Changes of serum and urine microbiota and metabolite spectrum in patients with diabetic kidney disease

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Abstract

Background: Diabetic kidney disease (DKD) is a common and potentially fatal consequence of diabetes. In the long run, it can lead to chronic renal failure or end-stage renal disease. Many studies have found that gut microorganisms play an important role in maintaining human body homeostasis, and that their metabolites are linked to a variety of chronic disorders, including renal and cardiovascular disease.

Methods: We employed 16S rRNA gene sequencing to identify bacteria species in DKD patients and healthy people.

Results: The findings indicated that the DKD had a distinct gut microbiota from the HC. Taxonomic investigations indicated that the DKD microbiome had less alpha diversity than a control group. Proteobacteria and Acidobacteria phyla increased in the DKD, while Firmicutes and Bacteroidetes decreased significantly ($P<0.05$). Acidobacteria and Acidobacteria were the most prevalent microbiota in the DKD, as determined by the LEfSe plot. Changes in the intestinal microbiota of DKD also had an effect on the makeup of metabolites. Short-chain fatty acids (SCFAs) and protein-bound uremic toxins (PBUTs) were shown to be specific. Then we discovered that arginine and proline metabolism was the primary mechanism involved in the regulation of diabetic kidney disease.

Conclusions: The findings of this study place the serum and urine microbiota of DKD patients into a functional context and identify the most abundant microbiota (Proteobacteria and Acidobacteria) in DKD. Metabolites of arginine may have a significant impact in people with DKD.

Introduction

Diabetic kidney disease (DKD) is a global health hazard that has steadily increased in prevalence over the last few decades. With the ongoing improvement of people's lifestyles and living standards, DKD has become a prevalent consequence of diabetes, estimated to impact 40% of diabetic patients (1). DKD not only contributes greatly to the development of end-stage renal disease (ESRD) (2) but also raises the chance of developing (3). The glomerular filtration rate (GFR) and urine albumin excretion rate (AER) are routinely employed to diagnose DKD, and several biomarkers have been connected with the disease. They still lack the specificity and sensitivity necessary for early diagnosis (4). Despite efforts to slow the progression of DKD, no significant advances in the management of people with DKD have been made.

The gut microbiota is primarily composed of bacteria and viruses, but also includes archaea, fungi, and unicellular eukaryotes. The gut microbiota is crucial for maintaining host homeostasis and for the development of a variety of disorders, including obesity, diabetes, liver disease, cancer, and DKD (5–8). With the advancement of research into the impacts of bacteria in the human intestine, the human gut microbiota is being considered as a source of innovative medicines (9). DKD can impair the function of the intestinal barrier and result in flora migration, resulting in endotoxemia and inflammatory reactions and hastening the decline of renal function.

Although the gut microbiome has been examined in a DKD mice model, the role of the intestinal flora in human beings remains unknown. The current study examined alterations in the makeup and function of the gut microbiome of 19 DKD patients and 15 healthy volunteers undergoing standard physical examination.

Materials And Methods

Study cohort and recruitment of subjects

To examine the gut microbiota and metabolites in DKD patients, we recruited 19 patients diagnosed with the disease and 15 healthy participants for a traditional physical examination at Jinan University's First Clinical Medical College. The following criteria were used to identify patients with DKD: over the age of 18 and has been diagnosed with type 2 diabetes. All healthy volunteers were over the age of 18 and had no history of sickness. The subjects were divided into two groups: those with diabetic kidney disease (DKD) and those with healthy controls (HG). Each patient was thoroughly informed about the goal of the trial and the experimental procedures. They all agreed to engage in the program on their own will and signed the informed consent form.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Jinan University's First Affiliated Hospital.

Metabolite extraction from serum samples

Following an overnight period, all participants were assessed the following morning (≥ 8 h). All patients had their peripheral blood and urine samples taken concurrently. 100 µL of the sample was transferred to an EP tube. After adding 400 L of extract solution (1: 1 acetonitrile-methanol-water, comprising an isotopically labeled internal standard mixture), the samples were vortexed for 30 seconds, sonicated for 10 minutes in an ice-water bath, then incubated at -40 °C for 1 hour to precipitate proteins. The material was then centrifuged at 12000 revolutions per minute for 15 minutes at 4 °C. The supernatant was then transferred to a new glass vial and analyzed. The quality control (QC) sample was created by combining an equal quantity of each sample's supernatants.

DNA extraction and 16S rRNA gene sequencing

We extracted genomic DNA from the samples using a PowerSoil DNA Isolation Kit (Qiagen, Germany). Then, we amplified the V3 and V4 sections of the 16S rRNA gene using universal primers (forward: 5'-ACTCCTACGGGAGGCAGCA-3'; reverse: 5'GGACTACHVGGGTWTCTAAT-3'). Following amplification, polymerase chain reaction products were combined with AMPure XP beads (Beckman Coulter, UK), and fragments screened and cleaned. The amplicons were quantified using a Qubit fluorometer (Invitrogen, USA) and sequenced using the Illumina HiSeq platform following purification and library building (Illumina, California, USA).

