*TMR Integrative Medicine*

# The variety of intestinal flora in Korean medicine Sasang people
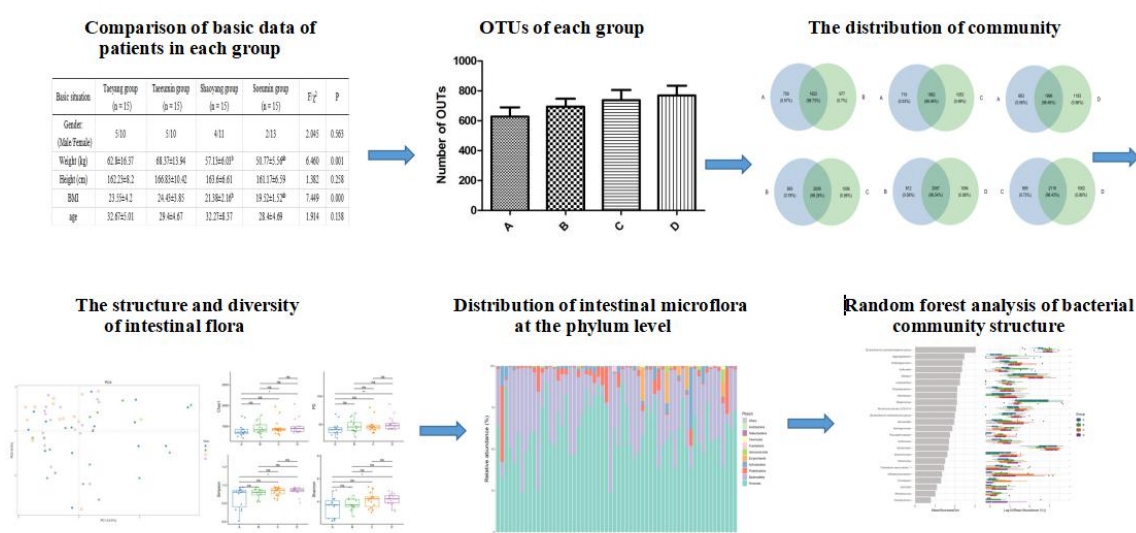
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## Highlights

The Sasang medicine of the Korean medicine is originated from *Dong Yi Shou Shi Bao Yuan* established by Mr. Lee Jima. It emphasizes that in the aspect of image discrimination, the patient's constitution, appearance, temperament, hobbies and so on are observed and understood through observation and inquiry methods, then, combined with the comprehensive reasoning and judgment of pulse condition, people are divided into four types: Taeyang (more Yang), Taeeum (more Yin), Shaoyang (less Yang) and Soeum (less Yin), according size, strong or weak of human organs, howeve, the concrete mechanism remains currently unknown yet and affects its academic development. Traditional Chinese medicine is primarily metabolized through the intestinal metabolism, and intestinal flora plays an important role in the metabolism of traditional Chinese medicine. New research is showing that closely associated with intestinal flora constitution. Therefore, it is believed that there is a certain correlation between the Sasang constitutions of the Korean medicine and intestinal flora. The current study explored and analyzed the diversity and difference of the intestinal flora in the Sasang Constitutions of the Korean medicine and the distribution regularities of the flora among these four constitutions, and provide new ideas for the distinguishing research of the four constitutions of the Korean medicine.

## Abstract

**Background:** To confirm that the four constitutions of the Korean medicine is closely related to the intestinal flora, and explore the influence of specific intestinal microbes on these four populations. **Methods:** We collect stool samples of four different physiques of the Taeeum, Soeum, Taeyang, and Shaoyang. Based on 16S rDNA amplicon sequencing, SPSS 18.0 statistical software and GraphPad Prism 8.0 mapping software, the body weight, body mass index, cholesterol and intestinal microcommunity composition of the four groups were analyzed and compared. **Results:** The results found that the body weight and body mass index of the Taeeum group were significantly higher than the Shaoyang group and the Soeum group, the body weight, cholesterol and body mass index of the Taeyang group were significantly higher than the Soeum Group. In the analysis of gut microbiota, the richness and diversity of intestinal microorganisms and the abundance ratio of posterior firmicutes to bacteroides were all different in the four populations, and there were 24 gut microbiota with significant differences ($P < 0.05$). Among them, the abundance of *[Eubacterium] coprostanoligenes* group of Taeyang people, Taeeum people, Shaoyang people, and Soeum people were 3.71, 9.62, 9.36 and 9.33, respectively. **Conclusion:** *[Eubacterium] coprostanoligenes* group may be related to the differences in cholesterol content of the four populations. Similarly, the firmicutes/bacteroidetes abundance ratio may also be related to the differences in body weight, blood glucose and blood lipid among the four groups.
**Keywords:** Korean medicine; Sasang people; Intestinal flora; Variety

## Background

The Sasang medicine of the Korean medicine is originated from *Dong Yi Shou Shi Bao Yuan* established by Mr. Lee Jima. It emphasizes that in the aspect of image discrimination, the patient's constitution, appearance, temperament, hobbies and so on are observed and understood through observation and inquiry methods. Then, combined with the comprehensive reasoning and judgment of pulse condition, people are divided into four types: Taeyang (more Yang), Taeeum (more Yin), Shaoyang (less Yang) and Soeum (less Yin), according size, strong or weak of human organs [1, 2]. It is featured as the medication view that some traditional Chinese medicine can work as prevention and treatment to one of the four constitutions of the Korean medicine, while they can not cure or, instead, results in severe side effect on some others.

