 1A](#)). The sequencing samples obtained were sufficient for further taxon identification on the basis of the rarefaction curve ([Figure 1B](#)) and species accumulation curve ([Figure 1C](#)). We found no significant difference in species diversity (Shannon and Simpson indices) or species richness [Chao1 and abundance-based coverage estimator (ACE)] in the Student's *t* tests.

We also identified 10 main bacterial phyla ([Figure 1D](#)), among which Firmicutes, Bacteroidetes, Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia constituted over 93% of the bacteria in all 16S rRNA sequence groups. Our data also showed that the relative abundance of Acidobacteria was higher in patients with IgAN (control: 9%; IgAN: 11%; P=0.04; [Figure 1E](#)), and that *Lactobacillus* (control: 16%; IgAN: 10%) levels decreased in the IgAN group at the genus level according to the Wilcoxon rank sum test (P=0.00776183; [Figure 1F](#)).

We used a Metastats analysis to predict the core microbiota at different levels of bacterial classification between the IgAN and control groups (P<0.05; LDA >4; [Figure 2A](#); [Table 1](#)) and cladograms to describe the evolutionary relationship between microorganisms based on LDA >4 ([Figure 2B](#)). The results showed that the abundance of the phylum Acidobacteria increased in patients with IgAN. By contrast, patients with IgAN exhibited a loss of *Lactobacilli* (order: Lactobacillales; family: *Lactobacillaceae*; genus: *Lactobacillus*) and *Bacilli* at the class level. In summary, the microbiome of patients with IgAN compared with HCs expressed a disorder of the microbial community which may lead to the disorder of immune mechanisms (21).

