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Supplemental Appendix 1

Inclusion and exclusion criteria of participants and therapeutic strategy

Patients with IgA nephropathy (IgAN) were recruited from Xijing hospital of the Fourth Military Medical University in Xi'an Shannxi province (Model cohort, n=127) and the First Affiliated Hospital of Zhengzhou University in Henan province of China (Cross-region cohort, n=56).

Patients with primary membranous nephropathy (MN) were recruited from Xijing hospital of the Fourth Military Medical University in Xi'an Shannxi province of China (n=40) in our previous published study[1].

Inclusion and exclusion criteria for patients

Inclusion criteria:

Patients were to: I) have biopsy-proven primary IgAN; II) be between 18–70 years of age, and III) have an estimated glomerular filtration rate (eGFR) from 15 to 120 ml/min/1.73 m^2.

Exclusion criteria:

Patients: who I) received immunosuppressive therapy before biopsy; II) had received antibiotics or probiotics within three months before enrollment; III) had comorbidities (including diabetes mellitus, other autoimmune diseases, acute or chronic gastrointestinal diseases, cancer, and acute infection); and IV) patients with <6 months follow-up.

Healthy controls

Healthy volunteers in the model cohort were sex-, age-, and BMI-matched with IgAN patients from the Physical Examination Center of Xijing hospital in Xi'an Shannxi province (n=86) and from a published paper of Chinese population[2] with the consent and authorization of the first author (n=41). Healthy volunteers in the cross-region cohort were from The First Affiliated Hospital of Zhengzhou University in Henan province of China (n=56).

Inclusion and exclusion criteria for healthy controls

Inclusion criteria:

Volunteers were to have: I) normal kidney and liver function, blood, fecal, and urine tests, fasting blood glucose, blood lipids, and blood pressure; and II) be between 18–70 years of age.

Exclusion criteria:

Volunteers who: I) were administered antibiotics or probiotics within three months before enrollment; II) had history of any chronic disease or acute infection, and III) did not want to donate stool specimens.

Therapeutic strategy

Clinical pathology conferences were held in the Department of Nephrology, Xijing Hospital to decide the optimal treatment for patients with IgAN according to their clinical symptoms, pathological changes and KDIGO guideline (2012)[3]. Patients with IgAN with 24-hour proteinuria more than 0.75g/day after receiving the optimal supportive treatment for at least three months were considered for immunosuppressive therapy, which mainly included: corticosteroid combined with cyclophosphamide (CS+CTX) or corticosteroid combined with mycophenolate Mofetil (CS+MMF). Prednisone was administered at a dose of 30-50 mg/day for 2 months, and then tapered by 20% each month for the next 4 months; CTX was prescribed orally at 50 mg/day or intravenously 0.8g/month for 6 months; MMF was prescribed for 6 months at 1.0 or 1.5 g/day for patients with body weight <50 or ≥50 kg, respectively; Renin-angiotensin system inhibitors were administered by all patients with IgAN during the study.

Definition of clinical outcomes

We defined clinical remission as patients who achieved either partial clinical remission or complete clinical remission.

- Partial clinical remission was defined as 24-hour proteinuria reduced to less than 50% of the baseline and maintained at less than 1.0g/d.
- Complete clinical remission was defined as 24-hour proteinuria reduced to less than 0.2g/d, and renal function that remained stable (eGFR reduction < 30% of the baseline) [4].

References

1 Dong R, Bai M, Zhao J, Wang D, Ning X, Sun S. A Comparative Study of the Gut Microbiota Associated With Immunoglobulin a Nephropathy and Membranous Nephropathy. Front Cell Infect Microbiol 2020;**10:557368.**

2 Ren Z, Fan Y, Li A, Shen Q, Wu J, Ren L*, et al.* Alterations of the Human Gut Microbiome in Chronic

Kidney Disease. Adv Sci (Weinh) 2020;**7**:2001936.

3 Beck L, Bomback AS, Choi MJ, Holzman LB, Langford C, Mariani LH*, et al.* KDOQI US commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis. American journal of kidney diseases : the official journal of the National Kidney Foundation 2013;**62**:403-41.

