The gut microbiome in microscopic polyangiitis with kidney involvement: common and unique alterations, clinical association and values for disease diagnosis and outcome prediction

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Background: Microscopic polyangiitis (MPA) is an autoimmune disease characterized by frequent kidney involvement. Imbalance of intestinal flora has been found implicated in multiple immune-mediated disorders. However, the profiling and the role of the gut microbiome in MPA remains unclear.

Methods: We performed 16S rRNA amplicon sequencing on fecal samples from 71 MPA patients with kidney involvement (35 at incipient active stage, 36 at remissive stage) and 34 healthy controls (HCs). Microbial diversity and abundance were compared among the three cohorts. The correlation between altered microbes and clinical indices were investigated. Two random forest models based on the profiling of the gut microbiome were constructed for the diagnosis of MPA.

Results: Two α-diversity indices, including Simpson and Shannon index, were decreased in MPA patients ($P<0.001$), especially in those with active disease ($P=0.001$). β-diversity analysis showed biased microbial composition among the three groups. Genus *Actinomyces* and *Streptococcus* were more abundant in both MPA cohorts than those in HCs, while genus *Subdoligranulum*, *Eubacterium hallii*, *Ruminococcaceae UCG013*, *Eubacterium ventriosum*, *Dorea* and *Butyricicoccus* were more abundant in HCs than those in both MPA cohorts. All the 6 genera with decreased abundance belong to short-chain fatty acids (SCFA)-producing taxons. Besides, 1 and 2 operational taxonomic units (OTUs) were enriched in patients with active MPA who needed dialysis at sampling and in patients who progressed to end-stage renal disease during follow up, respectively. Furthermore, the model for diagnosis of MPA incorporated 6 OTU markers and achieved an AUC of 93.45% (95% CI, 88.15–98.74%). Similarly, the model for predicting disease activity incorporated 11 OTU markers and achieved an AUC of 90.71% (95% CI, 82.49–98.94%).

Conclusions: Alteration of intestinal flora existed in MPA patients with kidney involvement and was characterized by increased abundance of genus Actinomyces and Streptococcus and decreased abundance of 6 SCFA-producing genera. Gut microbial profiling combined with machining-learning methods showed potentials for diagnosing MPA and predicting disease activity.

Keywords: Microscopic polyangiitis (MPA); anti-neutrophil cytoplasmic antibody (ANCA); gut microbiome; kidney; diagnosis

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Introduction

Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis is an autoimmune disorder characterized by multi-systemic involvement (1). The annual incidence is estimated to 21.8 and 22.6 per million in the United Kingdoms and Japan, respectively (2). Microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA) are two major phenotypes with evident differences in clinical manifestation, pathological features and geographical distribution (3,4). In China, MPA is more common than GPA (5). Notably, more than 90% of MPA patients suffer from kidney involvement (6). In addition, kidney involvement is associated with increased mortality, and advanced renal injury at diagnosis can predict poor renal prognosis (7).

Gut bacteria are essential for the homeostasis of mucosal immunity and the integrity of intestinal barrier (8). Disturbed gut community has been linked to many diseases, including type 2 diabetes (9), atherosclerotic cardiovascular disease (10,11), hepatocellular carcinoma (12), systemic lupus erythematosus (SLE) (13) and chronic kidney disease (CKD) (14-16). Accumulating evidence suggests that certain bacteria may be implicated in the development of ANCA associated vasculitis. Firstly, GPA patients usually have upper respiratory tract involvement and are frequently affected by infective events during relapse (17). Secondly, chronic nasal carriage of *Staphylococcus Aureus* in GPA correlates with increased frequency of relapse and maintenance of trimethoprim-sulfamethoxazole seems useful in patients with upper airway-limited GPA and history of frequent relapses (18,19). In mechanism, studies showed that two bacteria-derived peptides could trigger autoimmunity to autoantigen for ANCA and induce glomerulonephritis in mice (20,21). However, the characteristics of the gut microbiome of MPA have not been investigated, and its role in the development of MPA remains unknown.

Renal biopsy is valuable for the diagnosis and evaluation of MPA related renal injury. However, due to its invasive characteristics, its application is greatly limited. ANCA test is helpful for the diagnosis of MPA, but ANCA is negative in about 15% of patients (22). Thus, more noninvasive tools are urgently needed for the diagnosis and evaluation of MPA. Intriguingly, with advances on genetic sequencing and artificial intelligence, the gut microbiome has been investigated, and its changes were closely related to various diseases, such as hepatocellular carcinoma (12), juvenile idiopathic arthritis and SLE (13).

In this cross-sectional study, we performed 16S rRNA amplicon sequencing to profile the gut microbiome of MPA patients, and further explored its connection with clinical indices, as well as its potential as a diagnosis tool applying machine-learning methods. We present the following article in accordance with the MDAR and STROBE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-1315).

Methods

Study population and sample collection

Patients with incipient active MPA (aMPA), inactive MPA (inMPA), and age and gender-matched healthy controls (HCs) were recruited from 2017 to 2019 at the first affiliated hospital, Zhejiang University School of Medicine. MPA was diagnosed according to 2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitis (23). To minimize the heterogeneity of population, we enrolled only myeloperoxidase (MPO)-ANCA positive patients with kidney involvement. Patients with acute infection, diabetes mellitus, obesity, cancer and other autoimmune disorders (such as inflammatory bowel disease, rheumatoid arthritis and SLE), as well as those who took probiotics or antibiotics within one month before enrollment and at sampling were excluded.

The demographic and laboratory data were obtained through electronic medical record system. Birmingham Vasculitis Activity Score (BVAS) (24) was used to assess MPA activity, and BVAS =0 indicated remission of disease. The patients in aMPA cohort were prospectively followed up for 6 to 12 months. Events of death or end-stage renal disease (ESRD) were recorded.

For the aMPA cohort, fresh fecal samples were collected during in-patient care. For the inMPA and HC cohorts, the samples were collected at clinical visit or during health examination. All samples were frozen at −80 ℃ within one hour after collection. Our study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the ethics committee of the First Affiliated Hospital, Zhejiang University School of Medicine (approval No. 2017-694). Informed consents were available from all participants before sampling.

DNA extraction, library preparation and sequencing

Total genomic DNA was extracted from all fecal samples

using the PowerSoil® DNA Isolation Kit (MO BIO, USA) following the manufacturer's instructions. PCR was then performed to amplify the V3-V4 variable region of 16S rRNA gene. The 16S amplicon library was constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) and sequenced on an Ion S5TM platform at Zhejiang Institute of Microbiology (Hangzhou, China).

Sequence processing

All sequencing reads were filtered by the Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1) (25) and then aligned with GOLD database by UCHIME algorithm to eliminate chimera sequences (26). The obtained reads were subsequently clustered into different Operational Taxonomic Units (OTUs) according to similarity greater than 97% by Uparse 8.1 (27). Sequences with the highest frequency in each OTU were screened as representative sequences. For further taxonomic annotation, representative sequences were aligned with SILVA reference database (28) by uclust (29) at 90% threshold.

Analysis of microbial diversity and comparison of taxonomic abundance

α-diversity including bacterial richness (Ace and Chao1) and diversity (Shannon and Simpson) were calculated by QIIME. Non-metric multi-dimensional Scaling (NMDS), a typical β-diversity analysis, was performed by vegan package. Analysis of similarity (Anosim) was further conducted to statistically compare the community structure among the three groups. Relative abundance of phyla and genera greater than 0.01% were compared between any two groups using Wilcoxon rank sum test with "Benjamini-Hochberg" adjusted P values.

Correlation between altered genera and clinical indices, differential analysis on OTUs

Spearman correlations between the abundance of differential genera and clinical indices in MPA patients were calculated using the psych package 1.9.12. The obtained matrix with "Holm" adjusted P values was visualized by corrplot package 0.84. In order to find OTU markers for severe kidney damage and predicting renal prognosis, we compared the OTU abundance in aMPA patients based on their initial status on dialysis and final status on kidney survival. This differential analysis was performed using ALDEx2 package 1.18.0 and visualized by ggplot2 package 3.3.1.

Microbial community-based classifier models

In order to evaluate the feasibility of gut microbiome in assisting diagnosis of MPA, we implemented a 10-fold cross-validation approach on random forest models (randomForest package 4.6-14) (30) using the profile of OTU abundance from aMPA and HCs. For predicting disease activity, the same method was adopted using the profile of OTU abundance from aMPA and inMPA. The error curves of 5 trials of 10-fold cross-validation were averaged. The minimum averaged error plus the corresponding standard deviation (SD) was set as the cutoff. The optimal set of OTU markers was selected based on two terms: cross-validation error less than the cutoff and the least OTU numbers. Next, the probability of MPA or active disease was compared between groups using the optimal set of OTUs. The ROC curve was drawn using pROC package 1.16.1.

Statistical analysis

For continuous variables, normally distributed data was expressed as mean ± SD and skewed distributed data was expressed as median with interquartile range (IQR). Categorical variables were expressed as percentage. Difference between two groups was compared by Wilcoxon rank sum test or unpaired *t*-test. Multiple group comparisons were conducted by Kruskal-Wallis test and P values were adjusted using "Benjamini-Hochberg" method or "Holm" method. A difference with $P<0.05$ was considered a statistically significance. All statistical analysis was performed using R 3.6.1 or SPSS 22.0.

Results

Clinical and laboratory characteristics of all participants

A total of 71 different patients with MPA (35 with active MPA and 36 with inactive MPA) and 34 HCs met our inclusion criteria (Figure S1). The clinical, laboratory and histopathological findings of all participants were detailed in Table S1. There was no significant difference in age, gender and BMI among these groups (*Table 1*). Both aMPA and inMPA patients had higher levels of white blood cell ($P<0.001$, 0.001, respectively) and serum creatinine (both P

Table 1 The clinical characteristics and laboratory results of all enrolled participants (n=105)

Parameter	HC (n=34)	inMPA (n=36)	aMPA (n=35)
Demographic characteristic			
Age, yr	58 [53–63]	61 [55–65]	61 [55–68]
Male, n [%]	12 [35]	20 [56]	19 [54]
BMI, kg/m^2	21.37±2.1	22.75±3.76	21.70±3.46
Laboratory parameter			
WBC, 10^{12}/L	5.4±1.1	6.8±2.2**	7.6±2.7**
Hb, g/L	138±18	122±20**	83±18**##
PLT, 10^9/L	216±42	203±54	189±90*
Scr, mmol/L	59 [52–66]	129 [97–167]**	330 [206–533]**##
Up, g/24 h	–	0.62±0.48	2.03±1.21##
CRP, mg/L	–	2.3 [1.1–3.7]	7.6 [4.0–15.2]##
ESR, mm/h	–	16 [9–30]	66 [39–97]##
ANCA titre, UI/mL	–	39 [15–57]	62 [37–97]##
BVAS score	–	0 [0–0]	16 [13–18]##
Disease course [Mo]		28 [13–51]	Newly diagnosed
Immunosuppressive drugs at sampling, n [%]			
Steroid	–	25 [69]	23 [66]
MMF	–	23 [64]	3 [9]##
AZA	–	4 [11]	2 [6]
CTX	–	0 [0]	3 [9]
Rituximab	–	0 [0]	2 [6]

*, P<0.05, versus HC; **, P<0.01, versus HC; ##, P<0.01, versus inMPA; Wilcoxon test between any two groups and Kruskal-Wallis test among three groups. Data are expressed as median [IQR], mean [SD], or n [%] as appropriate. aMPA, active microscopic polyangiitis; ANCA, anti-neutrophil cytoplasm antibody; AZA, acetazolamide; BMI, body mass index; BVAS, Birmingham Vasculitis Activity Score; CRP, C-reactive protein; CTX, cyclophosphamide; ESR, erythrocyte sedimentation rate; HC, healthy control; Hb, hemoglobin; inMPA, inactive microscopic polyangiitis; MMF, mycophenolate mofetil; PLT, platelet; Scr, serum creatinine; Up, urine protein; WBC, white blood cell.

values <0.001), and lower hemoglobin level (both P values <0.001) compared with HCs. aMPA patients had higher levels of C-reactive protein, erythrocyte sedimentation rate, MPO-ANCA titre, serum creatinine, urine protein and BVAS compared with inMPA patients (all P values <0.001 except 0.004 for MPO-ANCA titre). Ten out of 35 aMPA patients had severe renal injury requiring dialysis during the initial hospitalization. For immunosuppressive treatment, the proportion of patients receiving steroid therapy was similar in aMPA group and inMPA group, while more inMPA patients received mycophenolate mofetil (MMF) compared with aMPA patients (64% versus 9%, P<0.01). At the final follow-up, 18 out of 34 aMPA patients progressed to ESRD, and one aMPA patient died of respiratory and renal failure during hospitalization.