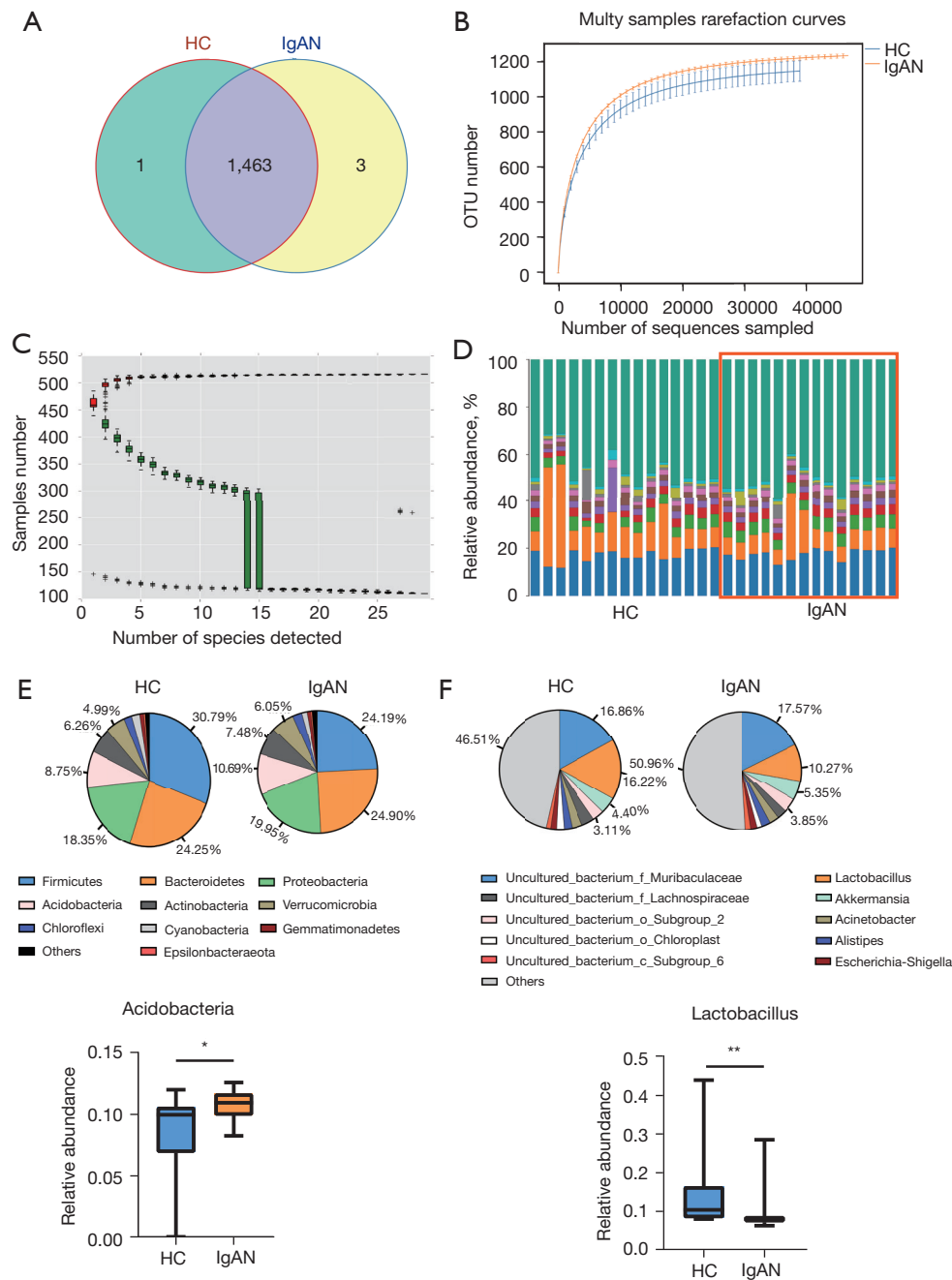


Figure 1 Microbiota diversity analysis. (A) 1,464 OTUs in the healthy control group and 1,466 OTUs in the IgAN group. (B) Rarefaction curves of microbiota from urine samples. (C) Species accumulation curve of microbiota from urine samples. (D) Relative abundance of dominant groups at the phylum level of each specimen. (E) Compared with the proportion of relative bacterial abundance from the IgAN and HC groups at the phylum (E) and genus level (F). *, $P < 0.05$; **, $P < 0.01$. HC, healthy control; IgAN, immunoglobulin A nephropathy; OTUs, operational taxonomic units.

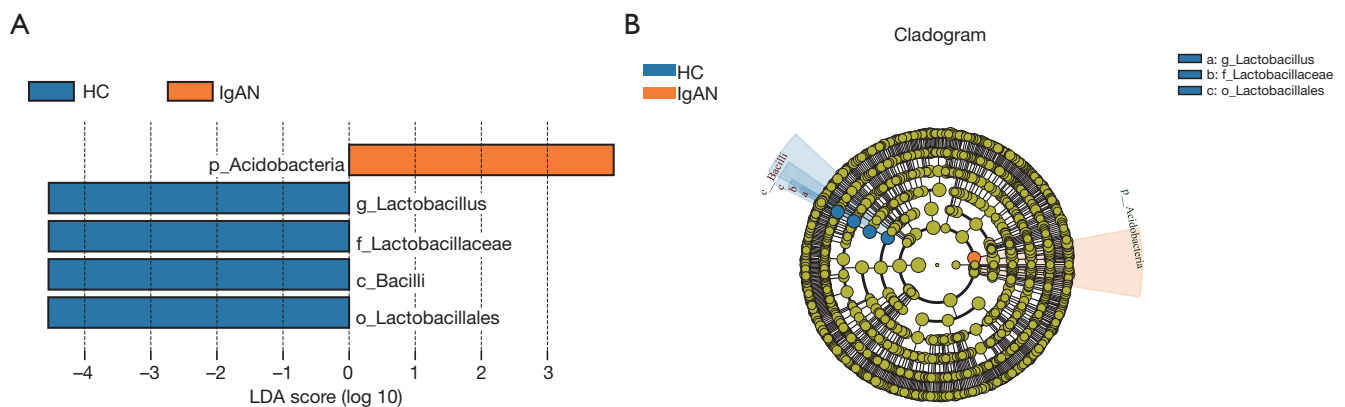


Figure 2 The LDA and LEfSe. (A) Influence of differences between the IgAN group and HC group. (B) Cladogram of the phylogenetic distribution of microbes. HC, healthy control; IgAN, immunoglobulin A nephropathy; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size.

