Differences in the Microbial Composition of Hemodialysis Patients Treated With and Without β-Blockers

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Research

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Abstract

Background:

β-blockers are commonly prescribed medications to treat cardiovascular disease and prevent sudden cardiac death during hemodialysis. Beyond the medication effects on the host, no study has investigated the impact of β-blocker on the gut microbiota in hemodialysis patients.

Results:

The β-blocker users had a higher proportion of diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, heart failure, cerebrovascular disease, and concurrent medications than non-users. After propensity score (PS) matching, there were no differences in comorbidities and concomitant medications. The α-diversity (Simpson index) increased in β-blocker users with a distinct β-diversity (Bray-Curtis Index) compared to non-users in the full cohort and PS-matched cohort. In the linear discriminative analysis effect size analysis and zero-inflated Gaussian fit model, there was a significant enrichment in the genus *Flavonifractor* in β-blocker users compared to non-users in the full cohort and PS-matched cohort; this was confirmed by random forest analysis.

Conclusions:

Hemodialysis patients using β-blocker used had a different gut microbiota composition compared to non-users, in particular, the *Flavonifractor* genus was significantly increased with β-blocker treatment. These findings highlight the significant impact of β-blockers on the gut microbiome in hemodialysis patients. Further research is warranted regarding the mechanisms and their clinical consequences.

Background

The gut microbiota has a crucial role in metabolic, nutritional, physiological, defensive and immunological processes in the human body, with its composition linked to human health and the development of diseases.[1, 2] Changes in this microbial equilibrium, that is, dysbiosis, promotes and influences the course of many intestinal and extra-intestinal diseases.[3–5] In addition to genetic and environmental factors, several common medications (e.g., proton pump inhibitors, nonsteroidal anti-inflammatory drugs, atypical antipsychotics, selective serotonin reuptake inhibitors, antibiotics, statins, and antidiabetic drugs) are associated with the specific gut microbiota composition.[6–12] Indeed, drug-microbiome-host interactions are complex and multifactorial, impacting host metabolism[13, 14], hence, should be part of the core phenotype set for human gut microbiota research.[15]

Patients with chronic kidney disease (CKD) have altered gut microbiota, with a bidirectional causal effect relationship.[16, 17] Among the cardiovascular preventive drugs for patients with end-stage renal disease

(ESRD), β-blockers are commonly prescribed in higher cardiovascular risk patients to prevent sudden cardiac death.[18, 19] Beyond the clinical effect of β-blockers in ESRD patients, they also have a potential impact on gut microbiota.[6, 15] Besides, the benefit of beta-blockers may be attributed to preventing the activity of the gut microbe-generated metabolite, such as phenylacetylglutamine.[20] However, limited study has investigated the impact on ESRD patients. Herein, we evaluate the gut microbiota composition of β-blocker users and non-users in Taiwanese hemodialysis patients.

Methods

Study participants

The study protocols were approved by the Ethics Committee of Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20160095 and KMUHIRB-E(I)-20180118) and Taipei Tzu Chi Hospital (07-X01-002). All participants provided written informed consent. Hemodialysis (HD) patients were recruited from the dialysis unit of Taipei Tzu Chi Hospital and Kaohsiung Medical University Hospital in Taiwan from August 2017 to February 2018. Eligible participants were those who received regular HD three times per week, 3.5–4 hours with high-flux dialyzers. Participants with active malignancies or participants who were prescribed antibiotics within three months before enrollment were excluded. Fecal samples were collected from 193 stable HD patients and analyzed by high-throughput 16S ribosomal RNA gene sequencing to compared participants with and without β-blocker treatment. All β-blocker users were prescribed for at least one month.

Comorbidity, laboratory, and clinical variables

All baseline characteristics of sociodemographic data, age, sex, dialysis vintage, arteriovenous shunt type, comorbidities, medications, and biochemical data were collected in the built-in electronic health care system. Blood samples were collected after overnight fasting through the arteriovenous fistula or graft before scheduled HD sessions. The biochemical data included serum values for hemoglobin, albumin, high sensitivity C reactive protein, total cholesterol, low-density lipoprotein, triglycerides, ion calcium, and phosphate from routine blood samples obtained within 30 days before stool sample collection. Diet was evaluated by a licensed dietitian using a modified short-form food frequency questionnaire. Diabetes was defined as HbA1C 6.5% or higher, or use of oral antidiabetic agents or insulin. Hypertension was defined as 140/90 mmHg or higher or taking blood pressure-lowering drugs. A history of myocardial infarction or documented by coronary angiography, class III or IV congestive heart failure, or a cerebrovascular accident were defined as cardiovascular disease.

Fecal sample collection and bacterial 16S rRNA amplicon sequencing and processing

All stool samples were frozen immediately after collection by each participant, then delivered in cooler bags to the laboratory (Germark Biotechnology, Taichung, Taiwan) within 24 hours. A QIAamp DNA Stool Mini Kit (Qiagen, MD, USA) was used to extract DNA from fecal samples. Barcode-indexed PCR primers (341F and 805R) were used to create an amplicon library by amplifying the variable regions 3 and 4 (V3-

V4) of the 16S rRNA gene.[21] The amplicons were sequenced (300 bp paired-end) using an Illumina MiSeq sequencer at the same time in the same laboratory to avoid batch effects (Germark Biotechnology, Taichung, Taiwan). The 16S-amplicon pipeline was adapted from 16S Bacteria/Archaea SOP v1 of Microbiome Helper workflows.[22] Paired-End reAd mergeR (PEAR; version 0.9.8)[23] was used to merge paired-end reads to raw reads, then filtered low-quality reads by thresholds of sequence length ≥400 bp and quality score of 90% bases of reads ≥20. Quantitative Insight Into Microbial Ecology (QIIME; version 1.9.1) software was used to select operational taxonomic units (OTU).[24] The SILVA (version 123) 16S database[25, 26] was applied to cluster OTUs and assign taxonomy using UCLUST algorithm[27] with a 97% sequence identity threshold. Reads were dereplicated and singletons were discarded. The final OTU table was rarefied into minimum sequencing depth in the data set.

