Alteration of the gut microbiota associated with childhood obesity by 16S rRNA gene sequencing

Xiaowei Chen¹,², Haixiang Sun¹, Fei Jiang¹,², Yan Shen¹,², Xin Li¹, Xueju Hu¹, Xiaobing Shen¹,² and Pingmin Wei¹,²

¹ Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, China
² Department of Epidemiology and Health Statistics, School of Public Health, Southeast University, Nanjing, China

ABSTRACT

Background. Obesity is a global epidemic in the industrialized and developing world, and many children suffer from obesity-related complications. Gut microbiota dysbiosis might have significant effect on the development of obesity. The microbiota continues to develop through childhood and thus childhood may be the prime time for microbiota interventions to realize health promotion or disease prevention. Therefore, it is crucial to understand the structure and function of pediatric gut microbiota.

Methods. According to the inclusion criteria and exclusion criteria, twenty-three normal weight and twenty-eight obese children were recruited from Nanjing, China. Genomic DNA was extracted from fecal samples. The V4 region of the bacterial 16S rDNA was amplified by PCR, and sequencing was applied to analyze the gut microbiota diversity and composition using the Illumina HiSeq 2500 platform.

Results. The number of operational taxonomic units (OTUs) showed a decrease in the diversity of gut microbiota with increasing body weight. The alpha diversity indices showed that the normal weight group had higher abundance and observed species than the obese group (Chao1: \( P < 0.001 \); observed species: \( P < 0.001 \); PD whole tree: \( P < 0.001 \); Shannon index: \( P = 0.008 \)). Principal coordinate analysis (PCoA) and Nonmetric multidimensional scaling (NMDS) revealed significant differences in gut microbial community structure between the normal weight group and the obese group. The linear discriminant analysis (LDA) effect size (LEfSe) analysis showed that fifty-five species of bacteria were abundant in the fecal samples of the normal weight group and forty-five species of bacteria were abundant in the obese group.

In regard to phyla, the gut microbiota in the obese group had lower proportions of Bacteroidetes (51.35%) compared to the normal weight group (55.48%) (\( P = 0.030 \)). There was no statistical difference in Firmicutes between the two groups (\( P = 0.436 \)), and the Firmicutes/Bacteroidetes between the two groups had no statistical difference (\( P = 0.983 \)). At the genus level, Faecalibacterium, Phascolarctobacterium, Lachnospira, Megamonas, and Haemophilus were significantly more abundant in the obese group than in the normal weight group (\( P = 0.048 \), \( P = 0.018 \), \( P < 0.001 \), \( P = 0.040 \), and \( P = 0.003 \), respectively). The fecal microbiota of children in the obese group had lower proportions of Oscillospira and Dialister compared to the normal weight group (\( P = 0.002 \) and \( P = 0.002 \), respectively).

How to cite this article Chen X, Sun H, Jiang F, Shen Y, Li X, Hu X, Shen X, Wei P. 2020. Alteration of the gut microbiota associated with childhood obesity by 16S rRNA gene sequencing. PeerJ 8:e8317 http://doi.org/10.7717/peerj.8317
Conclusions. Our results showed a decrease in gut microbiota abundance and diversity as the BMI increased. Variations in the bacterial community structure were associated with obesity. Gut microbiota dysbiosis might play a crucial part in the development of obesity in Chinese children.

Subjects Bioinformatics, Microbiology, Gastroenterology and Hepatology

Keywords Gut microbiota, 16S rRNA gene sequencing, Childhood obesity, Bacterial compositions, Alpha diversity, Beta Diversity

INTRODUCTION

Obesity has become a global epidemic in the industrialized and developing world (Luke et al., 2014; Popkin, Adair & Ng, 2012). The World Health Organization’s briefing on obesity showed that global obesity is on a rapid upward trend and has doubled since 1980, with more than 40 million obese children in the world (Women, 2011). Obesity is associated with serious health risks, raising great concerns about multiple comorbidities occurring with obesity, including musculoskeletal disorders, type 2 diabetes, cardiovascular diseases, nonalcoholic fatty liver disease, and certain cancers (Dietrich & Hellerbrand, 2014; Farni et al., 2014; Global Burden of Metabolic Risk Factors for Chronic Disease Collaboration, 2014; Pettitt et al., 2014; Prospective Studies Collaboration, 2009; Wormser et al., 2011; Saydah et al., 2014). Due to the health and economic burden brought by the rising BMI, obesity has been included as a global noncommunicable diseases (NCD) target, halting the rise of obesity in 2025 to its 2010 level (Kontis et al., 2014).