Table 1 Differential microbes at different levels of biological classification in Metastats and LEfSe analyses

Bacteria	Metastats analysis		Wilcoxon test	LEfSe analysis	
	HC (mean)	IgAN (mean)		HC (LDA value)	IgAN (LDA value)
Phylum					
Acidobacteria	8.64%	10.70%	0.04024279	0.036180617	4.00270126
Class					
Bacilli	17.50%	12%	0.04468533	4.547905826	0.04468533
Order					
Lactobacillales	17.20%	11.60%	0.04024279	4.551476082	0.04024279
Family					
Lactobacillaceae	15.70%	10.10%	0.00776183	4.544844194	0.00776183
Genus					
Lactobacillus	15.70%	10.10%	0.00776183	4.543791671	0.00776183

LEfSe, linear discriminant analysis effect size; HC, healthy control; IgAN, immunoglobulin A nephropathy; LDA, linear discriminant analysis.

Changes of serum metabolites in patients with IgAN

We performed an LC-MS/MS analysis of the serum metabolite alterations in patients with IgAN (VIP >1; $P < 0.05$), and found 168 differential metabolites (Table S3), divided into several classes, such as peptides, lipids, amino acids, and carbohydrates. Amino acids, peptides, organic heterocyclic compounds, and lipids accounted for the majority of major alterations in the differential metabolites of IgAN. We also found that amino acids and peptides showed an upward trend (Figure 3A), while lipids and lipid-like molecules in the serum metabolites of patients with

IgAN showed a descending trend (Figure 3B). Cellobiose, homocitrulline, apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)], prolylhydroxyproline, 24-epibrassinolide, butyrylcarnitine, guanidinosuccinic acid, formiminoglutamic acid, and diethanolamine were the metabolites with a VIP >2 and log fold change >2, and thus were suspected to be the core metabolites in patients with IgAN (Figure 3C). In addition, we found that the histidine metabolism pathway was statistically different ($P < 0.05$; impact value >0.1) through an enrichment analysis in Metaboanalyst v. 5.0 (Figure 3D).