Richness and diversity of microbial community

The sequencing data from 105 fecal samples were clustered into 1,754 different OTUs, of which 40% were shared by the three groups (*Figure 1A*). The community richness assessed by Ace and Chao1 index showed no significant difference among the groups, except that the Chao1 index of the aMPA group was lower than that of the inMPA group (*Figure 1B,1C*). The community diversity assessed by Simpson and Shannon index was lowest in the aMPA group

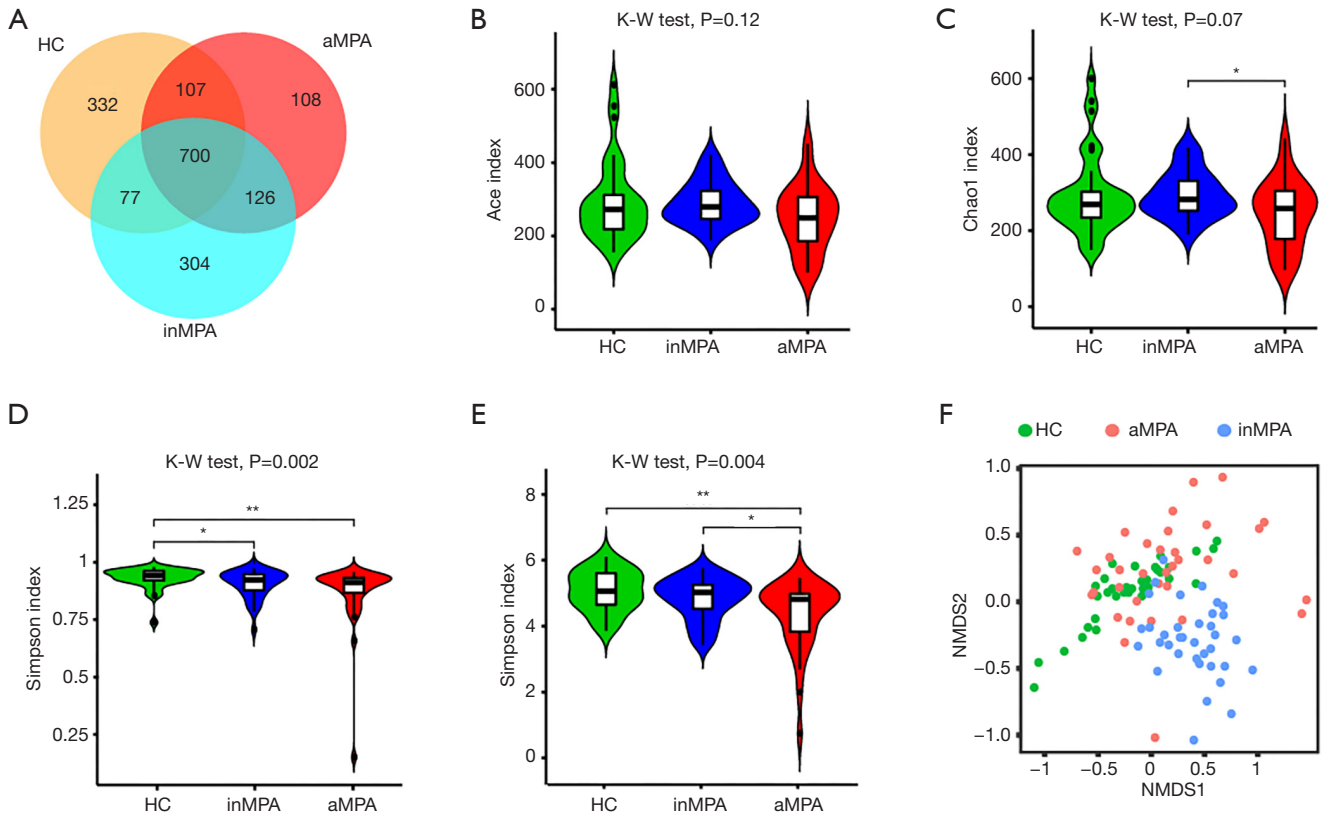

Figure 1 The diversity of gut microbiome in patients with active MPA (aMPA, n=35), inactive MPA (inMPA, n=36) and healthy controls (HCs, n=34). (A) A venn diagram showing overlaps of 1,754 clustered OTUs among the three groups. The α-diversity assessed by richness indices [Ace (B) and Chao1 index (C)] and diversity indices [Simpson (D) and Shannon index (E)] was compared among multiple groups by Kruskal-Wallis (K-W) test and between any two cohorts by Wilcoxon rank sum test. (F) β-diversity, calculated by Non-metric multidimensional scaling (NMDS) (stress=0.18), displayed the dissimilarities in microbial composition of all samples, and illustrated a biased community distribution among different groups. *P<0.05; **P<0.01.

and highest in the HCs (*Figure 1D,1E*). In addition, distinct microbial composition among the groups was initially illustrated by NMDS plot (*Figure 1F*, stress =0.18) and further confirmed by pairwise comparisons in ANOSIM (all P values =0.001 between any two groups).

Composition of microflora and differential taxa

After removing unassigned OTUs, we annotated the remaining 1,562 OTUs into 24 phyla, 37 classes, 86 orders, 149 families and 354 genera. At phylum level, the most common taxa were phylum *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria* (*Figure 2A*, Figure S2). At genus level, the most common taxa were genus *Bacteroides*, *Faecalibacterium*, *Blautia*, *Streptococcus*, *Escherichia Shigella* (*Figure 2B*, Figure S3).

Next, the abundance of phyla or genera greater than 0.01% was compared between each pair of groups. Three phyla—*Actinobacteria*, *Fusobacteria* and *Epsilonbacteraeota*, were significantly higher in the inMPA group than those in the HCs group (Figure S4A, Table S2, adjusted P<0.001, 0.003, <0.001, respectively). However, there was no statistical difference of phylum abundance between the aMPA group and the HCs group. At genus level, two genera including *Actinomyces* and *Streptococcus* were more abundant in the aMPA group (adjusted P=0.001 and 0.04) and the inMPA group (adjusted P<0.001, 0.002) than those in HCs group, while six genera including *Subdoligranulum*, *Eubacterium hallii*, *Ruminococcaceae UCG013*, *Eubacterium ventriosum*, *Dorea* and *Butyricicoccus* were more abundant in the HCs group than those in the aMPA group (adjusted P=0.01, 0.02, 0.01, 0.02, 0.007 and 0.004, respectively) and

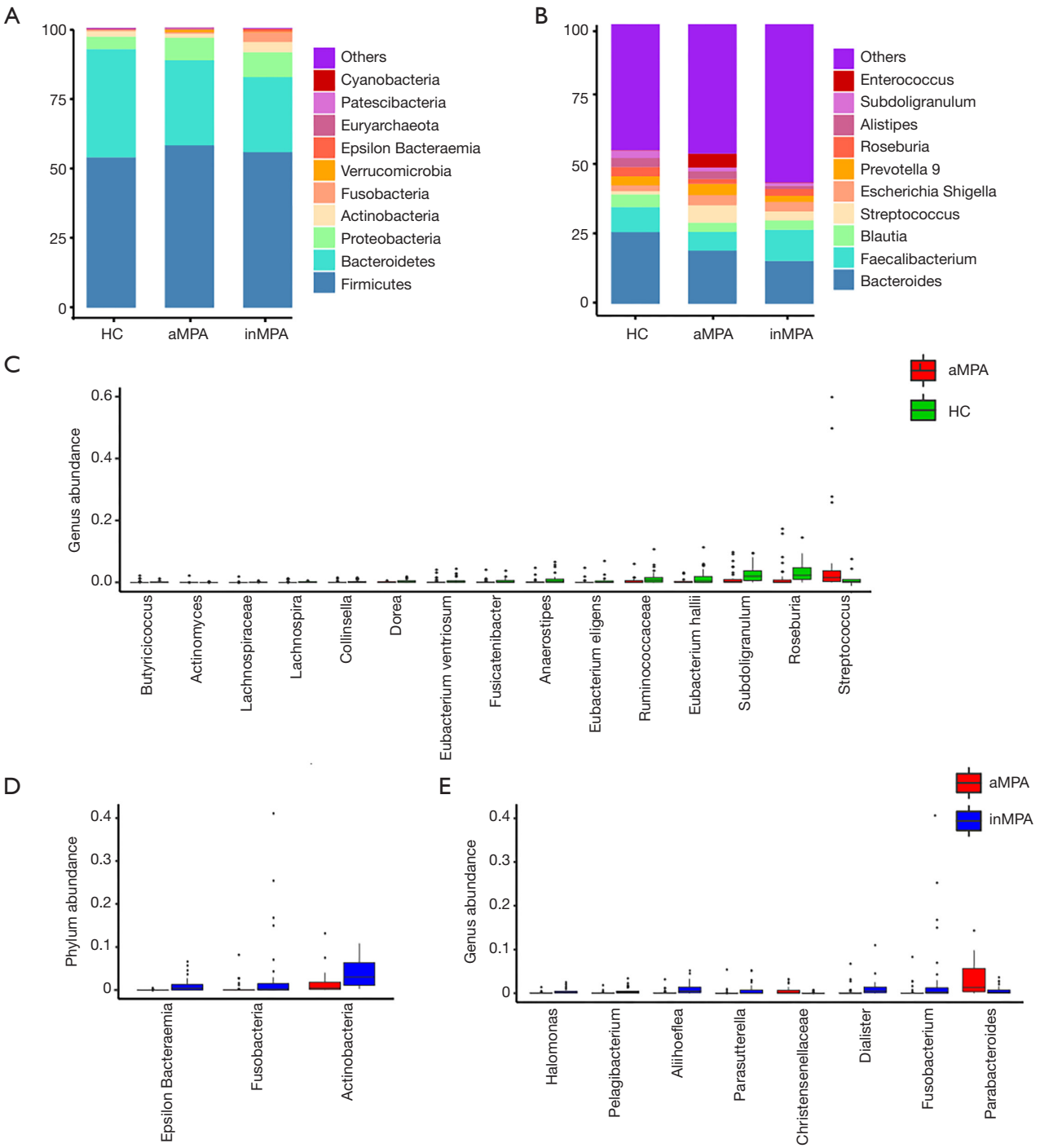

Figure 2 Microbial composition and differential bacterial abundance at phylum and genus levels in fecal samples from patients with active MPA (aMPA, n=35), inactive MPA (inMPA, n=36) and healthy controls (HCs, n=34). Top 10 abundant phyla (A) and genera (B) in fecal samples from the three groups. (C) The abundance of significantly different genera between aMPA samples and HC samples. (D) The increased abundance of phyla in inMPA samples versus aMPA. (E) The abundance of significantly different genera between aMPA and inMPA samples. The microbial abundance was compared by Wilcoxon rank sum test and adjusted using Benjamini-Hochberg method. The boxes and lines inside represent the 95% CI and median, respectively.