16s rRNA data analysis

Trimmomatic version 0.33 (10) was used to filter the original data's quality, and Cutadapt version 1.9.1 (11) was used to locate and eliminate primer sequences. Additionally, reads were combined using FLASH version 1.2.11 (12). Then, using UCHIME software version 8.1 (18), we were able to eliminate chimeric sequences and obtain high-quality clean tags. We assigned sequences with a similarity greater than 97 percent to the same operational taxon (OTU) using USEARCH version 10.0 (13) . Based on the 16S SILVA online database release 132 (14), the Naive Bayes classifier was used to perform taxonomic annotations on the feature sequence. The investigation of alpha diversity was carried out using the QIIME2 software (https://qiime2.org/). We calculated beta diversity using unweighted principal coordinate analysis (PCoA). Linear discriminant analysis (LDA) of effect size was used to compare species between groups and to quantify the influence of each species' abundance (http://huttenhower.sph.harvard.edu/lefse/) (15). To determine the difference in microbial community abundance between the two sets of samples, we utilized the Metastats program (http://metastats.cbcb.umd.edu/) to conduct a T-test on the species abundance data between the groups.

Liquid chromatography-tandem mass spectrometry data acquisition

The LC-MS/MS studies were conducted utilizing a UHPLC system (Vanquish, Thermo Fisher Scientific) equipped with a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm) and a Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). The liquid contained two phases: mobile phase A (25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water, pH = 9.75) and mobile phase B (acetonitrile). 3 μL of each sample was put into the system, and the temperature of the auto-sampler was set at four degrees Celsius. The QE HFX mass spectrometer was chosen because of its capacity to acquire MS/MS spectra using the information-dependent acquisition (IDA) mode under the control of the acquisition software (Xcalibur, Thermo). In this mode, the acquisition software constantly examines the complete scan MS spectrum. The following conditions were set for the ESI source: sheath gas flow rate of 30 Arb, auxiliary gas flow rate of 25 Arb, capillary temperature of 350 °C, full MS resolution of 60000, MS/MS resolution of 7500, collision energy of 10/30/60 in NCE mode, and spray voltage of 3.6 kV (positive) or -3.2 kV (negative).

Metabolomics data preprocessing and annotation

The QE platform's ionization source was ESI in both positive and negative ion modes. The raw data were converted to the mzXML format using ProteoWizard and processed using an in-house peak identification, extraction, alignment, and integration tool created in R and based on XCMS. Then, metabolite annotation was performed using an in-house MS2 database (Biotree DB). The annotation cutoff was set at 0.3.

Statistical analyses

The R language was used to create a rarefaction curve based on the number of sequences extracted and the diversity index of the relevant OTUs. We estimated the richness using the Chao index during the discovery phase by calculating the alpha diversity based on the microbial profiles. The Shannon and Simpson indices were used to determine the diversity. A high value for the Chao and Shannon indices

suggested that the sample contained a greater degree of diversity. These findings were utilized to investigate the influence of various phenotypic variables.

A Venn diagram can be used to illustrate the number of common and unique species (for example, OTUs) found in the LR, HR, and NR categories. We used a R language tool to construct a Venn diagram.

Beta diversity analysis of interindividual variability was performed using the principal coordinates analysis (PCoA) approach at the genus level, employing weighted UniFrac and Bray-Curtis dissimilarity. The difference metabolites were categorized into pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Results

In general condition surveys, there was no discernible difference between two groups (e.g., age, body mass index, and blood pressure). Proteinuria, serum albumin, creatine, and urea nitrogen levels were significantly different between the HC and DKD groups after 24 hours ($P < 0.05$). We acquired 2,685,485 high-quality 16S rDNA readings from the 34 samples, with a median read count of 79,974. (range from 79,726 to 80,313). After quality control, the sample averaged 419bp in length. 1467 OTUs were obtained following taxonomic assignment (Fig. 1A).

Bacterial OTUs and diversity analyses

The Venn diagram revealed three distinct OTUs in the DKD group and one distinct OTU in the HC group. Both groups shared 1463 OTUs (Fig. 1B). The rarefaction curves indicated that the depth and coverage of the sequencing were sufficient (Fig. 1C). The Shannon and Simpson indices demonstrated statistically significant variations in alpha diversity ($P < 0.01$), however the Chao1 ACE index did not differ significantly between DKD and HC (Fig. 1D-G). For beta diversity, PCA, PCoA, and NMDS plots were used to compare the microbial communities from each DKD and HC group, and the results indicated a distinct separation of the two groups.

Abundance distribution and differential analysis of different biologic classifications

At the phylum level, ten major groups of bacteria have been found, including Firmicutes, Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria (Fig. 2A). Proteobacteria had a greater relative abundance in the DKD group (median value: 28.00%) than in the HC group (median value: 28.00%). Firmicutes were lower in the DKD group (median value: 18.20%) than in the HC (median value: 30.80%). Additionally, we quantified the relative abundance of OTU at the class, order, family, genus, and species levels and highlighted the top ten microbial groups (Supplementary Figure S1). We used LEfSe analysis to determine the difference between the HC and DKD groups (Fig. 2B). Six microorganisms were overrepresented in the HC group samples (all LDA values (log10) > 4) (Fig. 2C). Firmicutes was the most prevalent microbiota at the phylum level in DKD when compared to HC. Bacilli and Clostridia were substantially more abundant than Acidobacteriia in the HC group, but Acidobacteriia were more abundant

in the DKD group. Lactobacillales and Clostridiales were the most prevalent microbiota at the order level in the HC. At the family level, Lactobacillaceae and Lachnospiraceae were notably numerous in the HC. Lactobacillus was the most abundant microbiome in the HC.

