Analysis of microbiota in patients with Acute Cerebral Infarction

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Abstract

Background and Aims: Recent evidence suggests that the microbiota is associated with almost all major types of diseases, including cardiovascular diseases. Yet its role in Acute Cerebral Infarction remains unexplored. It is important to understand the diversity and distribution of gut microbiota (GM) in patients with Acute Cerebral Infarction, and the role that GM plays in this type of disease.

Methods: To elucidate whether gut microbiota composition differs between patients with acute cerebral infarction and healthy controls, we performed pyrosequencing of the gut microbiota on 40 individuals: 31 with Acute Cerebral Infarction and 9 control individuals. We applied linear regression to calculate the correlation between gut flora and disease risk factors. Finally, KEGG functional enrichment analysis was conducted to examine the correlation between gut flora and Acute Cerebral Infarction.

Results: The overall microbial structure was similar in controls and patients, but the control group had higher relative presence of *Blautia obeum* while the presence of *Streptococcus infantis* and *Prevotella copri* were relatively high in the patient group. Using linear regression, we found that *Blautia obeum* was negatively associated with white blood cell count and *Streptococcus infantis* was positively correlated with creatinine and Lipoprotein. The KEGG pathway analysis indicated that the bio-pathways including methane metabolism, lipopolysaccharide synthesis, bacterial secretion and flagellar assembly of the gut microbiota in the patient group was expressed differently than that of the controls. We identified three differentially expressed gut microbial functions in Acute Cerebral Infarction and found four bacterial pathways that might be related to the development of this disease.

Conclusions : Our study identified three abnormally-expressed bacteria -- Blautia obeum, Streptococcus infantis and Prevotella copri -- in patients with ischemic stroke compared to healthy controls. It reveals a correlation of these bacterial species to Acute Ischemic Stroke as they relate to disease factors and functional pathways. These findings may shed light on the treatment of ischemic stroke because gut microbiota could serve as a potential therapeutic approach for the treatment of cardiovascular and metabolic diseases.

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Introduction

Stroke caused 6.2 million deaths globally[1], making it the second leading cause of death. Cerebral infarction, also known as ischemic stroke, accounting for 87 percent of all stroke cases[2], occurs when the arteries that supply blood to the brain are blocked or narrowed so that blood flow is interrupted. The main cause of ischemic stroke is atherosclerosis, which results in the development of fatty deposits lining the vessel walls. Tissue plasminogen activator (TPA) [3] is a common treatment for ischemic stroke, but many

patients do not access brain-saving treatment because they fail to arrive at the hospital within the limited time that treatment can be effective. Thus it is critical to identify stroke and to seek treatment immediately to have the best possible chance of full recovery.

Dysbiosis of the gut microbiota has not only been shown to contribute to the development of the immune system[4], but also, remarkably, to the development of the Central Nervous System (CNS) [5]. Researchers have identified variation in the composition of gut microbiota in strokes[6-8] but only a few experimental studies focusing on the role of gut microbiota in cerebral ischemia have been published. The link between gut flora and acute cerebral infarction remains to be established, so the exact mechanisms of how gut microbiota are involved in stroke are yet to be determined.

To elucidate this relationship, we conducted the described study in which 40 samples (31 from patients with Acute Ischemic Stroke and nine from controls) were analyzed for 16S rRNA genes by pyrosequencing to detect differences in gut microbial composition and to explore the correlation between gut flora and Acute Ischemic Stroke using linear regression and KEGG enrichment analysis.

Methods and Results

Methods

1. Sample Collection

Fecal samples were collected from patients with Acute Cerebral Infarction from the First Affiliated Hospital of Nanjing Medical University, from February 2018 to May 2018. There was no negative control for age or gender groups; instead, the age range was set from 40 to 80 years. The main inclusion criteria for the study group was Acute Cerebral Infarction as diagnosed by a neurologist. All patients underwent Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) scan to assess ischemic lesions. The large-artery atherosclerotic subtype of atherosclerosis was determined according to the TOAST classification system. Radiological evidence of intracerebral hemorrhage, a definitive cause of stroke or TIA, is not associated with atherosclerosis (e.g., carotid dissection, perivascular programmed stroke, cardiac stroke or other TOAST subtypes), serious comorbidity, or a medical condition within one month prior to admission (i.e., patients with congestive heart failure, respiratory failure, renal failure or severe liver dysfunction, or those taking probiotics or antibiotics, were excluded from the study group). The control group consisted of asymptomatic participants (according to physical examination and self report of no acute disease). The control group excluded participants who had taken antibiotics or probiotics within a month prior to admission, or with a history of cerebrovascular disease. In addition, we exclude interference from diabetes and coronary heart disease. Color Doppler imaging, transcranial Doppler ultrasound were performed in all control groups to determine cardiovascular status. The trial was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University, also the experiment was obtained informed consent from patients in hospital.