Modern research has found that 1,014 microorganisms live in the human body, which is 10 times the total number of human cells. Its equivalent to an important "organ", which plays an irreplaceable role in human health, and most of them parasitic in the intestine [3, 4]. The micro-ecosystem of the interdependence of the intestinal flora and the human body is involved in the physiological, biochemical, pathological and pharmacological processes of the human body [5], such as, regulate nutrition and energy metabolism, increase the host's adaptability to the environment, regulate the induction and maturation of the host's immune system and enhance the body's ability to prevent infectious diseases [6, 7]. The active ingredients of many Chinese medicines need to be metabolized by the intestinal flora before they can produce the metabolites with strong pharmacological activity [8]. Therefore, the intestinal flora plays a vital role in the process of drug metabolism. Considering the selectivity of different constitutions of Korean medicine for medicine may be highly related to the intestinal flora.

Korean medicine believes that the quality of the human body is innate and has obvious genetic characteristics. The formation of its composition also determines the diet preference and body shape of the four-physical quality Sasang people. It was found that the formation of intestinal microflora was also influenced by heredity and dietary structure. Amuguli et al. found that there were significant differences and specificities in the intestinal microflora structure of the four normal humoral groups of Uygur medicine [9]. Li et al. also confirmed that the change of intestinal microflora structure in patients with Yang deficiency constitution of traditional Chinese medicine affects their metabolic function through microecology and metabonomics [10]. Therefore, it is further speculated that the formation of the Sasang people is closely related to intestinal flora.

This study is based on 16S rDNA amplicon sequencing to determination the DNA sequence of the intestinal flora of Korean medicine Sasang populations, set a fingerprint of the intestinal flora, and a biological evolution tree, and use multivariate statistical methods to explore the diversity and difference of intestinal florain medical Korean medicine Sasang people, as well as the distribution of intestinal flora among the four kinds of people, to confirm relationship between the formation of the four constitutions of the Korean medicine and the intestinal flora, and the influence of specific intestinal microbes on these four constitutions.

## Methods

### Study subjects

Eighty-five patients were selected from the medical center of Yanbian hospital of traditional Chinese medicine Yanji hospital of traditional Chinese medicine and Yanbian University (Approval Number: YB-2016001). Based on the key points of Korean Sasang medicine doctor Li Jima (1837–1900) in *Dong Yi Shou Shi Bao Yuan* and the standard of the quality table of Korean medicine people, "The specification of sasang constitutional scale in traditional korean medicine", including the appearance, body shape, walking gait, psychological status, eating habits, health and pathological characteristics. According to the results of the body quality table, they were divided into four groups (15 in each group), namely, Taeyang (A), Taeeum (B), Shaoyang (C) and Soeum group (D). Twenty-five volunteers who did not meet the key points of the Sasang medicine people were excluded.

### Inclusion criteria

① Age 18–55 years, regardless of gender.
② No persistent or infectious gastroenteritis or gastric or intestinal ulcer.
③ Hose who have not performed gastrointestinal surgery (including cholecystectomy and appendectomy).
④ No chronic diarrhea or chronic constipation.
⑤ No history of gastrointestinal bleeding or irritable bowel syndrome.
⑥ Have not been treated with antibiotics or microecological agents in the last three months.
⑦ Sign the informed consent of the project.

### Exclusion criteria

① Pregnant or lactating women.
② Have had cosmetic surgery.
③ Those with abnormal detection indicators and clear diagnosis of the disease.

### General information and sample collection

Collect volunteer age, gender, height, weight, blood glucose and cholesterol content indicators, and

calculate body mass index (BMI) = weight/height$^2$ (kg/m$^2$) according to height and weight values. Collect the Sasang people who meet the selection criteria in the morning about 1 g of stool sample, put it in 5 mL sterile centrifuge tube, and save at -80℃ for future use.

### Intestinal flora total DNA extraction

Total DNA was extracted from Stool with Stool Genomic DNA Extraction Kit (Solarbio, China). Take 0.2 g of feces sample in a 1.5 mL centrifuge tube, add 600 ul Solution A, place on ice for 10 minutes, 12,000 r/min, centrifuge for 1 min. Remove the supernatant, add 400 ul of Solution B, 20 ul of 10 mg/mL RNase A, 20 ul of 10 mg/mL proteinase K to the pellet, vortex for 30 s, in a 65℃ water bath for 30–60 min, invert several times during this period, and centrifuge at 12,000 r/min for 2 min. Take the supernatant in a 1.5 mL centrifuge tube, add 600 ul Solution C, 12,000 r/min, centrifuge for 2 min, and take the supernatant. Pipette the supernatant into the DNA adsorption column, put it at room temperature for 2 min, and centrifuge at 12,000 r/min for 1 min (repeatable once). Pour out the waste liquid in the collection tube, add 500 ul rinse solution to the adsorption column, and centrifuge at 12,000 r/min for 1 min (repeat once). Pour off the waste liquid in the collection tube, put the adsorption column back into the collection tube, and centrifuge at 12,000 r/min for 1 min. Take out the adsorption column and dry it at room temperature for a few minutes (depending on the season, climate and other factors). Put the adsorption column into a new centrifuge tube, add 100 ul of eluent (preheated at 65℃), and centrifuge at 12,000 r/min for 1 min. Add the liquid in the centrifuge tube to the adsorption column again, and centrifuge at 12,000 r/min for 1 min. The fecal microbial DNA solution is in the centrifuge tube.