Identification of metabolites in serum samples

We used score plots and the principal component analysis (PCA) model to examine data. PCA was used to differentiate the DKD and HC groups. The orthogonal projections to latent structures-discriminant analysis (OPLS-DA) score chart revealed that the two sets of samples were statistically different and that the samples were essentially within the 95 percent confidence interval (Hotelling's T-squared ellipse). Additionally, the permutation test for OPLS-DA demonstrated that the model is robust and does not exhibit overfitting. 58 differential metabolites were identified in serum samples using the screening criteria VIP > 2 and log fold change > 2. (Supplementary Table S1). The DKD group identified Organic acids and derivatives, Organoheterocyclic compounds, Organic oxygen compounds, Lipids, Lipid-like molecules, and Benzenoids.

Metabolic pathway analysis of the differential metabolites.

To gain a better understanding of the differential metabolites' activities, we performed KEGG analysis on the differential metabolites in the DKD (Fig. 3A). The major metabolic pathways were those for Arginine and proline, Beta-alanine, and Glycine, serine, and threonine.

Discussion

The intestines of healthy persons are colonized by a diverse array of bacteria, with Firmicutes and Bacteroides accounting for the majority of the intestinal flora (16). The intestinal microbial ecology is involved in the digestion and absorption of nutrients, energy metabolism, immunological control, and a variety of other physiological functions (17–19). According to studies, the occurrence and progression of diseases alters the gut flora.

There is mounting evidence that DKD patients' gut bacteria are changed (20, 21). Our findings indicated that the DKD and HC groups had significantly different microbial counts and relative abundances. Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Actinobacteria accounted for the majority of the microbial content in all samples in this investigation, which was consistent with earlier findings. Firmicutes and Bacteroidetes are the two major bacterial groupings found in healthy humans, together accounting for around 90% of the phylum level (22). Interestingly, we discovered that the DKD group possessed a larger relative abundance of Proteobacteria, Acidobacteria, and Actinobacteria, but lacked Firmicutes and Bacteroidetes. Nosratola D.Vaziri found that Actinobacteria, Firmicutes, and Proteobacteria were abundant in patients with ESRD than healthy controls (23). Numerous studies have demonstrated that the number of proteobacteria increases at the phylum level in obese and diabetic patients, while the Bacteroidetes phylum decreases (24, 25). Proteobacteria rise promotes the formation

of Lipopolysaccharides (LPS), which results in an increase in pro-inflammatory factors and an inflammatory response (26).

Meanwhile, we detected a considerable decrease in the relative abundance of Lactobacillus at the genus level in the DKD, while Escherichia-Shigella increased. Lactobacillus is high in probiotics, which aid in the maintenance of the intestinal functional barrier's integrity. Tae-Hee Lee et al. confirmed that BP121 boosted the number of good intestinal flora Lactobacillus and decreased kidney inflammation, oxidative stress, and uremic toxins (27). Lactobacillus may create the helpful organic acid lactate, which is metabolized in the colon to butyric acid (28). As a result, we hypothesized that reducing Lactobacillus would potentially result in an increased inflammatory response. Additionally, butyrate contributes to the reduction of the pro-inflammatory effects of LPS activation. Escherichia-Shigella was shown to be abundant in stool samples of patients with DKD and reduced renal function after crossing the intestinal epithelial barrier, according to a study (29). Escherichia coli may create more indoxyl sulfate (IS) in people with advanced chronic kidney disease (30).

More and more data from trials in mice and humans suggests that metabolites produced by the gut microbiota play a critical role in the development and progression of renal disorders. Numerous studies have demonstrated that trimethylamine-oxide (TMAO), short-chain fatty acids (SCFAs), protein-bound uremic toxins (PBUTs), bile acids (BAs), tryptophan-derived metabolites, and branched-chain amino acids (BCAAs) were abundant in DKD patients, all of which play a significant role in the progression of DKD. We observed an increasing rise in several organic nitrogen compounds, including TMPO, L-Carnitine, and Choline, in serum samples from DKD patients. Trimethylamine (TMA) is primarily generated from choline, phosphatidylcholine, and L-carnitine by intestinal bacteria and is subsequently oxidized to TMAO in the liver by monooxygenase 3 enzymes before being distributed to various tissues or eliminated via the kidney (31). The TMAO pathway was the first to establish a relationship between gut microbe-produced compounds and the risk of cardiovascular and renal illness (32). High TMAO levels were reported to worsen DKD in animal models, and supplementation with TMAO inhibitors (3,3-dimethyl-1-butanol) was found to mitigate the exacerbation of DKD symptoms (20, 33). Posada-Ayala et al. detected seven distinct metabolites in s from 16 chronic kidney disease patients and 15 healthy controls, with chronic kidney disease patients having higher plasma TMAO levels, consistent with our finding (34).

