Gut microbiota serve as a risk predictor of weight gain in schizophrenia patients with antipsychotic medication

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Abstract

Background: Emerging evidence indicates that gut microbial dysbiosis is associated with the development of antipsychotic-induced overweight/obesity in schizophrenia (SZ). We aim to determine the taxonomic composition and metabolites profiling of “obesogenic” microbiota in SZ patients after chronic antipsychotic medication.

Results: Our retrospective survey identified two groups of population separated by BMI, with 1/3 patients developing overweight/obesity after chronic antipsychotics treatment. Then, based on the 16S rRNA sequencing and multi-omics analyses, we found that SZ patients with overweight/obesity had lower relative abundances of the signature bacteria genera such as *Bacteroides*, *Parabacteroides*, *Akkermansia*, and *Clostridium* when compared to individuals with normal BMI. Further investigation revealed dysregulated energy expenditure and nutritional metabolism coupled with severe clinical indices and decreased levels of beneficial metabolites, e.g. indole-3-carboxylic acid, and propionic acid. The signature bacteria were positively associated with host metabolic homeostasis, according to the redundancy and co-occurrence analysis. Moreover, leveraging the data from first-episode drug-naïve SZ (FSZ) patients at 1-month and 1-year follow-up, both artificial neural network and random forest classifier-based prediction models demonstrated a strong ability of microbial profiles to predict antipsychotic-induced weight gain. Importantly, FSZ patients with a higher relative abundance of *Parabacteria distasonis*are less susceptible to antipsychotic-induced weight gain.

Conclusion: Gut microbiota could serve as a noninvasive approach to predict antipsychotic-induced weight gain, guiding clinical antipsychotics administration and developing novel therapeutic strategies for the weight management in SZ.

Background

Schizophrenia (SZ) is the most disabling psychiatric disorder with a lifetime prevalence of about 1% [1], threatening public health for a long time. Several studies have proposed the pathogenesis of SZ, among which the neurotransmitter hypothesis was the most cited explanation [2]. Based on this hypothesis, the antipsychotic has developed to become the most widely used first-line drug in treating SZ which can effectively attenuate the psychotic symptoms. Despite the great medical cost along with the medical care, frequently taking the drug would lead to antipsychotic-induced metabolic disorder, such as hyperglycemia, hyperlipidemia or obesity, which have risen to be one of the most concerning side effects. According to clinical studies and observations, antipsychotic-induced overweigh/obesity is more common in SZ patients than in healthy controls, affecting approximately 40%-60% of the SZ population [3]. Obesity not only is associated with high risk of type II diabetes, cardiometabolic disease and even early mortality, but also have an impact on cognitive function and psychiatric outcomes in SZ [4, 5]. However, the exact mechanisms underlying this high prevalence of overweight/obesity in SZ patients are not fully understood and no effective treatments have yet been developed [6].

In recent years, the "gut-brain axis" researches have drawn increasing attention, in particular the interaction between gut microbiota and host metabolism. In early 2005, a pioneering study observed a decrease of phylum *Bacteroides* and an increased in phylum *Firmicutes* in genetically obese mice, positioning a role of microbial dysbiosis in obesity [7]. Furthermore, it has been reported that comparing to other host microbiome, obesity-associated microbiome have more advantages in gaining weight and storing energy [8, 9]. Furthermore, gut microbiota is involved in food digestion, nutrient absorption and energy metabolism, which can convert dietary nutrients into various metabolites such as short-chain fatty acids (SCFAs) and indole, which could then regulate appetite and eating behavior of the host after being absorbed into the blood [10]. Furthermore, therapeutic drugs can strongly affect the gut microbiome [11], and recent studies have proposed that the antipsychotics may also interact and reshape the microbial profiles, mediating the development of antipsychotics-induced weight gain [12, 13]. In line with this hypothesis, preliminary findings in germ-free mice suggested that olanzapine could induce weight gain and promote a shift towards “obesogenic” microbial profile only after the colonization of gut microbiota (i.e., increased *Firmicutes* while decreased *Bacteroides*) [14]. Similar findings were also observed in antipsychotic-treated human studies for both children and adults [15, 16]. With the advance of risperidone treatment, the gut microbial composition was significantly altered, and the gradual increase in *Firmicutes/Bacteroides* ratio was significantly correlated with the increase in BMI [16]. In a prospective study of 24-week risperidone treatment targeted in first-episode drug-naïve SZ (FSZ) patients, Yuan et al. [15] also revealed a gradual increase of BMI and glucolipid metabolic indices, accompanied by dynamic alterations in gut microbial characteristics. All the evidence proved that microbial profile is associated with antipsychotic-induced weight gain and metabolic disturbance.

Recently, two randomized clinical trials demonstrated that probiotics plus dietary fiber can effectively reduce the antipsychotic-induced weight gain and metabolic disturbance in SZ patients [17, 18]. And such favorable effects were linked to an increased abundance of gut microbiota [18]. However, the exact microbial composition that constitute the “obesogenic” microbial profile in SZ patients with antipsychotic-induced weight gain remain unknow. Despite the fact that previous studies on antipsychotic-induced weight gain have revealed an increase in the *Firmicutes/Bacteroidetes* ratio in SZ [16, 18], the existing evidences focused primarily on taxonomic composition, without addressing the compositional nature such as microbial function and metabolites. Microbial functionality and metabolomics should also be considered for correctly recording the host-microbiome interaction, because various microbial compositions may induce a similar phenotype due to functional redundancy [19]. Moreover, whereas the beneficial effects of probiotics on weight reduction have been extensively examined in individuals with diabetes and obesity, they remain significantly understudied in those with SZ [20–22]. As a result, it is critical to explore the particular abnormalities in the gut microbiome of SZ patients with antipsychotic-induced weight gain.

In the present study, we sought to determine the precise taxonomic composition and metabolites profiling that constitutes to the “obesogenic” microbial profile after chronic administration of antipsychotic drugs. It is necessary to understand how these perturbations to the microbiome and the metabolites promote the development of overweight/obesity in SZ patients. To address these questions, the retrospective

investigation was performed to assess the BMI changes in chronically antipsychotic-treated SZ patients, followed by using 16S rRNA amplicon sequencing, PICRUSt2 and metabonomic analysis to characterize gut microbial structure, function and metabolites profiling in a cross-sectional study. Finally, for the first time, the artificial neural network (ANN) model was utilized to assess whether the altered gut microbiome could be used as a simple, and non-invasive approach for predicting the risk of antipsychotic causing weight gain in SZ patients.

Methods

Study Design And Participants

This study consisted of three parts: the retrospective survey, cross-sectional and validation cohort. In the retrospective part, we investigated the BMI changes in chronically antipsychotic-treated SZ patients who were hospitalized at the Third Affiliated Hospital of Sun Yat-sen University from January 2017 to December 2018. Through preliminary screening and telephone follow-up, 147 SZ patients were included in this survey (Fig. 1A). In the cross-sectional part, we recruited an extra 60 chronically antipsychotic-treated SZ patients, including 22 patients with overweight or obesity (SZ-O) and 38 patients with normal weight (SZ-N). As shown in Tab. S1, the two groups were well matched in demographic characteristics. In the validation cohort part, 22 first-episode drug-naïve SZ (FSZ) patients were further recruited, and 18 patients completed 1-month as well as 1-year follow-up on BMI changes after antipsychotic treatment.

The chronically antipsychotic-treated SZ patients were defined as having a disease duration > 2 years and receiving regular antipsychotic treatment for at least 1 year. The FSZ patients were defined as having a first episode (illness duration ≤ 6 years) and not previously treated with antipsychotics or other psychotropic medication. The BMI was calculated as weight/height^2 (kg/m^2) and grouped referenced by the criteria of World Health Organization [23]: 18.5 kg/m^2 < BMI < 25 kg/m^2 for normal weight; BMI ≥ 25kg/m^2 for overweight or obese. All the patients in the retrospective and cross-sectional part had a normal weight prior to antipsychotic treatment, and all the participants in the validate cohort had an initial BMI < 25 kg/m^2. The study protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. Informed consent was gained from all participants before any procedures were performed.

Inclusion criteria included: (1) met the diagnostic criteria for schizophrenia according to the International Classification of Disease the tenth edition (ICD-10); (2) aged 16–49 years; (3) Family members or legal guardians of patients fully understood the study contents and had given informed consent.