### Polymerase chain reaction amplification

Real-time quantitative polymerase chain reaction (PCR) (Bio-Rad, USA) and Ready-to-Use PCR Kit (BBI, China) were performed DNA amplification. PCR primers: 5'→3': 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'); PCR reaction system (25 µL) includes: 0.25 U Platinum® Pfx DNA polymerase, 2.5 µL 10×Pfx amplification buffer, 0.5 mM MgSO$_4$, each primer 6.25 pmol, four deoxynucleotides 300 µM each, 20 ng total bacterial DNA as template Amplification procedure: 94℃ 3 min; 94℃ denaturation 1 min, annealing 1 min (annealing temperature) (from 65℃ to 57℃, decrease 1℃ every 2 cycles, anneal at 56℃ for 1 min, 55℃ for 1 min), extend at 72℃ for 1 min, perform 20 cycles; finally extend at 72℃ for 6 min. PCR product with 1.2% agarose Gel electrophoresis detection, and purification using Gel/PCR DNA Fragments Extraction Kit. PCR products were measured using Pico Green kit

concentration. 30 ng of each sample was mixed for equal volume, and then Gel/PCR DNA Fragments Extraction Kit was used.

Three replicates were performed for each sample, and each PCR reaction was terminated during the linear amplification period. After the PCR was completed, the PCR products of the same sample were mixed and then subjected to electrophoresis detection. The gel recovery kit was used to cut the gel to recover the PCR product, and the buffer was eluted. Recycle target DNA fragments.

### DNA fragment analysis

A library (Illumina TruSeq DNA PCR-Free Sample Prep Kit (FC-121-3001/3003)) was established to assess the diversity of the sample communities and operating classification units (OTUs) at different levels. The unique sequence was compared with the online Nast multi aligner, and OTUs were divided according to the sequence similarity.

According to the out-abundance information of each sample library, the diversity of the sample library and the diversity of the sample library under different out levels are evaluated. Through the calculation of alpha diversity, reflect the abundance and diversity of intestinal flora of each sample, so as to evaluate the abundance and diversity of intestinal flora structure of each the four constitutions Sasang people. UniFrac and Principal Component Analysis (PCA) were used to compare the diversity and difference of intestinal microflora structure of different people in Korean medicine Sasang people, and the specificity of intestinal microflora in Korean medicine Sasang people was identified on the basis of random forest analysis.

### Statistics

SPSS 18.0 and GraphPad prism 8.0 were used for data analysis. According to the normal distribution data, the measurement data are expressed by means ± standard deviation, the single factor analysis of variance is used for the comparison between groups, and least significant difference (LSD) method is used for the comparison between groups, which is statistically significant. Data that do not conform to the normal distribution are all represented by the median (quartile), and the comparison between groups is performed by Kruskal-Wallis test. The number of cases (percentage) and $\chi^2$ test expressed by the classified and counted data were used in the control group. The difference was statistically significant ($P < 0.05$).

## Results

### Comparison of the basic data of patients in each group

Comparison of basic data of patients in each group (Table 1). There were some differences in gender,

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height, age, and blood glucose of the patients in the groups, but the differences were not statistically significant ($P > 0.05$ for all). There were statistic differences in body weight, BMI, and cholesterol in each group ($P < 0.05$ for all). Further comparisons of statistically significant indicators using LSD method showed that the weight and BMI of the Taeeum group were significantly higher than those of the Soeum group; the weight, cholesterol and BMI of the Taeyang group was significantly higher than the Soeum group, and the differences were statistically significant ($P < 0.05$).

**Analysis of intestinal flora**

Through the detection of fecal bacterial sequences in 60 patients, 1980225 sequences were obtained. The average sequence length is 295 bp, 20 phylum level, 132 family level, 343 genus level, 4,974 OTUs. There is no significant difference in the number of distributions in each group of OTUs, and the average number of OTU distributions is Taeyang group (627.5) < Taeeum group (627.5) < Shaoyang group (737.6) < Soeum group (768.9) (Figure 1). The number of OTUs was used to make a Venn diagram for community

analysis between groups (Figure 2). The common species in each group, such as 1,922 kinds of Taeyang group and Taeeum group, and the independent species in Taeyang group and Taeeum groups, such as 759 and 977 independent species in Taeyang group and Taeeum groups. In order to evaluate the structure of intestinal microbial community, we calculated the richness and evenness of intestinal microbial community in each group. Chao1 diversity index and phylogenetic diversity (PD) index of each group were Taeyang group < Taeeum group < Shaoyang group < Soeum group, and there was significant difference between Taeyang group and Soeum group ($P < 0.05$). Compared with the Taeeum group, Shaoyang group and Soeum group, the Shannon richness index of the Taeyang group decreased, and the richness difference between the Taeyang group and the Soeum group was statistically significant ($P < 0.05$); Simpson evenness index was used to measure the diversity of intestinal flora, and the diversity of each group was Taeyang group < Taeeum group < Shaoyang group < Soeum group, and the difference between Taeyang group and Soeum group was statistically significant ($P < 0.05$) (Table 2 and Figure 3).