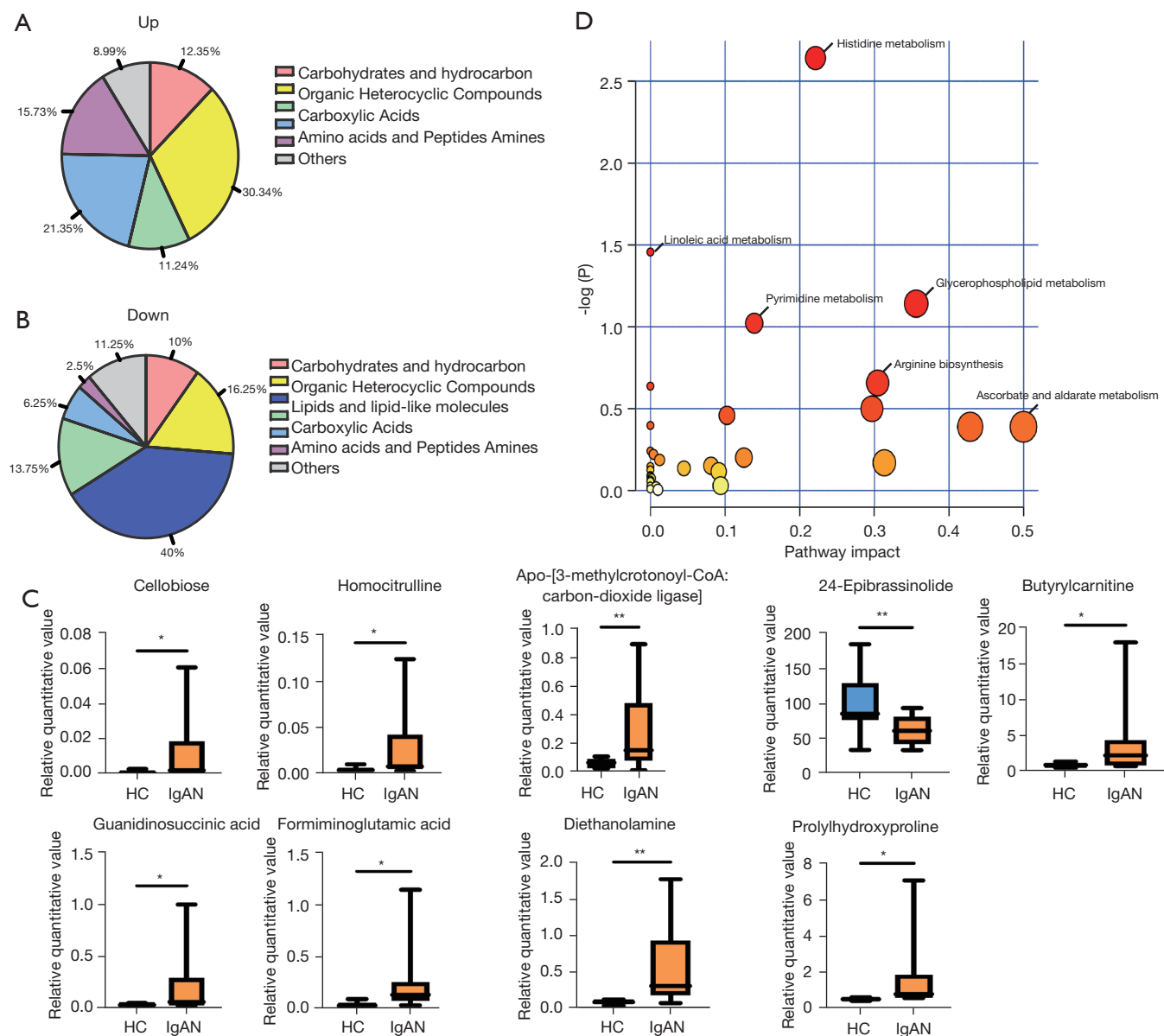


Figure 3 The analysis in changes of serum metabolite and pathway enrichment. (A) An increase in the serum metabolites of patients with IgAN. (B) A decrease in the serum metabolites of patients with IgAN. (C) The 9 core metabolites in serum samples were differentially expressed. (D) Metabolic pathway enrichment of serum samples. *, $P < 0.05$; **, $P < 0.01$. HC, healthy control; IgAN, immunoglobulin A nephropathy.

Correlation between altered microbiome and metabolism

We clarified the relationship between the microbiome and differential metabolites through a Spearman correlation analysis (Figure 4A) and found that 12 different metabolites were negatively correlated with *Lactobacillus* and 19 different metabolites were positively correlated with *Lactobacillus*

(Table S4). Interestingly, *Lactobacillus*, which was positively correlated with guanidinoacetic acid, has a negative correlation with apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)] and diethanolamine in core metabolites ($r > 0.5$; $P < 0.05$). The disturbance of *Lactobacillus* number may affect the changes of these metabolites.