Considering that fecal samples were collected in the cross-sectional and validation sections for gut microbial analysis, exclusion criteria were further added in these two parts: (1) had a history of other mental illnesses, such as mood disorders, anxiety, eating disorders, substance abuse and mental retardation; (2) have taken any antibiotics, probiotics, or prebiotics within 1 month before sampling; (3) had a history of serious or chronic physical disease (heart failure, thyroid dysfunction, chronic hepatitis,

metabolic disease history, metabolic comorbidities such as hypertension and diabetes); (4) had a history of allergy, hormone therapy or received immune agent treatment in the past 3 months; (5) had gastrointestinal surgery, gastroscopy, colonoscopy or gastrointestinal barium meal examination in the past 1 year; (6) experienced major life events or changes in the living environment and diet in the past 1 month; (7) pregnant or lactating women.

Clinical Assessments And Sample Collections

The SZ was diagnosed by a chief physician using the mini-SCID procedure according to the ICD-10 diagnostic criteria. The positive and negative syndrome scale (PANSS) and clinical global impression-severity of disease (CGI-SI) were combined to assess the patient's symptom severity; medication adherence rating scale (MARKS) was used to assess the patients' medication compliance; and the international physical activity questionnaire-short vision (IPAQ-SV) was used to measure the physical activity. Additionally, a self-made questionnaire was used to obtain general data including age, gender, ethnicity, years of education, illness duration, previous treatment with antipsychotics (type, dose and duration) and mood stabilizer valproate with obvious effect on weight gain, and lifestyle (smoking, drinking history, eating habits). The weight, height, waist and hip circumferences, and blood pressure of all subjects were tested and collected by a specific investigator. Subjects were weighed in a fasting state in light clothing with footwear removed. For FSZ patients, we collected the BMI data at baseline as well as 1-month and 1-year follow-up after antipsychotic treatment.

Further, we also obtained the biological samples from patients in the cross-sectional and validation cohort. Fecal samples were collected in sterile plastic containers and immediately stored at -80℃ prior to further processing. Blood samples were drawn from fasting individuals by venipuncture between 08:00 am and 10:00 am and stored at − 80°C after centrifugating.

Measurement Of Clinical Metabolic Indices

Serum interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-17 (IL-17), tumor necrosis factor- α (TNF- α), lipopolysaccharide (LPS), adiponectin (ADP) and leptin were measured by using the enzyme-linked immunosorbent assay kits (ABclonal Technology Co.,Ltd.). The level of fasting blood glucose (FBG) was measured by biochemical kit (DiaSys Diagnostics Systems (Shanghai) Co.,Ltd.), triglyceride (TG), total cholesterol (TC), apolipoprotein A (APOA) and apolipoprotein B (APOB), lipoprotein a (Lipoprotein a, Lpa) were measured by biochemical kit (Maccura Biotechnology Co.,Ltd.). High density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were measured by biochemical kit (Sekisui Medical Co., Ltd.). All the experimental procedures were followed with corresponding specifications.

16s Rrna Amplicon Sequencing And Analysis

The 16sRNA sequencing data were obtained from all participants of the cross-sectional and validation cohorts. Bacterial DNA was extracted from approximately 200 mg fecal material using the E.Z.N.A. ®Stool DNA Kit (D4015, Omega, Inc., USA) following the manufacturer's instructions. The V3–V4 regions of the bacterial 16S rRNA gene were amplified by PCR using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). PCR amplification was performed in a total volume of 25 μL reaction mixture containing 25 ng of template DNA, 12.5 μL PCR Premix, 2.5 μL of each primer, and PCR-grade water to adjust the volume. The PCR products were confirmed with 2% agarose gel electrophoresis. The amplicons were purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The amplicon pools were prepared for sequencing and the size and quantity of the amplicon library were assessed on Agilent 2100 Bioanalyzer (Agilent, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on NovaSeq PE250 platform according to the manufacturer's recommendations. Furthermore, based on the DATA2 algorithm and Greengenes database, we obtained high-quality amplicon sequence variants (ASVs) and transferred them into relative abundance at the phylum, class, order, family, genus and species levels to perform taxonomy profile analysis.

Downstream bioinformatics analysis, including α-diversity (Shannon and Simpson index) and β-diversity (based on Bray-Curtis and Binary Jaccard distance), structure plot, redundancy analysis (RDA), random forest model, correlation analysis, co-occurrence analysis, sankey plot were performed with “EasyMicroPlot” package of R[24]. Here, the core bacteria were defined as having a relative abundance higher than the min-relative abundance and presenting a proportion above the min-ratio of the samples in any group. To make a more comprehensive comparison and screen key bacteria to the greatest extent, we set up 3 sets of filtering conditions (the min-relative abundance was set to 0.001, and the min-ratio was set to 0.5, 0.6, 0.7, respectively). In addition, to probe the microbial metabolism and predict metagenome functional content from the marker gene, PICRUSt2 was utilized to explore differences of the KEGG pathway between groups [25].

Fecal And Serum Metabonomic Analysis

We performed fecal and serum metabonomic analyses on 30 samples from the cross-sectional cohort (15 SZ-N participants, 15 SZ-O participants) and 10 samples from the validation cohort, respectively. An ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) system (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA) was used to perform the fecal and serum metabonomic analysis. The raw data files generated by UPLC-MS/MS were processed using the MassLynx software (v4.1, Waters, Milford, MA, USA) to perform peak integration, calibration, and quantitation for each metabolite. The orthogonal Partial Least Squares Project to Latent Structure Discriminant Analysis (OPLS-DA) was used to visually discriminate SZ-O subjects from SZ-N. By analysis of OPLS-DA loadings, the differential metabolites responsible for discriminating between the two groups were identified with variable importance plot (VIP) values of greater than 1.0, and *p*-values of less than

0.05. IPA software (Qiagen, Redwood City, CA, USA) was used to uncover the predicted molecular pathways and biological functions of the identified differentially expressed metabolites.

Artificial Neural Network (Ann) Prediction Model

To identify whether the microbiome data could predict weight gain outcomes under antipsychotic treatment, we applied an ANN prediction model to predict BMI changes after 1-month antipsychotic treatment based on the microbiome data at baseline in the FSZ patients. In this validation cohort, we also tested the ability of this model in predicting metabolic indices at baseline. Of note, to minimize human intervention and incorporate more objective data, we included all the bacterial species of which relative abundance higher than 0.001. The ANN was performed on python 3.6.1 with the pyTorch, sklearn, pandas, and numpy packages. The optimized parameters, including learning rate, activation function, layers, number of neurons and dropout, were selected by grid search and cross-validation.

Statistical Analyses

For all demographic and clinical data, the statistical analyses were performed using the IBM SPSS Statistics for Windows Version 26.0 (IBM Corp., Armonk, NY), and the statistical significance threshold was set to $p < 0.05$. The continuous variables were summarized by means ± standard deviations or median (upper and lower quartile), and analyzed by independent t-test, Wilcoxon signed rank test or Wilcoxon rank sum test. The categorical variables were summarized using frequencies, and analyzed by Chi-square test or Fisher exact test. Correlation analyses were conducted using spearman correlation coefficient.

Results

Identification Of The Interpatient Heterogeneity In Antipsychotics-induced Weight Gain

Although antipsychotic medications are largely responsible for the high prevalence of overweight/obesity among SZ, most previous studies were cross-sectional designs and did not take consideration of the patients' BMI prior to prescribing the medication [26–28]. To investigate the incidence of overweight/obesity under chronic antipsychotic treatment, we conducted a retrospective study in chronically antipsychotic-treated SZ patients with normal BMI at the initial. After screening, 147 out of 1685 eligible patients were included in the present analysis (Fig. 1A). Among these participants, 49 patients have developed overweight or obesity (BMI ≥ 25kg/m^2), and the other 98 patients remained at normal weight (18.5 kg/m^2 < BMI < 25 kg/m^2). According to the current BMI, we divided the patients into two subgroups: overweight or obese (SZ-O), and normal weight (SZ-N), as shown in Table 1. The age, gender, initial BMI, and antipsychotic medication were not significantly different between the two subgroups. In comparison to the SZ-N group, participants in SZ-O group had a higher body weight, as

indicated by current BMI and abnormal BMI increment. Although BMI was significantly increased after 1–2 years of antipsychotic medication, the SZ-N group was more resistant to the antipsychotic medication-induced body weight increase (Fig. 1B). Thus, the retrospective survey revealed heterogeneity in weight gain among patients on equivalent antipsychotic medication, implying that other confounding factors may also contribute to the difference.