**Table 1 Comparison of basic data of patients in each group**

| Basic situation | Taeyang group (n = 15) | Taeeum group (n = 15) | Shaoyang group (n = 15) | Soeum group (n = 15) | $F/\chi^2$ | $P$ |
|---|---|---|---|---|---|---|
| Gender (male/female) | 5/10 | 5/10 | 4/11 | 2/13 | 2.045 | 0.563 |
| Weight (kg) | 62.8 ± 16.37 | 68.37 ± 13.94 | 57.13 ± 6.03[b] | 50.77 ± 5.56[ab] | 6.460 | 0.001 |
| Height (cm) | 162.23 ± 8.2 | 166.83 ± 10.42 | 163.6 ± 6.61 | 161.17 ± 6.59 | 1.382 | 0.258 |
| Body mass index | 23.55 ± 4.2 | 24.43 ± 3.85 | 21.38 ± 2.16[b] | 19.52 ± 1.52[ab] | 7.449 | 0.000 |
| Age (year) | 32.67 ± 5.01 | 29.4 ± 4.67 | 32.27 ± 8.37 | 28.4 ± 4.69 | 1.914 | 0.138 |
| Blood glucose (mmol/L) | 5.22 ± 0.49 | 5.29 ± 0.48 | 5.13 ± 0.4 | 4.96 ± 0.36 | 1.637 | 0.191 |
| Cholesterol (mmol/L) | 1.31 ± 0.55 | 1.06 ± 0.52[a] | 1.12 ± 0.27 | 0.88 ± 0.27[a] | 3.185 | 0.031 |

Note: a is compared with the Taeyang group, b is compared with the Taeeum group, $P < 0.05$.

**Table 2 Structure and diversity of intestinal flora in each group**

| | Chao1 | PD | Simpson | Shannon |
|---|---|---|---|---|
| A-Taeyang | 800.87 ± 295.96 | 41.53 ± 11.37 | 0.86 ± 1.12 | 3.54 ± 0.91 |
| B-Taeeum | 924.61 ± 290.83 | 47.46 ± 11.99 | 0.91 ± 0.05 | 3.84 ± 0.61 |
| C-Shaoyang | 949.69 ± 374.60 | 48.0 ± 12.19 | 0.93 ± 0.05 | 4.11 ± 0.66 |
| D-Soeum | 983.88 ± 379.73[a] | 51.10 ± 12.71[a] | 0.93 ± 0.05[a] | 4.15 ± 0.51[a] |

Note: [a] compared with the Taeyang group, $P < 0.05$. PD, phylogenetic diversity.
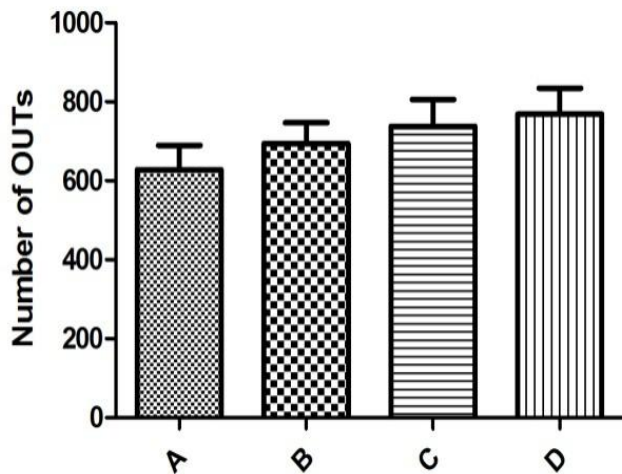
**Figure 1 Comparison of operating classification units of each group.** In the analysis of operating classification units, the operating classification units distribution of each group is Taeyang group < Taeeum group < Shaoyang group < Soeum group ($P > 0.05$).

We used PCA to assess phylogenetic differences within the intestinal flora (Figure 4). The intestinal microflora of Shaoyang group and Soeum group, Taeyang group and Taeeum group were almost distributed in the same quadrant, while few of the intestinal microbiota of the Shaoyang group, the Soeum group and the Taeyang groupwere in the same quadrant, and few of the Taeeum group were in the same quadrant as the Soeum group and Shaoyang group.

To assess the changes in specific intestinal flora, we compared the relative abundance of the phylum levels identified by sequencing in the four groups (Figure 5). Abundant Chlamydomonas and bacteroides in the Taeyang group, Taeeum group, Shaoyang group and Soeum group respectively, Chlamydomonas ratio and Bacteroides group. The intestinal flora of each group were mainly distributed in the Firmicutes and Bacteroidetes, and the abundance of the Firmicutes and Bacteroidetes in the Taeyang group, Taeeum group, Shaoyang group and Soeum group were 55.04% and 37.97%, 67.83 % and 25.84%, 59.50% and 32.15%, 59.32% and 30.02%, and the abundance ratios of the four groups of Firmicutes and Bacteroidetes were 1.45, 2.62, 1.85 and 1.98.