the inMPA group (adjusted P=0.002, 0.001, <0.001, <0.001, 0.008 and 0.001, respectively) (*Figure 2C*, Figure S4B, Table S3). Among the above six decreased genera in the MPA groups, *Subdoligranulum*, *Ruminococcaceae UCG013* and *Butyricicoccus* belong to family *Ruminococcaceae* and the other three belong to family *Lachnospiraceae*. Both *Ruminococcaceae* and *Butyricicoccus* belong to order *Clostridiales*, which is a short-chain fatty acids (SCFA)-producing taxon. Compared with the inMPA group, the aMPA group had lower abundances of *Actinobacteria*, *Fusobacteri*a and *Epsilonbacteraeota* at phylum level (*Figure 2D*, Table S2, adjusted P=0.003, 0.02, <0.001, respectively), lower abundances of *Fusobacterium*, *Dialister*, *Parasutterella*, *Aliihoeflea*, *Halomonas* and *Bacteroides* at genus level (*Figure 2E*, Table S3, adjusted P=0.008, <0.001, 0.038, <0.001, <0.001, <0.001, respectively) and higher abundances of *Parabacteroides* and *Christensenellaceae* at genus level (*Figure 2E*, Table S3, adjusted P=0.04, 0.01).

Correlation between altered genera, OTUs and clinical indices

Fourteen differential genera among the three groups were correlated with 9 clinical indices (*Figure 3A*). Of note, most identified taxa were corelated with serum albumin (71%), creatinine (79%) and blood urea nitrogen (64%), which partly indicated the severity of renal damage. The absolute values of the corresponding correlation coefficients were 0.33–0.49, 0.34–0.63, 0.34–0.58, respectively (Table S4). Genus *Actinomymyces*, *Aliihoeflea*, *Dialister*, *Halomonas and Pelagibacterium* were positively corelated with serum albumin and hemoglobin, and negatively corelated with serum creatinine and blood urea nitrogen. Genus *Actinomyces* was negatively corelated with BVAS, while *Butyricicoccus* was positively corelated with BVAS.

Differential analysis of OTU abundance showed that OTU-5, OTU-13, OTU-19, OTU-27, OTU-60, OTU-71, OTU-367 and OTU-1026 were significantly decreased and OTU-42 was significantly increased in patients receiving initial dialysis (n=9) by Wilcoxon test (*Figure 3B*). Consistently, the 5 decreased OTUs and another 2 decreased OTUs belong to family *Lachnospiraceae* and *Prevotellaceae* respectively (Table S5), both of which play an important role in SCFA production. In addition, the levels of OTU-150 and OTU-182 were increased with an absolute value of effect size ≥0.5 in the patients progressing to ESRD during follow up (*Figure 3C*). However, no significant difference existed when *P* values were adjusted by "Benjamini Hochberg" method.

OTU markers-based models in initial diagnosis and activity evaluation of MPA

To assess the diagnostic efficiency of the gut microbiome for MPA, we incorporated OTU feature tables of the aMPA samples and the HC samples into random forest models. Five trials of 10-fold cross-validation on random forest models picked the optimal set of 6 OTU markers (*Figure 4A*). All these 6 OTU markers belong to Phylum *Firmicutes* and 4 of them belong to family *Lachnospiraceae*. The random forest model based on this set of OTUs verified a significantly increased predicted possibility of MPA in the aMPA samples versus the HC samples (*Figure 4B*, $P=5.7\times10^{-10}$, Wilcoxon test), and the AUC of ROC reached 93.45% (*Figure 4C*, 95% CI, 88.15–98.74%). We also constructed a model to distinguish the aMPA samples from the inMPA samples to evaluate disease activity with the same method. Finally, 11 OTU markers were picked (*Figure 4D*). This optimal set of OTUs also contributed to a model with a significantly increased predicted possibility of active disease in the aMPA samples versus the inMPA samples (*Figure 4E*, $P=3.8\times10^{-9}$, Wilcoxon test), and the AUC of ROC reached 90.71% (*Figure 4F*, 95% CI, 82.49–98.94%).

Discussion

MPA is a systemic inflammatory disease usually accompanied by severe renal damage (1,4,31). With advances in high-throughput sequencing, the landscape of previously uncultured microorganisms is gradually unveiled (32), and the significant role of the gut microbiome in a variety of disorders, including immune-mediated diseases (13,33,34) and CKD (15) has been illustrated. In the present study, we reported the profiling of the gut microflora in MPA patients, and further explored its clinical association and value in disease classification.

First, our results revealed that MPA patients had intestinal dysbiosis, which seemed to partially recover considering a significant rise of Shannon and Chao1 indices in inMPA versus aMPA. However, β-diversity analysis showed biased community constitution among the three groups, implying that the gut microflora of remissive MPA patients may be interfered by other cofounding factors.

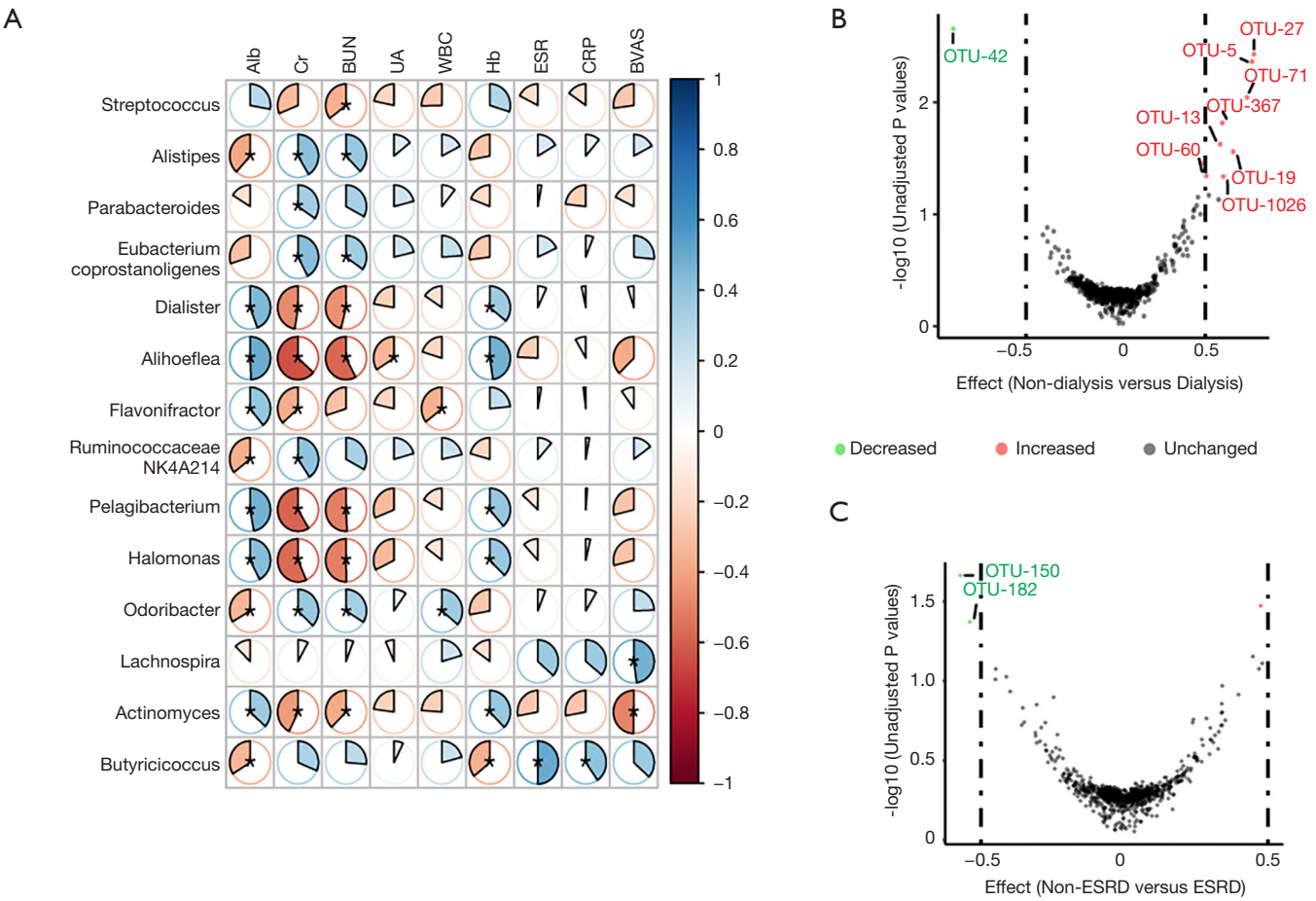

Figure 3 The genus and OTU markers in association with disease-related indices, severity of kidney impairment and renal prognosis. (A) Correlations between the abundance of the 14 markedly altered genera and clinical parameters. The color and area of the pie charts represent the positive (blue) or negative (red) correlation and the scale of correlation coefficient, respectively, and asterisks inside denote "Holm" adjusted P values <0.05. Volcano plots of the differential analysis on OTU abundance of incipient patients with active MPA between those with initial dialysis or not (B) and between those progressing into ESRD or not (C). The red and green points indicate increased and reduced abundance in non-dialysis or non-ESRD group, respectively, that reach a significance difference with unadjusted P values <0.05 by Wilcoxon test. The cut-off of effect size, i.e., the ratio of median difference between two groups and median of the largest difference within two groups, is set to 0.5. An absolute effect size of 0.5 or greater denotes an OTU marker for differentiation of subgroups. ESRD, end-stage renal disease.

Actually, gut microbiota has been proven susceptible to multiple elements, such as age (35), dietary habit (36), illness (16,37), and use of antibiotics or non-antibiotic drugs (38). Chen *et al.* (39) found that RA patients receiving methotrexate and hydroxychloroquine had an increase in species richness and diversity. In the present study, we didn't identify an association between use of immunosuppressants at sampling and microbial richness or diversity. It may be due to a small population, a wide variety of course and concurrent use of other drugs.