Numerous PBUTs (for example, indoxyl sulfate, phenyl sulfate, and phenylacetylglutamine) are derived from the results of microbial metabolism of food chemicals in the intestine, including aromatic amino acids, tyrosine, phenylalanine, and tryptophan (35). Barrios et al. established that indoxyl-sulfate, p-cresyl-sulfate, and phenylacetylglutamine were all early indicators of renal function decrease (36). Numerous investigations have demonstrated that phenyl sulfate may impair the barrier function of the glomerular basement membrane, resulting in increased urine protein levels (37). Increased serum phenylacetylglutamine levels have been shown to be an independent risk factor for cardiovascular disease (38, 39), implying that DKD patients in this study may be at an increased risk of developing cardiovascular disease. Certain uremic toxins, such as Phenylacetylglutamine, N-acetyl-L-arginine, Methylguanidine, and Guanidinosuccinic acid, can be found as metabolites in patients with end-stage

renal failure. They demonstrated an upward trend in DKD in our study, implying that it may advance to ESRD. These uremic solutes have the potential to impair endothelium repair following injury and to cause direct vascular damage, hence increasing the risk of cardiovascular problems.In our study, they showed an uptrend in the DKD, which suggested that it might progress to ESRD. These uremic solutes have the potential to impair endothelium repair following injury and to cause direct vascular damage, hence increasing the risk of cardiovascular problems.

We analyzed the KEGG database to gain a better understanding of the probable function of DKD intestinal flora. We discovered an enrichment in arginine and proline metabolism in serum samples from DKD patients. Citrulline, L-Arginine, D-Proline, Hydroxyproline, Guanidoacetic acid, Creatinine, 5-Guanidino-2-oxopentanoate, 4-Aminobutyraldehyde, and 5-Aminopentanoic acid were all constituents of this pathway. Arginine and its metabolites are involved in a variety of metabolic processes. In adults, glutamine and proline are metabolized to citrulline in the colon via pyrroline-5-carboxylate (P5C), and subsequently to arginine in the kidneys. L-arginine is a precursor to the formation of nitric oxide (NO), polyamines, and agmatine. These metabolites play a role in the progression of renal disease (40, 41). Additionally, NO metabolism is critical for endothelial dysfunction in DKD (42). Although animal models of kidney disease have demonstrated that supplementing with L-arginine is good for diabetic nephropathy, the mechanism by which it works is yet unknown (43). Numerous traditional Chinese remedies, including Cicada Cordyceps Polysaccharide, Tangshen Recipe, and Shenyankangfu Pian, have demonstrated that DKD can be treated by modifying the intestinal microbiota's composition or function.

Our research was not without flaws. The experiment used blood samples for differential metabolite analysis and did not include pee samples for comparison. We used the 16S RNA gene sequencing technique to determine bacterial taxa at a low resolution (genus) level in order to investigate the gut microbiota. As a result, the absence of comprehensive characterization of the entire microbiome precluded us from examining the taxonomic and functional potentials of species and subspecies.

Conclusion

In general, we detected significant variations in the composition and function of microbial communities in the guts of DKD patients. The DKD group had fewer total bacteria in their stool than healthy controls, and their gut flora shifted from Firmicutes to Proteobacteria and Acidobacteria. The gut flora was found to be associated with DKD's inflammatory state and renal function. The accumulation of metabolites stimulates the immune system continually, resulting in increased production of inflammatory factors and kidney damage. Additionally, we noticed a correlation between arginine and proline metabolism and DKD. Patients with diabetic nephropathy may need to adapt their diet and arginine intake correctly, which may bring additional therapy options.

Abbreviations

DKD, diabetic kidney disease; ERSD, end-stage renal disease; GFR, glomerular filtration rate; AER, albumin excretion rate; SCFAs, Short-chain fatty acids; PBUTs, protein-bound uremic toxins; OUT, operational taxon; PCoA, principal coordinate analysis; LDA, linear discriminant analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes;

Declarations

Ethics approval and consent to participate

Approved by the Ethics Committee of the First Affiliated Hospital of Jinan University (KY-2021-017), all samples were obtained from the First Affiliated Hospital of Jinan University. We fully explained the purpose and experimental procedures to each patient. They all volunteered to participate in the program and signed the informed consent.

Consent for publication

Not applicable.

Data availability statement

The mass spectrometry proteomics data have been deposited to the 10.6084/m9.figshare.19127585

Competing interests

we declare that the research was conducted without any commercial or financial relationships.

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Authors' contributions

YY, Li designed the study, acquired all datasets, and wrote the manuscript. CM, WD, XZ analyzed the data. WC collected the specimens. LY interpreted the data. FL, YD, and DT supervised the project and revised the manuscript. All authors contributed to the article and approved the submitted version.