In order to further analyze the four groups of samples with significant differences in the types of bacteria, we classified and analyzed four groups of samples found in intestinal flora, and found four groups of samples with significant differences in 24 kinds of bacteria. Random forest analysis of the significantly different species showed that [eucharacterium] coprostanoligenes group may be the main target of the four people differences (Figure 6), and the abundance of the bacteria of Taeyang people, Taeeum group, Shaoyang group, and Soeum group were 3.71, 9.62, 9.36 and 9.33, respectively. In general, these results can reflect the differences of intestinal flora among the four people.
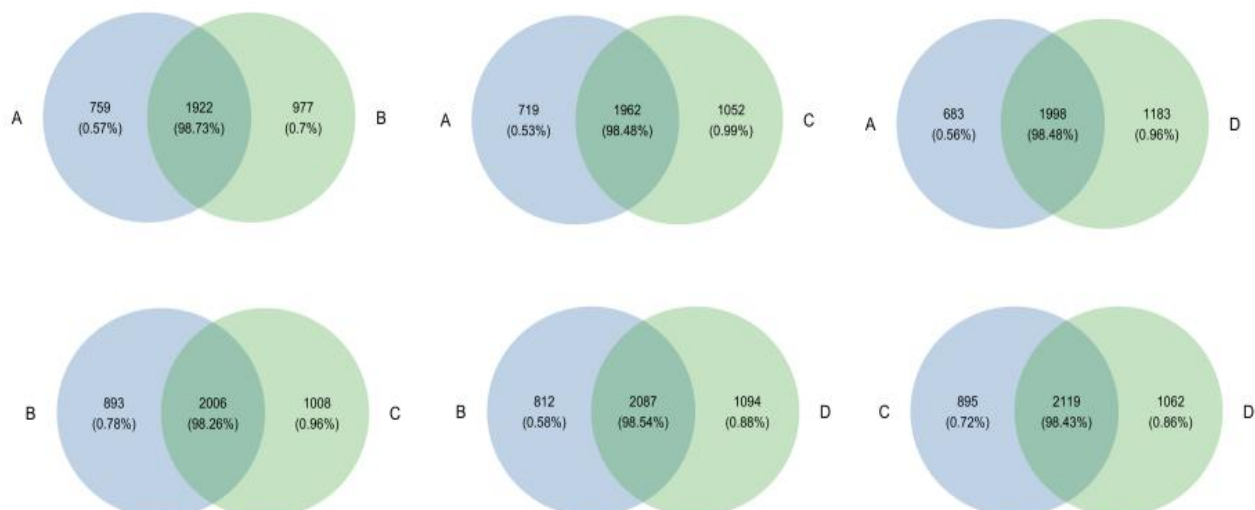


**Figure 2 Shared operating classification units analysis of different groups.** In the community analysis between groups, the communities of each group are distributed with unique communities, and the same communities also exist. A, Taeyang; B, Taeeum; C, Shaoyang; D, Soeum.
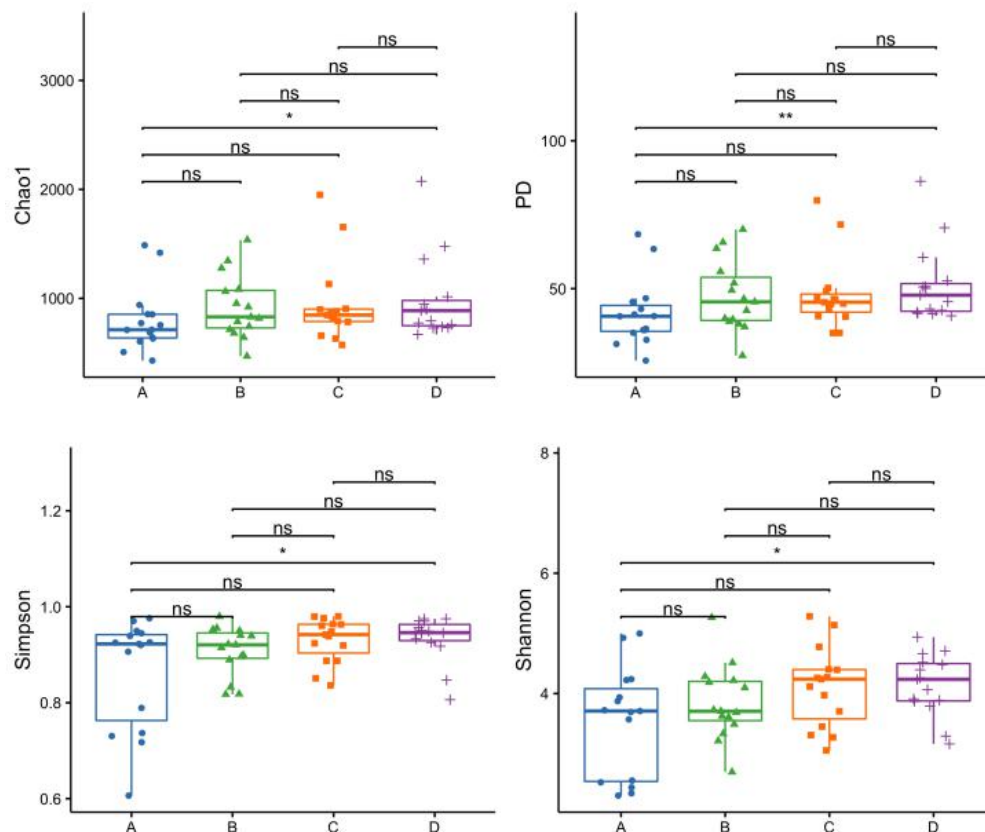
**Figure 3 Structure and diversity of intestinal flora in each group.** Chaol and phylogenetic diversity indicate the diversity of species; Shannon represents the richness of species; Simpson measures the diversity of intestinal communities. A, Taeyang (blue); B, Taeeum (green); C, Shaoyang (orange); D, Soeum (purple).
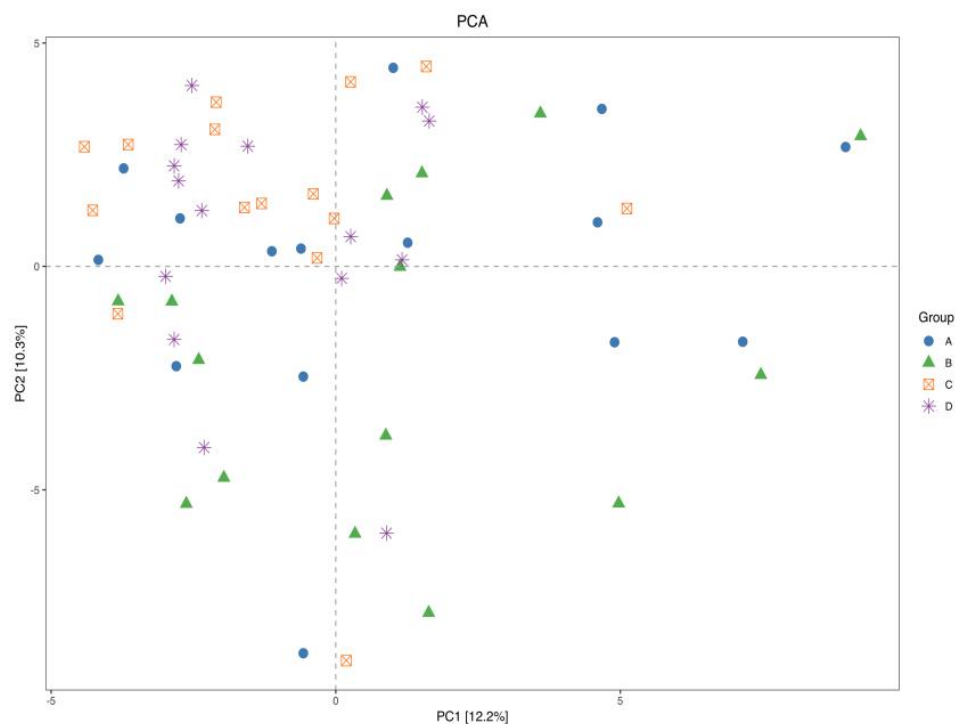