4 Lv J, Zhang H, Wong MG, Jardine MJ, Hladunewich M, Jha V*, et al.* Effect of Oral Methylprednisolone on Clinical Outcomes in Patients With IgA Nephropathy: The TESTING Randomized Clinical Trial. Jama 2017;**318**:432-42.

Supplemental Appendix 2

Alteration of gut microbiota in patients with IgAN before and after receiving corticosteroids (CS) combined with cyclophosphamide (CTX) or mycophenolate mofetil (MMF)

Patients who received CS+CTX therapy

Patients with IgAN treated with CS+CTX were comparable to the HC group with respect to alpha diversity of baseline gut microbiota and no significant differences were observed between patients who did or did not achieve clinical remission (responders and non-responders) (Shannon index, responders versus HCs, p=0.301; non-responders versus HCs, p=0.796). After receiving the CS+CTX therapy for 6 months, the alpha diversity of the gut microbiota increased in responders and non-responders, and was significantly higher than the HC group (Chao index, responders versus HC, p=0.001; non-responders Versus HC, p=0.007).

With respect to beta diversity, the responders and non-responders were comparable at baseline and were distinct from that of HCs (Adonis test, responders versus HCs, p=0.0002; non-responders versus HCs, p=0.033). After 6 months of CS+CTX, responders showed a striking change in the bacterial composition of the gut microbiota (Adonis test, baseline versus post-treatment, p=0.0132), which was not observed in non-responders (Adonis test, baseline versus post-treatment, p=0.804).

Notably, the abundance of phylum *Proteobacteria* reduced significantly after 6 months of CS+CTX therapy in responders (0.182±0.047 at baseline versus 0.069±0.047 post-treatment, P=0.036), and reached a level of abundance that similar to HCs (0.069±0.047 in responders versus 0.0327±0.007 in HCs, p=0.228). However, no similar reduction was observed in non-responders post-treatment (0.174±0.086 at baseline versus 0.135±0.085 post-treatment, p=0.489).

The abundance of class *Gammaproteobacteria* declined significantly in responders after 6 months of CS+CTX therapy (0.181±0.047 at baseline versus 0.108±0.042 post-treatment, p=0.037), and reached levels of abundance that were similar to HCs

(0.108±0.042 in responders versus 0.033±0.007 in HCs, p=0.208). No similar reduction was observed in non-responders (0.173±0.086 at baseline versus 0.135±0.085 post-treatment, p=0.546).

The abundance of order *Enterobacterales* declined significantly in responders after 6 month of CS+CTX therapy (0.165±0.047 at baseline versus 0.058±0.040 post-treatment, p=0.007), and reached levels of abundance that were similar to HCs (0.058±0.040 in responders versus 0.025±0.006 in HCs, p=0.315). No similar reduction was observed in non-responders (0.158±0.083 at baseline versus 0.127±0.081 post-treatment, p=0.605).

The abundance of family *Enterobacterales* declined significantly in responders after 6 months of CS+CTX therapy (0.165±0.047 at baseline versus 0.086±0.041 post-treatment, p=0.007), and reached levels of abundance that were similar to HCs (0.086±0.041 in responders versus 0.021±0.006 in HCs, p=0.314). No similar reduction was observed in non-responders (0.159±0.084 at baseline versus 0.127±0.082 post-treatment, p=0.605).

The abundance of genus *Escherichia_Shigella* declined significantly in responders after 6 months of CS+CTX therapy (0.160±0.047 at baseline versus 0.041±0.020 post-treatment, p=0.001), and reached levels of abundance that were similar to HCs (0.041±0.020 in responders versus 0.011±0.003 in HCs, p=0.152). No similar reduction was observed in non-responders (0.153±0.082 at baseline versus 0.101±0.063 post-treatment, p=0.666).

Patients who received CS+MMF therapy

Patients with IgAN treated with CS+MMF were comparable to the HC group with respect to alpha diversity of baseline gut microbiota, and no significant differences were observed between patients who did or did not achieve clinical remission (responders and non-responders) (Shannon index, responders versus HCs, p=0.671; non-responders versus HCs, p=0.606). After receiving CS+MMF for 6 months, the alpha diversity of the gut microbiota increased in responders (Shannon index, baseline versus post-treatment, p=0.006), but remained unchanged in non-responders (Shannon

index, baseline versus post-treatment, p=0.208).