Fresh stool samples were collected in the morning to avoid surface contamination and urine contamination. Internal stool samples of 3-5g were collected with a clean sterile spoon and were placed in a sterile, air tight tube (QIAGEN). In an anaerobic environment, the sample was preserved immediately in a liquid nitrogen tube to avoid repeated freezing and thawing. After dry ice transportation, the sample was stored at -80 ℃. Fecal stool collection in the patient group was completed within 48 hours of admission.

2. PCR Amplification and Pyrosequencing of Bacterial 16S rRNA Genes

Bacterial DNA was extracted from fecal samples according to manufacturer instructions using the Fecal DNA Extraction Kit (QIAGEN). The V4 variable region of bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using bar code primers 514F (GTGCCAGCMGCCGCGTAA) and 805R (GGACTACHVGGGTWTCTAAT). The PCR cycle conditions were: initial denaturation at 94C for two minutes; denaturation at 94C for 30 seconds; denaturation at 52C for 30 seconds; denaturation at 72C for 45 seconds; and extension at 72C for five minutes. Each 25μL reaction consisted of 0.5μL of template DNA, 2.0μL of dNTP Mix (2.5 mmol/L; TaKaRa), 2.5μL of TaKaRa 10Ex Taq buffer ($Mg2^+$ free), 1.5μL of $Mg2^+$ (25 mmol/L), 0.25μL of TaKaRa Ex Taq DNA polymerase (2.5 units), 0.5μL of 10 lmol/L bar code primer 514F, 0.5 μL of 10 μmol/L primer 805R, and 17.25μL of double-distilled water. All dilution was carried out with sterile double distilled water.

All PCR products were combined and sent to Beijing Genomic Institute for sequencing using the Illumina Miseq (PE 150), according to manufacturer protocol[23].

3. Statistical Analysis

The tag number of each taxonomic rank (species) or OTU in the samples were summarized in a profiling table or histogram, drawn with R(v3.1.1) software. The nonparametric Wilcoxon Rank-Sum test was used to identify OTU among different groups in QIIME. In order to determine the differences between the two groups not reported in QIIME, the OTU:s of $P<0.1$ in QIIME analysis were classified into generic levels, and then statistical tests were implemented using Graphpad prim6. A chi-square test was used for categorical variables. A value of $P<0.05$ was considered statistically significant in the comparison groups.

Results

1. Composition of Gut Flora in Patients with Acute Cerebral Infarction and in Controls

In this study, a total of 40 blood and fecal samples were collected from 31 patients and nine healthy controls. Clinical characteristics of the whole population are shown in Table 1.

To investigate whether gut microbiota composition differed between the study population of patients with cerebral infarction and healthy controls, we performed sequencing of the V4 region of the 16S ribosomal RNA gene from fecal samples. After filtering for quality, a total of 1,670,914 reads were included for downstream analysis and an average of 41,772± 293 sequences (SD) were assigned to each sample.

PLS-DA was performed in order to further distinguish between groups. This was achieved by rotating PCA components such that a maximum separation between classes was obtained, and to understand which variables carry the class separating information. The PCA components were calculated to analyze the composition of the gut microbiota in patients with Acute Cerebral Infarction and in the control group (figure1A). However, the diversity of species richness (represented by Chao, observed species, ace), and richness and evenness (represented by Shannon and Simpson indexes) of the microbial community indicated that no significant difference in gut flora diversity could be identified between these two groups (Figure 1B).

2. Gut Bacterial Species Variations in Acute Cerebral Infarction Patients and Controls

In contrast, taxa abundance in gut microbiota showed significant differences at the species level between patients with Acute Cerebral Infarction and healthy controls. The bacterial species with the highest relative abundances were *Blautia*, *Streptococcus* and *Prevotella*. The most remarkable difference was found in *Blautia obeum*. The presence of *Blautia obeum* is relatively lower in the patient group while *Streptococcus infantis* and *Prevotella copri* are relatively higher in ischemic stroke patients compared to the control group (Figure 2.)

3. Correlation between Gut Microbial Taxa at the Species Level and Acute Ischemic Stroke

After identifying three abnormally-expressed gut microbiota species, we analyzed whether there were associations between bacteria and cardiovascular risk factors using linear regression. Findings are indicated in Table 2. They show that *Blautia* was negatively associated with white blood cell count. In contrast, *Streptococcus* showed positive correlation with creatinine and Lipoprotein, whereas *Prevotella* had no significant relation to any disease risk factors.

To predict the abundance of gene families and related functional pathways of microbial communities in fecal contents, KEGG functional pathway analysis, a predictive metabolism approach, was performed based on the 16S rRNA gene sequencing and Green Genes database (Figure.3). Results suggested that many bacterial pathways involved in methane, lipopolysaccharide, secretion and flagella functions were significantly modulated in Acute Cerebral Infarction.