**Figure 4 PCA analysis of intestinal flora in different groups.** A, Taeyang (blue); B,Taeeum (green); C, Shaoyang (orange); D, Soeum (purple).
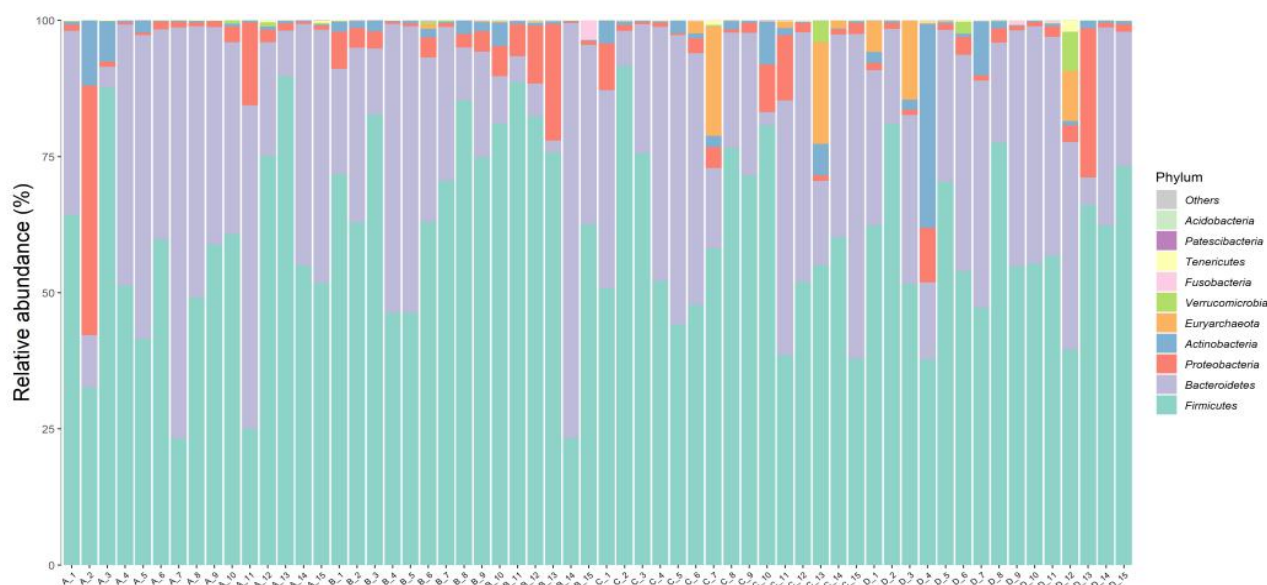
**Figure 5 Distribution of intestinal microflora at the phylum level for each sample.** In the phylum level analysis, the intestinal flora of each group was mainly distributed in the Firmicutes and Bacteroidetes, and the abundance ratios of Firmicutes and Bacteroides of each group were 1.45, 2.62, 1.85 and 1.98, respectively. A, Taeyang; B, Taeeum; C, Shaoyang; D, Soeum; "other" includes less than 1% of the phylum in all samples.