Propensity score matching

Propensity score (PS) matching[28, 29] was performed to balance confounders between the comparisons of interest (i.e., β-blocker users versus non-users) and minimize the confounding by indication resulting from nonrandom treatment study. Using a logistic regression model, β-blocker use was accessed to estimate the propensity to receive a β-blocker for each participant based on potential confounders, including age, sex, dialysis vintage, vascular access type, Bristol stool scale, dietary intake, comorbidities (diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, heart failure, cerebrovascular disease, and parathyroidectomy history), concomitant drugs used (including ACEI [angiotensin converting enzyme inhibitors]/ARB [angiotensin-receptor blockers], glucose-lowering drugs [such as sulfonylurea, dipeptidyl peptidase-4 inhibitors, insulin], statin, calcium carbonate, and proton pump inhibitors), and clinical laboratory data (hemoglobin, albumin, total cholesterol, triglyceride, high sensitivity C reactive protein [hsCRP], sodium, potassium, total calcium, phosphate, parathyroid hormone, serum iron, ferritin, normalized protein catabolic rate [nPCR], and single pool Kt/V). In this study, 193 hemodialysis patients were enrolled, including 83 β-blocker users and 110 non-users (full cohort). PS-matched (1:1) analysis was used to match participants with β-blocker treatment (N=62) to participants without β-blocker treatment (N=62) (PS-matched cohort, **Figure 1**).

Statistical and bioinformatics analyses of microbiota

The study design is presented in **Figure 1**. Demographic characteristics are shown as the mean, median, or frequency, with differences between β-blocker users and non-users determined using an independent T-test or chi-squared test, as appropriate. A rarefaction curve was built to prevent methodological artifacts originating from variations in sequencing depth. The α-diversity (Shannon index and Simpson index) was estimated with the R “vegan” package and calculated using the *p*-value by the Kruskal-Wallis test. The β-diversity (measured by Bray-Curtis dissimilarity) was visualized through a Principal Coordinates Analysis (PCoA) and calculated using homogeneity of group dispersions by Permutational Analysis of Multivariate Dispersions (PERMDISP) to evaluate the difference/similarity of bacterial communities between the groups.[30]

Co-occurrence analysis was used to determine the relationships within communities, with core microbiome analysis performed at the genus level using MicrobiomeAnalyst[31], in which sample prevalence and relative abundance cut off values were set at 20 and 0.2%, respectively. For visualization of the internal interactions and further measurement of the microbial community, SparCC was used to calculate the Spearman correlation coefficient with the corresponding *p*-value between every two taxa. Microbiota community structure was assessed by co-occurrence networks built by the Sparse Correlations for Compositional data (SparCC) algorithm.[32] The *p*-values were estimated using a bootstrap procedure with 100 random permutations and iterations for each SparCC calculation, and correlation matrices were computed from the resampled data matrices. Only OTUs with correlation scores greater than 0.4 and *p*-value less than 0.05 were categorized into co-abundance groups (CAGs); these coefficients were also used to assess the length of edges on the network. An undirected network, weighted by SparCC correlation magnitude, was generated using bioinformatics tools in MicrobiomeAnalyst.[31]

The bacterial OTU difference between β-blocker users and non-users by the linear discriminant analysis (LDA) of effect size (LEfSe) analysis with more than 0.1% relative abundance and present in >30% of samples. The LEfSe analysis employed the non-parametric factorial Kruskal-Wallis test or Wilcoxon rank-sum test and LDA to identify differentially abundant taxa between the groups. Only taxa with LDA score greater than two or less than two at a *p*-value <0.05 were considered significantly enriched. All statistical tests are two-tailed, and a *p*-value < 0.05 was considered statistically significant. The random forest method[33] was performed to determine a ranked list of all bacterial taxa to identify the most predictive bacterial community to classify β-blocker users and non-users. The random forests is a supervised learning algorithm ranking OTUs based on their ability to discriminate among the groups, while accounting for the complex interrelationships in high dimensional data. The MetagenomeSeq method was also used to evaluate differential abundance in sparse marker-gene survey data using a zero-inflated Gaussian (ZIG) fit model to account for undersampling and sparsity in OTU count data after normalizing the data through cumulative sum scaling (CSS).[34] Finally, the log-transformed read counts difference of the top selected genera from the ZIG fit model between β-blocker users and non-users was analyzed in the full and PS-matched cohorts.

Co-occurrence and random forest analyses were performed by MicrobiomeAnalyst.[31] The other statistical analyses were performed using R statistical software (version 3.5.1) and STATA statistical software (version 14).

Results

Patient characteristics

Patient characteristics are shown in **Table 1**, with those receiving β-blockers having a higher proportion of diabetes, hypertension, dyslipidemia, coronary artery disease, heart failure, cerebrovascular disease, and more commonly used ACEI/ARB, glucose-lowering drugs (such as dipeptidyl peptidase-4 inhibitors or

insulin) and statin. PS matching resulted in 62 matched pairs with balanced baseline characteristics (**Table 1**).

Gut microbiota profile differs in HD patients with and without β blocker treatment

The rarefaction curves were close to asymptotic based on the number of OTUs observed. To represent the microbiome community with enough coverage, the rarefaction curves reached saturation at a cutoff point of 45,000 sequences per sample (**Supplementary Figure 1**). Compared to the gut microbiota composition and structure between β-blocker users and non-users, no substantial differences were observed in the relative abundance proportion in the full and PS-matched cohorts (**Supplementary Figure 2**). Hemodialysis patients taking β-blockers had a higher α-diversity and a distinct β-diversity compared to non-users in the full and PS-matched cohorts (**Figure 2**). The core microbiome was *Bacteroides* in hemodialysis patients (**Supplementary Figure 3A**), with a similar core microbiome in β-blocker users and non-users (**Supplementary Figure 3B**).