Microbial fermentation of indigestible carbohydrates can lead to the generation of SCFAs (8). Bacteria derived SCFAs have versatile functions on human physiology, such as weight control, glucose homeostasis and immunity modulation (8,37). In the present study, several SCFA-producing microbes were markedly decreased in MPA patients. Among these bacteria, Genus *Roseburia*, *Eubacterium hallii*, *Anaerostipes* and *Butyricicoccus* were implicated in the production of butyrate (8,40). Butyrate is essential for intestinal homeostasis and has immune-

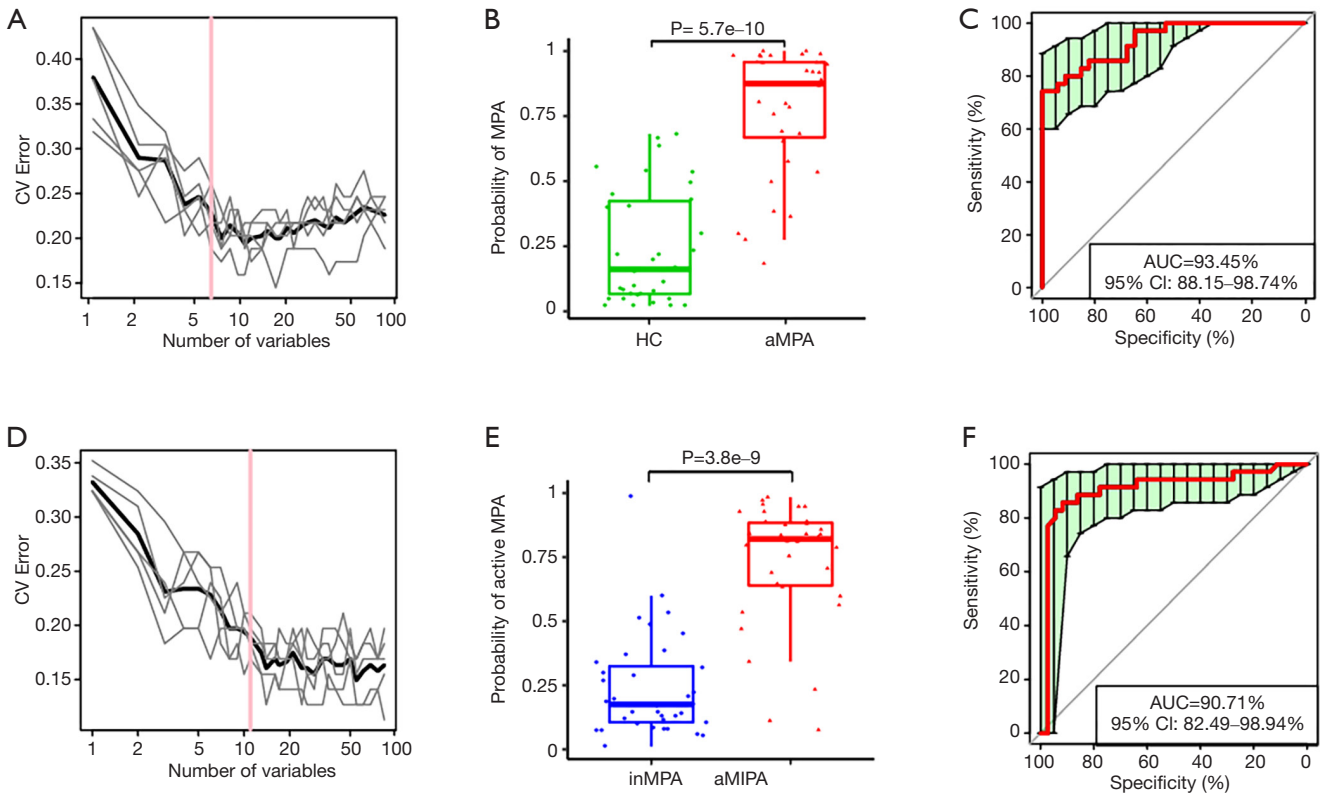

Figure 4 Gut microbial profiling-based models for diagnosing MPA and predicting disease activity. (A) Plots of five trials of cross-validation (CV) error on random forest models to differentiate patients with active MPA (aMPA, n=35) from healthy controls (HCs, n=34). The optimal set of markers comprises 6 OTUs (pink line). The black curve represents the average CV error of the five trials (grey lines). (B) The predicted probability of MPA significantly higher in aMPA samples than in HCs samples ($P=5.7\times10^{-10}$, Wilcoxon test) in the optimal set of OTUs in A. (C) Receiver operating characteristic curve (ROC) for the selected 6 OTU markers. The AUC is 93.45 and 95% CI is 88.15–98.74%. (D) Plots of cross-validation error on the same model to differentiate Ampa (n=35) from in active MPA (inMPA) (n=36). The optimal set of markers comprises 11 OTUs (pink line). (E) The predicted probability of active disease significantly higher in aMPA samples than in inMPA samples ($P=3.8\times10^{-9}$, Wilcoxon test) in the optimal set of OTUs in D. (F) ROC for the selected 11 OTU markers. The AUC is 90.71 and 95% CI is 88.15–98.74%.

suppressive and anti-inflammation effects (8,37). In addition, 7 out of 8 non-dialysis enriched OTUs belonged to SCFA-producing bacteria, suggesting decreased production of SCFA may underlie the development of renal injury in MPA.

On the other hand, CKD and related alterations of commensals could impair the integrity of intestinal barrier, promote translocation of toxic compounds into circulation, and thereby induce systemic inflammation and immune paralysis (15). Wang and colleagues (16) illustrated that two ESRD-enriched species *Eggerthella lenta* and *Fusobacterium nucleatum*, could increase uremic toxins production and aggravate renal fibrosis and oxidative stress in a CKD rat model. Consistently, our data showed that genus *Eggerthella* was enriched at initial stage in those who progressed to ESRD later. In addition, genus *Fusobacteria*, *Actinobacteria* and *Epsilonbacteraeota* were increased in inMPA compared with aMPA and HCs, indicating alteration of these microbes might be owing to a chronic disease course. Besides, the abundance of phylum *Proteobacteria*, a major source of bacterial Lipopolysaccharide (LPS), was significantly higher in remissive MPA patients than HCs. Notably, LPS has been found elevated in CKD patients (11) and instrumental to augment glomerular damage in a murine model of anti-MPO glomerulonephritis (41). Herein, we also identified 5 new genera *Actinomymyces*, *Aliihoeflea*, *Dialister*, *Halomonas* and *Pelagibacterium* related to hemoglobin, two genera *Flavonifractor*, *Odoribacter* related to white blood cell and

one genus *Aliihoeflea* related to uric acid. Since anemia, hyperuricemia and microinflammation were common complications in CKD (42,43), these taxa may act as potential indictors for progression of kidney disease in MPA.

Intriguingly, a common pattern of intestinal dysbiosis had been described in several immune mediated inflammatory diseases (IMIDs), including Crohn's disease, ulcerative colitis, multiple sclerosis and RA (33). Specifically, increased abundance of genus *Actinomyces*, *Eggerthella*, *Clostridium III*, *Faecalicoccus*, and *Streptococcus* and decreased abundances of genus *Gemmiger*, *Lachnospira*, and *Sporobacter* were observed in all disease cohorts versus HCs. Particularly, the alterations of *Actinomyce*, *Eggerthella*, *Streptococcus* and *Lachnospira was* in line with our results. Therefore, the common pathogenic or beneficial microbes may be involved in the pathogenesis of paralleled diseases, and therapy targeting these microbes would be promising and suitable for wide application.

Though *S. aureus* was strongly linked to GPA from previous clinical and experimental studies (18,19), there still lacked convincing evidences for a connection between *S. aureus* and MPA, including this preliminary study. Recently, Gu *et al.* (44) identified a peptide from *Actinomyces* species which could induce crescentic nephritis in two murine models of anti-glomerular basement membrane (anti-GBM) disease via epitope mimicry. *Actinomyces* are gastrointestinal commensals which could lead to actinomycosis, a chronic granulomatous infectious disorder, when the mucosal integrity is disrupted (45). However, there was no clue of increased *Actinomyces* in MPA or in CKD. In the current study, genus *Actinomyces* were significantly increased in both aMPA and inMPA cohorts. Considering that ANCA presented in approximately 35% of patients with anti-GBM disease (46), whether *Actinomyces* could trigger anti-MPO immunity needs more basic research and epidemiological trials to verify. Besides, we also found an elevation of *Streptococcus* in both active and inactive MPA groups. Elevated intestinal *Streptococcus* has been described in patients with IgA nephropathy (47). Frequent mucosal carriage of *Streptococcus* may induce a glycosylation deficiency in IgA1, which is an important pathogenetic mechanism of IgA nephrology (48). It's interesting that IgA class ANCA also play a pathogenetic role in ANCA associated vasculitis (49), thus it's reasonable to speculate that *Streptococcus* may aggravate MPA in an IgA-dependent way. So, increased exposure of certain detrimental flora to intestinal immune system may activate specific immune response which underlies the pathogenesis of MPA.

Using gut microbiome as non-traumatic diagnostic tools has been attempted in compelling studies. Ren *et al.* (12) established a diagnostic model applying 30 microbial markers that validated strong diagnosis potential for early and advanced hepatocellular carcinoma. Li and colleagues (13) verified that the gut microbiome could also be used to distinguish active SLE from remissive SLE. Herein we established two models using machine-learning methods (12,30) to diagnose incipient MPA and predict activity level of MPA. The AUC reached more than 90% in both two models, indicating a good performance on classification.

Our study had several limitations. First, GPA patients were not enrolled due to the low incidence in China (5). Second, the proportion of kidney biopsy was relatively low, so the severity of renal injury couldn't be fully assessed. Third, considering the old onset age of MPA, potential cardiovascular and metabolic diseases may interfere the gut microbial community. Four, most participants lived in one province, so whether the specific alterations in this study are applicable to other population remains to be determined.

In conclusion, our study revealed dysbiosis of the gut microbiome in MPA patients, particularly in patients with active disease. The alterations of microbial community in MPA demonstrated a combined composition of the disordered microbes verified in IMIDs and CKD, and a tendency toward loss of SCFA-producing bacteria. Furthermore, we established two random forest models based on gut microbial markers, showing the potential of diagnosing MPA and evaluating disease activity.

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Footnote

Reporting Checklist: The authors have completed the MDAR and STROBE reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-1315

Data Sharing Statement: Available at https://dx.doi.org/10.21037/atm-21-1315

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-1315). 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. Our research was conformed with the Declaration of Helsinki (as revised in 2013) and was approved by the ethics committee of the First Affiliated Hospital, Zhejiang University School of Medicine (approval No. 2017-694). Informed consents were available from all participants before sampling.