## Discussion

According to the theory of Korean medicine, the physical quality of Sasang people is determined by the innate endowment, which directly related to the next generation. Some genetic characteristics of Korean Sasang medicine has been listed as follows: the offspring of the two people of the Soeum type would only be of Soeum type or Taeeum type, while the offspring of two people of the Taeeum type would only be of Taeeum type. It is suggested that the Korean medicine Sasang people have an obvious genetic characteristic [11]. At the same time, the formation of their physical quality also determines the diet preference of Sasang people. For example, Shaoyang people usually like raw and cold drinks, Taeyang people like cold food, Soeum people like warm food, and Taeeum people like fatty food, such as cheese, beef, butter and other high-calorie food. The visceral situation of the Taeeum people is characterized by "large liver and small lungs". "Large liver" means that the liver absorbs too much and induces a strong appetite in the Taeeum people. The Taeeum people usually eat fat and strong taste. "Small lung" means that the vital capacity is relatively inadequate. In addition, Taeeum people usually does not like exercise, so it is easy to form an obese body [12]. In addition, a study on the BMI indicators of the Sasang people of the Korean medicine found that the proportion of obese people is the highest among the Taeeum people, and the BMI level of the Taeeum people is higher than

that of the Shaoyang and Soeum people [13]. Similarly, our results show that the weight and BMI of Taeeum people are the highest among the four groups, and they are all Taeeum group > Taeyang group > Shaoyang group > Soeum group. Therefore, we believe that the physical characteristics of Taeeum people led to the result.

According to statistics, there are 1,000 to 1,150 kinds of bacteria in the human intestine. Averagely, about 160 dominant bacteria exist in each person's intestine, which are also shared by the vast majority of individuals [14]. Intestinal micro-ecological studies have found that the formation of intestinal flora is influenced by genetics and dietary structure. There are certain heredity and differences in the intestinal flora among human beings. The genetic characteristics are directly proportional to the intestinal flora [15]. Therefore, there are differences among intestinal flora of Sasang people because of their life habits and inheritance. And our results also show that the microbial communities of the four populations are different. In the analysis of the structural diversity of the intestinal flora (Figure 3 and Table 2), it was observed that the Chaol index, the PD index, the Shannon index and the Simpson index of each group were different, especially there are significant differences between the Taeyang group and the Soeum group in each index. In addition, in the PCA chart (Figure 4), it also shows that there are differences in the composition of the microbial community in each group.

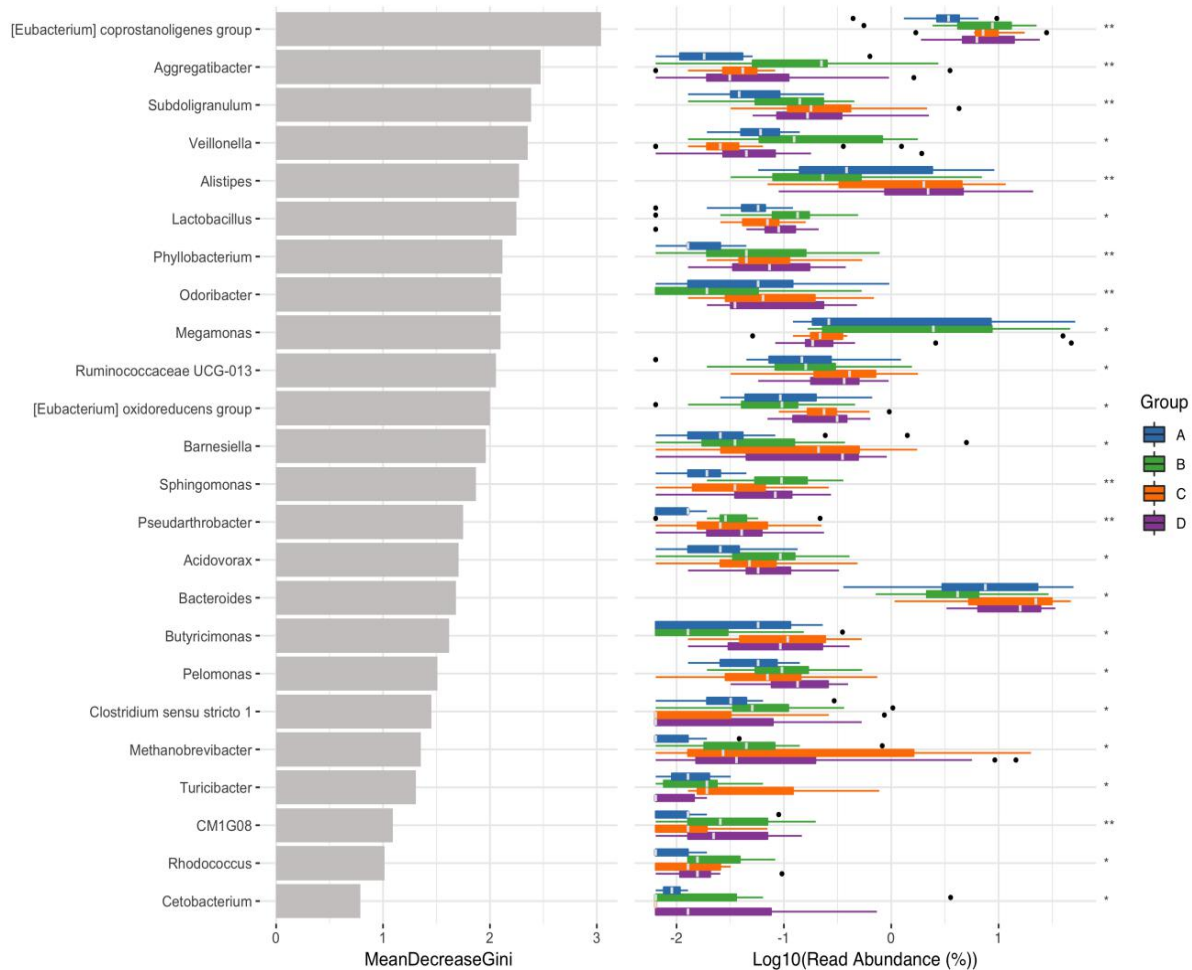In our study, we collected feces from the Taeyang,