Functional analysis showed that the methane metabolism of these three bacteria was inhabited in patients with acute IS. But lipopolysaccharide synthesis was enhanced in patients with acute IS. In addition, acute ischemic stroke may be positively correlated with bacterial secretion and flagellar assembly in these three gut microbiota.

Discussion

Pyrosequencing of 40 fecal samples revealed that while the overall microbial structure was similar in controls and patients with Acute Cerebral Infarction, the patient group had a higher relative abundance of the bacterial species *Streptococcus infantis* and *Prevotella copri* and lower abundance of *Blautia obeum*.

The intestinal microbiome has been further implicated in the pathogenesis of multiple diseases such as obesity, depressive disorder, chronic ileal inflammation, liver disease, and atherosclerosis.[9-15] For example, the metabolism by intestinal microbiota of dietary L-carnitine, a nutrient in red meat, was demonstrated to promote atherosclerosis and lead to cardiovascular disease risk by producing trimethylamine and trimethylamine-N-oxide.[15] However, its role in cerebral infarction is not well understood, with no published reports linking gut flora with clinical data of Acute Ischemic Stroke.

To address this void, the present study sought to determine whether the composition of gut microbial community differed between Acute IS patients and healthy controls, and whether gut microbial taxa correlates with risk factors for ischemic stroke. In terms of microbiota composition, no significant difference was identified between the patient group and the control group. But the control group showed a higher prevalence of *Blautia obeum* (0.315%) in the gastrointestinal system than the disease group (0.115%).

Blautia obeum is a species of anaerobic, gram-positive bacteria found in the gut and that is recognized as being dominant in the human colon[16]. It has been reported that *B. obeum*, along with other relevant taxa, play an important role both in the recovery process from *V. cholerae* infection and microbiota maturation in children. [17] There has been speculation about the possibility that some of these bacteria may be helpful in the 'repair' of gut microbiota in individuals whose gut communities have been 'wounded' through a variety of insults, including enteropathogen infection[17]. In addition, D. Hatziioanou et. al. identified and characterized a gene cluster from the human gut isolate *Blautia obeum* A2-162 which encodes the novel nisin-like peptides NsoA1-3 and NsoA4. Moreover, the antimicrobial activity of the host strain could be detected in the presence of trypsin. [18] Thus, based on our discovery, *B. obeum* may function as an anti-stroke factor and therefore its role in Acute Ischemic Stroke deserves thorough investigation.

We also found that both *Streptococcus infantis* and *Prevotella copri* were expressed more in acute ischemic stroke patients than in healthy people. *Streptococcus infantis* is reported to cause minocycline resistance -- its genetic basis is due to tet(S) present on a novel low copy number plasmid flanked by IS1216 elements, which likely mediate its excision. [19] *Prevotella* are members of the oral, vaginal, and gut microbiota and are often recovered from anaerobic infections of the respiratory tract. Overgrowth of *Prevotella* in other diseases has been demonstrated, such as hypertension[20] and chronic inflammatory disease.[21] When compared to strict commensal bacteria, *Prevotella* exhibits increased inflammatory properties, and studies indicate that *Prevotella* predominantly activate the toll-like receptor 2, leading to production of Th17-polarizing cytokines by antigen presenting cells, including interleukin-23 (IL-23) and IL-1. Furthermore, the expansion of *Prevotella copri* is correlated with enhanced susceptibility to arthritis, and the colonization of mice revealed the ability of *P. copri* to dominate the intestinal microbiota, resulting in an increased

sensitivity to chemically induced colitis. [22] There is growing evidence linking *Prevotella copri* to human diseases.[22] Our study revealed that these two bacteria were found in relatively low abundance in Acute Ischemic Stroke; whether they exert an indirect influence on the pathogenesis of this disease remains to be determined.

The correlation between gut flora and disease risk factors was also analyzed, using linear regression. The data revealed a negative correlation between *Blautia*'s and WBC, and a positive correlation between *Streptococcus* and creatinine and Lipoprotein. Combined with previous findings, it may be concluded that *Blautia* may support the immune system, while *Streptococcus* may contribute to the development of Acute Ischemic Stroke.

Finally, we compared the differences of microbial bio-pathway enrichment between the patient group and control group by KEGG enrichment analysis. The analysis revealed that methane metabolism inhabited patients with acute IS, while lipopolysaccharide synthesis, secretion pathways and the flagellar assembly process were enhanced in these patients.

In conclusion, this study identified three abnormally-expressed bacteria -- *Blautia obeum*, *Streptococcus infantis* and *Prevotella copri* -- in patients with ischemic stroke compared to healthy controls. It also revealed a correlation of these bacterial species to Acute Ischemic Stroke as they relate to disease factors and functional pathways. These findings may shed light on the treatment of ischemic stroke because gut microbiota could serve as a potential therapeutic approach for the treatment of cerebrovascular and metabolic diseases.