Specific microbial taxa differences between β-blocker users and non-users

Discriminant analysis using LEfSe identified the significant differentiating taxa between study groups. In the full cohort, the genera *Ruminococcus 2*, *Collinsella*, *Ruminococcaceae UCG-004*, *Ruminiclostridium 5*, *Anaerotruncus*, *Eisenbergiella*, and *Flavonifractor* were enriched in β-blocker users compared to non-users (**Figure 3A**). In the PS-matched cohort, the enriched genera were *Faecalibacterium*, *Subdoligranulum*, *Tyzzerella*, *Pantoea*, *Lachnospiraceae UCG-004*, and *Flavonifractor* were found (**Figure 3B**). Using random forest models for taxonomy prediction, the top three ranked genera to discriminate between β-blocker users and non-users were *Parabacteroides*, *Flavonifractor*, and *Ruminococcaceae UCG-004* in the full cohort (**Figure 4A**), *Prevotella 9*, *Flavonifractor*, and *Tyzzerella* in the PS-matched cohort (**Figure 4B**).

To reduce the effect of zero-inflation in the microbiome data, we performed the MetagenomeSeq algorithm integrating the CSS method and a statistical model based on the ZIG distribution to improve the power for differential abundance analysis of the microbiome data. Evaluating the significant difference in genus taxonomy between β-blocker users and non-users, we found eight genera differences in the full cohort and PS-matched cohort (**Supplementary Table 1**). There were three different genera (*Flavonifractor*, *Tyzzerella*, and *Prevotellaceae NK3B31 group*) in both the full and PS-matched cohort s(**Figure 5A**). Focusing on the ZIG fit model to predict specific genera, there was an increased *Flavonifractor* genus in β-blocker users compared to non-users using a classical univariate test (Kruskal-Wallis test) in the full (p = 0.023) and PS-matched cohorts (p = 0.01) (**Figure 5B**). However, no differences were found in *Tyzzerella* or *Prevotellaceae NK3B31 group* (**Figure 5B**).

Discussion

In the present study, hemodialysis patients treated with β-blockers had increased α-diversity and a distinct β-diversity compared to non-users. The microbial communities contained higher levels of *Bacteroidetes* and lower levels of *Firmicutes* in all hemodialysis patients, which is similar to CKD rat microbial

communities[35] and in a human CKD microbiota study.[36] Co-occurrence analysis revealed no difference in keystone taxa *Bacteroides* between β-blocker users and non-users. Overall, there was an enriched genus *Flavonifractor* in β-blocker users in the full and PS-matched cohorts. Furthermore, LEfSe analysis, random forest algorithm, ZIG fit model, and univariate test all confirmed this difference between groups.

β-blocker use was associated with a higher α-diversity than non-users in hemodialysis patients, which was linked to a favorable healthy state.[37] Increased α-diversity has been associated with foods generally considered as healthy, such as plant consumption or red wine.[38–40] Furthermore, commonly used medications such as antibiotics or proton pump inhibitors can decrease gut α-diversity.[41] Regarding the specific taxonomy of the gut microbiome, the genus *Flavonifractor* was enriched in β-blocker users in both the full and PS-matched cohorts. *Flavonifractor* is associated with several diseases, such as obesity,[42] atrial fibrillation,[43] coronary artery disease,[44] major depressive disorder,[45] and bipolar disorder[46], and medications (antidiabetic drugs, such as Metformin and Glucagon-like peptide 1 Receptor agonist[47]). It can convert quercetin or other flavonoids into acetic acid and butyric acid[48] and is also correlated with oxidative stress and inflammation.[46] Taken together, *Flavonifractor* is significantly less abundant in the subjects with obesity,[42] atrial fibrillation,[43] or coronary artery disease,[44] thus, the increased abundance of *Flavonifractor* by β-blocker treatment may have a potential benefit in cardiovascular disease via gut microbiota regulation. However, an enriched *Flavonifractor* in major depressive disorder[45] and bipolar disorder[46] could also link the relationship between β-blockers and major depressive disorder or bipolar disorder.[49]

We also identified a potential link between β-blocker use and the genus *Tyzzerella* in the PS-matched cohort. Importantly, *Tyzzerella* was enriched in those with a high cardiovascular risk profile.[50] However, the small sample size limited the potential association between β-blocker use and *Tyzzerella* in univariate analysis, so more extensive studies are needed to confirm this association. Regarding the link between β-blocker and microbiota changes, a chimera mouse model suggested bone marrow beta1/2 adrenergic receptor signaling can regulate host-microbiota interactions, leading to the generation of novel anti-inflammatory treatments for gut dysbiosis.[51] Therefore, depletion of this sympathetic regulation in bone marrow promotes beneficial shifts in gut microbiota associated with gut immune suppression.[51] It is proposed that beta-blockers may provide a beneficial microbiome in such conditions.

In the present study, we compared the differences in the microbiota between β-blocker users and non-users using PS matching analysis. Since β-blocker intake is highly correlated with age, cardiovascular risk, comorbidities, and concurrent medication, each factor represents a relevant confounder for microbiome analyses.[15, 52] Most observational studies have controlled for possible confounding variables, but even rigorous data adjustment cannot eliminate the risk of bias. PS matching is an alternative to reduce the effect of influencing factors on gut microbiota analysis,[28, 29]; thus, we selected variables of interest as potential confounders, then performed PS matching to reduce the effects of these deviations and confounding variables to conduct a reasonable comparison between groups. The intestinal microbiota was affected by various factors, including demographic data, comorbidities,

concomitant medications, and clinical laboratory data, and the application of PS matching eliminated confounding factors. Using PS analysis, there was still a higher α-diversity and different β-diversity in β-blocker users compared to non-users. We also identified six genera associated explicitly with the β-blocker user in the LEfSe analysis, four top-ranked genera in random forest analysis, and eight genera in ZIG fit model analysis. Although there some differences in bacterial associations with β-blocker use in our full (before PS matching) and PS-matched cohorts, we investigated the taxa represented in both the full and PS-matched cohort. For example, our full cohort analysis found that β-blocker treatment was associated with the genus *Ruminococcaceae UCG-004* in LEfSe analysis, random forest algorithm, and ZIG fit model analysis but was attenuated after PS matching, suggesting that *Ruminococcaceae* abundance may be more strongly associated with other confounding variables, such as comorbidities or concomitant medications, which was accounted for in the PS models.