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References

1. Chen Y, Lee K, Ni Z, He JC. Diabetic Kidney Disease: Challenges, Advances, and Opportunities. Kidney Dis (Basel). 2020;6(4):215–25.
2. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bragg-Gresham J, Balkrishnan R, et al. US Renal Data System 2018 Annual Data Report: Epidemiology of Kidney Disease in the United States. Am J Kidney Dis. 2019;73(3 Suppl 1):A7-A8.
3. Sabanayagam C, Chee ML, Banu R, Cheng CY, Lim SC, Tai ES, et al. Association of Diabetic Retinopathy and Diabetic Kidney Disease With All-Cause and Cardiovascular Mortality in a Multiethnic Asian Population. JAMA Netw Open. 2019;2(3):e191540.
4. Maiti AK. Development of Biomarkers and Molecular Therapy Based on Inflammatory Genes in Diabetic Nephropathy. Int J Mol Sci. 2021;22(18).
5. Lazar V, Ditu LM, Pircalabioru GG, Picu A, Petcu L, Cucu N, et al. Gut Microbiota, Host Organism, and Diet Trialogue in Diabetes and Obesity. Front Nutr. 2019;6:21.
6. Tilg H, Adolph TE, Gerner RR, Moschen AR. The Intestinal Microbiota in Colorectal Cancer. Cancer Cell. 2018;33(6):954–64.
7. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine. 2020;51:102590.
8. Fang Q, Liu N, Zheng B, Guo F, Zeng X, Huang X, et al. Roles of Gut Microbial Metabolites in Diabetic Kidney Disease. Front Endocrinol (Lausanne). 2021;12:636175.
9. Cani PD. Human gut microbiome: hopes, threats and promises. Gut. 2018;67(9):1716–25.
10. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20.
11. Kechin A, Boyarskikh U, Kel A, Filipenko M. cutPrimers: A New Tool for Accurate Cutting of Primers from Reads of Targeted Next Generation Sequencing. J Comput Biol. 2017;24(11):1138–43.
12. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–63.
13. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996–8.
14. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581–3.
15. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
16. Zaky A, Glastras SJ, Wong MYW, Pollock CA, Saad S. The Role of the Gut Microbiome in Diabetes and Obesity-Related Kidney Disease. Int J Mol Sci. 2021;22(17).
17. Mertowska P, Mertowski S, Wojnicka J, Korona-Glowniak I, Grywalska E, Blazewicz A, et al. A Link between Chronic Kidney Disease and Gut Microbiota in Immunological and Nutritional Aspects.

Nutrients. 2021;13(10).

18. Malard F, Dore J, Gaugler B, Mohty M. Introduction to host microbiome symbiosis in health and disease. Mucosal Immunol. 2021;14(3):547–54.
19. Lee MC, Hsu YJ, Ho HH, Kuo YW, Lin WY, Tsai SY, et al. Effectiveness of human-origin Lactobacillus plantarum PL-02 in improving muscle mass, exercise performance and anti-fatigue. Sci Rep. 2021;11(1):19469.
20. Fang Q, Zheng B, Liu N, Liu J, Liu W, Huang X, et al. Trimethylamine N-Oxide Exacerbates Renal Inflammation and Fibrosis in Rats With Diabetic Kidney Disease. Front Physiol. 2021;12:682482.
21. Li L, Ma L, Fu P. Gut microbiota-derived short-chain fatty acids and kidney diseases. Drug Des Devel Ther. 2017;11:3531–42.
22. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol. 2015;21(29):8787–803.
23. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, et al. Chronic kidney disease alters intestinal microbial flora. Kidney Int. 2013;83(2):308–15.
24. Zhang Q, Yu H, Xiao X, Hu L, Xin F, Yu X. Inulin-type fructan improves diabetic phenotype and gut microbiota profiles in rats. PeerJ. 2018;6:e4446.
25. Moreno-Navarrete JM, Serino M, Blasco-Baque V, Azalbert V, Barton RH, Cardellini M, et al. Gut Microbiota Interacts with Markers of Adipose Tissue Browning, Insulin Action and Plasma Acetate in Morbid Obesity. Mol Nutr Food Res. 2018;62(3).
26. Verhaar BJH, Prodan A, Nieuwdorp M, Muller M. Gut Microbiota in Hypertension and Atherosclerosis: A Review. Nutrients. 2020;12(10).
27. Lee TH, Park D, Kim YJ, Lee I, Kim S, Oh CT, et al. Lactobacillus salivarius BP121 prevents cisplatininduced acute kidney injury by inhibition of uremic toxins such as indoxyl sulfate and pcresol sulfate via alleviating dysbiosis. Int J Mol Med. 2020;45(4):1130–40.
28. Lee SM, Kim N, Nam RH, Park JH, Choi SI, Park YT, et al. Gut microbiota and butyrate level changes associated with the long-term administration of proton pump inhibitors to old rats. Sci Rep. 2019;9(1):6626.
29. Tao S, Li L, Li L, Liu Y, Ren Q, Shi M, et al. Understanding the gut-kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. Acta Diabetol. 2019;56(5):581–92.
30. Wu IW, Lin CY, Chang LC, Lee CC, Chiu CY, Hsu HJ, et al. Gut Microbiota as Diagnostic Tools for Mirroring Disease Progression and Circulating Nephrotoxin Levels in Chronic Kidney Disease: Discovery and Validation Study. Int J Biol Sci. 2020;16(3):420–34.
31. Janeiro MH, Ramirez MJ, Milagro FI, Martinez JA, Solas M. Implication of Trimethylamine N-Oxide (TMAO) in Disease: Potential Biomarker or New Therapeutic Target. Nutrients. 2018;10(10).
32. Velasquez MT, Centron P, Barrows I, Dwivedi R, Raj DS. Gut Microbiota and Cardiovascular Uremic Toxicities. Toxins. 2018;10(7):287.