Microbial Changes Of Diversity And Composition In Sz-o Patients

Recent studies have found a potential link between gut bacteria and aberrant BMI after antipsychotic medication. Two randomized clinical trials demonstrated that modulating the gut milieu with probiotics and dietary fiber could effectively ameliorate antipsychotics-induced weight gain for SZ patients [17, 18], suggesting a potential link between microbial dysbiosis and metabolic disorders. Hence, we conducted a cross-sectional study to compare the gut microbiota between SZ-O and SZ-N groups. A total of 60 SZ patients were recruited in the study, and the demographic and clinical characteristics were summarized in Tab. S1-2. Rarefaction measurement of Shannon and Simpson indices indicated that sequencing depth was sufficient for downstream analysis (**Fig. S1**). At the phylum level, both the SZ-N and SZ-O groups were predominantly enriched with *Firmicutes* and *Actinobacteria* (Fig. S2A-B). Patients in SZ-O group had a higher *Firmicutes*/*Bacteroidetes* (F/B) ratio than those in SZ-N group (Fig. S2C). Although α-diversity such as Simpson and Shannon indices was not significantly different between two groups (Fig. 2A & Fig. S3A-B), the principal coordinates analysis (PCoA) based on Bray-Curtis and Binary Jaccard distance at the genus level indicated a significant separation between two groups (Fig. 2B-C & Fig. S3C-D). In addition, structure plot at the genus level showed significant difference in bacterial composition between two groups (Fig. 2D). Using the core bacteria at the species level with the redundancy analysis (RDA), it was discovered that core bacteria explained 87.45% of the variability in metabolic data under the min-ratio of 0.5 (Permutation number = 999, $p = 0.023$) (Fig. 2E). Consistent results were also obtained under the min-ratio of 0.6 and 0.7 (Fig. S4), indicating that core bacteria were tightly correlated with metabolic indices, despite the steady decline in explanation and the model's stability (reflected by the increased *p*-value of the model) might be due to a drop in the number of core bacteria. Overall, the gut microbiota differed considerably between the SZ-N and SZ-O groups and correlated with host metabolism, while the precise differential gut microbial composition and the relationships with host metabolism still remain unknown.

The random forest algorithm was performed to identify the signature bacteria between the SZ-O and SZ-N group. We identified 8 signature bacteria that were responsible for the discriminating between the two groups at min-ratios of 0.5, 0.6 and 0.7. They were V7 (*A.muciniphila, Akkermansia muciniphila*), V12 (*C.aldenense, Clostridium aldenense*), V20 (*B.uniformis, Bacteroides uniformis*), V27 (*Bacteroidaceae Bacteroides*), V29 (*Porphyromonadaceae Parabacteroides*), V39 (*P.distasonis, Parabacteroides distasonis*), V41 (*C.citroniae, Clostridium citroniae*), V52 (*B.ovatus, Bacteroides ovatus*) (Fig. 2F & Fig. S5). Notably, the relative abundance of the 8 signature bacteria were significantly higher in the SZ-N than in the SZ-O group, and these bacteria mainly belonged to the genus *Bacteroides* (*B.uniformis*,

Bacteroidaceae Bacteroides, *B.ovatus*), *Parabacteroides* (*P.distasonis*; *Porphyromonadaceae Parabacteroides*), *Akkermansia* (*A.muciniphila*), *Clostridium* (*C.aldenense*, *C.citroniae*). In addition, the spearman correlation analysis was conducted to explore the connection between 8 signature bacteria and BMI as well as clinical metabolic indices. As shown in Fig. 2G & Tab. S4, all the signature bacteria (*B.uniformis, B.ovatus, Bacteroidaceae Bacteroides, P.distasonis, Porphyromonadaceae Parabacteroides, A.muciniphila, C.aldenense, C.citroniae*) were negatively correlated with BMI (all $p < 0.05$). Both *B.uniformis* and *C.citroniae* were negatively correlated with TG (r = -0.39, $p = 0.003$; r = -0.28, $p = 0.039$; respectively). In contrast, *Bacteroidaceae Bacteroides* and *C. citroniae* were positively correlated with HDL-C (r = 0.27, $p = 0.047$; r = 0.40, $p = 0.002$; respectively). In addition, *A.muciniphila*, *Bacteroidaceae Bacteroides* and *C.aldenense* were negatively correlated with LPS (r = -0.28, $p = 0.043$; r = -0.28, $p = 0.042$; r = -0.35, $p = 0.010$; respectively). Taken together, our findings imply that antipsychotics-induced changes in gut microbial composition are directly linked to weight gain and metabolic disruption in SZ-O patients.

Functional Change On Gut Microbiota In Sz-o Patients

Given the possibility of functional redundancy within bacteria [19], we performed the KEGG-based PICRUSt analysis for microbial function prediction. The variations between the two groups were attributed to 32 different KEGG pathways in total (Wilcoxon rank sum test, $p < 0.05$) (Fig. 3A **& Tab. S5**). These differential pathways were mainly involved in glucose metabolism, lipid metabolism and amino acid metabolism.

Compared with SZ-N group, the Glycolysis/Gluconeogenesis (ko00010), Fatty acid degradation (ko00071), Purine metabolism (ko00230), Pyrimidine metabolism (ko00240), Cysteine and methionine metabolism (ko00270), Tyrosine metabolism (ko00350), Glutathione metabolism (ko00480), Starch and sucrose metabolism (ko00500), Vitamin B6 metabolism (ko00750), Nicotinate and nicotinamide metabolism (ko00760), Ribosome biogenesis in eukaryotes (ko03008), Proteasome (ko03050) were upregulated in SZ-O patients. While the Citrate cycle (TCA cycle) (ko00020), Fructose and mannose metabolism (ko00051), Steroid hormone biosynthesis (ko00140), Valine, leucine and isoleucine degradation (ko00280), Glycosaminoglycan degradation (ko00531), Linoleic acid metabolism (ko00591), Biotin metabolism (ko00780), Limonene and pinene degradation (ko00903), Carotenoid biosynthesis (ko00906), Flavonoid biosynthesis (ko00941), Metabolism of xenobiotics by cytochrome P450 (ko00980), Protein processing in endoplasmic reticulum (ko04141), NOD-like receptor signaling pathway (ko04621) were downregulated. These data imply that metabolism-related pathways in SZ-O patients are dramatically changed.

Alterations In Fecal And Serum Metabolites In Sz-o Patients

To further investigate the metabolic outputs, a HPLC-MS/MS-based metabolomic analysis was used to detect 3 and 27 differential metabolites in fecal and serum samples, respectively. In serum metabolites, the SZ-O group displayed enrichment in 24 metabolites and depletion in 3 metabolites (arachidonic acid,

3-methyladipic acid and glutarylcarnitine) (Fig. 3B). These altered metabolites were mainly involved in amino acid metabolism (Alanine, Tyrosine, Aminoadipic acid, Valine, Isoleucine, Leucine), bile acid biosynthesis (GCA, GCDCA, GCDCA-3S), and lipid metabolism (2-Hydroxy-3-methylbutyric acid, 3-Methyladipic acid, Arachidonic acid, Citramalic acid) (Fig. 3C). Patients in the SZ-O group were characterized by decreased levels of 3 fecal metabolites (indole-3-carboxylic acid, propionic acid and valeric acid) (Fig. 3D), which are mostly involved in propanoate metabolism (Fig. S6).

We further explore the associations between the metabolites and signature bacteria. The relationships between 8 signature bacteria and metabolites were summarized in Fig. 3E & Tab.S6. In serum metabolites, the arachidonic acid was positively correlated with *A. muciniphila* (r = 0.430, $p = 0.020$) and *C. aldenense* (r = 0.516, $p = 0.004$); 3-methyladipic acid was positively correlated with *A. muciniphila* (r = 0.383, $p = 0.040$); and glutarylcarnitine were positively correlated with *P. distasonis* (r = 0.390, $p = 0.037$). Other serum metabolites (GCDCA, GCA, Alanine, Leucine, Isoleucine, Homovanillic acid, Valine, Tyrosine, Isovalerylcarnitine, 2-Methylbutyroylcarnitine, Citramalic acid, GCDCA-3S, Aminoadipic acid, Hydroxypropionic acid) were found to be more abundant in SZ-O group, indicating negative relationships with 8 signature bacteria (all $p < 0.05$). Altogether, the metabolomics further underpinned the gut microbial functions disrupted by weight gain in SZ-O patients.