In addition, there were more zeros than expected under the assumption of Poisson or negative binomial distributions for microbiome OTU counts, known as zero-inflation. One popular strategy to circumvent the zero-inflation problem is to add a pseudo-count[53]; however, this assumption may not be appropriate due to the large extent of structural zeros due to physical absence. Moreover, the choice of the pseudo-count is arbitrary, and the clustering results can be highly dependent upon the choice.[54] Thus, CSS was developed for microbiome sequencing data and a zero-inflated model was used to model read counts that have an excess of zeros.[34, 55, 56] In CSS, raw counts are divided by the cumulative sum of counts up to a percentile determined using a data-driven approach to capture the relatively invariant count distribution for a dataset. To solve the zero-inflation issue, we applied the ZIG fit model and calculated the CSS. Interestingly, the four genera in the full (*Ruminococcaceae UCG-004*, *Ruminiclostridium 5*, *Anaerotruncus*, and *Flavonifractor*) and PS-matched cohorts (*Flavonifractor*, *Tyzzerella*, *Faecalibacterium*, *Subdoligranulum*) overlapped in the LEfSe analysis and ZIG fit model analysis.

Several limitations should be mentioned. First, cross-sectional studies only provide an impression of the relative abundance of bacterial taxa at a single time point, so causal inference cannot be addressed. Second, the microbiota was assessed with a fecal sample, which may differ from microbiota from other parts of the intestine. Besides, 16S rRNA sequencing is limited as it cannot differentiate viable from non-viable bacteria. A significant portion of the taxa identified by sequencing may not be metabolically active. Thus, further study is needed to investigate various samples, such as small intestine or colon mucosal bacteria. Finally, the study was performed in Asia HD patients, whose diet is different from other populations, so the results regarding dietary effects on gut microbiome should be interpreted with caution.

Conclusions

This study demonstrated that the composition of the gut microbiota was different in HD patients treated with β-blockers, with an increased α-diversity and genus *Flavonifractor.* These findings support the additional benefits of β-blocker treatment, which may mediate the microbiota in HD patients. However, the functional relevance of the β-blocker induced microbial differences is unclear. Hence, larger prospective

treatment naïve studies are warranted to understand the impact of β-blockers on the gut microbiome of CKD patients and their implications for health and disease.

Abbreviations

PS, propensity score; CKD, chronic kidney disease; ESRD, end-stage renal disease; HD, hemodialysis; QIIME, Quantitative Insight Into Microbial Ecology; OTU, operational taxonomic units; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin-receptor blockers; hsCRP, high sensitivity C reactive protein; nPCR, normalized protein catabolic rate; PCoA, Principal Coordinates Analysis; PERMDISP, Permutational Analysis of Multivariate Dispersions; SparCC, Sparse Correlations for Compositional data; LEfSe, linear discriminant analysis of effect size; ZIG, zero-inflated Gaussian; CSS, cumulative sum scaling;

Declarations

Ethics approval and consent to participate

This study protocols were approved by the Ethics Committee of Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20160095 and KMUHIRB-E(I)-20180118) and Taipei Tzu Chi Hospital (07-X01-002).

Consent for publication

Not applicable.

Availability of data and material

The dataset using in the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Tables

Table 1: Baseline characteristics of hemodialysis patients with and without β blocker treatment

Baseline characteristics	Before propensity score matching			After propensity score matching		
	β blocker users (N = 83)	β blocker non-users (N = 110)	*P* value	β blocker users (N = 62)	β blocker non-users (N = 62)	*P* value
Age (years)	64.3 ± 11.4	65.4 ± 11.2	0.511	64.7 ± 11.6	66.3 ± 11.8	0.446
Male	49 (59.0%)	57 (51.8%)	0.318	37 (59.7%)	28 (45.2%)	0.106
Dialysis vintage (months)	86.24 ± 56.53	96.54 ± 63.21	0.243	93.22 ± 57.61	85.4 ± 55.67	0.444
Arteriovenous fistula	75 (90.4%)	99 (90.0%)	0.934	57 (91.9%)	57 (91.9%)	>0.999
Comorbidities						
Diabetes mellitus	45 (54.2%)	34 (30.9%)	0.001	24 (38.7%)	30 (48.4%)	0.277
Hypertension	80 (96.4%)	87 (79.1%)	<0.001	59 (95.2%)	59 (95.2%)	>0.999
Dyslipidemia	31 (37.3%)	24 (21.8%)	0.018	16 (25.8%)	15 (24.2%)	0.836
Coronary artery disease	34 (41.0%)	22 (20.0%)	0.001	21 (33.9%)	18 (29.0%)	0.562
Heart failure	22 (26.5%)	15 (13.6%)	0.025	14 (22.6%)	11 (17.7%)	0.502
Cerebrovascular disease	31 (37.3%)	24 (21.8%)	0.018	5 (8.1%)	8 (12.9%)	0.379
Parathyroidectomy history	7 (8.4%)	18 (16.4%)	0.104	6 (9.7%)	6 (9.7%)	>0.999
Medications						
ACEI/ARB	29 (34.9%)	24 (21.8%)	0.043	23 (37.1%)	15 (24.2%)	0.119
Glucose lowering drugs	34 (41.0%)	23 (20.9%)	0.003	20 (32.3%)	19 (30.6%)	0.847
Sulfonylurea	14 (16.9%)	13 (11.8%)	0.317	6 (9.7%)	11 (17.7%)	0.192
Dipeptidyl peptidase 4 inhibitors	28 (33.7%)	13 (11.8%)	<0.001	17 (27.4%)	11 (17.7%)	0.198