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Author contributions

L H provided project design, data collection, statistical analysis, writing and revision of articles; T W helped patient screening, sample collection, and data collection; Q W helped in data analysis and article modification, X D helped sample Statistical analysis of data collection and data;F S, D L and X Q participated in patient screening, sample collection, and data collection; L Y helped in research ideas, project design, interpretation of materials, revision of articles, and funding. Q W provided research ideas, project design, data collection, analysis and interpretation of materials, drafting, revision of manuscripts, statistical analysis and supervision of this research.

Conflict interest

The authors report no relationships that could be construed as a conflict of interest.

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284

285 **Table 1. Characteristics of the study participants**

286 CAD, coronary artery disease; HBP, high blood pressure; HDL, high-density lipoprotein; HLP,
287 hyperlipidemia; LAA, large-artery atherosclerosis; LDL, low-density lipoprotein; TC, total cholesterol; TG,
288 triglycerides; GLU, glucose; WBC, white blood cell count; Cr, creatinine; UA, uric acid;
289 LP(a),Lipoprotein(a). Median and inter-quartile range where applicable. Significant differences between
290 groups were analyzed with the: a, Chi-square test; b Kruskal Wallis Test; c, Student's t test.

291 **Table 2. Correlation between gut microbial taxa at the Species level and disease risk factors**

292 P-values and r2-values for linear regression. A + or - indicates positive or negative association, respectively.
293 N.S indicates not significant.

294 **Figure 1. Comparison of a-diversity between the gut microbiota of patients and controls.**

295 A: OTU Based PLS-DA Analysis. Orange triangles represent samples (intestinal microbiota) from patients;
296 blue circles represent samples of controls. B: Five indices were used to represent the a-diversity (observed
297 species, chao, ace, Shannon's diversity, Simpson diversity.) N indicates healthy people and P indicates
298 patients.

299 **Figure 2. Analysis of the variation of intestinal gut in species level in controls and IS patients**

300 A: The relative abundance of each species in the intestinal gut in each sample from IS patients and
301 controls.B: The average relative abundance of three gut microbiota (Blautia boeum, Streptococcus infantis,
302 Prevotella copri) between patient group and control group. $P<0.05$ was considered statistically significant
303 in the comparison groups. N indicates normal people and P indicates patients.

304 **Figure 3. Pathway abundance analysis of microbial taxa**.

305 A: KEGG functional pathway abundance analysis of the gut microbiota B: Average pathway abundance of
306 a gut bacterium N indicates normal people and P indicates patients.

307

Figure 1(on next page)