With respect to beta diversity, responders and non-responders were comparable at baseline and were similar to HCs (Adonis test, non-responders versus responders versus HCs, p=0.165).

After 6 months of CS+MMF, responders showed a striking change in the bacterial composition of the gut microbiota compared to baseline (Adonis test, baseline versus post-treatment, p=0.002), which was not observed in non-responders (Adonis test, baseline versus post-treatment, p=0.436).

The baseline abundance of genus *Escherichia_Shigella* was remarkably higher in responders and non-responders at baseline compared to HCs (0.077±0.032 in responders versus 0.011±0.003 in HCs, p=0.041; 0.031±0.012 in non-responders versus 0.011±0.003 in HCs, p=0.036).

The abundance of genus *Escherichia_Shigella* declined significantly in responders after 6 months of CS+MMF compared to baseline (0.077±0.032 at baseline versus 0.035±0.016 post-treatment, p=0.558), and reached levels of abundance that were similar to HCs (0.035±0.016 in responders versus 0.011±0.003 in HCs, p=0.174). Conversely, non-responders appeared to experience an increase in the abundance of genus *Escherichia_Shigella* post-treatment (0.031±0.012 at baseline versus 0.069±0.051 post-treatment, p=0.666).

Figure S1. Phylogenetic profiles of the gut microbiome between IgAN and healthy controls (Discovery set; IgAN=84, HC=84).

A. Average compositions of the bacterial community in both groups at the phylum level. The component diagram shows that *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* accounted on average for up to 98% of the relative abundance, and *Proteobacteria* is obviously more abundant in IgAN than HC.

B. Average compositions of the bacterial community in both groups at the class level. The component diagram shows that *Clostridia*, *Bacteroidia*, *Gammaproteobacteria*, *Negativicutes*, *Actinobacteria*, *Bacilli* and *Verrucomicrobiae* accounted on average for up to 98% of the relative abundance, and *Gammaproteobacteria* is obviously more abundant in IgAN than HC.

C. Average compositions of the bacterial community in both groups at the order level.

The component diagram shows that *Bacteroidales*, *Oscillospirales*, *Lachnospirales*, *Enterobacterales*, *Veillonellales−Selenomonadales*, *Bifidobacteriales*, *Acidaminococcales*, *Erysipelotrichales*, *Lactobacillales*, *Burkholderiales* and *Verrucomicrobiales* ccounted on average for up to 98% of the relative abundance, and *Enterobacterales* is obviously more abundant in IgAN than HC.