Insulin	17 (20.5%)	10 (9.1%)	0.024	9 (14.5%)	8 (12.9%)	0.794
Statin	29 (34.9%)	17 (15.5%)	0.002	17 (27.4%)	12 (19.4%)	0.289
Calcium carbonate	67 (80.7%)	94 (85.5%)	0.382	51 (82.3%)	50 (80.6%)	0.817
Proton pump inhibitors	13 (15.7%)	10 (9.1%)	0.163	9 (14.5%)	7 (11.3%)	0.592
Clinical laboratory data						
Hemoglobin (g/dl)	10.62 ± 1.14	10.71 ± 1.41	0.650	10.6 ± 1.05	10.74 ± 1.49	0.555
Albumin (g/dl)	3.52 ± 0.51	3.56 ± 0.46	0.538	3.53 ± 0.46	3.54 ± 0.47	0.902
Total cholesterol (mg/dl)	154.01 ± 33.75	161.89 ± 33.62	0.109	151.94 ± 33.57	163.51 ± 35.30	0.064
Triglyceride (mg/dl)	140.52 ± 103.77	129.61 ± 90.35	0.437	136.21 ± 105.99	131.14 ± 95.51	0.780
High sensitivity CRP (mg/dl)	2.15 ± 4.65	2.5 ± 4.21	0.589	2.45 ± 5.23	2.21 ± 3.95	0.779
Sodium (mmol/l)	136.92 ± 2.68	137.07 ± 2.62	0.700	137.19 ± 2.80	136.64 ± 2.44	0.241
Potassium (mmol/l)	4.73 ± 0.68	4.61 ± 0.62	0.195	4.77 ± 0.66	4.65 ± 0.65	0.294
Total calcium (mg/dl)	9.15 ± 0.86	9.29 ± 0.94	0.277	9.19 ± 0.92	9.25 ± 0.86	0.683
Phosphate (mg/dl)	5.08 ± 1.21	4.95 ± 1.24	0.453	5.16 ± 1.15	5.09 ± 1.35	0.768
Parathyroid hormone (pg/ml)	376.53 ± 338.79	383.5 ± 278.13	0.876	394.16 ± 370.62	357.29 ± 245.84	0.515
Serum iron (μg/dl)	63.57 ± 26.73	65.85 ± 21.16	0.508	63.94 ± 26.61	67.52 ± 22.93	0.424
Ferritin (ng/ml)	567.53 ± 549.64	496.67 ± 377.33	0.291	534.93 ± 330.67	538.54 ± 413.54	0.957
nPCR (g/kg/day)	1.12 ± 0.21	1.16 ± 0.27	0.326	1.12 ± 0.20	1.18 ± 0.28	0.180
Single pool Kt/V	1.67 ± 0.27	1.65 ± 0.27	0.591	1.67 ± 0.28	1.68 ± 0.27	0.817
Dietary intake (serving/day)						

Meat	0.86 ± 0.57	0.82 ± 0.53	0.652	0.86 ± 0.57	0.74 ± 0.52	0.241
Vegetable	2.01 ± 1.09	1.86 ± 1.11	0.265	2.05 ± 1.06	1.91 ± 1.18	0.499
Fruit	0.93 ± 0.72	0.95 ± 0.72	0.583	0.86 ± 0.63	0.89 ± 0.75	0.837
Bristol stool scale	3.94 ± 1.86	3.74 ± 1.76	0.448	4 ± 1.78	3.71 ± 1.67	0.352

* ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers; CRP, C reactive protein; nPCR, normalized protein catabolic rate