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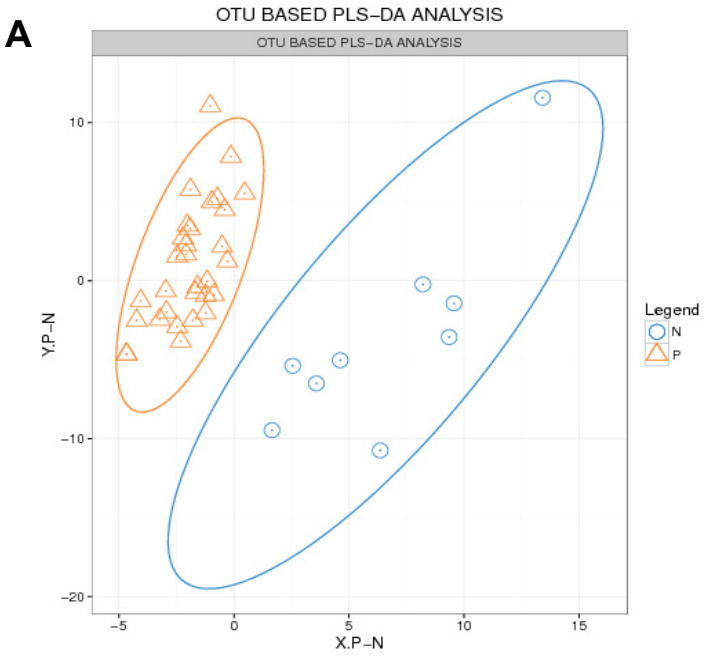
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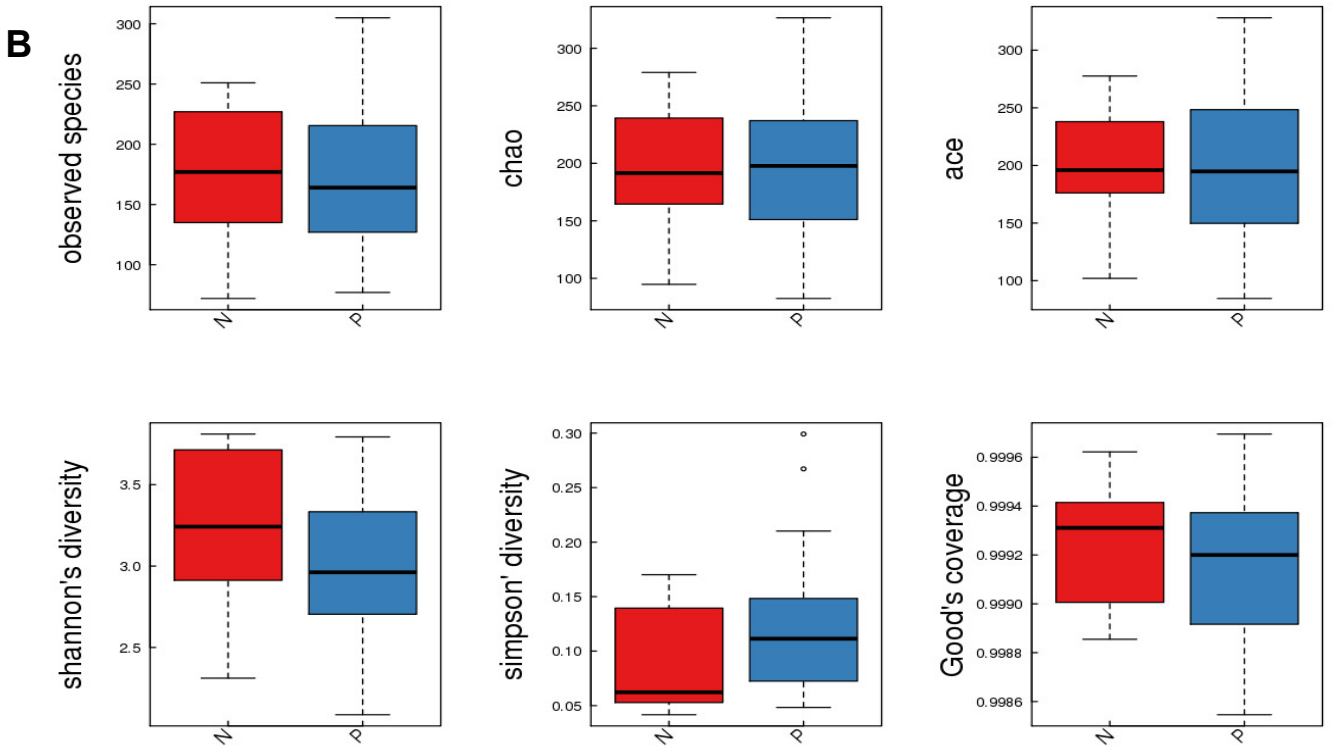
B
observed species
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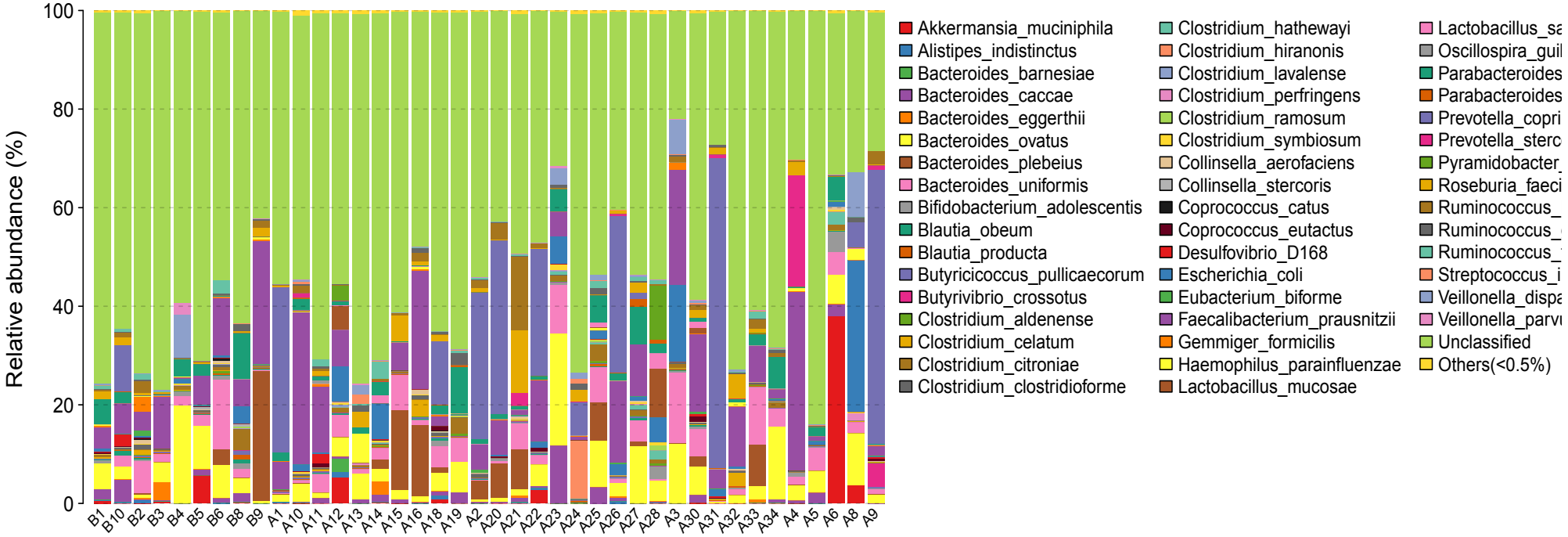
Good's coverage
N
P