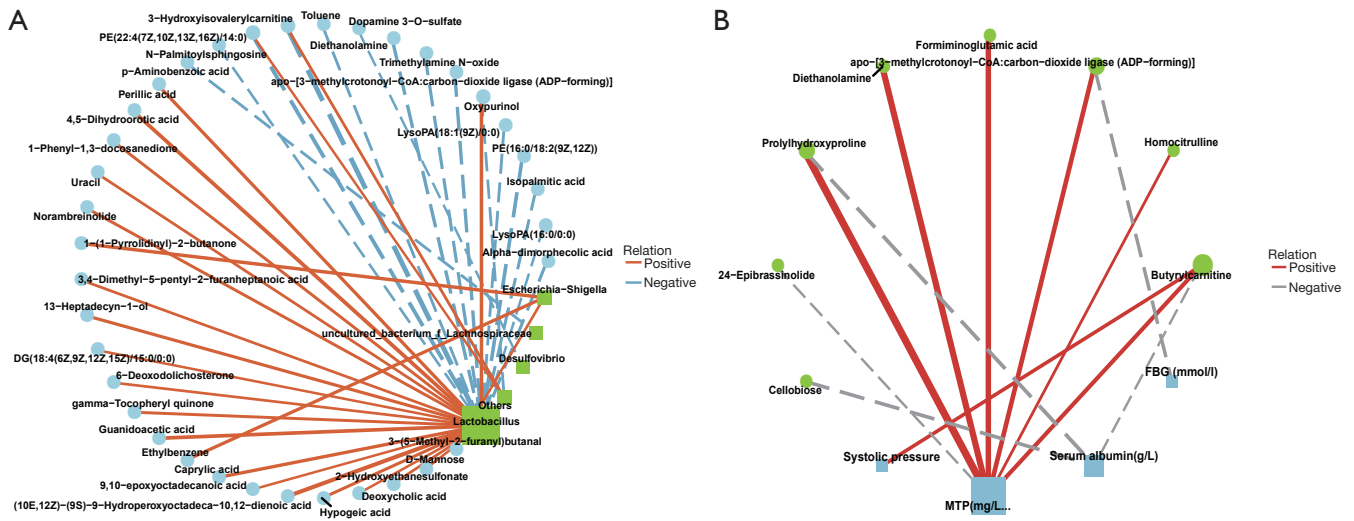


Figure 4 Correlation between the microbiome, metabolites, and clinical indicators in patients with immunoglobulin A nephropathy ($|r| > 0.5$). (A) The relationship between microbiome and differential metabolites according to Spearman correlation analysis. (B) The relationship between core metabolites and clinical indicators according to Spearman correlation analysis. rho, degree of the relationship between 2 variables.

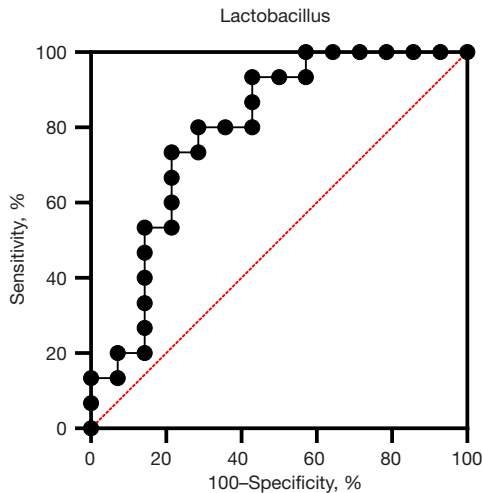


Figure 5 ROC curve. ROC, receiver operating characteristic.

Correlation analysis between metabolites, microbiome, and clinical symptoms

Using Spearman correlation analysis, we assessed the relationship between metabolites, microbiome, and clinical indicators (Figure 4B). We found that some core metabolites including homocitrulline, apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)], butyrylcarnitine, formiminoglutamic acid (FIGLU), diethanolamine, and prolylhydroxyproline had a

positive correlation with mili-total protein (MTP), whereas cellobiose, butyrylcarnitine, and prolylhydroxyproline had a negative correlation with serum albumin, with 24-epibrassinolide being negatively correlated with MTP ($P < 0.05$; $|r| > 0.5$). *Lactobacillus* was also negatively correlated with MTP and urea nitrogen but positively correlated with serum albumin (Table S5). There was also no significant difference in core metabolites when the Oxford classification of IgAN was used. *Lactobacillus* and core metabolites were determined to be predictors of IgAN by an area under the curve (AUC) analysis. *Lactobacillus* showed an excellent prediction ability for the diagnosis of IgAN in the ROC curve (AUC > 0.79 ; 95% CI: 0.6176–0.9633; Figure 5).