33. Sun G, Yin Z, Liu N, Bian X, Yu R, Su X, et al. Gut microbial metabolite TMAO contributes to renal dysfunction in a mouse model of diet-induced obesity. Biochem Biophys Res Commun. 2017;493(2):964–70.
34. Posada-Ayala M, Zubiri I, Martin-Lorenzo M, Sanz-Maroto A, Molero D, Gonzalez-Calero L, et al. Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease. Kidney International. 2014;85(1):103–11.
35. Mair RD, Sirich TL, Plummer NS, Meyer TW. Characteristics of Colon-Derived Uremic Solutes. Clin J Am Soc Nephrol. 2018;13(9):1398–404.
36. Barrios C, Beaumont M, Pallister T, Villar J, Goodrich JK, Clark A, et al. Gut-Microbiota-Metabolite Axis in Early Renal Function Decline. PLoS One. 2015;10(8):e0134311.
37. Kikuchi K, Saigusa D, Kanemitsu Y, Matsumoto Y, Thanai P, Suzuki N, et al. Gut microbiome-derived phenyl sulfate contributes to albuminuria in diabetic kidney disease. Nat Commun. 2019;10(1):1835.
38. Poesen R, Claes K, Evenepoel P, de Loor H, Augustijns P, Kuypers D, et al. Microbiota-Derived Phenylacetylglutamine Associates with Overall Mortality and Cardiovascular Disease in Patients with CKD. J Am Soc Nephrol. 2016;27(11):3479–87.
39. Nemet I, Saha PP, Gupta N, Zhu W, Romano KA, Skye SM, et al. A Cardiovascular Disease-Linked Gut Microbial Metabolite Acts via Adrenergic Receptors. Cell. 2020;180(5):862–77 e22.
40. Rodriguez-Gomez I, Moliz JN, Quesada A, Montoro-Molina S, Vargas-Tendero P, Osuna A, et al. L-Arginine metabolism in cardiovascular and renal tissue from hyper- and hypothyroid rats. Exp Biol Med (Maywood). 2016;241(5):550–6.
41. Elbassuoni EA, Ragy MM, Ahmed SM. Evidence of the protective effect of l-arginine and vitamin D against monosodium glutamate-induced liver and kidney dysfunction in rats. Biomed Pharmacother. 2018;108:799–808.
42. Tessari P. Nitric oxide in the normal kidney and in patients with diabetic nephropathy. J Nephrol. 2015;28(3):257–68.
43. Popolo A, Adesso S, Pinto A, Autore G, Marzocco S. L-Arginine and its metabolites in kidney and cardiovascular disease. Amino Acids. 2014;46(10):2271–86.