Figure 2(on next page)

Analysis of the variation of intestinal gut in species level in controls and IS patients

A: The relative abundance of each species in the intestinal gut in each sample from IS patients and controls.B: The average relative abundance of three gut microbiota (Blautia boeum, Streptococcus infantis, Prevotella copri) between patient group and control group. $P<0.05$ was considered statistically significant in the comparison groups. N indicates normal people and P indicates patients.

A


Relative abundance (%)
100
80
60
40
20
0
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Akkermansia_muciniphila
Alistipes_indistinctus
Bacteroides_barnesiae
Bacteroides_caccae
Bacteroides_eggerthii
Bacteroides_ovatus
Bacteroides_plebeius
Bacteroides_uniformis
Bifidobacterium_adolescentis
Blautia_obeum
Blautia_producta
Butyricicoccus_pullicaecorum
Butyrivibrio_crossotus
Clostridium_aldenense
Clostridium_celatum
Clostridium_citroniae
Clostridium_clostridioforme
Clostridium_hathewayi
Clostridium_hiranonis
Clostridium_lavalense
Clostridium_perfringens
Clostridium_ramosum
Clostridium_symbiosum
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Collinsella_stercoris
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Faecalibacterium_prausnitzii
Gemmiger_formicilis
Haemophilus_parainfluenzae
Lactobacillus_mucosae
Lactobacillus_sa
Oscillospira_guil
Parabacteroides
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Prevotella_sterc
Pyramidobacter_
Roseburia_faeci
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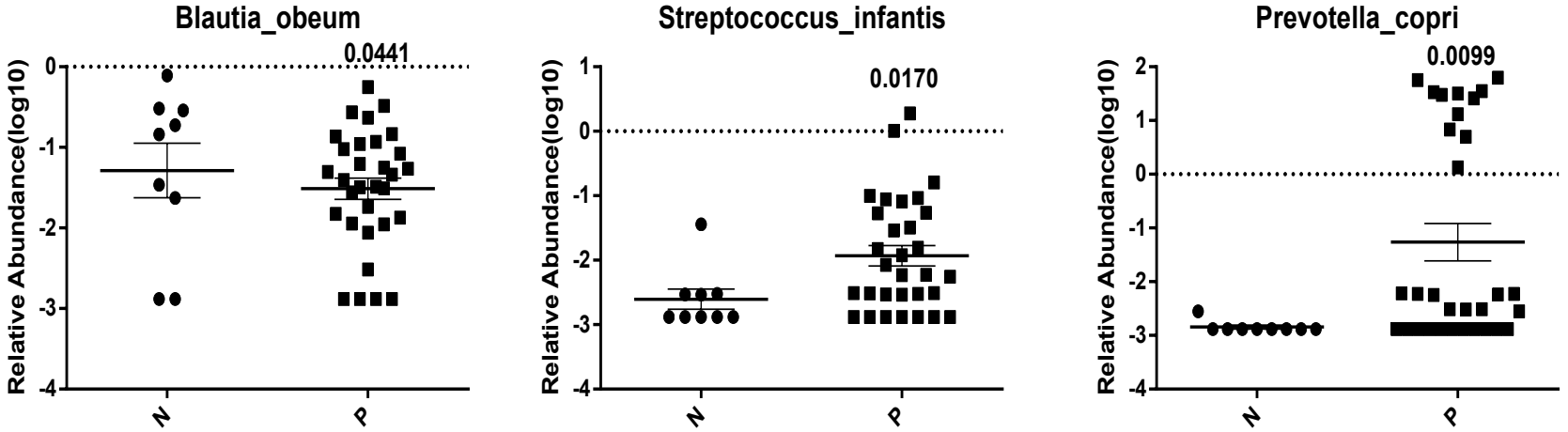
Veillonella_parvu
Unclassified
Others(<0.5%)

B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-2
-3
-4
N
P
Streptococcus_infantis
0.0170
Relative Abundance(log10)
1
0
-1
-2
-3
-4
N
P
Prevotella_copri
0.0099
Relative Abundance(log10)
2
1
0
-1
-2
-3
-4
N
P

Figure 3(on next page)

Pathway abundance analysis of microbial taxa.

A: KEGG functional pathway abundance analysis of the gut microbiota B: Average pathway abundance of a gut bacterium N indicates normal people and P indicates patients.