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Supplementary

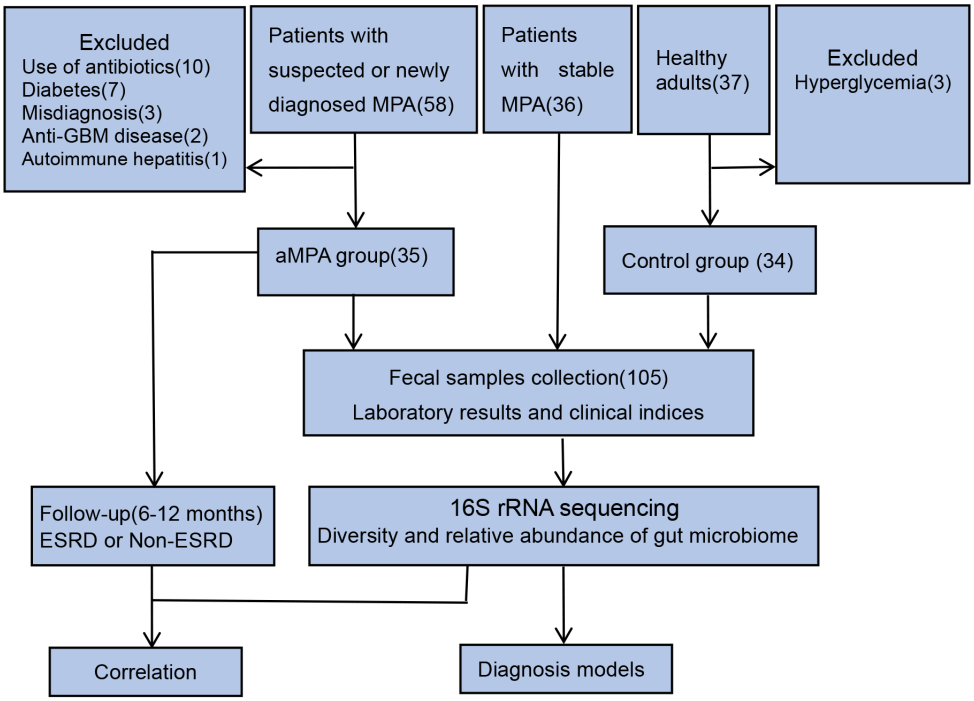

Figure S1 Flowchart of the study. ESRD, end stage renal disease.

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Table S1 The clinical characteristics and laboratory parameters of all enrolled individuals

Sample ID	Group	Age	Gender	Height (cm)	Weight (kg)	BMI (kg/m2)	Alb (g/l)	Cr (μmol/l)	BUN (mmol/l)	UA (μmol/l)
P1	aMPA	56	male	170	73	25.26	30	513	30.55	433
P2	aMPA	68	female	158	55	22.03	26	220	12.09	214
P3	aMPA	67	male	170	79	27.34	31	716	24.11	414
P4	aMPA	54	male	165	55	20.20	40	626	52.73	463
P5	aMPA	59	male	160	50	19.53	29	671	33.51	425
P6	aMPA	41	female	165	74	27.18	33	258	21.49	184
P7	aMPA	70	female	160	42	16.41	30	61	5.67	234
P8	aMPA	57	female	157	60	24.34	31	295	15.34	314
P9	aMPA	67	female	150	62	27.55	32	279	18.87	505
P10	aMPA	66	male	167	61	21.87	37	438	29.49	481
P11	aMPA	60	female	147	29	13.42	35	483	15.68	228
P12	aMPA	68	male	168	61	21.61	34	176	11.91	410
P13	aMPA	54	male	168	66	23.38	36	151	9.89	422
P14	aMPA	54	male	164	61	22.68	37	336	30.21	359
P15	aMPA	68	male	163	50	18.82	33	599	20.5	399
P16	aMPA	73	female	163	52	19.57	32	191	11.98	289
P17	aMPA	61	male	168	60	21.26	33	409	17.61	476
P18	aMPA	73	female	150	43	19.11	33	671	17.72	300
P19	aMPA	63	female	155	50	20.81	29	230	8.2	240
P20	aMPA	64	female	160	55	21.48	31	462	16.71	466
P21	aMPA	62	male	173	63	21.05	36	295	14.03	493
P22	aMPA	40	male	173	81	27.06	34	356	22.76	412
P23	aMPA	60	male	169	66	23.11	37	974	38.81	600
P24	aMPA	35	female	165	47	17.26	38	202	9.56	529
P25	aMPA	68	male	168	74	26.22	39	330	27.28	580
P26	aMPA	71	female	150	49	21.78	27	552	19.33	505
P27	aMPA	58	female	159	43	17.01	25	699	29.3	492
P28	aMPA	64	male	167	57	20.44	37	131	7.6	393
P29	aMPA	54	female	165	63	23.14	32	130	10.5	256
P30	aMPA	55	male	155	45	18.73	34	321	16.4	369
P31	aMPA	26	female	158	46	18.43	40	84	8.45	270
P32	aMPA	67	male	165	63	23.14	32	491	26.11	453
P33	aMPA	49	male	171	59	20.18	29	209	10.85	440
P34	aMPA	56	male	168	79	27.99	36	118	6.44	291
P35	aMPA	74	female	150	45	20.00	32	580	22.52	574
R1	inMPA	60	male	171	59	20.18	30	160	10.28	326
R2	inMPA	38	male	172	75	25.35	38	94	6.65	419
R3	inMPA	68	male	168	78	27.64	40	330	23.62	693
R4	inMPA	58	male	165	46	16.90	43	137	13.6	469
R5	inMPA	61	male	175	60	19.59	36	198	16.94	380
R6	inMPA	73	male	160	56	21.88	42	196	10.86	259
R7	inMPA	63	female	160	43	16.80	40	69	3.77	185
R8	inMPA	57	male	173	81	27.06	42	104	6.29	394
R9	inMPA	66	male	167	50	17.93	41	101	5.64	364
R10	inMPA	57	female	172	57	19.27	43	110	6.84	452
R11	inMPA	53	female	150	55	24.44	29	178	15.12	391
R12	inMPA	28	female	166	54	19.60	50	57	4.34	265
R13	inMPA	26	female	157	43	17.44	41	95	8.95	366
R14	inMPA	65	female	158	53	21.23	42	101	6.72	363
R15	inMPA	63	male	173	63	21.05	43	174	11.14	334
R16	inMPA	48	male	174	68	22.46	34	173	14.62	443
R17	inMPA	46	female	158	51	20.43	45	159	16.13	380
R18	inMPA	61	male	168	55	19.49	43	81	6.11	294
R19	inMPA	51	male	175	80	26.12	41	111	3.98	337
R20	inMPA	63	male	167	50	17.93	41	120	11.23	381
R21	inMPA	61	female	160	70	27.34	46	127	10.57	391
R22	inMPA	40	female	168	74	26.22	44	89	7.7	356
R23	inMPA	71	female	156	60	24.65	42	67	6.54	283
R24	inMPA	45	male	160	65	25.39	41	193	11.55	324
R25	inMPA	55	female	154	70	29.51	43	166	9.6	324
R26	inMPA	58	female	164	68	25.28	44	83	5.12	351
R27	inMPA	64	male	164	64	23.80	40	146	7.1	365
R28	inMPA	67	female	158	67	26.84	35	148	8.9	303
R29	inMPA	71	female	152	64	27.70	41	144	12.51	179
R30	inMPA	62	female	150	58	25.78	44	76	7.44	149
R31	inMPA	66	male	172	53	17.92	42	185	11.95	301
R32	inMPA	64	male	175	75	24.49	40	112	6.51	411
R33	inMPA	72	male	162	58	22.10	35	98	8.45	393
R34	inMPA	65	female	159	44	17.40	33	169	14.09	372
R35	inMPA	67	male	157	60	24.34	37	131	10.4	503
R36	inMPA	57	male	170	79	27.34	37	148	12.6	323
C1	HC	47	female	161	55	21.22	52	64	4.03	276
C2	HC	62	female	161	54	20.83	51	49	4.34	223
C3	HC	56	female	164	57	21.19	44	49	4.4	221
C4	HC	54	female	165	54	19.83	45	54	4.88	221
C5	HC	41	female	160	44	17.19	45	48	5.46	263
C6	HC	62	male	168	57	20.08	44	78	3.95	334
C7	HC	44	female	166	54	19.60	46	47	5.5	231
C8	HC	52	female	159	51	20.17	43	51	6.01	292
C9	HC	63	male	162	59	22.48	46	60	4.39	229
C10	HC	62	female	152	45	19.48	45	62	5.53	322
C11	HC	61	male	166	72	26.13	45	64	4.92	296
C12	HC	50	male	177	64	20.43	46	56	6.31	296
C13	HC	46	female	167	59	21.16	41	60	4.89	259
C14	HC	45	male	172	75	25.35	46	68	5.41	265
C15	HC	52	female	161	48	18.71	43	49	4.19	287
C16	HC	72	female	150	50	22.22	48	43	4.9	199
C17	HC	69	male	179	68	21.22	44	76	4.06	243
C18	HC	64	male	178	75	23.67	52	53	5.7	342
C19	HC	62	female	161	56	21.60	48	62	3.1	242
C20	HC	59	male	170	70	24.22	40	66	5.21	224
C21	HC	66	female	160	56	21.88	44	69	6.91	417
C22	HC	55	female	155	52	21.64	42	66	5.24	273
C23	HC	58	female	164	58	21.56	42	49	4.45	362
C24	HC	55	female	160	52	20.31	44	66	4.5	449
C25	HC	55	male	168	70	24.80	47	54	4.5	305
C26	HC	61	female	172	68	22.99	47	54	4.5	305
C27	HC	70	male	169	69	24.16	45	77	4.54	367
C28	HC	67	female	165	50	18.37	48	87	4.6	351
C29	HC	41	male	170	70	24.22	49	76	5	389
C30	HC	66	male	170	62	21.45	49	70	8.81	273
C31	HC	58	female	162	52	19.81	43	58	4.26	234
C32	HC	68	female	170	55	19.03	43	56	4.03	116
C33	HC	56	female	160	50	19.53	48	55	3.6	238
C34	HC	55	female	162	53	20.20	46	51	4.76	262