Obesity prevails among children and results in a global problem regarding children’s general health and well-being. With high prevalence of childhood obesity, many children suffer from obesity-related complications (Han, Lawlor & Kimm, 2010). The pathogenesis of obesity is complicated, with multiple factors involved. Apart from genetic and nutritional factors, a new factor has been recently identified related to the onset and progression of obesity—the gut microbiota (Ridaura et al., 2013; Sonnenburg & Sonnenburg, 2014). As 500-1,000 species of microbes lives in the gastrointestinal tract, the gut microbiota is an ecosystem in itself (Karlsson et al., 2013a; Karlsson, Nookaew & Nielsen, 2014). The number of microbial genes in the gut microbiota is at least 150-fold larger than that of the human genes inside in human body (Qin et al., 2010).

Up to now, many studies have demonstrated that the gut microbiota, as the largest and most complex microecosystem inside the human body, plays extremely important roles in health and disease (Guyton & Alverdy, 2017; Leung et al., 2016; Lathrop et al., 2011; Nicholson et al., 2012; Sandhu et al., 2017; Tang & Hazen, 2016; Tremaroli & Backhed, 2012). The gut microbiota genome maintains normal physiological and metabolic functions in the human body, aiding food digestion through significantly enriching genes in metabolizing carbohydrates, vitamins, short-chain fatty acids and amino acids (Gill et al., 2006). For a long period, the microorganisms of the gut microbiota stay in the mutualistic symbioses and create a balanced and stable intestinal microsystem. This balanced microsystem prepare the host to adapt to special conditions, while the dysbiosis of
this microsystem shall cause the compositional and functional imbalance in the intestinal microorganisms (Lynch & Pedersen, 2016).

In recent years, a growing number of researchers have paid attention to the gut microbiota and explored its functions in the pathogenesis and regulation of metabolic disorders. It has been suggested that the microbiota continues to develop through childhood and children may be the best candidate for microbiota interventions to realize health promotion or disease prevention (Hollister et al., 2015). Therefore, it is essential to foster a preliminary understanding of the pediatric gut microbiota. However, due to limited information of the pediatric gut microbiota regarding its structure and function, the mechanism and the degree of gut microbiota contributing to the development of childhood obesity have not yet been elucidated. As a result, a gut microbiota study in the context of obesity is needed to explain the relationship between the obesity epidemic and the gut microbiota.

Though it is difficult to cultivate the anaerobic gut microbiota in the laboratory, new genome sequencing techniques enable us to collect and analyze information of human gut microbiota from the perspectives of microbial composition and function (MetaHIT Consortium, 2011; Qin et al., 2010). In our study, we recruited children from a same geographic area to minimize variations irrelevant to obesity. Our research goal was to evaluate gut microbial biodiversity between obese and normal weight children, aged between 6-11 years old, using 16S rRNA gene sequencing. We expect that our findings could have some reference meanings in preventing and treating childhood obesity.

**MATERIALS AND METHODS**

**Ethics statement**

The study was conducted in accordance to the Declaration of Helsinki revised in 2013 and approved by the ethics committee of Zhongda Hospital, Southeast University (approval number: 2017ZDSYLL109-P01). Participation in this study was voluntary, and all parents gave written informed consent.

**Research object**

A total of 51 volunteers of both sexes (27 males and 24 females) were recruited from Nanjing, China. Subjects were strictly categorized into the normal weight group ($n = 23$) or the obese group ($n = 28$) according to the inclusion and exclusion criteria.