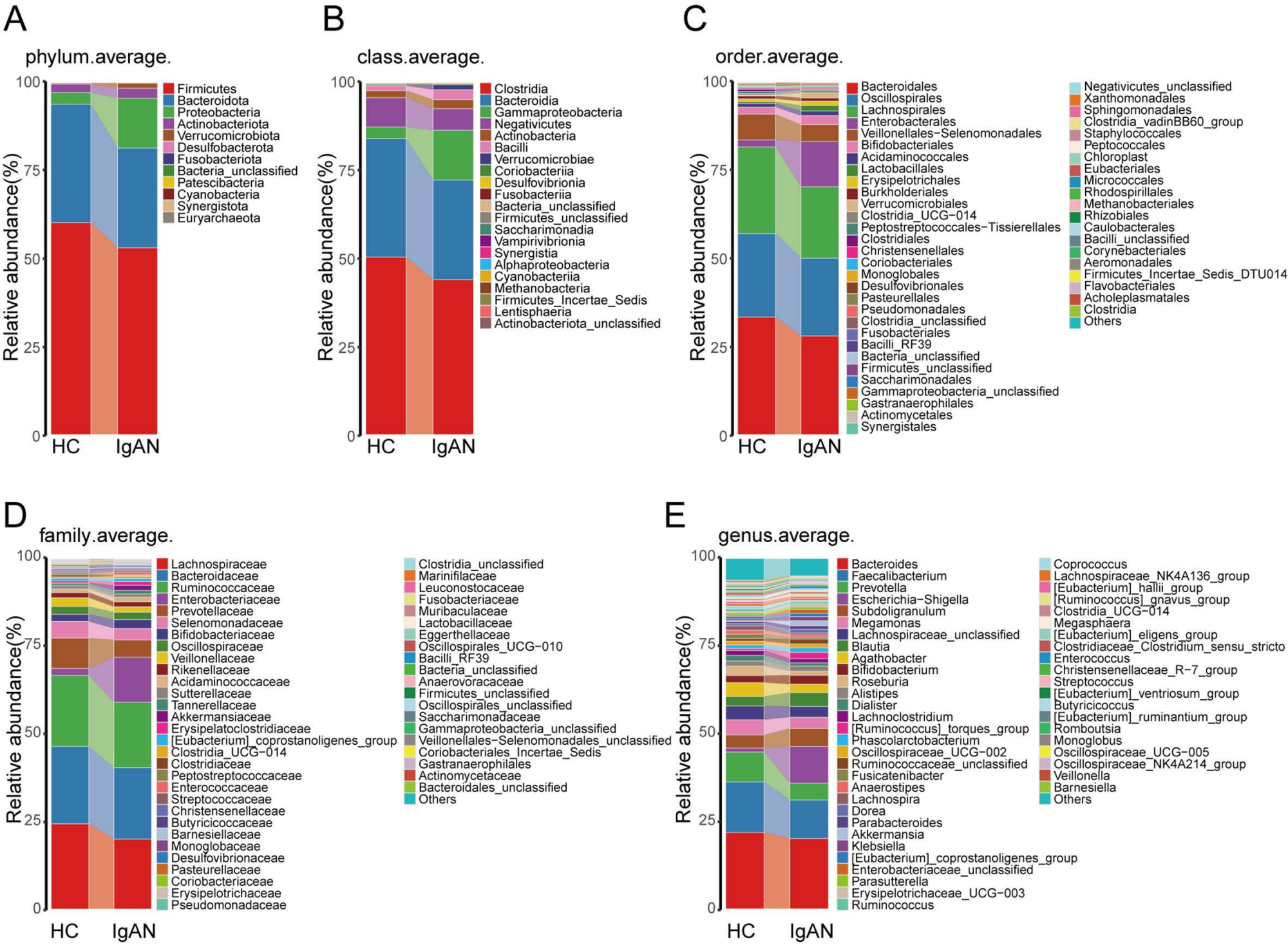
D. Average compositions of the bacterial community in both groups at the family level.

The component diagram shows that *Bacteroidaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Prevotellaceae*, *Enterobacteriaeace*, *Selenomonadaceae*, *Oscillospiraceae*, *Bifidobacteriaceae*, *Veillonellaceae*, *Rikenellaceae*, *Acidaminococcaceae*, *Sutterellaceae*, *Akkermansiaceae*, and *Tannerellaceae* accounted on average for up to 98% of the relative abundance, and *Enterobacteriaceae* is obviously more abundant in IgAN than HC.

E. Average compositions of the bacterial community in both groups at the genus level.

The component diagram shows *that Escherichia−Shigella* is obviously more abundant in IgAN than HC.