**Figure 6 Random forest analysis of bacterial community structure.** The abscissa of the left picture is the average decrease value of Gini index, the ordinate is the classification information of the genus, and the right picture is the box plot of the abundance of taxa of different groups. The * on the right represents the difference between groups (Kruskal-Wallis rank sum test) the degree of significance (***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$). From top to bottom, the importance of thegroups are decreasing in order. A, Taeyang(blue); B, Taeeum (green); C, Shaoyang (orange); D, Soeum (purple).

Taeeum, Shaoyang and Soeum types and explored the composition of bacterial species based on the abundance of OTUs in 16S rRNA gene sequence as well as the species diversity analysis. We found some similarities and differences among the four types. The intestinal flora is mainly composed of 6 categories, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria and Verrucomicrobia [16]. We observed that the intestinal flora of these four types of Sasang people was also composed of the six bacteria categories mentioned above. And these quences of Firmicutes and Bacteroides accounted for about 90% of the total number. The ratio of Firmicutes and Bacteroides (F/B) are closely related to body weight [17], and an increase in the abundance ratio of F/B may lead to excessive immune responses and excessive diet, promote energy accumulation in the body, and cause obesity [18, 19]. Therefore, the abundance ratio

of F/B is also an obvious indicato of obesity and type 2 diabetes [20]. In the analysis of the structure of the intestinal flora (Figure 5), the abundance ratios of F/B in the four populations are Taeeum group > Soeum group > Shaoyang group > Taeyang group. And in the comparison of the data of the four groups (Table 1), BMI, body weight and blood glucose concentration are Taeeum group > Taeyang group > Shaoyang group > Soeum group. As characteristic description of the four groups of people mentioned that Taeeum people like to eat greasy and heavy-tasting foods, and do not like exercise, so they are easy to form an obese body. When BMI is greater than 23 kg/m$^2$, the risk of diabetes in the population increases significantly. Therefore, we believe that the formation of the Taeeum body physique is closely related to the abundance ratio of F/B, and the differences in the physique of other people are also related to the ratio of F/B.

The food we in take daily requires gut microbes to process it, from which nutrients and energy are extracted. There is "individual difference" in the transformation and absorption of food components among different people, which is largely due to the differences in intestinal microbes and the related metabolic enzymes [21, 22]. *[Eubacterium] coprostanoligenes* group is a kind of faecal sterol-producing eubacterium that breaks down cholesterol into faecal sterols that cannot be absorbed. The proportion of faecal sterols excreted can be up to 50% [23]. This means that people with faecal sterol-producing eubacterium in the gut absorb cholesterol less than half of what they eat from high-cholesterol foods than others. We found that the content of *[Eubacterium] coprostanoligenes* group in the feces of the four constitution types was as follows: Taeyang < Shaoyang < Soeum < Taeeum. Meanwhile, the blood cholesterol content in the four types was as follows: Soeum < Taeeum < Shaoyang < Taeyang. Above results indicated that the difference in cholesterol content among the four types of Sasang people may be relative to the abundance of *[Eubacterium] coprostanoligenes* group.

## Conclusion

In conclusion, we found that the difference in gut microbial composition among the Sasang people in Korean medicine. The abundance ratio of Firmicutes and Bacteroidetes was as follows: Taeeum > Soeum > Shaoyang > Taeyang. There are 24 gut microbes with significant difference among the four types, and the *[Eubacterium] coprostanoligenes* group may be a key factor in distinguishing the four types, as well as regulating their life habits and inheritance. Therefore, this research focused on intestinal microbes of different populations in the perspective of Korean Sasang medicine, may provide a theoretical reference for the searching of new microbial markers and the impact of intestinal flora on physical quality. It may provide a reference for physical quality. In this way, early intervention treatment can be carried out to achieve "prevention before disease", which provides a basis for future health management and clinical individualized diagnosis.

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