Cross-talk Between Gut Microbiota And Host Metabolic Homeostasis

To gain a better understanding of the relationships between gut microbiota and host metabolism, the co-occurrence network and sankey plot were used to investigate the interactions between core bacteria, KEGG pathways, fecal/serum metabolites, and clinical metabolic indicators. Signature bacteria generated extensive and complicated relationships with both metabolic indices and KEGG pathways, according to the co-occurrence network **(**Fig. 4A **& Fig. S7**). All of the signature bacteria were found in the network's intersection area and had a high node relevance, suggesting that signature bacteria acted as prominent leaders in co-occurrence network. Furthermore, positive correlations among signature bacteria were observed, indicating that those signature bacteria may interact with one other and have dominant effects on host metabolism (Fig. S8 & Tab. S7). All the signature bacteria except *P. distasonis* (V39) were also found to be positively correlated with *Lactobacillaceae Lactobacillus* (V24) (Fig. S8 & Tab. S7). The consistent results of co-occurrence analysis obtained under the min-ratio of 0.6 and 0.7 were also explored (Fig. S7). The sankey plot showed that 8 signature bacteria were significantly correlated with clinical metabolic indices through 19 KEGG pathways and 17 metabolites (Fig. 4B). In conclusion, both the co-occurrence network and sankey plot highlight the complexities of interactions between the signature bacteria and the host metabolism, which are mediated by the KEGG pathways and fecal/serum metabolites.

Microbiota-based Prediction On Weight Gain And Metabolic Outcomes

According to the findings, the gut microbiota composition may play an important role in the development of antipsychotic-induced weight gain. The validation group included 18 FSZ patients with 1-month and 1-year antipsychotic medication follow-up to evaluate if the gut microbiota could be used as a simple and noninvasive approach to predict the likelihood of antipsychotic-induced weight gain. The demographic and clinical characteristics were summarized in Tab. S3. With this, we developed an artificial neuron network (ANN) prediction model based on the relative abundance of gut bacteria at baseline to evaluate the weight gain potential and metabolic indices prior to antipsychotic therapy. By integrating BMI into the ANN model, this model resulted in a high coefficient of determination for BMI at 1month (R^2 = 0.70) and BMI change within 1month (R^2 = 0.61). Meanwhile, at baseline, the ANN model had a high coefficient of determination for BMI (R^2 = 0.67), FBG (R^2 = 0.79), TC (R^2 = 0.73), LDL-C (R^2 = 0.72), TG (R^2 = 0.67), HDL-C (R^2 = 0.63), APOA (R^2 = 0.77), ADP (R^2 = 0.80), IL-17 (R^2 = 0.77), TNF-α (R^2 = 0.65) (Fig. 5). The results with an R^2 score ≤ 0.6 were summarized in Fig. S9. Furthermore, the relative abundance of *P. distasonis* at baseline negatively correlated with BMI changes within one year (Fig. S10). Moreover, a random forest classification algorithm with a fivefold cross-validation was also used to determine the predictive performance of the gut bacteria and metabolites at baseline in predicting the risk of antipsychotic-induced overweight/obesity. When compared to models utilizing either gut microbiota or metabolites, the RF model employing using gut microbiota in combination with metabolites demonstrated a higher accuracy in identifying patients with normal weight or overweight/obesity (Fig. S11). Furthermore, as compared to the initial BMI, the follow-up BMI at 1 month and 1 year in the FSZ group were considerably higher (Fig. S12). Taken together, our data highlight the potential role of signature gut bacteria in predicting the antipsychotic-induced weight gain in SZ patients with chronic antipsychotic medication.

Discussion

Antipsychotics is known to be able to induce overweight or obesity in SZ, but the precise mechanism underlying the high prevalence remains unknown. According to preclinical data, the gut microbiota may contribute to the development of antipsychotic-induced weight gain. However, further investigation is required to determine the exact taxonomic composition of the “obesogenic” microbial profile in SZ patients receiving chronical administration of antipsychotic. In this study, by investigating the microbial characteristics in SZ patients, we identified the key differences in both microbial taxonomic composition and function for patients who developed overweight/obesity after chronic antipsychotic treatment or those who maintained normal weight. Moreover, an ANN model was constructed for the first time based on the baseline microbial profiles, and it demonstrated its capacity in predicting the risk of weight gain prior to initiating antipsychotic treatments. Importantly, the lower abundance of *P. distasonis* at baseline was confirmed to be a prime predictor for the development of overweight/obesity after 1-year antipsychotic treatments. Taken together, these findings imply that the gut microbiome is essential for understanding the susceptibility and resistance to antipsychotics-induced overweight/obesity in SZ patients. Most importantly, the microbial profiles at baseline may develop into a straightforward and effective approach to predict the risk of antipsychotics-induced weight gain, guiding the clinical prescription of antipsychotic in clinical practice.

In the present study, we firstly conducted a retrospective survey in chronically antipsychotic-treated SZ patients who had a normal initial BMI before antipsychotics treatment. According to the findings, up to one-third of patients who received long-term antipsychotic treatment became overweight or obesity, while the others presented stronger resistance to the weight gain. To clarify such interpatient heterogeneity in antipsychotics-induced weight gain, we further conducted a comprehensive comparison of the gut microbial characteristics between those patients who developed overweight/obesity and those who maintained at normal weigh in a cross-sectional study. *Firmicutes* and *Bacteroidetes*, two dominant bacteria in human gut communities at the phylum level, make up more than 70% of the entire gut microbiota [29]. Additionally, there is proof that an increased *Firmicutes/Bacteroidetes* (F/B) ratio was directly linked to the emergence of obesity [7]. Although we also observed elevated F/B ratios in SN-O compared to SZ-N patients in our investigation, two phyla of *Firmicutes* and *Actinobacteria* predominated in both groups. Since *Firmicutes* and *Bacteroidetes* have also been reported as two dominant phyla in first-episode drug-naïve SZ patients [30], this discrepancy in the present study could be due to disease chronicity and prolonged exposure to antipsychotics medication [11]. Furthermore, significant differences were also detected in β-diversity between the two groups, and the core bacterial species explained 87.45% of the variability in clinical metabolic indices. The robust random forest algorithm was then carried out to screen the signature bacteria between two groups. Eight signature bacteria were identified, including members of genus *Bacteroides* (*B.uniformis*, *B.ovatus*, *Bacteroidaceae Bacteroides*), *Parabacteroides* (*P. distasonis*; *Porphyromonadaceae Parabacteroides*), *Akkermansia* (*A.muciniphila*), *Clostridium* (*C.aldenense*, *C.citroniae*). Notably, the relative abundance of all the signature bacteria was negatively correlated with BMI in the SZ-N group, indicating that reduction of signature bacteria in SZ-O group may contributed to antipsychotic-induced weight gain.

Previous studies have demonstrated that members of genus *Bacteroides*, *e.g. B. uniformis* and *B. ovatus* were proved to improve the metabolic and immune dysfunction in high-fat diet-induced obese mice [31, 32]. The administration of *B. uniformis* or *B. ovatus* not only significantly lower level of serum cholesterol, triglyceride and fasting blood glucose, but also improve insulin and leptin resistance [31, 33]. Additionally, the relative abundance of *Bacteroidaceae Bacteroides* at baseline has also been reported to be an effective predictor for weight loss efficacy under short-term low-carbohydrate diet intervention [34]. Consistent with these findings, we also found that the *Bacteroides* genera were negatively correlated with serum level of TG and LPS, whereas positively correlated with serum level of HDL-C. Other than the member of genera *Bacteroides*, *A. muciniphila* is a significant bacterial species in human gut, and was considered to be a promising candidate for next generation probiotics [35]. In previous studies, *A. muciniphila* was reported to strengthen intestinal barrier and reduce the serum level of LPS [36–39], and supplementation with *A. muciniphila* was proved to be safe and well-tolerated to improve metabolic disturbance and obesity-related complications [36, 40, 41]. In line with these findings, the present study also showed a negative correlation between *A. muciniphila* and serum LPS. Similarly, *P. distasonis* was also defined as one of the 18 core members of the human gut microbiome, which has been shown to be in relatively low abundance in the obese individuals [42]. *P. distasonis* has been demonstrated to affect the serum bile acid profiles [43, 44], an essential molecules not only promoting the absorption of fat-