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Sample ID	TG (mmol/l)	Cho (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	Glu (mmol/l)	WBC (×10E9/l)	Neu (×10E9/l)	Hb (g/l)	Plt (×10E9/l)
P1	1.64	4.46	1.03	2.45	3.89	8.7	6.3	71	166
P2	1.88	3.32	0.73	1.98	3.81	7.6	6.3	65	154
P3	0.84	2.61	0.86	1.35	4.56	12.7	11.4	76	107
P4	0.39	2.93	1.07	1.74	8.64	7	6.3	59	104
P5	1.06	2.7	0.68	1.52	5.95	5.4	4.6	60	77
P6	1.62	3.44	1.75	1.16	4.58	8.7	8	87	169
P7	0.66	3.28	1.01	1.98	4.26	6.9	5.8	70	308
P8	1.36	2.91	1.03	1.4	4.8	4.8	3.8	72	172
P9	1.17	4.87	1.62	2.89	4.21	14.4	12.9	78	395
P10	1.69	4.98	1.02	3.1	4.04	3	1.4	79	148
P11	2.97	4.55	0.96	2.34	3.99	6.1	4.3	81	137
P12	0.68	3.57	0.96	2.22	4.46	3.8	2.3	91	151
P13	0.95	4	0.99	2.54	3.73	8.4	5.7	91	356
P14	1.44	5.43	2.2	2.36	4.99	7.4	6.1	98	125
P15	0.52	3.06	1.29	1.51	3.43	6.9	5.6	76	88
P16	1.77	4.51	1.88	1.99	3.68	4.8	4	86	116
P17	0.78	2.42	1.02	1.04	4.31	8.7	6.6	92	107
P18	1.31	3.09	0.77	1.65	4.32	8.2	7.1	49	192
P19	1.39	3.53	0.67	2.12	5.15	11.1	9.1	78	232
P20	0.79	5.88	1.45	4.01	8.63	9.6	8.9	83	239
P21	2.29	4.13	0.79	2.51	4.44	9	6.3	85	307
P22	2.46	5.47	0.92	4.44	4.53	8.3	5.8	104	258
P23	1.88	3.1	0.76	1.69	4.49	5.3	4.3	70	98
P24	1.41	4.46	1.31	2.38	4.61	9.2	6	107	220
P25	1.67	6.15	1.29	4.01	5.47	9.2	7.9	82	246
P26	1.58	3.79	0.74	1.93	3.92	11.2	8	83	406
P27	1.2	2.82	0.67	1.83	6.71	3.8	2.5	57	81
P28	1.68	4.31	1	2.64	4.26	6.2	3.8	99	190
P29	1.18	3.44	0.99	1.97	7.18	5.5	4.1	81	211
P30	1.34	4.12	1.57	2.06	3.57	10	6.8	88	209
P31	1.78	6.03	2.6	2.96	4.86	11.2	6.1	138	166
P32	0.65	4.55	1.34	2.81	5.41	5.9	5.5	77	210
P33	0.81	3.42	1.03	1.83	3.56	3.7	2.3	92	125
P34	1.45	4.26	0.59	2.72	6.39	9.3	6.6	134	288
P35	1.54	4.22	1.69	1.63	3.58	3.7	3.4	82	47
R1	0.52	3.96	1.9	1.87	3.85	7.6	4.5	96	177
R2	1.95	5.2	1.12	3.38	4.24	7.7	4.7	154	246
R3	4.86	5.67	1.27	2.84	4.85	6.6	4.5	113	77
R4	1.53	5.37	1.88	2.83	6	6	3.8	133	201
R5	0.86	5.46	2.12	3.13	4.77	4.6	2.5	143	168
R6	1.94	4.75	1.5	2.78	5	10.1	6.7	144	256
R7	0.66	4.15	2.81	1.1	5.18	3.4	1.7	115	181
R8	1.35	2.86	0.71	1.73	5.48	6.7	3.5	140	173
R9	1.87	5.41	1.34	1.86	4.98	5.1	3.4	143	182
R10	1.14	4.27	1.73	2.07	4.98	4	2.4	123	192
R11	2.28	8.35	2.46	4.88	3.47	5.2	3.4	82	257
R12	1.18	3.14	1.23	1.48	4.23	5.8	3.6	134	224
R13	3.26	6.62	1.62	3.61	4.45	6.6	4.3	118	241
R14	0.76	4.33	2.62	1.49	4.49	4.3	2.5	120	120
R15	1.39	6.31	1.65	4.09	4.6	8.1	4.8	121	256
R16	0.7	3.46	1.42	1.83	4.07	7.1	6.6	88	171
R17	2.06	4.25	1.61	1.82	4.44	5.1	3.3	113	226
R18	1.79	4.3	1.17	2.29	4.66	6.9	5	136	201
R19	1.81	5.23	1.08	3.57	6.27	6.1	4.1	164	191
R20	0.86	3.53	1.05	2.26	5.23	11.8	9.4	106	184
R21	1.98	7.51	1.7	5.23	4.6	11.6	7.7	114	334
R22	2.44	5.4	1.93	2.6	5.39	8.2	5.6	106	227
R23	2.3	5.62	1.56	3.17	5.02	10.5	4.8	132	265
R24	2.64	5.64	1.1	3.49	4.58	5.8	2.5	144	210
R25	1.96	6.17	1.43	3.72	4.7	7.5	6.9	99	221
R26	2.09	4.61	1.03	2.86	7.14	5.5	3.1	123	172
R27	1.43	5.51	1.27	3.9	4.63	7.4	5.2	156	230
R28	1.36	4.68	1.89	2.41	4.81	5.6	3.5	118	95
R29	2.25	4.81	1.58	2.69	4.26	7.3	5.4	121	214
R30	1.32	3.74	1.25	2	5.62	7.3	4.6	123	317
R31	1.46	4.55	1.57	2.53	5.06	6.1	3.9	145	129
R32	1.46	4.84	1.11	3.08	4.73	4.7	2.6	127	162
R33	0.81	5.15	1.72	2.92	4.16	5.2	3.5	96	240
R34	1.61	4.3	1.24	2.32	3.8	2.1	1	108	146
R35	1.47	5.66	2.81	2.42	3.64	9	7.9	107	203
R36	2.57	4.28	0.9	2.29	3.71	10.7	9.3	88	226
C1	1.17	4.31	1.42	2.63	4.77	4.7	2.5	136	284
C2	1.66	4.98	1.75	2.53	5.54	4.4	2.3	149	129
C3	1.41	5.12	1.33	3.15	4.51	5.3	2.8	123	217
C4	0.72	4.64	1.6	2.64	5.01	5.5	3.8	127	217
C5	1.14	3.63	1.35	1.89	4.89	5.3	3.2	138	227
C6	1.69	4.22	1.23	2.67	4.19	7.4	4	157	175
C7	1.4	4.28	1.05	2.59	4.86	4.5	2.6	129	283
C8	1.64	5.17	1.23	3.36	5.02	5.5	3.39	129	233
C9	1.49	4.43	0.95	2.73	5.22	6.8	4.06	160	255
C10	0.68	4.87	1.98	2.76	5.61	4	2.49	121	152
C11	0.63	5.22	1.28	2.42	3.99	5	2.6	159	286
C12	0.87	4.52	1.68	3.1	5.27	4.4	1.8	146	247
C13	0.89	4.7	1.54	2.96	5.07	3.9	2.3	125	176
C14	1.01	4.46	1.38	2.58	4.42	4.5	2.8	150	230
C15	1.28	5.2	2.18	3.25	4.83	5.2	2.7	120	210
C16	0.8	5.47	1.45	2.65	4.72	5.8	3.27	130	180
C17	0.78	4.75	1.73	2.85	4.88	4.3	2.1	138	170
C18	0.35	4.52	2.36	1.95	5.59	3.9	1.93	136	215
C19	0.9	3.8	1.52	2.05	5.28	3.9	1.5	138	147
C20	0.87	5.1	1.33	3.37	3.97	5.6	3.8	133	233
C21	1.53	5.75	1.37	3.48	5.76	5.3	3.16	143	179
C22	0.97	5.25	1.62	3.14	3.81	5	56	146	189
C23	1.7	5.05	1.54	3.1	3.9	9.5	55.3	139	232
C24	0.92	4.47	1.09	2.16	3.43	6.1	3.3	155	236
C25	0.98	4.4	1.42	3.11	4.86	4.9	1.7	126	230
C26	0.98	4.4	1.42	3.11	4.86	4.9	1.7	126	230
C27	1.71	4.84	1.38	2.6	5.2	6.2	3.83	150	215
C28	1.9	4.46	1.05	2.54	4.41	5.4	3.6	155	178
C29	0.97	5.08	1.61	2.88	4.66	5.3	3	161	210
C30	1.32	6.02	1.97	3.29	4.96	5	2.9	112	234
C31	0.66	3.25	1.43	1.49	5.39	6.1	2.8	143	293
C32	0.72	2.99	1.35	1.42	4.65	6.6	3.4	141	273
C33	2.05	4.67	2.31	4.54	5.8	5.1	2.6	123	185
C34	2.29	5.01	2.37	4.31	4.1	6.6	3.9	133	180

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Sample ID	Kidney biopsy	GC (%)	GS (%)	MPO-ANCA	UP (g/d)	URBC (/μl)	ESR (mm/h)	CRP (mg/l)	Course (Mo)	Dialysis	BVAS	ESRD after induction treatmet	Immunosuppressive drug at sampling
P1	yes	66.67	0.00	46.1	2.35	663.5	79	8.6	Incipient	no	20	no	Steroid
P2	yes	30.77	15.38	34.3	0.26	36	45	7.05	Incipient	yes	24	no	Steroid
P3	no	/	/	70.6	0.33	2761	36	18.4	Incipient	yes	24	no	Steroid/CTX
P4	no	/	/	30.5	2.16	792.4	62	22.55	Incipient	no	16	yes	Steroid
P5	no	/	/	42.6	2.44	863.1	140	137.11	Incipient	yes	14	yes	Steroid
P6	yes	50.00	25.00	100	1.94	21.5	40	3.2	Incipient	no	12	no	Steroid
P7	yes	28.95	5.26	11	0.55	338.8	140	77.3	Incipient	no	16	no	no
P8	yes	60.53	23.68	71.3	2.02	743.2	120	4.5	Incipient	no	17	no	no
P9	no	/	/	21.9	1.38	114	24	10	Incipient	no	17	no	Steroid
P10	yes	22.58	67.74	167.1	4.6	568.3	95	0.76	Incipient	no	12	yes	Steroid
P11	no	/	/	11.2	/	/	99	12	Incipient	yes	20	yes	Steroid
P12	yes	12.50	12.50	165.8	3.26	596.3	24	2.8	Incipient	no	16	no	Steroid/MMF
P13	yes	15.15	6.06	44.2	1.06	354.8	34	4.2	Incipient	no	21	no	MMF
P14	no	/	/	17.6	1.76	12.9	12	5	Incipient	no	14	yes	Steroid/CTX
P15	no	/	/	512.9	1.62	2426.7	66	8.5	Incipient	no	12	die	Steroid
P16	yes	15.79	52.63	477.1	1.98	558.7	100	53	Incipient	no	16	yes	Steroid/CTX
P17	no	/	/	70.3	1.45	890.9	40	7.9	Incipient	yes	18	yes	no
P18	no	/	/	354.6	0.32	507.9	140	102	Incipient	yes	17	yes	Steroid/AZA
P19	yes	33.33	12.50	57.6	2.14	574.5	115	160	Incipient	no	18	yes	Steroid
P20	no	/	/	93.3	3.96	1243.6	117	3.9	Incipient	no	12	yes	Steroid
P21	yes	7.41	66.67	38.7	1.43	162.8	92	8.2	Incipient	no	16	no	Steroid/AZA
P22	yes	30.00	10.00	81.2	5.21	810.6	54	4.8	Incipient	no	21	yes	Steroid
P23	no	/	/	56.2	1.92	137.4	30	0.4	Incipient	yes	12	yes	Steroid
P24	yes	5.00	55.00	30.8	3.26	312	58	3.8	Incipient	no	16	no	RTX
P25	yes	3.33	56.67	69.1	1.76	246	72	4.8	Incipient	no	12	yes	Steroid
P26	yes	80.95	19.05	41.1	1.83	915.6	71	24.3	Incipient	yes	24	yes	no
P27	no	/	/	152.4	3.03	1180.4	66	2.3	Incipient	yes	12	yes	Steroid
P28	yes	36.42	27.78	1099.7	1.17	688.8	20	4.36	Incipient	no	15	no	MMF
P29	yes	18.92	8.11	12.3	1.15	134.9	88	9.8	Incipient	no	13	no	Steroid
P30	yes	15.38	69.23	100	3.1	113.2	49	2	Incipient	no	17	yes	Steroid
P31	yes	3.45	34.48	48.98	0.58	119.4	7	3.1	Incipient	no	10	no	no
P32	yes	45.83	37.50	63.08	2.45	804.5	46	4.12	Incipient	no	16	yes	no
P33	yes	14.29	25.00	62.9	0.73	603.3	38	7.6	Incipient	no	12	no	no
P34	yes	52.63	5.26	23.8	3.92	3045.5	78	66.6	Incipient	no	14	no	RTX
P35	no	/	/	62.3	1.8	1021.3	101	11.6	Incipient	yes	20	yes	Steroid
R1	yes	23.08	30.77	31.23	0.55	17.8	21	1.28	7	no	0	/	Steroid/MMF
R2	no	/	/	59.34	1.07	41.3	2	4.2	24	no	0	/	Steroid/MMF
R3	no	/	/	15.17	1.2	23.9	13	0.47	8	yes	0	/	Steroid/AZA
R4	yes	16.13	3.23	55.23	0.86	113.1	51	2.18	41	no	0	/	Steroid
R5	yes	0.00	71.43	67.39	0.25	13.2	2	0.33	36	no	0	/	MMF
R6	yes	48.00	36.00	3.61	0.9	3.2	4	1.3	45	no	0	/	MMF
R7	yes	57.89	21.05	47.68	0.08	28.8	5	3.3	48	no	0	/	Steroid
R8	yes	52.63	5.26	10.97	0.54	21.8	32	4.76	13	no	0	/	no
R9	no	/	/	61.11	0.05	35.6	3	2.4	53	no	0	/	Steroid
R10	yes	17.39	56.52	31.49	0.03	12.6	9	1.59	56	no	0	/	Steroid
R11	yes	75.00	18.75	8.54	0.89	100	33	1.46	6	yes	0	/	Steroid/AZA
R12	no	/	/	19.12	0.03	2.2	14	0.5	28	no	0	/	Steroid/MMF
R13	yes	32.00	12.00	44.5	0.49	12.3	24	0.46	39	no	0	/	Steroid/MMF
R14	no	/	60.00	55.69	0.08	10	17	0.56	81	no	0	/	Steroid/MMF
R15	yes	7.41	66.67	16.02	1.18	22.1	37	1.1	15	no	0	/	Steroid/MMF
R16	no	/	/	34.8	0.64	99.7	14	2	6	no	0	/	MMF
R17	yes	61.54	30.77	48.18	1.14	15	27	0.21	117	yes	0	/	MMF
R18	no	/	/	96.59	0.15	36	9	2.69	26	no	0	/	Steroid
R19	yes	10.53	52.63	75.51	0.48	1.1	10	3.59	98	no	0	/	MMF
R20	no	/	/	15.34	1.47	23.5	36	7.63	99	no	0	/	MMF
R21	no	/	/	75.17	0.13	3.9	40	2.67	41	no	0	/	Steroid/AZA
R22	yes	60.53	23.68	28.61	1.38	137.2	31	3.23	14	no	0	/	Steroid/MMF
R23	yes	76.67	3.33	54.06	0.15	26.3	24	2.21	31	yes	0	/	MMF
R24	yes	31.25	37.50	49	1	1.6	14	0.83	48	no	0	/	MMF
R25	yes	75.76	15.15	13.07	1.06	83.9	16	4.2	8	no	0	/	Steroid/MMF
R26	yes	14.29	10.71	8.45	0.06	1.5	10	4.1	19	no	0	/	Steroid/AZA
R27	yes	25.00	25.00	80.91	0.08	37.4	4	0.99	63	no	0	/	MMF
R28	yes	56.25	9.38	36.01	0.78	1.8	30	0.42	29	no	0	/	Steroid/MMF
R29	yes	7.69	53.85	83.38	0.76	47	16	3.91	79	no	0	/	Steroid/MMF
R30	no	/	/	41.04	0.23	2.9	16	8.63	22	no	0	/	MMF
R31	yes	6.25	50.00	67.67	0.63	4.7	5	1.78	74	no	0	/	Steroid/MMF
R32	yes	33.33	5.56	17.51	0.13	86	23	12	15	no	0	/	Steroid
R33	yes	30.00	10.00	10.41	0.14	19.9	52	10.82	7	no	0	/	Steroid
R34	yes	22.22	55.56	7.4	1.36	42	20	2.8	16	no	0	/	Steroid
R35	yes	3.33	56.67	9.7	1.53	32.7	8	2.7	8	no	0	/	Steroid/MMF
R36	yes	30.00	10.00	48.3	0.81	65.1	36	3.02	9	no	0	/	Steroid/MMF