IgAN, IgA nephropathy; HC, healthy controls**.**


A
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Relative abundance(%)
100
75
50
25
0
HC
IgAN
Firmicutes
Bacteroidota
Proteobacteria
Actinobacteriota
Verrucomicrobiota
Desulfobacterota
Fusobacteriota
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Patescibacteria
Cyanobacteria
Synergistota
Euryarchaeota
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Bacteroidia
Gammaproteobacteria
Negativicutes
Actinobacteria
Bacilli
Verrucomicrobiae
Coriobacteriia
Desulfovibrionia
Fusobacteriia
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Vampirivibrionia
Synergistia
Alphaproteobacteria
Cyanobacteriia
Methanobacteria
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Lentisphaeria
Actinobacteriota_unclassified
C
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Bacteroidales
Oscillospirales
Lachnospirales
Enterobacterales
Veillonellales−Selenomonadales
Bifidobacteriales
Acidaminococcales
Lactobacillales
Erysipelotrichales
Burkholderiales
Verrucomicrobiales
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Clostridiales
Christensenellales
Coriobacteriales
Monoglobales
Desulfovibrionales
Pasteurellales
Pseudomonadales
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Fusobacteriales
Bacilli_RF39
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Firmicutes_unclassified
Saccharimonadales
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Gastranaerophilales
Actinomycetales
Synergistales
Negativicutes_unclassified
Xanthomonadales
Sphingomonadales
Clostridia_vadinBB60_group
Staphylococcales
Peptococcales
Chloroplast
Eubacteriales
Micrococcales
Rhodospirillales
Methanobacteriales
Rhizobiales
Caulobacterales
Bacilli_unclassified
Corynebacteriales
Aeromonadales
Firmicutes_Incertae_Sedis_DTU014
Flavobacteriales
Acholeplasmatales
Clostridia
Others
D
family.average.
Lachnospiraceae
Bacteroidaceae
Ruminococcaceae
Enterobacteriaceae
Prevotellaceae
Selenomonadaceae
Bifidobacteriaceae
Oscillospiraceae
Veillonellaceae
Rikenellaceae
Acidaminococcaceae
Sutterellaceae
Tannerellaceae
Akkermansiaceae
Erysipelatoclostridiaceae
[Eubacterium]_coprostanoligenes_group
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Clostridiaceae
Peptostreptococcaceae
Enterococcaceae
Streptococcaceae
Christensenellaceae
Butyricicoccaceae
Barnesiellaceae
Monoglobaceae
Desulfovibrionaceae
Pasteurellaceae
Coriobacteriaceae
Erysipelotrichaceae
Pseudomonadaceae
Clostridia_unclassified
Marinifilaceae
Leuconostocaceae
Fusobacteriaceae
Muribaculaceae
Lactobacillaceae
Eggerthellaceae
Oscillospirales_UCG−010
Bacilli_RF39
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Anaerovoracaceae
Firmicutes_unclassified
Oscillospirales_unclassified
Saccharimonadaceae
Gammaproteobacteria_unclassified
Veillonellales−Selenomonadales_unclassified
Coriobacteriales_Incertae_Sedis
Gastranaerophilales
Actinomycetaceae
Bacteroidales_unclassified
Others
E
genus.average.
Bacteroides
Faecalibacterium
Prevotella
Escherichia−Shigella
Subdoligranulum
Megamonas
Lachnospiraceae_unclassified
Blautia
Agathobacter
Bifidobacterium
Roseburia
Alistipes
Dialister
Lachnoclostridium
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Phascolarctobacterium
Oscillospiraceae_UCG−002
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Fusicatenibacter
Anaerostipes
Lachnospira
Dorea
Parabacteroides
Akkermansia
Klebsiella
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Ruminococcus
Coprococcus
Lachnospiraceae_NK4A136_group
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Clostridiaceae_Clostridium_sensu_stricto
Enterococcus
Christensenellaceae_R−7_group
Streptococcus
[Eubacterium]_ventriosum_group
Butyricicoccus
[Eubacterium]_ruminantium_group
Romboutsia
Monoglobus
Oscillospiraceae_UCG−005
Oscillospiraceae_NK4A214_group
Veillonella
Barnesiella
Others

Figure S2. The diagnostic power of the bacterial classifier for discriminate IgAN against MN (n=40).

A. The POD value of IgAN versus MN in the disease validation analysis.

B. The POD index achieved an AUC value of 0.6183 in the MN cohort as shown in ROC.

POD, probability of disease; CI, confidence interval; ROC, receiver operating characteristic; AUC, area under the curve. IgAN, IgA nephropathy; MN, membranous nephropathy**.**