soluble nutrients but also regulating the metabolic homeostasis of glucose and lipid [45]. Treatment with live *P. distasonis* was confirmed to alleviate obesity and obesity-related disturbance via secondary bile acid-activated FXR signaling and succinate-activated intestinal gluconeogenesis (IGN) in both ob/ob and diet induced obese (DIO) mice[43]. More recently, Mengci et al.[44] also reported that the depletion of *P. distasonis* during calorie restriction was closely related to decreased proportion of non-12α-hydroxylated secondary bile acids in mice, which is further responsible for the rapid weight rebound after calorie restriction. These results further support the potential metabolic benefits of *P. distasonis*, which was connected to the bile acids profiles. Meanwhile, *Porphyromonadaceae Parabacteroides* has previously been reported to generate butyrate, a component of SCFAs that exerts diverse metabolic benefits on host health [46]. Likewise, *Clostridium* is another beneficial genus known for its ability to produce SCFAs [47], which not only reduce the levels of fatty acids content in both liver and plasma, but also improve tissue insulin sensitivity [48]. In our study, we found a negative correlation of *Clostridium* (*C. aldenense* and *C. citroniae*) with serum levels of TG and LPS. Our findings are consistent with the previous report. Particularly, potential synergistic effects were also observed between *Lactobacillus* and signature bacteria. *Lactobacillus* is a typical probiotic that has been widely used to enhance intestinal barrier [49], reduce visceral fat mass [50, 51], maintain body weight [52], and improve glucose tolerance [53]. Recently, *Lactobacillus* has also been proved to be effective in curbing antipsychotics-induced weight gain in patients with SZ [17, 18]. Thus, these synergistic effects may further support the potential uses of "supplementation with *Lactobacillus*" in antipsychotic-induced weight gain by increasing the abundance of healthy bacteria. Along with these findings from previous and current attempts, we proposed that these signature bacteria may play a crucial role in the development of antipsychotic-induced weight gain in SZ patients, and such metabolic effects may be closely associated with the microbial function [54].

The fecal metabolome has been considered as functional readout of the gut microbiome for a long time. In our study, we identified three fecal metabolites that were enriched in SZ-N group relative to the SZ-O group, including indole-3-carboxylic acid, propionic acid and valeric acid. Specifically, indole-3-carboxylic acid is one of the microbiota-derived tryptophan metabolites, and known to exert profound effects on host physiology. Recent evidence has demonstrated its ability in activating the aryl hydrocarbon receptor (AhR) [55]. The activation of AhR can promote the production of interleukin-22 (IL-22), which plays a key role in the maintenance of epithelial barrier and immune function by inducing the production of antimicrobial peptides and tight junction proteins [56, 57]. Two of the main components of SCFAs, which were the byproducts of indigestible fiber fermentation from gut bacteria, are propionic acid and valeric acid [58]. As an important energy and signaling molecule connecting the gut microbiota with the host, SCFAs has multiple favorable effects on the host metabolic homeostasis [48]. A recent study showed that propionic acid could effectively reduce the body weight and lower fasting insulin levels in obese mice [59]. Likewise, valeric acid could reduce serum cholesterol levels in DIO rats, and the level of serum valeric acid was inversely correlated with the LDL-C/HDL-C ratio [60]. Taken together, patients with higher level of indole-3-carboxylic acid, propionic acid and valeric acid in feces are less susceptible to antipsychotic-induce weight gain. In other words, the decreased level of the three fecal metabolites may be a factor linked to antipsychotics-induced weight gain.

Beyond the identification of gut microbial metabolites, the KEGG-based PICRUSt analysis was also performed to interpret the potential functional change of signature bacteria. Compared with SZ-N group, a wide range of metabolic pathways were altered significantly in the SZ-O group. Importantly, some identified pathways were also confirmed by serum metabolome, and these differential microbial function and serum metabolites were consistently mapped into carbohydrate, amino acid and lipid metabolism. For instance, glycolysis/gluconeogenesis and TCA cycle are important biological processes in glucose homeostasis and energy expenditure, respectively. Here, we found that the glycolysis/gluconeogenesis pathway was relatively enriched in the SZ-O group, which was consistent with previous studies [45, 61]. Similarly, the TCA cycle is the site for terminal fat oxidation and gluconeogenesis, which has been found to be down-regulated in obese adults [61]. Liu et al. [62] reported genes involved in the TCA pathway were downregulated in obese subjects versus lean subjects, and the pathways involved in carbohydrate metabolism such as starch, sucrose and galactose metabolism were enriched in obese subjects, which were well consistent with our findings. In addition, our study also showed that the valine, leucine, and isoleucine degradation pathway was down-regulated in the SZ-O group, which is consistent with the finding that SZ-O subjects had higher serum levels of valine, leucine, and isoleucine. It is worth noting that the valine, leucine and isoleucine are three branched-chain amino acids (BCAAs) that have been implicated in obesity and T2DM [63, 64]. Furthermore, high levels of BCAAs were also associated with insulin resistance in both animal and human studies [65, 66]. The restriction of isoleucine or valine in diet has been proved to promote the metabolic health in mice, and higher level of dietary isoleucine was related to increased inflammation and BMI in humans [67–69]. Moreover, the linoleic acid metabolism pathway was also identified to be significantly lower in SZ-O group, which is consistent with the depleted level of serum arachidonic acid in SZ-O group. As an essential derivative of linoleic acid metabolism and one of the most important polyunsaturated fatty acids, arachidonic acid has been reported to exhibit anti-inflammatory properties that may help improve the insulin sensitivity in obesity [70–72]. This idea is supported by research that shows that obese and T2DM rats had lower levels of serum arachidonic acid [72].

Based on the aforementioned analyses, we speculated that the gut microbial changes may be responsible for the antipsychotic-induced weight gain in SZ patients. Clinically, overweight or obesity is a challenging issue that has to be solved urgently in SZ patients. Prior to initial treatment, it is essential to accurately predict the risk of weight gain caused by antipsychotics because patients who are more likely to gain weight may need to choose antipsychotics with lower metabolic risk and undergo more frequent monitoring of their metabolic indices to avoid metabolic syndrome. Thus, we further investigated whether it is feasible to predict the risk of antipsychotic-induced weight gain before initiating antipsychotic treatment based on the gut microbial characteristics at baseline. Here, we established an artificial neural network (ANN) model, a powerful deep learning model with obvious superiority in handling complex data. This model has already been used to improve the auxiliary diagnosis of insomnia disorder [73], predictors of catheter-related thrombosis in hospitalized infants [74], and estimate weight loss potential of short-term low-carbohydrate diet intervention in obese patients [75]. Our findings demonstrated that the ANN

model could well predict the BMI change following 1-month antipsychotic treatments, using the baseline microbiome profiles data of FSZ patients.

Meanwhile, this model could also effectively predict some metabolic indices prior to the antipsychotic treatment, suggesting that this bacteria-based model may have the potential to help patients assess their physical condition. Additionally, we also found that the microbiota and/or metabolites at baseline could effectively predict the risk of developing overweight/obesity in FSZ patients after 1-year antipsychotic treatments, with AUC of 0.79, 0.81 and 0.88, respectively. Moreover, the FSZ patients with higher abundance of *P. distasonis* at baseline exhibited a stronger resistance to antipsychotic-induced weight gain. Our findings are in line with a recent study [44] that supplementation with *P. distasonis* could ameliorate the rapid weight regain after calorie restriction. Notably, our study also showed that the reduction of *P. distasonis* was associated with a higher serum level of primary bile acids (GCA and GCDCA) in SZ-O group, which is consistent with previous findings of a higher level of total bile acids, primary bile acids and the ratio of primary to secondary bile acids in patients with non-alcoholic fatty liver disease (NAFLD) [76]. Thus, these results further support the potential role of *P. distasonis* in preventing antipsychotic-induced weight gain, and such metabolic effects are also closely associated with serum level of primary bile acids. Further investigations are required to verify whether *P. distasonis* is potent to improve or even reverse antipsychotics-induced weight gain and its association with bile acid profiles.

There are certain limitations in our study. Firstly, the comparison of gut microbiota between the two group was a cross-sectional design, which precluded causal inferences. Thus, longitudinal design is needed to determine whether the microbial characteristics occurred before or after the antipsychotics-induced overweight/obesity in SZ patients. Secondly, the study utilized 16s rRNA gene sequencing, which was insufficient to elaborate the relationships between gut microbiota and host metabolism at strain-level. Future studies should give deeper insight into the relationships via metagenomics and meta-transcriptomics. Thirdly, although we identified several key bacterial species which may have potential to ameliorate the antipsychotics-induced weight gain, in particular for *P. distasonis*, further animal experiments still need to be conducted to verify the findings.

Conclusion

In our study, we identified interpatient heterogeneity in the development of antipsychotic-induced weight gain in SZ patients. Those with overweight/obesity are characterized by disturbances of gut microbial diversity, composition, and function. Moreover, the gut microbiota at baseline can be predictors to assess antipsychotic-induced weight gain before the drug administration. Taken together, our study provided evidence that gut microbial dysbiosis may play an essential role in the development of antipsychotic-induced overweight/obesity in SZ. Furthermore, our findings also provide new avenues to guide the clinical application of antipsychotic treatment and to develop novel therapeutic tools to manage body weight for SZ patients.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the third Affiliated Hospital of Sun Yat-sen University (no. 2021-02-379). Informed consent was obtained from all participants.