Discussion

Interactions between genetics and the environment are thought to determine IgAN’s development (22,23). The microbiome is currently recognized as a key environmental factor that contributes to the development and progression of the disease. While sequencing adds the comprehensive understanding of microorganisms, the metabolomics based on mass spectrometry is a pivotal technology of detect small molecules produced by microbiome (24). Through the correlation analysis of microbiome and metabolomics, we can provide broader ideas and information for the

screening of disease markers. Most existing multiomics studies elaborate on changes in the serum metabolome and gut microbiome, and a few studies have tried to examine the alterations in the serum metabolome and urinary microbiome. It has long been thought that the UT was a sterile environment, but the introduction of new high-throughput sequencing technologies and improved culture schemes for microbiome research has proven that the UT is not sterile (12,25,26). It has also been reported that *Limosilactobacillus urinaemulieris* sp. nov. and *Limosilactobacillus portuensis* sp. nov. are present in the urine of healthy women (27). The complicated microbial communities of the human UT can now be analyzed using high-throughput molecular sequencing of bacterial 16S rRNA genes (28). Recent studies have reported that the bacterial taxa of the urinary microbiome have an important influence on diseases and homeostasis in the UT (29-31). For example, some studies indicated an association between lower urinary tract symptoms (LUTS), or incontinence, and the human urinary microbiome (32,33). The dysbiosis of several key urinary bacteria has also been found to be related to interleukin-8 (IL-8) in type 2 diabetes mellitus (T2DM) (34). We hypothesized that urinary microbiota and metabolites might also be a feasible detection tool for IgAN.

Based on alpha and beta diversity analyses, we found that bacterial species did not differ significantly between the IgAN group and the HC group, which is similar to findings in previous research (9). We also found that the amount and diversity of bacteria was different in patients with IgAN. It has been reported that the main bacterial taxa of healthy people (aged 22–51 years) are *Lactobacillus*, *Aerococcus*, *Klebsiella*, *Staphylococcus*, *Corynebacterium*, *Gardnerella*, *Streptococcus*, *Escherichia*, *Prevotella*, and *Enterococcus* (35). We found that the distributions of the major phyla in the UT (Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria) were roughly consistent with the results of previous human adult gut studies (36-38). There was a 1.24-fold increase in Acidobacteria at the phylum level ($P < 0.05$), a 1.9-fold increase in *Actinomyces* in the genus level ($P < 0.05$), and a 2.6-fold decrease in *Lactobacillaceae* ($P < 0.05$), but a 0.34-fold decrease in *Lactobacillus* ($P < 0.05$) in our Wilcoxon rank sum test, which indicated that the specific species of urinary microbiota in patients with IgAN was altered. Our Metastats analysis found that Actinobacteria was more abundant at the phylum level in patients with IgAN and that *Actinotignum* was a notable differential microorganism at the genus level ($P < 0.01$). A previous study using both 16S DNA and rRNA found that Actinobacteria of the main bacterial phyla

significantly differed in the fecal microbiota of patients with IgAN (10). A study revealed that *Actinotignum*, known for its proinflammatory features, could be an early polybacterial biofilm colonizer. *Actinotignum* usually presents in the UT as an opportunistic pathogen and coagent of various polymicrobial infections, but it has not been found in feces (39). Studies have also shown that *Actinotignum* could be associated with noninfectious conditions and diseases, such as chronic inflammation, prostatism, and bladder cancer (31,40-43). Therefore, we speculate that the increase of *Actinomyces* in urinary flora may be related to IgAN.