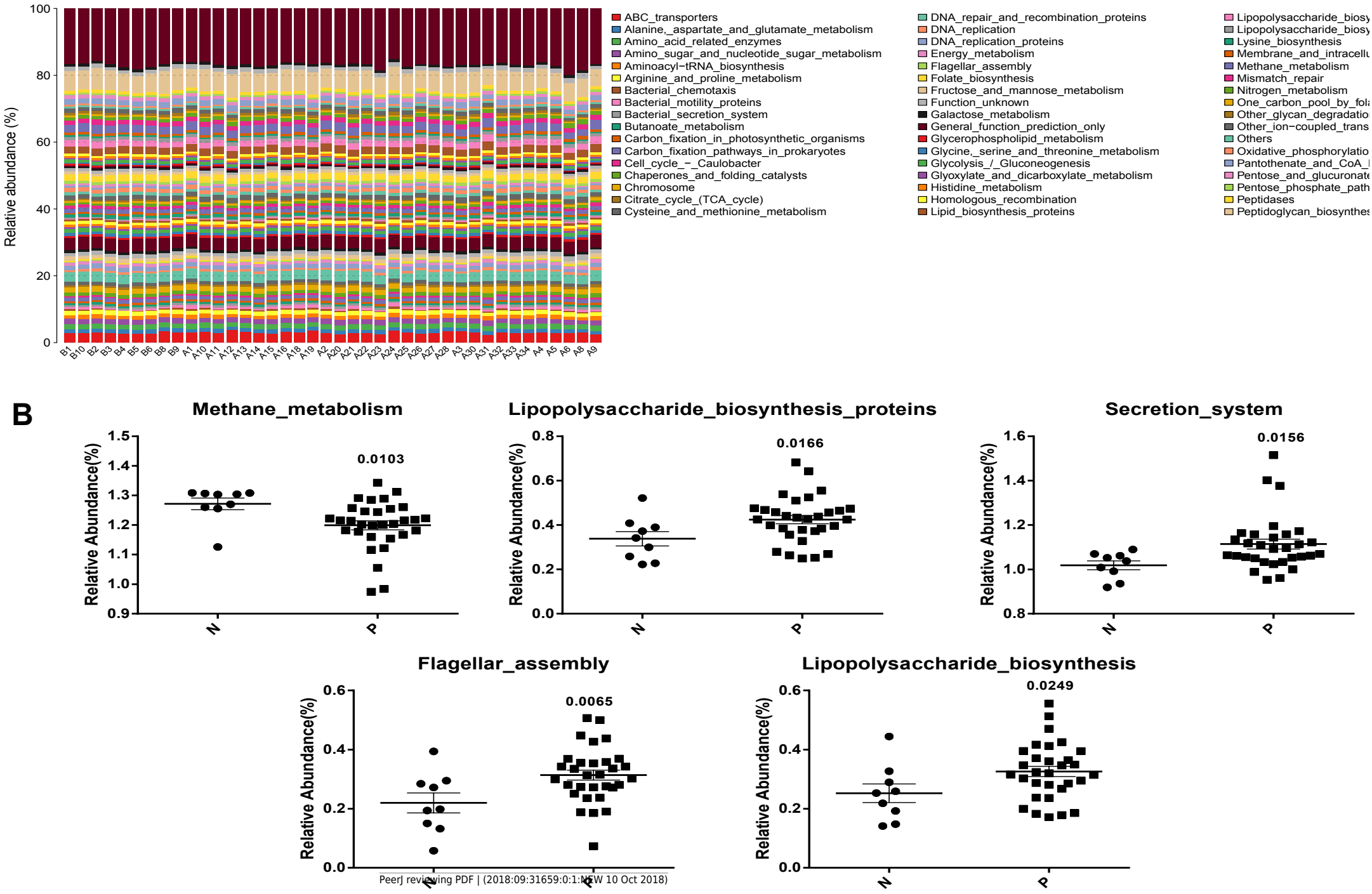
A
Relative abundance (%)
100
80
60
40
20
0
B1 B10 B2 B3 B4 B5 B6 B8 B9 A1 A10 A11 A12 A13 A14 A15 A16 A18 A19 A2 A20 A21 A22 A23 A24 A25 A26 A27 A28 A3 A30 A31 A32 A33 A34 A4 A5 A6 A8 A9
ABC_transporters
Alanine,_aspartate_and_glutamate_metabolism
Amino_acid_related_enzymes
Amino_sugar_and_nucleotide_sugar_metabolism
Aminoacyl−tRNA_biosynthesis
Arginine_and_proline_metabolism
Bacterial_chemotaxis
Bacterial_motility_proteins
Bacterial_secretion_system
Butanoate_metabolism
Carbon_fixation_in_photosynthetic_organisms
Carbon_fixation_pathways_in_prokaryotes
Cell_cycle_−_Caulobacter
Chaperones_and_folding_catalysts
Chromosome
Citrate_cycle_(TCA_cycle)
Cysteine_and_methionine_metabolism
DNA_repair_and_recombination_proteins
DNA_replication
DNA_replication_proteins
Energy_metabolism
Flagellar_assembly
Folate_biosynthesis
Fructose_and_mannose_metabolism
Function_unknown
Galactose_metabolism
General_function_prediction_only
Glycerophospholipid_metabolism
Glycine,_serine_and_threonine_metabolism
Glycolysis_/_Gluconeogenesis
Glyoxylate_and_dicarboxylate_metabolism
Histidine_metabolism
Homologous_recombination
Lipid_biosynthesis_proteins
Lipopolysaccharide_biosy
Lipopolysaccharide_biosy
Lysine_biosynthesis
Membrane_and_intracellu
Methane_metabolism
Mismatch_repair
Nitrogen_metabolism
One_carbon_pool_by_fola
Other_glycan_degradatio
Other_ion−coupled_trans
Others
Oxidative_phosphorylatio
Pantothenate_and_CoA_
Pentose_and_glucuronate
Pentose_phosphate_path
Peptidases
Peptidoglycan_biosynthes
B
Methane_metabolism
Relative Abundance(%)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.0103
N
P
Lipopolysaccharide_biosynthesis_proteins
Relative Abundance(%)
0.8
0.6
0.4
0.2
0.0
0.0166
N
P
Secretion_system
Relative Abundance(%)
1.6
1.4
1.2
1.0
0.8
0.0156
N
P
Flagellar_assembly
Relative Abundance(%)
0.6
0.4
0.2
0.0
0.0065
N
P
Lipopolysaccharide_biosynthesis
Relative Abundance(%)
0.6
0.4
0.2
0.0
0.0249
N
P