Availability of data and materials

All raw sequence data have been uploaded into the NMDC databases under accession number NMDC10018169.

Declaration of competing interest

These authors declare no conflict of interest.

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Authors' contributions

Yaxi Liu, Hui Wu, Bingdong Liu contributed equally to this work. Xiaoli Wu and Liwei Xie designed this study. Yaxi Liu, Hui Wu, Bingdong Liu, Shengyun Chen, Liujing Huang developed and optimized the methodology. Yaxi Liu, Hui Wu, Bingdong Liu, Shengyun Chen, Liujing Huang and Zhihong Liu collected and analyzed the data. Yaxi Liu, Hui Wu and Jie Wang drafted and edited the manuscript. Xiaoli Wu and Liwei Xie acquired the grants and review the manuscript.

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Tables

Table 1. Comparison of general demographic and clinical data between the SZ-N and SZ-O groups

Parameters	SZ-N (n=98)	SZ-O (n=49)	t/Z/X^2	*p* value
Gender (%)			0.67	0.412
Male	51 (52.0%)	29 (59.2%)		
Female	47 (48.0%)	20 (40.8%)		
Age (year)	24.50 (19.00, 31.25)	23.00 (20.50, 30.50)	-0.09[a]	0.926
Initial Height (cm)	165.00 (158.00, 171.25)	168.00 (158.00, 172.00)	-0.97[a]	0.332
Initial Weight (kg)	58.43 ± 6.96	60.28 ± 7.25	-1.50	0.135
Initial BMI (kg/m²)	21.48 (20.89, 22.57)	22.46 (21.08, 23.40)	-1.90[a]	0.057
Current Weight (kg)	60.20 ± 6.68	74.05 ± 9.36	-9.25	< 0.001
Current BMI (kg/m²)	22.29 (21.26, 23.44)	26.35 (25.65, 28.05)	-9.87[a]	< 0.001
BMI Increment (kg/m²)	0.37 (0.00, 1.73)	4.50 (3.10, 6.77)	-9.05[a]	< 0.001
Drug Dose (Chlorpromazine mg/d)	470.00 (300.00, 718.75)	437.50 (255.00, 600.00)	-0.95[a]	0.343
Antipsychotics (%)			1.00	0.607
High Metabolic Risk	63 (65.6%)	28 (57.1%)	1.00	0.318
Moderate Metabolic Risk	22 (22.9%)	14 (28.6%)	0.56	0.456
Low Metabolic Risk	11 (11.5%)	7 (14.3%)	0.24	0.625
Combined Use of Antipsychotics (%)			0.34	0.560
Yes	64 (66.7%)	35 (71.4%)		
No	32 (33.3%)	14 (28.6%)		
Valproate (%)			0.54	0.461
Yes	23 (23.7%)	9 (18.4%)		
No	74 (76.3%)	40 (81.6%)		

a: Wilcoxon rank sum test

Figures

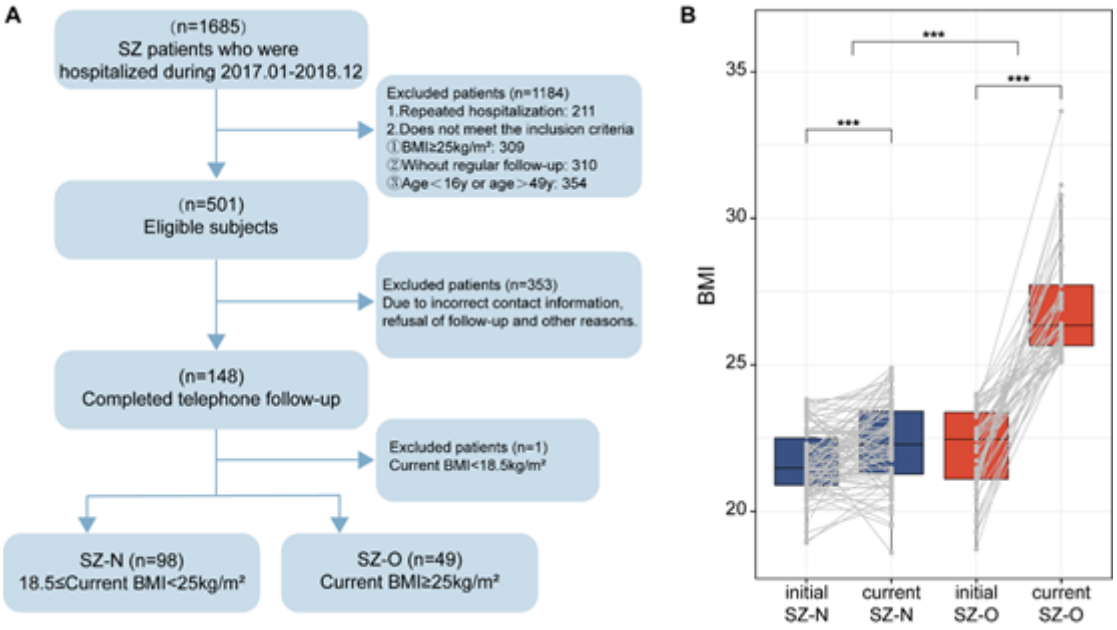

Figure 1

The retrospective survey in chronically antipsychotic-treated SZ patients. (A) Flow chart showed the whole retrospective study process. **(B)** Comparison between initial BMI and current BMI in the SZ-N and SZ-O groups (Wilcoxon signed rank test), and comparison of BMI increments between the two groups (Wilcoxon rank sum test). *p<0.05, **p<0.01, ***p<0.001. **(C)** Comparison of general demographic and clinical data between the SZ-N and SZ-O groups.