Figures

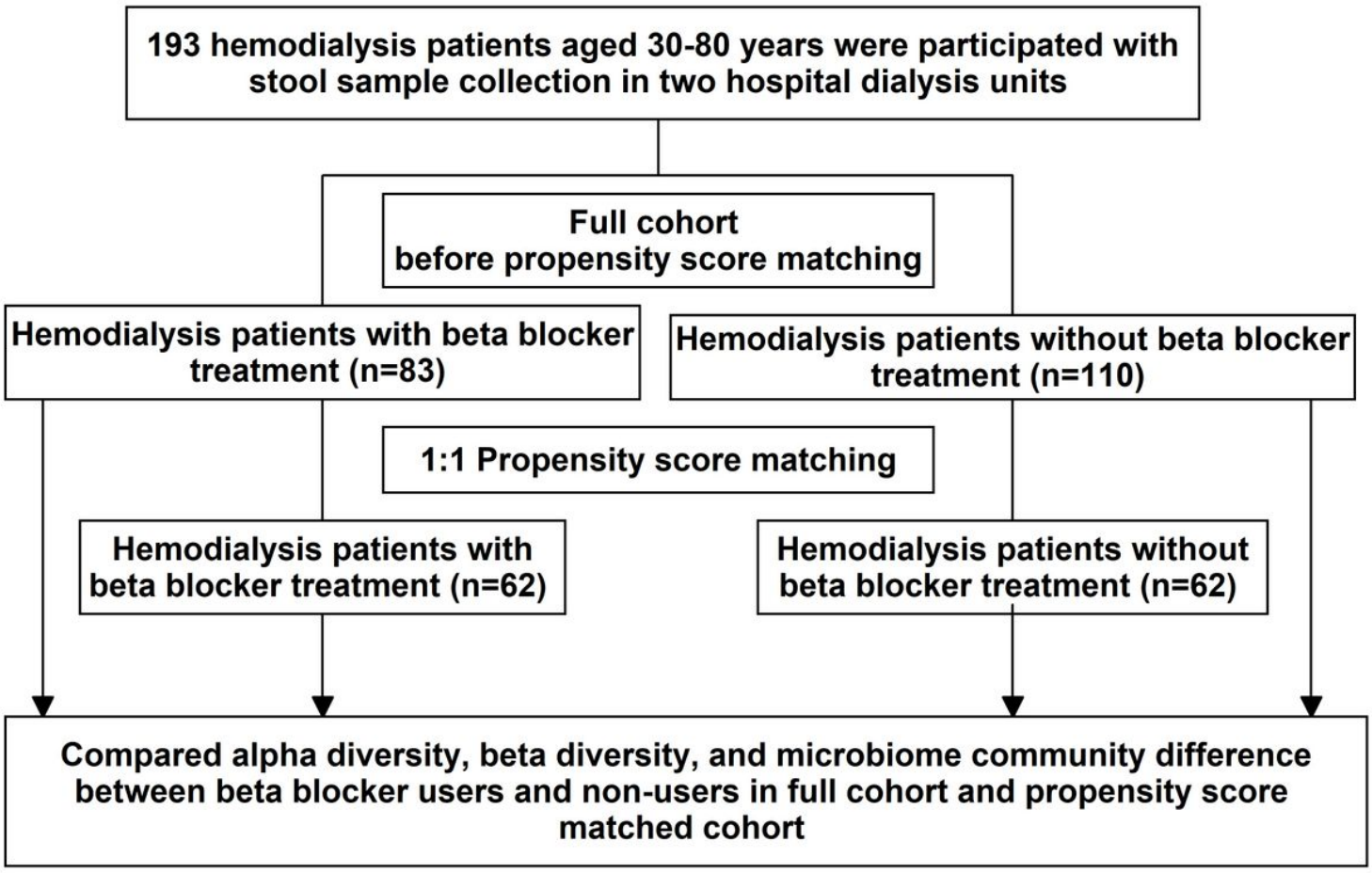

Figure 1

Study design.

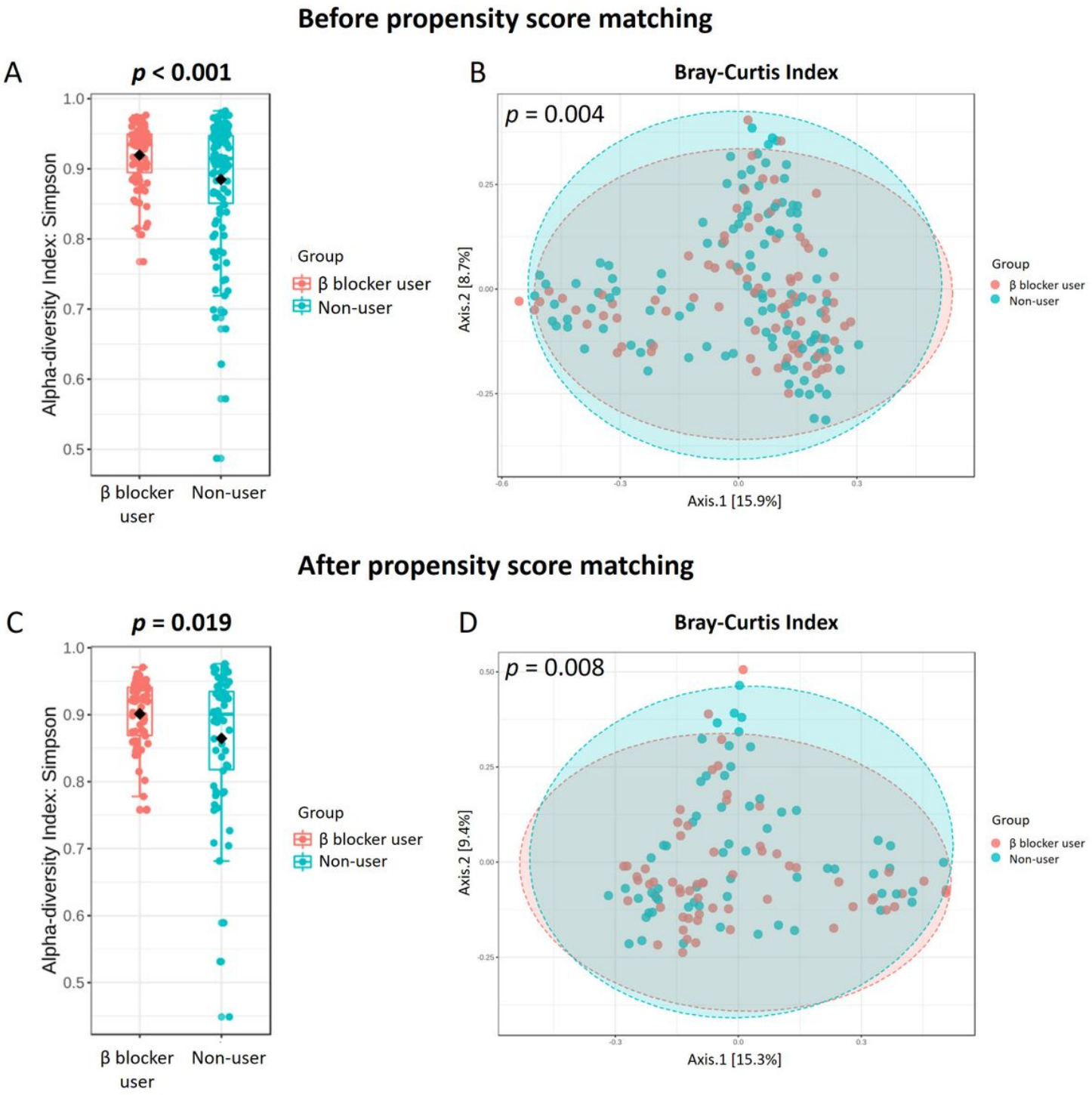

Figure 2

The α-diversity and β-diversity in hemodialysis patients with and without β blocker used in full cohort (A, B) and propensity score matching cohort (C, D).β blocker users had a higher α-diversity than β blocker non-users in full cohort (A) and propensity score matching cohort (C) β blocker users had a different β-diversity (Bray-Curtis index) compared to β blocker non-users in full cohort (B) and propensity score matching cohort (D). The β-diversity p-value was calculated using the homogeneity of group dispersions by the PERMDISP test.