Table 1(on next page)

Characteristics of the study population.

CAD, coronary artery disease; HBP, high blood pressure; HDL, high-density lipoprotein; HLP, hyperlipidemia; LAA, large-artery atherosclerosis; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; GLU□glucose; WBC, white blood cell count; Cr, creatinine; UA, uric acid; LP(a),Lipoprotein(a). Median and inter-quartile range where applicable. Significant differences between groups were analyzed with the: a, Chi-square test; b Kruskal Wallis Test; c, Student's t test.

Characteristics of the study population.

Variable	Patients	Controls	P Value
N	31	9	-
Male, n (%)	22（70.97）	6（66.67）	0.385[a]
Age (Median, IQR)	61 (40-94)	61 (53-69)	.238[b]
Current smoker(%)	19（61.29）	0	0.001[a]
Previous diabetes, n (%)	0	0	-
Previous CAD, n (%)	0	0	-
Previous HBP, n (%)	23（74.19）	2（22.22）	0.005[a]
Previous HLP, n (%)	11（35.48）	0	0.036[a]
Previous ischemic stroke(%)	1（3.23）	0	0.585[a]
TC, mmol/L	4.984 (2.87-12.33)	4.089 (3.13-5.13)	0.028[b]
HDL, mmol/L	1.056 (0.72-1.98)	1.437 (1.12-2.00)	0.002[b]
LDL, mmol/L	3.199 (1.65-7.42)	2.712 (1.980-3.610)	0.199[b]
TG, mmol/L	1.93 (0.79-6.26)	0.961 (0.510-1.260)	0.003[c]
GLU, mmol/L	5.132 (4.03-6.90)	4.646 (4.00-5.800)	0.178[b]
WBC, 10^9/L	6.733 (3.20-10.20)	4.932 (3.110-7.520)	0.023[b]
Cr, μmol/L	75.28 (42.30-128.5)	59.52 (46.80-72.00)	0.13[b]
UA,μmol/L	329.4 (31.00-510.0)	277.9 (194.0-407.0)	0.134[c]
LP(a),mg/L	325.8 (46.00-935.0)	74.89 (6.000-136.0)	0.003[b]

Table 1. Characteristics of the study participants

CAD, coronary artery disease; HBP, high blood pressure; HDL, high-density lipoprotein; HLP, hyperlipidemia; LAA, large-artery atherosclerosis; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; GLU，glucose; WBC, white blood cell count; Cr, creatinine; UA, uric acid; LP(a),Lipoprotein(a). Median and

inter-quartile range where applicable. Significant differences between groups were analyzed with the: a, Chi-square test; b Kruskal Wallis Test; c, Student's t test.

Table 2(on next page)

Correlation between gut microbial taxa at the Species level and disease risk factors

P-values and r^2-values for linear regression. A + or - indicates positive or negative association, respectively. N.S indicates not significant.

	TC	HDL	LDL	TG	GLU	WBC	Cr	UA	LP(a)
Blautia	N.S	N.S	N.S	N.S	N.S	P=0.04,r^2= 0.1053 -	N.S	N.S	N.S
Prevotella	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Streptococcus	N.S	N.S	N.S	N.S	N.S	N.S	p=0.0226,r^2=0.1328+	N.S	p=0.0494, r^2=0.1004 +

Table 2. Correlation between gut microbial taxa at the Species level and disease risk factors

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