We found that *Lactobacillus* at the genus level was lower in patients with IgAN compared to healthy controls, which is in agreement with a previous study that found that *Lactobacillus* genera to be higher in controls and *Lactobacillaceae* to be lower in patients with IgAN ($P < 0.05$) (10). It has also been reported that *Lactobacillus* can reduce inflammation of the kidney and also reduce injury in renal tubular epithelial cells. *Lactobacillus* may also protect the kidney through an independent mechanism against the interference of the original bacterial flora. *Lactobacillus* can decrease oxidative stress, reduce the proinflammatory response, and increase kidney function in immune responses. We speculate that *Lactobacillus* supplementation may work as a preventive approach against chronic kidney disease (CKD) (44).

Metabolites play an important role in the development and progression of renal diseases as mediators of bacterial functional activities (9). Previous studies have reported the alteration of amino acids, such as Met, Glu, and Pro, in the serum samples of patients with IgAN (45,46). This finding is in line with our research, which showed both increased differential metabolites (amino acids, oligopeptides, and amines) and decreased differential metabolites (medium-/long-chain fatty acids; *Figure 3A*). We hypothesize that the level of amino acids in serum was changed through the alteration of the tricarboxylic acid cycle and the increased hydrolysis of proteins in cell necrosis (10). We found that an increase of oligopeptides, rather than polypeptides, demonstrated bacterial proteolytic fermentation. Several studies have reported that protein assimilation, such as digestion and absorption, are impaired in patients with ESRD, suggesting that oligopeptides are more easily absorbed due to noncompetitive transport and low energy consumption (47-49). In addition, we found a decrease in lipids, especially in unsaturated fatty acids (e.g., arachidonic acid). Studies have shown that long-chain polyunsaturated fatty acids (LCPUFA) play a preventive and deferring role

in renal patients with cardiovascular disease (CVD), and that LCPUFA supplementation can extend the life of patients on long-term dialysis (50,51). A low dose of LCPUFA can also help lower estimated glomerular filtration rate (eGFR) (52). In our study, we found changes in differential metabolites in the serum samples of patients with IgAN as compared to healthy controls.

A previous study reported that the dysbiosis of urinary microbiota is related to proinflammatory chemokine IL-8 (34). Here, we studied the relationship between serum metabolites and urinary microbiota (Figure 4A). We found that some serum metabolites, such as trimethylamine-N-oxide (TMAO) and D-mannose, were related to *Lactobacillus* ($P < 0.05$; $|\text{rho}| > 0.5$). Several researchers reported that atherosclerosis, mediated by TMAO, could be an important cause of kidney and heart disease from dietary phosphatidylcholine (53). TMAO concentration is also associated with eGFR, prognosis, and even long-term survival in CKD (54,55). Some probiotics, such as *Lactobacillus*, have been shown to inhibit the synthesis of TMAO, reducing inflammatory signaling and improving renal function in CKD (56,57). Some studies suggest that D-mannose can inhibit bacterial adherence to uroepithelial cells (58,59). We speculate that *Lactobacillus* and D-mannose may work together to aid urethral immunity. KEGG analysis showed that histidine pathway was enriched in the serum of patients with IgAN. The microbiota can produce L-histidine through the histidine metabolic pathway, which is a potential biomarker of septic acute kidney injury (AKI) (60).

In this study, Spearman correlation analysis indicated a correlation between core metabolites, the microbiome, and clinical indicators in patients with IgAN. Similarly, homocitrulline, involved in CKD progression, has been found to be related to MTP (61); Butyrylcarnitine, which is positively correlated with MTP, was also shown to be a predictive biomarker in the diagnosis of renal cell carcinoma (RCC) (62); meanwhile, prolylhydroxyproline, which has also been positively correlated with MTP, may be a marker for cognitive impairment in patients receiving long-term maintenance dialysis (63). We also found that *Lactobacillus* was negatively correlated with urea nitrogen and MTP but positively correlated with serum albumin. This is consistent with previous studies which found that some *Lactobacilli* can reduce urea nitrogen and upregulate albumin levels (64,65). Our research still needs to be verified with larger samples, but using an ROC model, we found that *Lactobacillus* is a good predictor of IgAN (AUC > 0.79 ; Figure 5).