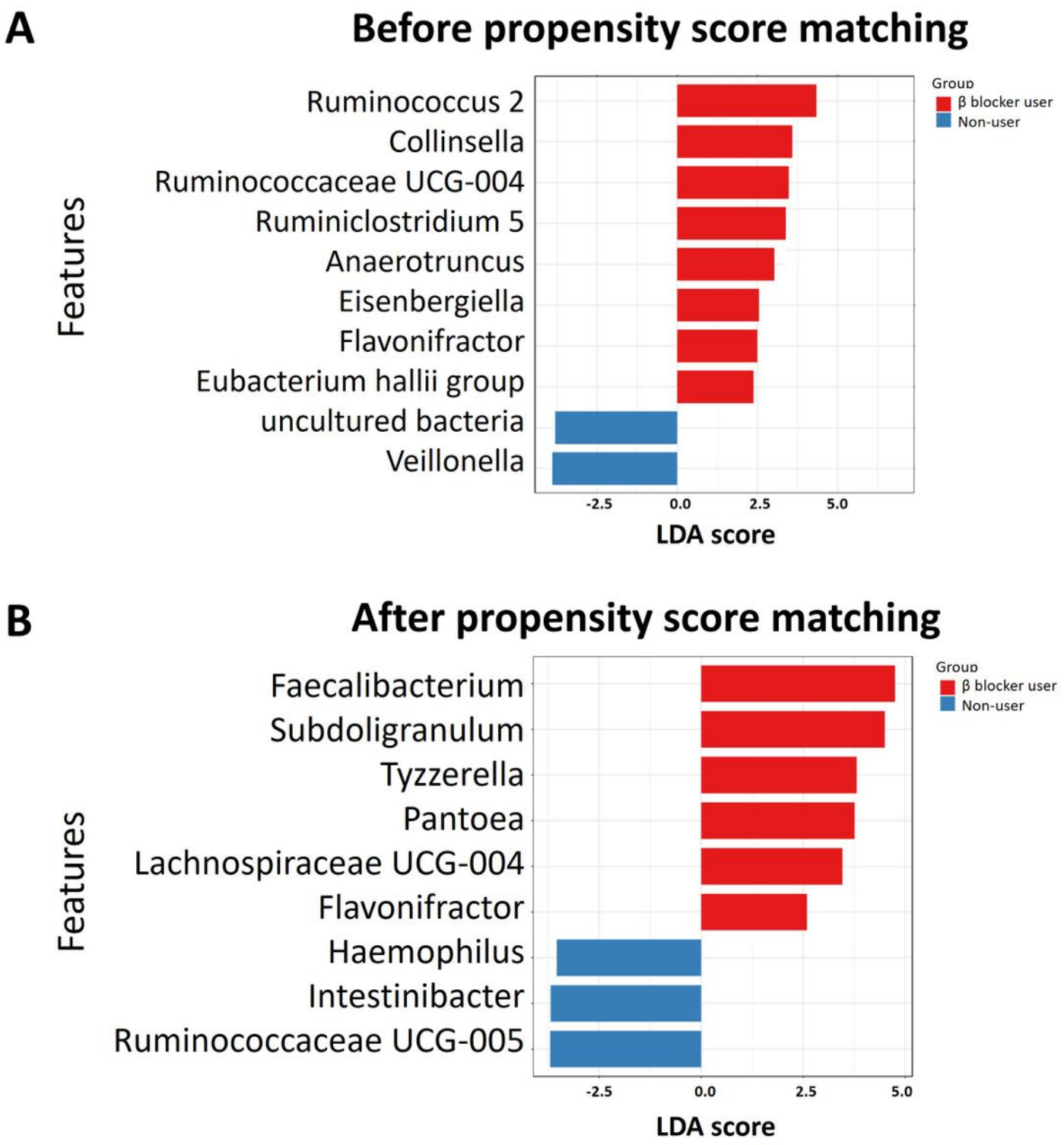

Figure 3

Taxonomic differences were detected between β blocker users and non-users in the full cohort (A) and propensity score matching cohort (B). Linear discriminative analysis (LDA) effect size (LEfSe) analysis between β blocker users (red) and nonusers (blue) with an LDA score > 2.0 or < -2 with p value >0.1 among β blocker users and non-users.