Figures

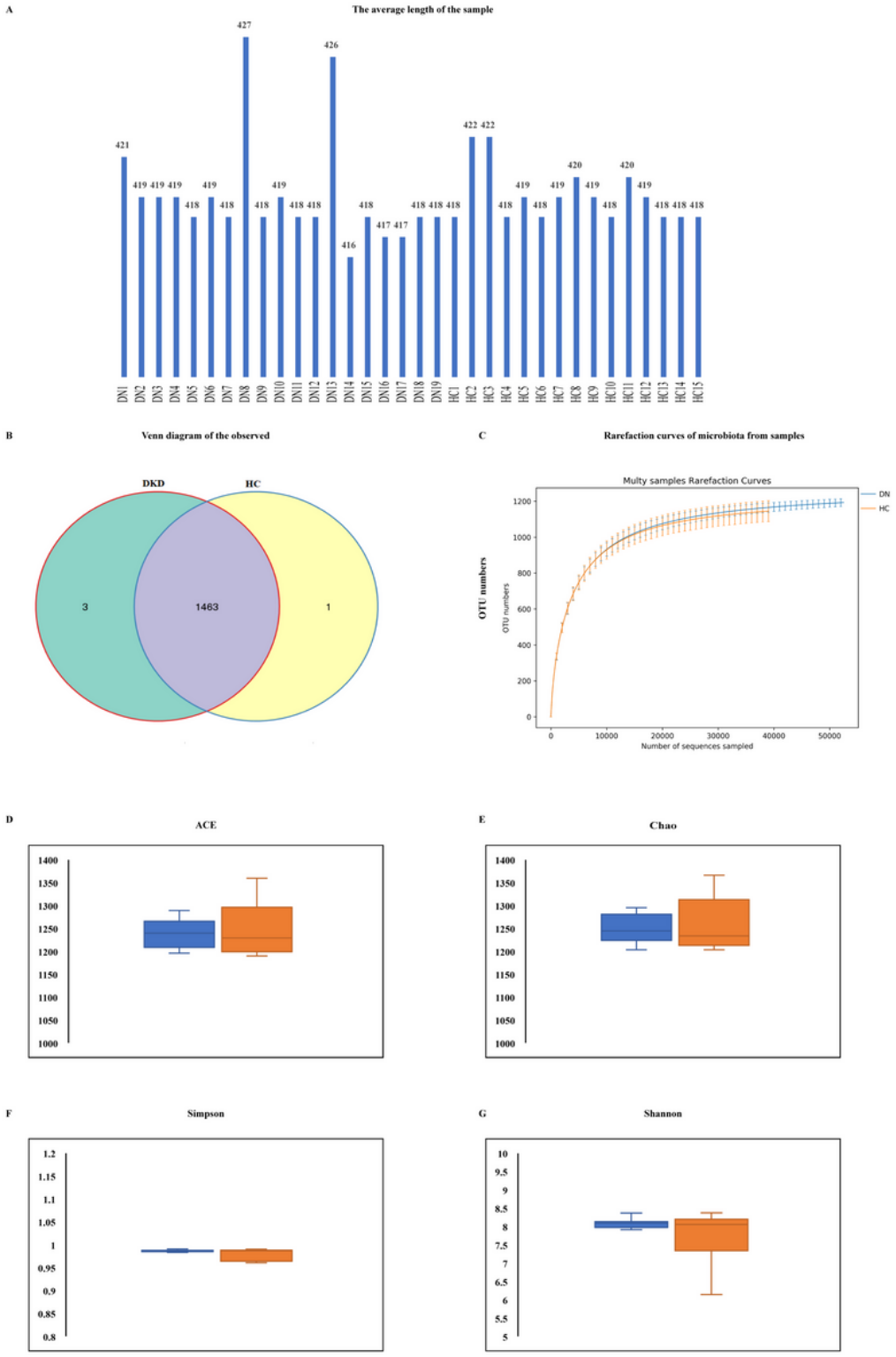

Figure 1

Microbiota diversity analysis. (A) The length of reading (B) Operational taxonomic units (OTUs) of diabetic kidney disease (DKD) and healthy control (HC) groups. C) Rarefaction curves of microbiota from serum samples. Means ±95% confidence intervals are shown. (D-G) Abundance-based coverage estimator (ACE), richness estimator (Chao1) and diversity indices (Simpson and Shannon) of microbiota from the serum samples of DKD and HC groups.