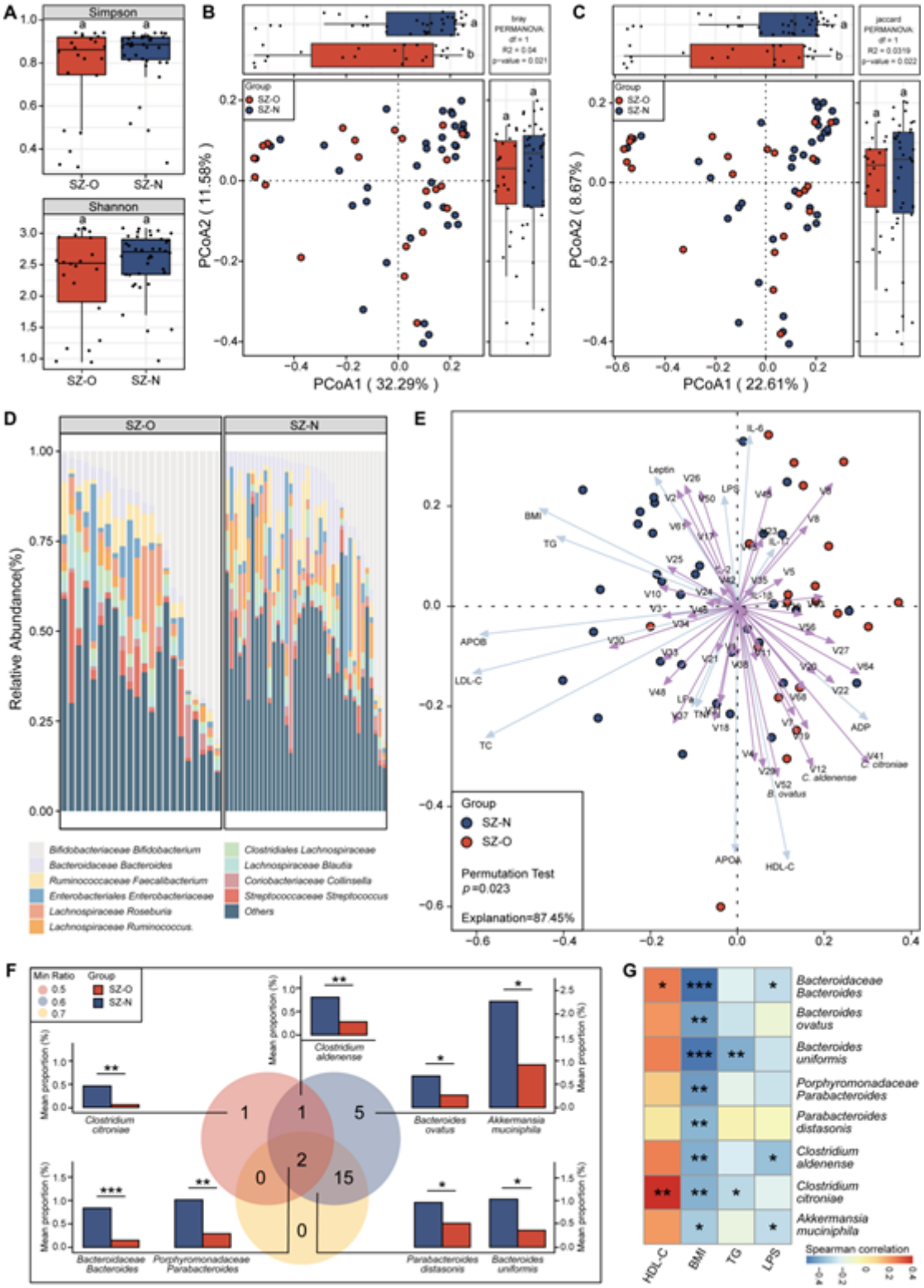

Figure 2

Characterization of gut microbial structure between the SZ-N and SZ-O groups and its relationships with metabolic indices. (A) Box plots showed no significant differences in α-diversity indices (Shannon and Simpson) between the SZ-N and SZ-O groups at the genus level. The horizontal lines in the box plots mean median values. The highest and the lowest boundaries of the box denote the 75% and 25% quartiles, and whiskers represent the lowest and highest values within 1.5 times the interquartile range

(IQR) from the 25% and 75% quartiles, respectively. Dots represent data points beyond the whiskers. The same letters on the whiskers indicate no significant difference between the groups; on the contrary, different letters indicate significant differences between the groups. Min-ratio=0.5. (**B & C)** The PCoA of β-diversity based on bacterial distribution at the genus level by Bray-Curtis and Binary Jaccard algorithm showed significant differences in gut taxonomic composition between the SZ-N and SZ-O groups. Min-ratio=0.5. (**D)** The overall composition and relative abundance of the bacterial community in each group at the genus level were different. **(E)** RDA showed core bacteria at the species level were strongly associated with metabolic indices. 3 bacterial species (*B. ovatus, Bacteroides ovatus*; *C. aldenense Clostridium aldenense*; *C. citroniae, Clostridium citroniae*) had major effect on metabolic data. Abbreviation: BMI, Body Mass Index; HDL-C, High Density Lipoprotein-Cholesterol; LDL-C, Low Density Lipoprotein-Cholesterol; TG, Triglyceride; TC, Total Cholesterol; APOA, Apolipoprotein A; APOB, Apolipoprotein B; Lpa, Lipoprotein a; IL-1β, Interleukin-1β; IL-2, Interleukin-2; IL-6, Interleukin-6; IL-17, Interleukin-17; TNF-α, Tumor Necrosis Factor-α; ADP, Adiponectin. Min-ratio = 0.5. **(F)** 8 signature bacterial species were confirmed under different min-ratio. Different colors of the circle represent different min-ratio, and the figures in the circles represent all the potential signature bacteria confirmed by random forest algorithm. The histogram showed the signature bacteria with statistical differences in relative abundance between the SZ-N and SZ-O groups; Wilcoxon rank sum test, *p<0.05 **p<0.01 ***p<0.001. **(G)** Spearman correlation analysis of the signature bacteria and metabolic indices. The panel is the scale of the spearman correlation coefficient, red and blue represent positive and negative correlations, respectively; *p<0.05 **p<0.01 ***p<0.001.

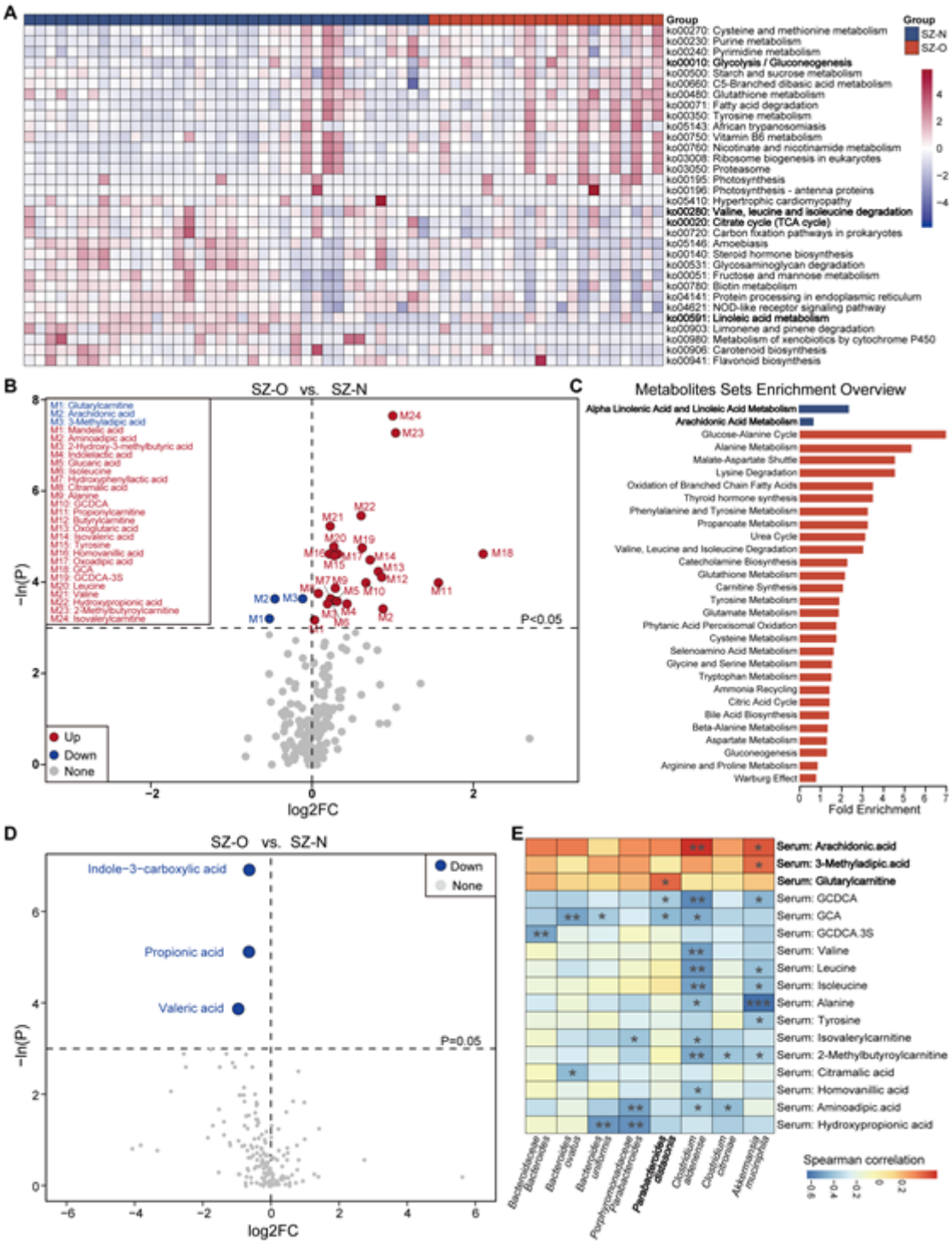

Figure 3

Characterization of gut microbial function between the SZ-N and SZ-O groups. (A) Heatmap of PICRUSt analysis showed significantly different KEGG pathways between the SZ-N and SZ-O groups. Wilcoxon rank sum test, $p<0.05$. (**B & C)** The differential serum metabolites and enriched pathways between the SZ-N and SZ-O groups. **(D)** The differential fecal metabolites between the SZ-N and SZ-O groups. **(E)** Spearman correlation analysis between the metabolites and signature bacteria. The panel is the scale of the spearman correlation coefficient, red and blue represent positive and negative correlations, respectively; *$p<0.05$, **$p<0.01$, ***$p<0.001$.