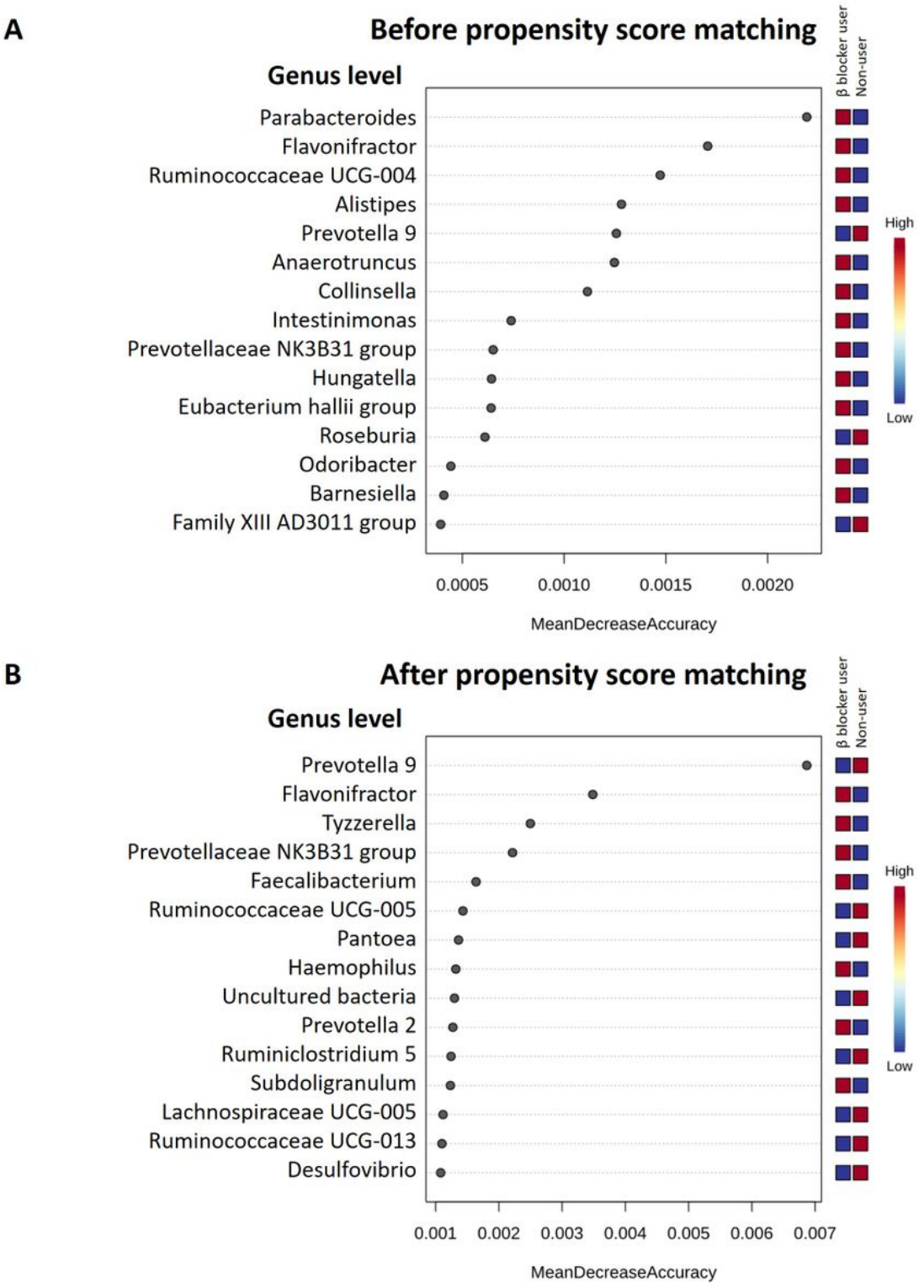

Figure 4

Determination of specific bacteria for discriminatory across hemodialysis patients with and without β blocker treatment in full cohort (A) and propensity score matching cohort (B). The discriminatory taxa were determined by applying Random Forest analysis using the genus-level abundance.

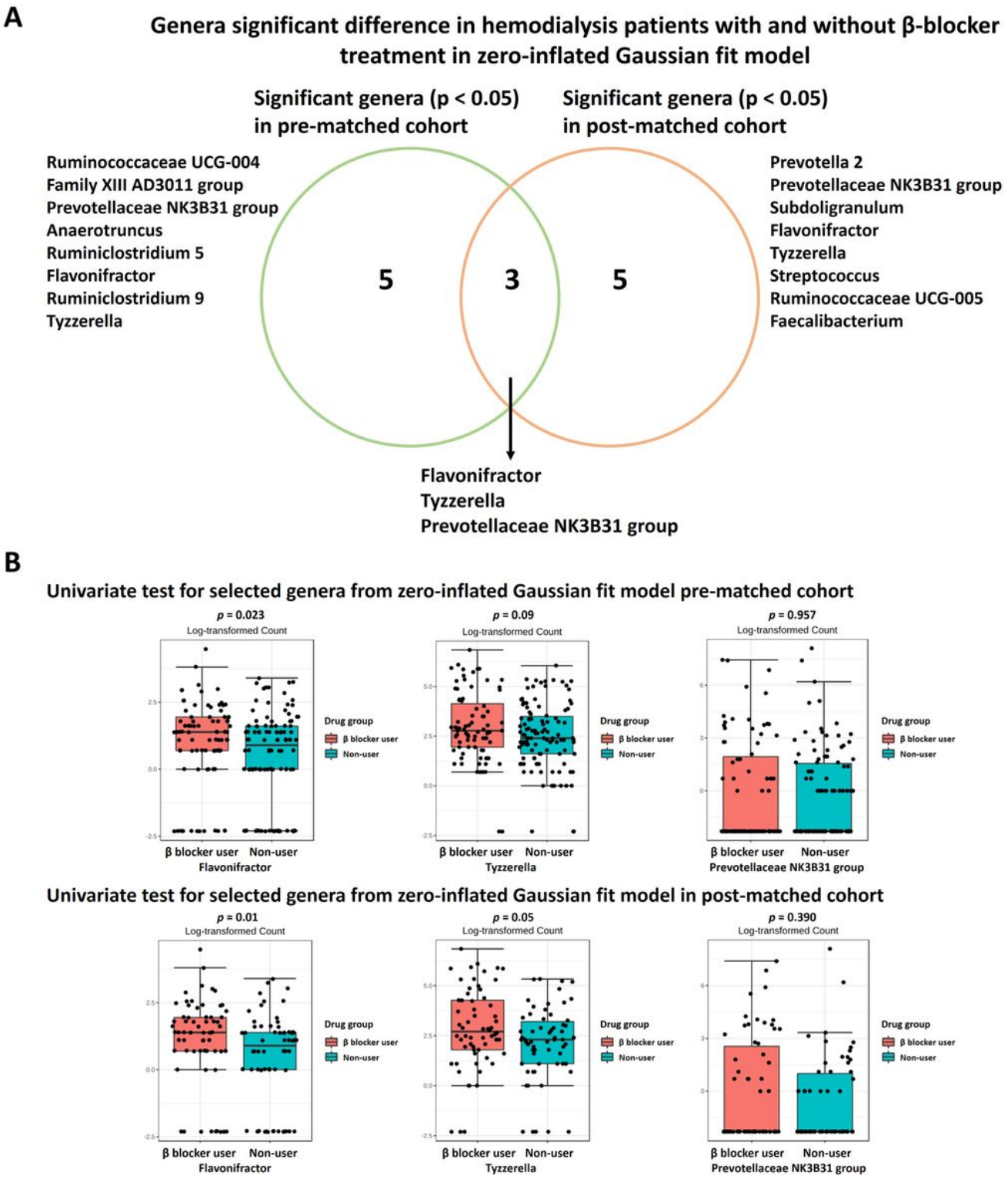

Figure 5

The genera difference between β blocker users and non-users in the full cohort and propensity score matching cohort using zero-inflated Gaussian fit model. (A) Venn diagram showed the different significant genera in the full cohort and propensity score-matched cohort. (B) Univariate test between selected genera from zero-inflated Gaussian fit model. Significance was considered for p < 0.05.

Supplementary Files

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