Inclusion criteria: (1) The age range for participation was 6-11 years old. (2) In accord with the determination standard of normal weight and obesity of children, the standard was “Body mass index cut-offs for overweight and obesity in Chinese children and adolescents aged 2-18 years” (Li et al., 2010) formulated by the Department of Growth and Development, Capital Institution of Pediatrics (Table 1). (3) Willing to participate in the study and obtain the consent of the guardian, voluntarily taking the subject and signing the informed consent form.

Exclusion criteria: (1) Antibiotics have been used in the past 4 weeks. (2) Gastrointestinal dysfunction, previous gastrointestinal disease history or diarrhea, abdominal distension, abdominal pain or constipation within the past 4 weeks. (3) Trauma, serious infection,
Table 1  Body mass index cut-offs for overweight and obesity in Chinese children and adolescents aged 2-18 years.

| Age (Years) | Boys          |          | Girls         |          |
|-------------|---------------|----------|---------------|----------|
|             | Overweight    | Obesity  | Overweight    | Obesity  |
| 2           | 17.5          | 18.9     | 17.5          | 18.9     |
| 3           | 16.8          | 18.1     | 16.9          | 18.3     |
| 4           | 16.5          | 17.8     | 16.7          | 18.1     |
| 5           | 16.5          | 17.9     | 16.6          | 18.2     |
| 6           | 16.8          | 18.4     | 16.7          | 18.4     |
| 7           | 17.2          | 19.2     | 16.9          | 18.8     |
| 8           | 17.8          | 20.1     | 17.3          | 19.5     |
| 9           | 18.5          | 21.1     | 17.9          | 20.4     |
| 10          | 19.3          | 22.2     | 18.7          | 21.5     |
| 11          | 20.1          | 23.2     | 19.6          | 22.7     |
| 12          | 20.8          | 24.2     | 20.5          | 23.9     |
| 13          | 21.5          | 25.1     | 21.4          | 25.0     |
| 14          | 22.1          | 25.8     | 22.2          | 25.9     |
| 15          | 22.7          | 26.5     | 22.8          | 26.7     |
| 16          | 23.2          | 27.0     | 23.3          | 27.2     |
| 17          | 23.6          | 27.5     | 23.7          | 27.6     |
| 18          | 24.0          | 28.0     | 24.0          | 28.0     |

and infectious diseases. (4) Hereditary obesity. (5) Drug-induced obesity. (6) Endocrine disorders and metabolic diseases.

Sample collection
Fecal samples were collected and well-sealed in sterile boxes, immediately frozen in a refrigerator and transported to school the next morning and stored at $-20\degree C$ before being transferred in insulating polystyrene foam containers to the Key Laboratory of Environmental Medical Engineering and Education Ministry, where samples were stored at $-80\degree C$ until further analysis.

DNA extraction and PCR amplification
Genomic DNA was extracted according to the specifications of TIANamp Stool DNA kit (TIANGEN, China, Cat. DP328), applied to all feces samples. The integrity and purity of DNA were detected by 1% agarose gel electrophoresis, while the concentration and purity of DNA were detected by NanoDrop One. The V4 region of the bacterial 16S rDNA was amplified by PCR with the specific primers 515F (5’-GTGCCAGCMGCGGCTACGG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) labeled in a 12 bp barcode. PCR amplification was conducted using primers with a barcode and Premix Taq under the following conditions: 5 min at 94 $\degree C$ for initialization, 30 s denaturation at 94 $\degree C$ for 30 cycles, 30 s annealing at 52 $\degree C$, and 30 s extension at 72 $\degree C$, and a final 10 min elongation at 72 $\degree C$. The fragment length and concentration of PCR products were detected by 1% agarose gel electrophoresis, and samples with a main band length in the range of 290-310 bp were selected. After comparing the concentrations of PCR products using GeneTools.
Analysis Software (Version 4.03.05.0, SynGene), the required volume of each sample was calculated according to the principle of equal quality, and each PCR product was mixed and recovered by the An E.Z.N.A.® Gel Extraction Kit (OMEGA, USA, Cat. D2500). TE buffer was used to elute and recover the target DNA fragments.