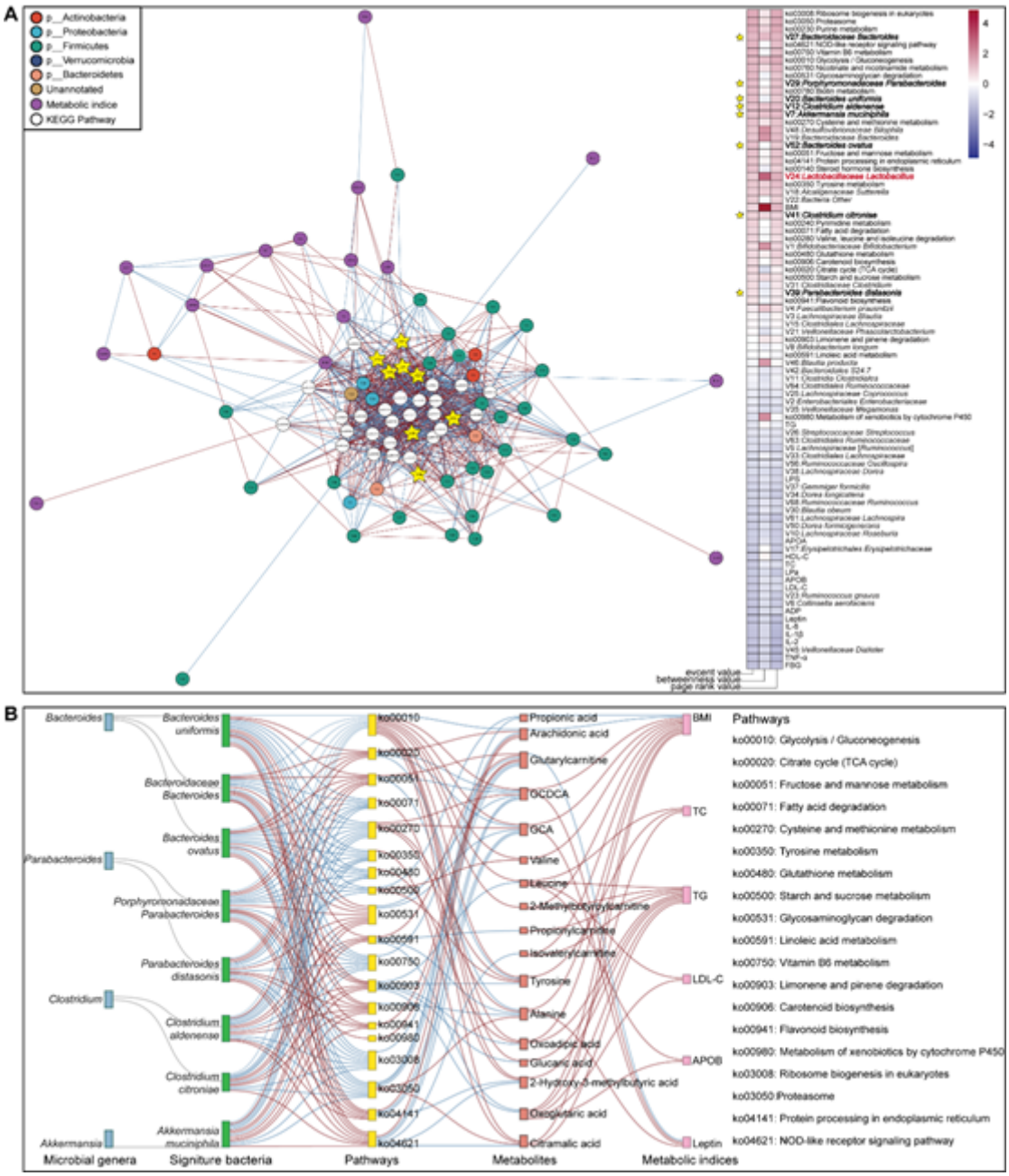

Figure 4

Cross-talk between gut microbiota and host metabolic homeostasis. (A) The co-occurrence networks reflected network interaction complexity. All bacterial nodes were colored at phylum level (isolated nodes were excluded), metabolic indices and KEGG pathways were stained uniformly, and edges were estimated by Spearman's rank correlation coefficient (p<0.05). Edges colored with red and blue represent positive and negative correlations, respectively. Signature bacteria were marked with yellow five-pointed star. The heatmap showed the importance of each node in the network calculated by three algorithms, and the transition from red to blue represent the gradual decrease of the node's importance. Min ratio=0.5. **(B)** Sankey plot showed complex interactions between the signature bacteria and metabolic indices meditated by KEGG pathways and fecal/serum metabolites. Connections colored with grey indicate the subordinate relationships between the signature bacteria and the microbial genera. Connections colored

with red and blue represent positive and negative correlations and were estimated by Spearman's rank correlation coefficient (p<0.05).

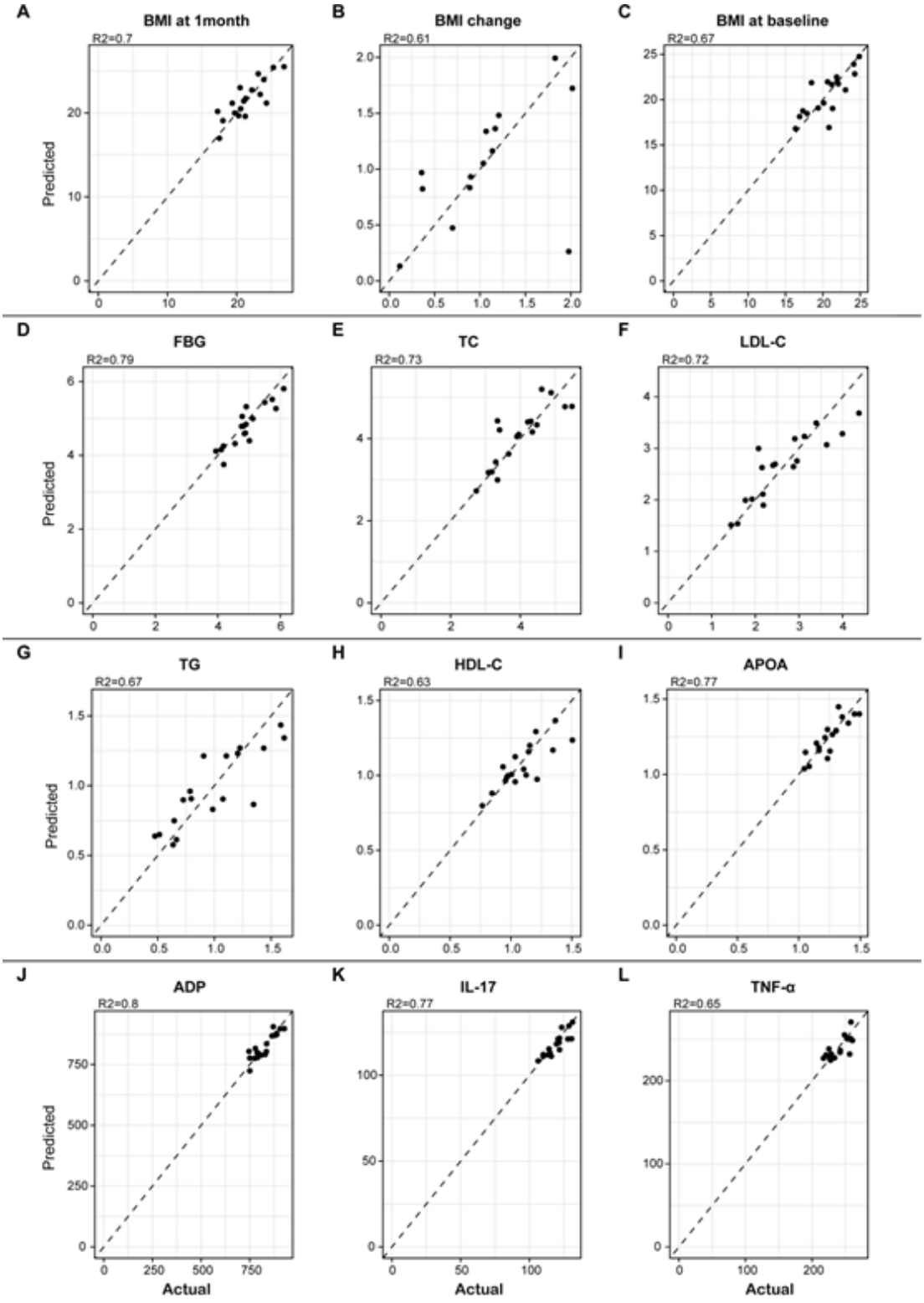

Figure 5

Gut bacteria-based prediction model on weight gain potential by artificial neuron network (ANN). Applying the relative abundance of gut bacterial species at baseline, the ANN was established to predict the weight

gain potential and clinical metabolic indices, including BMI at 1 month **(A)**, BMI change within 1 month **(B)**, and the BMI, FBG, TC, LDL-C, TG, HDL-C, APOA, ADP, IL-17 and TNF-α at baseline **(C to L)**. We presented these results on the basis of an R^2 score > 0.6.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.xlsx