AZA, acetazolamide; BVAS, Birmingham Vasculitis Activity Score; CTX, cyclophosphamide; ESRD, end-stage renal disease; GC, Glomerular crescents; GS, Global sclerosis; MMF, mycophenolate

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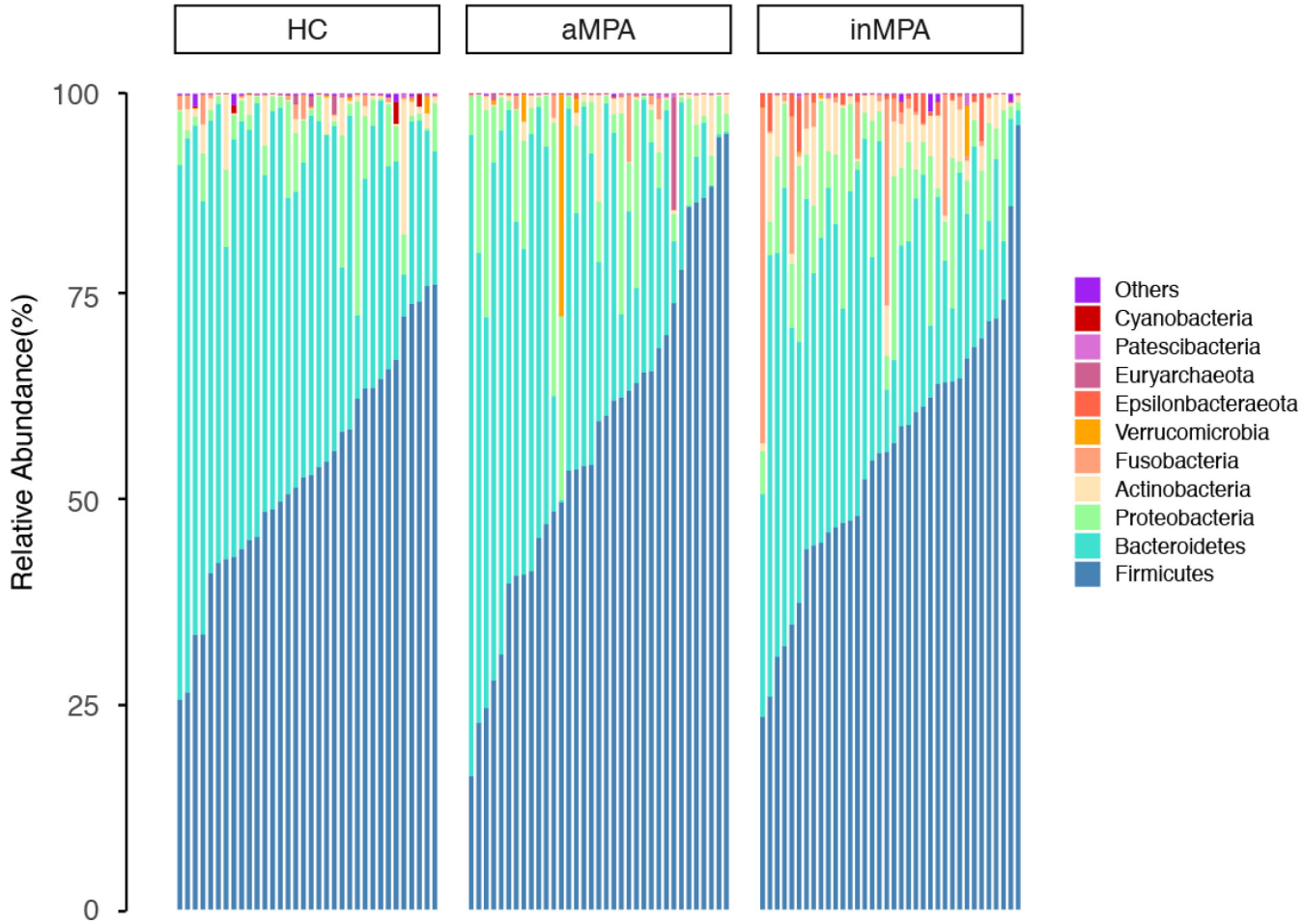

Figure S2 The bacterial composition at phylum level in all samples.

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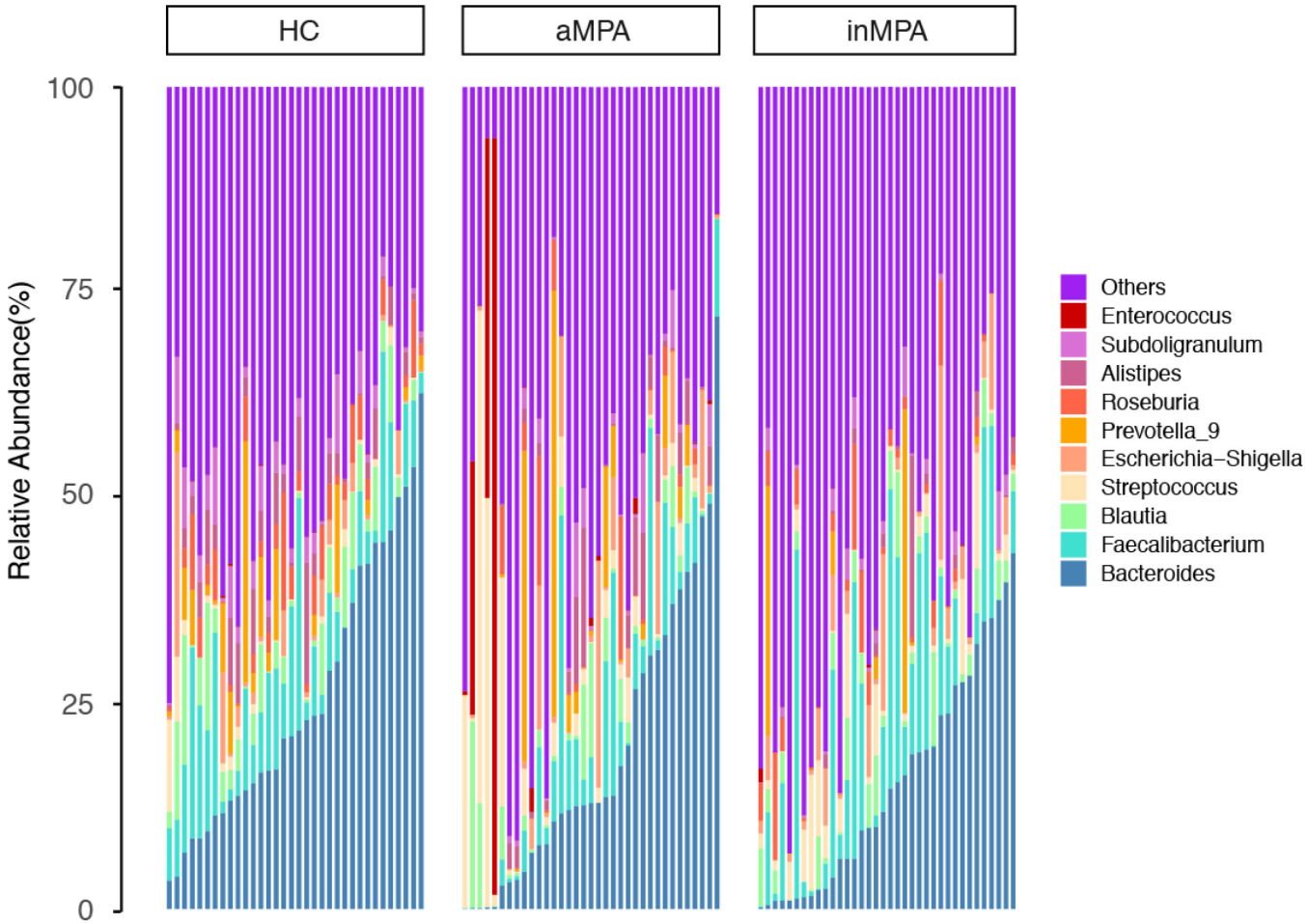

Figure S3 The bacterial composition at genus level in all samples.