Urine, a waste product, is created through the various metabolic endpoints, which eventually reach the bladder and kidneys. Urethral microbiota are affected by external factors, such as lifestyle, diet, and the environmental, and has ample time to interact with and alter these metabolisms filtered by blood through kidneys (7,31). Urethral microbiota affects the kidney through a host of mechanisms in this process, such as evading the protective factors of the host or inhibiting host IgA transport (66). Moreover, a study on sterile mice have shown that microbial deficiency is associated with an impaired immune system and behavioral or neurological diseases (67). Therefore, the changes in the urethral microbiota of patients with IgAN as compared with healthy controls may increase the risk of alterations in renal pathologies. We found a close relationship between urethral microbiota and serum metabolites. There may be a similar mechanism in the human body that causes the deposition of polymeric and hypogalactosylated IgA1 (Gd-IgA1) in the glomerular mesangium or capillary wall, leading to IgAN. Renal insufficiency changed the composition of serum metabolites, so we hypothesize that urethral microbiota interacts with serum metabolites.

Currently, the main treatments for IgAN are immunosuppressive drugs and systemic corticosteroids, but the minimal therapeutic effects and risk of side effects limit their value and application. Our research may provide a therapeutic approach including targeting microbiota modulation or restoration and maintenance of metabolic balance. A recent study of the identification of urinary microbiota using 16S rRNA and culturomics, proposed that the 64% of species in urinary microbiota, which originates from the gut, overlap with gut microbiota (68). The gut microbiota is helpful in maintaining the diversity of prokaryotes in the UT and this study highlighted reduction in recurrence of urinary tract infections after fecal microbiota transplantation (FMT) (69). Perhaps changing the imbalance of metabolites and microbiota could delay the occurrence of IgAN and reduce the impact of the disease. Some researchers have reported that the preventive supplement of D-Mannose and *Lactobacillus* effectively prevented UT infection (70-72). Furthermore, adjunctive probiotic supplementation may mitigate the increase of TMAO in proteolytic fermentation and decrease the risk of CVD and dementia (73). A reasonable supplement of probiotics and polyunsaturated fatty acids may be a key tool for preventing and treating IgAN.

Our study had some limitations. Our combined microbiome and metabolome analysis results would be more robust if the urinary differential metabolites had

been added to the experiment. The results of our study should also be verified on more samples. Now, with more convenient and faster sequencing technologies, we can detect the size and grandeur of microbial communities. Application of the microbiome and metabolome will become a powerful non-invasive target for precision medicine. However, we still lack understanding of the molecular functions encoded by microbial genes. In addition, the joint analysis of multi-omics will be a major trend. We will use the expanded quantitative urine culture (EQUC) to detect the low-abundance uropathogens or bacteria in the future (74,75). EQUC associates with sequencing tools, such as targeted amplicon sequencing, metagenomic sequencing and long-read sequencing, to further describe the urine microbiota and build an expanded genome sequencing isolate banking.

Conclusions

We observed a shift in the abundance of bacteria and metabolites in patients with IgAN. Our study clarified the relationship between serum metabolites, urine microbiota, and disease in those with IgAN, which will help to provide further research and develop new tools for preventing and delaying the occurrence of the disease.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5334/rc>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-5334/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was authorized and reviewed by the institutional review board of the First Affiliated Hospital of Jinan University (No. KY-2020-034) and conducted in accordance with the Declaration of Helsinki (as revised in 2013). These patients were informed of the study and signed an informed consent form.

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