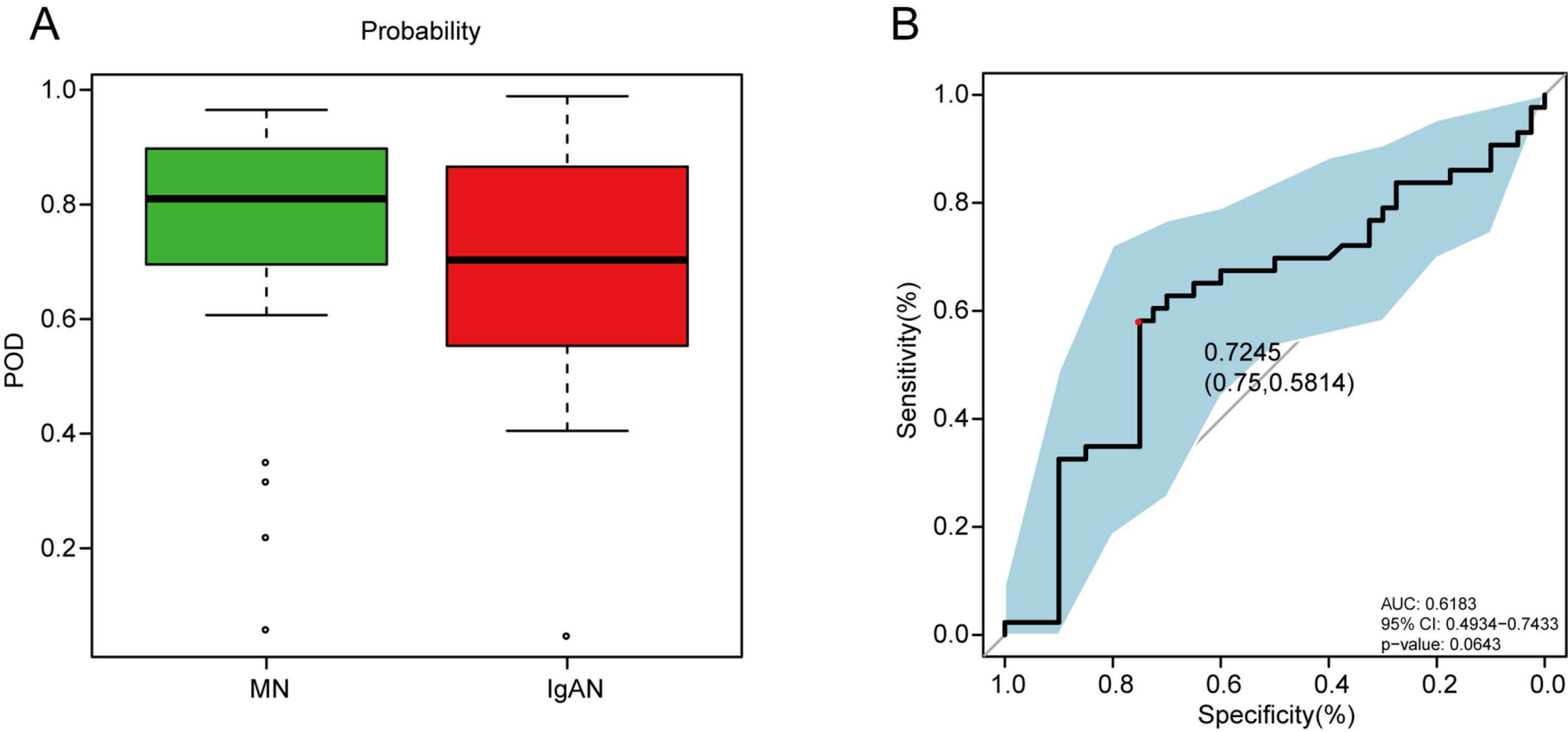
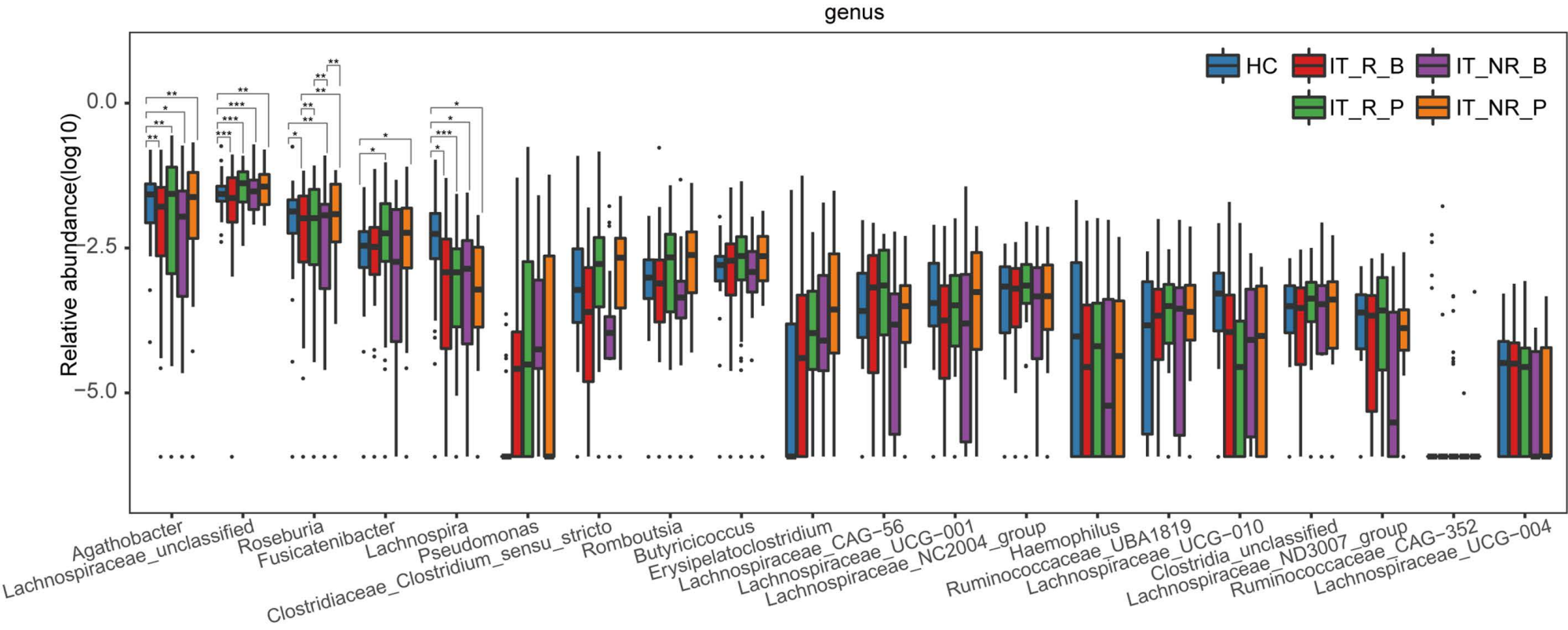