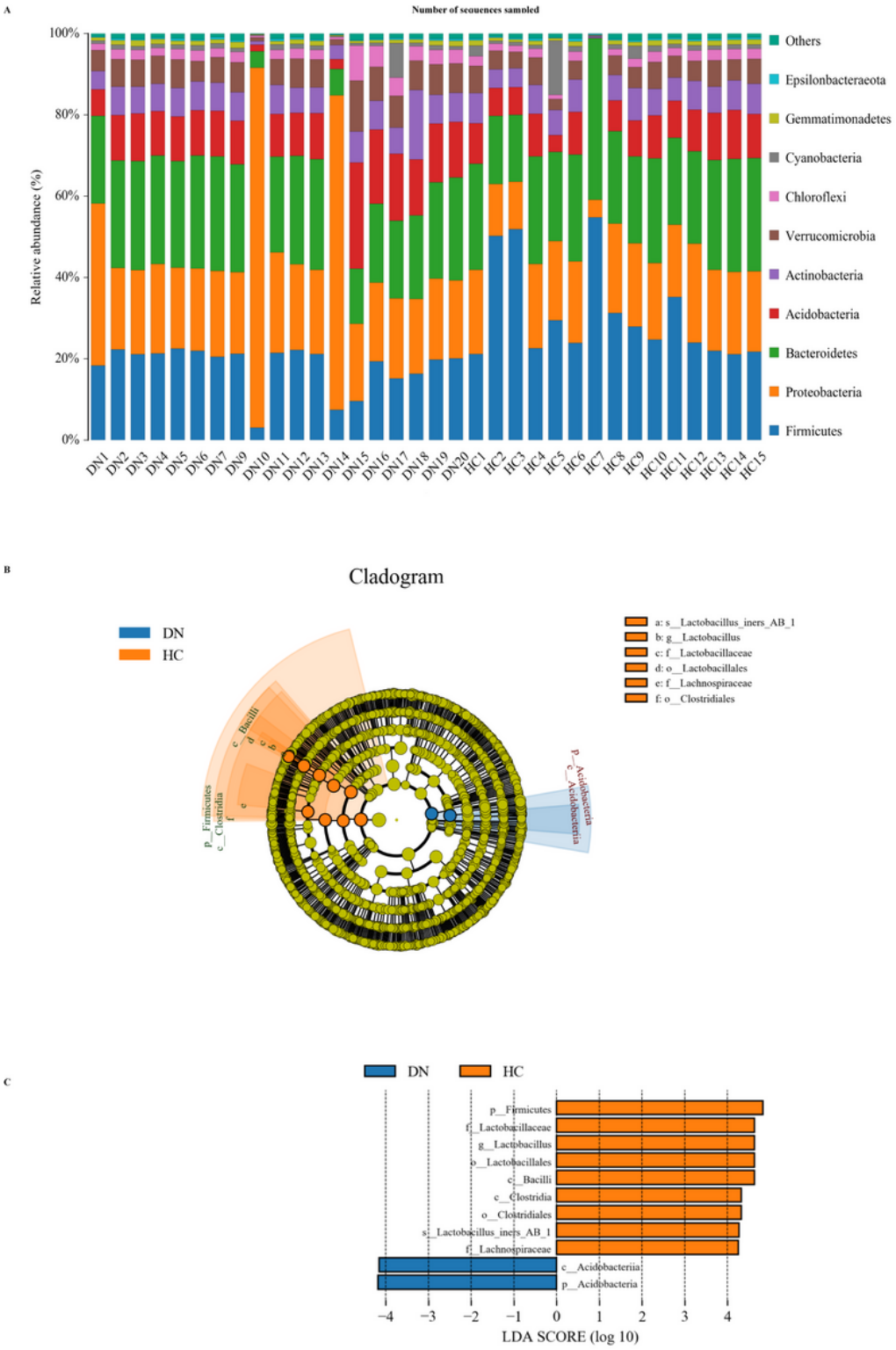

Figure 2

Bacterial Abundance Changes at Phylum Level and Linear Discrimination Analysis (LDA) (A) Ten major groups of bacteria in fecal samples from DKD and HC groups at the phylum level. (B) Cladogram of the phylogenetic distribution of microbes. Each circle represents a classification level from phylum to species from the inner to outer circles. The size of each circle is proportional to relative abundance. Microbes with no significant difference in abundance are shown in green. Microbes with an LDA value >4 in DKD and

HC groups are marked with orange and blue, respectively. (C) Differences in the relative abundance of bacteria in DKD and HC groups.

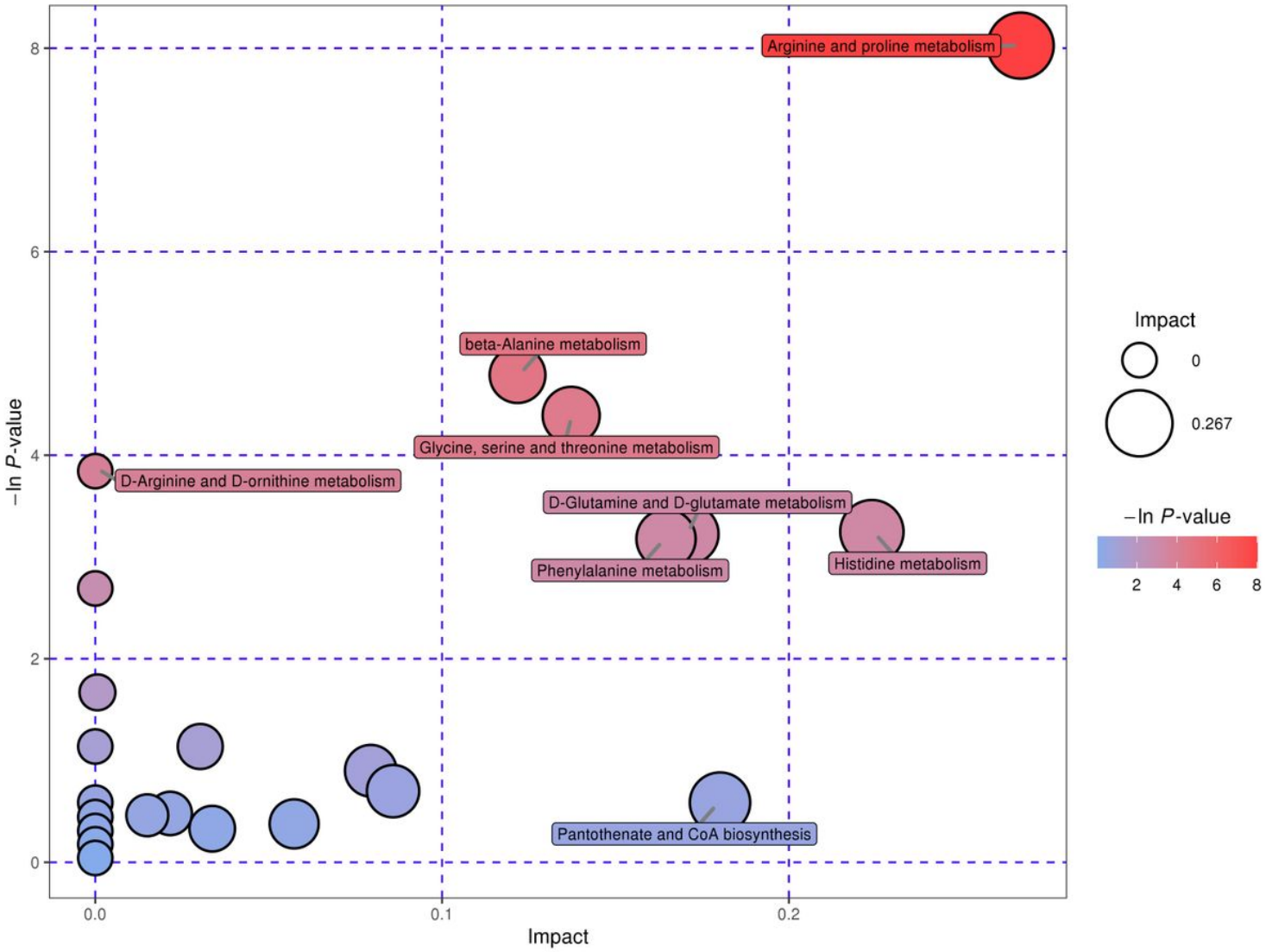

Figure 3

Metabolic pathway enrichment of serum samples. The major metabolic pathways were Arginine and proline, Beta-alanine, and Glycine, serine, and threonine.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable.xlsx
- SupplementaryFigureFV.docx