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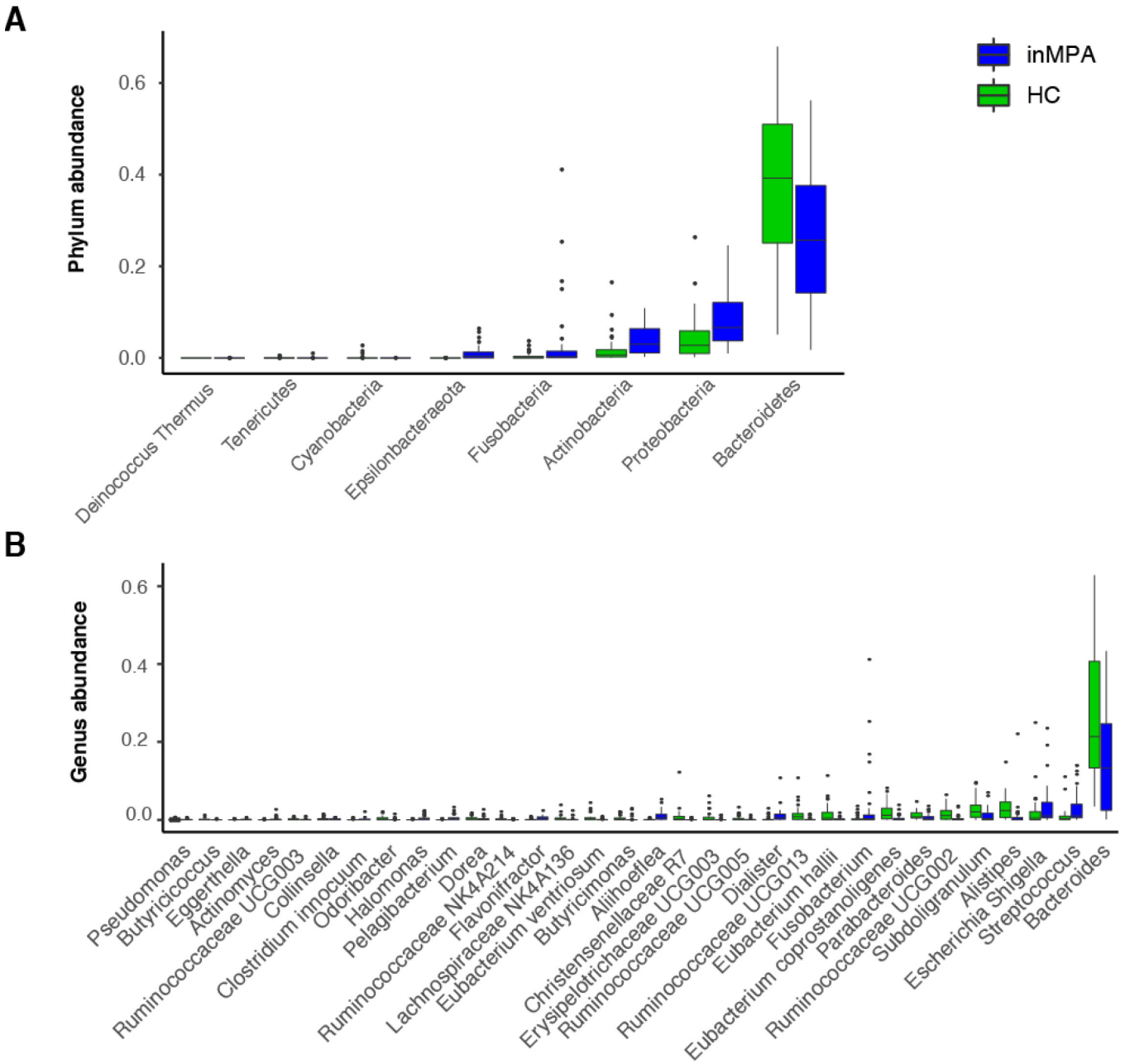

Figure S4 Differential bacterial abundance at phylum(A) and genus(B) level between inMPA and HC cohort. All taxon with significant inter-group difference (Wilcoxon rank sum test, P values adjusted by "Benjamini-Hochberg" < 0.05) are presented.

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Table S2 The phyla with differential abundance among aMPA, inMPA and HC cohorts

Comparison	Differential phyla	Enriched group	FDR
aMPA *vs.* inMPA	Actinobacteria	inMPA	<0.001
	Fusobacteria	inMPA	0.003
	Epsilonbacteraeota	inMPA	<0.001
HC *vs.* inMPA	Bacteroidetes	HC	0.02
	Proteobacteria	inMPA	0.003
	Actinobacteria	inMPA	0.003
	Fusobacteria	inMPA	0.02
	Epsilonbacteraeota	inMPA	<0.001
	Cyanobacteria	inMPA	0.007
	Tenericutes	HC	0.003
	Deinococcus Thermus	inMPA	0.04

Wilcoxon rank sum test with “Benjamini-Hochberg” adjusted P values

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Table S3 The genera with differential abundance among aMPA, inMPA and HC cohorts

Comparison	Differential genera	Enriched group	FDR
aMPA *vs.* HC	Streptococcus	aMPA	0.04
	Roseburia	HC	0.002
	Subdoligranulum	HC	0.01
	Eubacterium hallii	HC	0.02
	Ruminococcaceae UCG013	HC	0.01
	Eubacterium eligens	HC	0.02
	Anaerostipes	HC	0.01
	Fusicatenibacter	HC	0.03
	Eubacterium ventriosum	HC	0.02
	Dorea	HC	0.007
	Collinsella	HC	0.03
	Lachnospira	HC	0.004
	Lachnospiraceae UCG004	HC	0.02
	Actinomyces	aMPA	0.001
	Butyricicoccus	HC	0.004
aMPA *vs.* inMPA	Parabacteroides	aMPA	0.04
	Fusobacterium	inMPA	0.008
	Dialister	inMPA	<0.001
	Christensenellaceae R7	aMPA	0.01
	Parasutterella	inMPA	0.04
	Aliihoeflea	inMPA	<0.001
	Pelagibacterium	inMPA	<0.001
	Halomonas	inMPA	<0.001
HC *vs.* inMPA	Bacteroides	HC	0.02
	Streptococcus	inMPA	0.002
	Escherichia Shigella	inMPA	0.04
	Alistipes	HC	<0.001
	Subdoligranulum	HC	0.002
	Ruminococcaceae UCG002	HC	0.001
	Parabacteroides	HC	0.003
	Eubacterium coprostanoligenes	HC	<0.001
	Fusobacterium	inMPA	0.01
	Eubacterium hallii	HC	0.001
	Ruminococcaceae UCG013	HC	<0.001
	Dialister	inMPA	<0.001
	Ruminococcaceae UCG005	HC	0.003
	Erysipelotrichaceae UCG003	HC	0.002
	Christensenellaceae R7	HC	0.001
	Aliihoeflea	inMPA	<0.001
	Butyricimonas	HC	0.001
	Eubacterium ventriosum	HC	<0.001
	Lachnospiraceae NK4A136	HC	0.007
	Flavonifractor	inMPA	<0.001
	Ruminococcaceae NK4A214	HC	0.001
	Dorea	HC	0.008
	Pelagibacterium	inMPA	<0.001
	Halomonas	inMPA	<0.001
	Odoribacter	HC	<0.001
	Clostridium innocuum	inMPA	0.03
	Collinsella	HC	0.03
	Ruminococcaceae UCG003	HC	0.005
	Actinomyces	inMPA	<0.001
	Eggerthella	inMPA	0.02
	Butyricicoccus	HC	0.001
	Pseudomonas	inMPA	0.02

Wilcoxon rank sum test with “Benjamini-Hochberg” adjusted P values.

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Table S4 The correlation coefficient matrix between differential genera and clinical indices

R value	Clinical parameter								
	Alb	Cr	BUN	UA	WBC	Hb	ESR	CRP	BVAS
Genus									
Streptococcus	0.28	-0.31	-0.35	-0.22	-0.25	0.30	-0.18	-0.15	-0.27
Alistipes	-0.38	0.41	0.38	0.14	0.17	-0.28	0.17	0.11	0.17
Parabacteroides	-0.16	0.34	0.33	0.21	0.11	-0.19	0.03	-0.23	-0.18
Eubacterium coprostanoligenes	-0.30	0.42	0.35	0.21	0.24	-0.27	0.18	0.06	0.26
Dialister	0.44	-0.47	-0.46	-0.22	-0.16	0.36	0.07	-0.03	-0.05
Aliihoeflea	0.49	-0.63	-0.58	-0.34	-0.20	0.48	-0.24	-0.08	-0.37
Flavonifractor	0.39	-0.36	-0.30	-0.21	-0.35	0.23	0.03	-0.02	-0.10
RuminococcaceaeNK4A214	-0.35	0.41	0.33	0.21	0.22	-0.21	0.12	0.03	0.15
Pelagibacterium	0.47	-0.59	-0.51	-0.31	-0.17	0.39	-0.13	0.01	-0.29
Halomonas	0.42	-0.57	-0.50	-0.32	-0.15	0.37	-0.12	0.04	-0.29
Odoribacter	-0.33	0.37	0.34	0.10	0.36	-0.28	0.06	0.09	0.24
Lachnospira	-0.13	0.08	0.06	-0.07	0.21	-0.15	0.36	0.36	0.48
Actinomyces	0.37	-0.43	-0.38	-0.23	-0.24	0.38	-0.28	-0.28	-0.50
Butyricicoccus	-0.34	0.31	0.26	0.07	0.21	-0.36	0.50	0.40	0.37

Spearman correlations were calculated. Alb, albumin; BUN, Blood Urea Nitrogen; BVAS, Birmingham Vasculitis Activity Score;Cr, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; UA, uric acid; WBC, white blood cell.

Table S5 The OTU markers for initial dialysis and renal prognosis after induction treatment in active MPA

OTU ID	Phylum	Class	Order	Family	Genus	Enriched group
OTU-1026	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Non-dialysis
OTU-27	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	Non-dialysis
OTU-60	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Non-dialysis
OTU-5	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	Non-dialysis
OTU-367	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	Non-dialysis
OTU-71	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Fusicatenibacter	Non-dialysis
OTU-13	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	Non-dialysis
OTU-19	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	Non-dialysis
OTU-42	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	Dialysis
OTU-182	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	ESRD
OTU-150	Actinobacteria	Coriobacteriia	Coriobacteriales	Eggerthellaceae	Eggerthella	ESRD

ESRD, end stage renal disease

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