A
Probability
POD
1.0
0.8
0.6
0.4
0.2
MN
IgAN
B
Sensitivity(%)
Specificity(%)
1.0
0.8
0.6
0.4
0.2
0.0
0.7245
(0.75,0.5814)
AUC: 0.6183
95% CI: 0.4934−0.7433
p−value: 0.0643

Figure S3. Relative abundance of genera, which were differentially abundant in treatment-naïve patients with IgAN ((Discovery set; IgAN=84, HC=84)), before and after 6 months immunosuppressive therapy.

The horizontal bar within each box represents the median. The bottom and top of each box represent the 25th and 75th percentiles, respectively. The horizontal bar within each boxplot represents the median; the bottom and top of each box represent the 25th and 75th percentiles, respectively; The upper and lower whiskers extend to data no more than 1.5 × the IQR from the upper and lower edges of the box, respectively, and black dots represent outliers beyond the whiskers. IgAN, IgA nephropathy; HC, healthy controls**;** IT, immunosuppressive therapy; R, achieved clinical remission; NR, did not achieve clinical remission; B, before treatment; P, after treatment. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.


genus
HC
IT_R_B
IT_NR_B
IT_R_P
IT_NR_P
Relative abundance(log10)
0.0
−2.5
−5.0
Agathobacter
Lachnospiraceae_unclassified
Roseburia
Fusicatenibacter
Lachnospira
Pseudomonas
Clostridiaceae_Clostridium_sensu_stricto
Romboutsia
Butyricicoccus
Erysipelatoclostridium
Lachnospiraceae_CAG−56
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