**Sequencing and data processing**

Libraries were built according to the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® and sequenced on an Illumina HiSeq2500 platform (Caporaso et al., 2011), and then 250 bp paired-end reads were generated. Trimmomatic software (V0.33, http://www.usadellab.org/cms/?page=trimmomatic) (Bolger, Lohse & Usadel, 2014) was used to filter the quality of the raw reads data at both ends. At the same time, with reference to the barcode and primer information at both ends of the sequence, Mothur software (V1.35.1, http://www.mothur.org) (Schloss et al., 2009) was used to distribute the sequence to corresponding samples; the allowed mismatch number of barcodes was 2, and the maximum mismatch number of primers was 3. Then, after quality control, barcode and primers were removed to obtain paired-end clean reads. For double-ended sequencing data, it was necessary to splice each pair of paired-end reads using FLASH software (V1.2.11, https://ccb.jhu.edu/software/FLASH/) (Magoc & Salzberg, 2011) in terms of the overlap between paired-end reads to splice the paired reads into a sequence and to filter out nonconforming tags in order to collect the original spliced sequence (raw tags). The minimum overlap length was set to 10 bp and the maximum allowable mismatch ratio in the overlap region of the spliced sequence was set to 0.1. Mothur software was used to carry out quality control and filter the spliced sequences to obtain effective spliced fragments.

**OTU and species community analysis**

The USEARCH software (V8.0.1517, http://www.drive5.com/usearch/) (Edgar, 2010) was used to cluster all clean tags for all samples. By default, the sequence was clustered into an operational taxonomic unit (OTU) with 97% identity. Singleton OTUs were removed with usearch (http://www.drive5.com/usearch/manual/chimera_formation.html), and chimeric sequences were removed with UCHIME (http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar et al., 2011). The assign_taxonomy.py script and Ribosomal Database Project (RDP) Classifier method (Cole et al., 2007) in Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1) were used to obtain species annotation information. The number of valid tag sequences (No. of seqs) and the OTU taxonomic comprehensive information table (otu_table) were obtained by removing chloroplast and mitochondrial sequences as well as OTUs and the tags that could not be annotated at the set limit. Based on otu_table, R software (V2.15.3) (R Core Team, 2013) was used to calculate the annotation proportion of OTUs at each classification level, and the sequence of each sample at each classification level was obtained to form a column chart. All values greater than 0 in each column of values were recorded as 1 and summed, which was the total OTUs of each sample. On the basis of the normalized otu_table, the common or unique OTU analysis was conducted with the ggplot2 package in R software, and meanwhile the OTU triangulation was drawn using the ggtern package to show the
common or unique OTUs and their abundance between the two groups. The heatmap package in R software was used to carry out the cluster analysis between samples and species. With the phylogenetic relationship and relative abundance information of each OTU in the samples, the species annotation results of a single sample was visualized by KRONA software (http://sourceforge.net/projects/krona/) (Ondov, Bergman & Phillippy, 2011). Using GraPhlAn software (http://huttenhower.sph.harvard.edu/graphlan) (Asnicar et al., 2015), a single sample OTU annotation circle graph based on GraPhlAn was obtained. Representative OTU sequences with the top 50 overall relative abundance and genus classification information were selected, and Mafft software (Katoh et al., 2002) was used to carry out a multisequence comparison. FastTree software (http://microbesonline.org/fasttree/) (Price, Dehal & Arkin, 2009) was used to build trees simultaneously combining the relative abundance of each OTU and species annotation confidence information of representative sequences, and the ggtree software package (Yu et al., 2017) was used to carry out the visual display. OTU abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. Based on the normalized data, alpha diversity and beta diversity were all performed in the following paragraphs.

**Alpha diversity analysis**

According to the normalized OTU abundance table, the alpha_diversity.py script (http://huttenhower.sph.harvard.edu/graphlan) in the QIIME software package (version 1.9.1) (Caporaso et al., 2010; Kuczynski et al., 2011) was used to calculate four diversity indices (Chao et al., 2010). According to the OTU abundance table, the alpha_rarefaction.py script (http://qiime.org/scripts/alpha_rarefaction.html) in the QIIME software package was used to calculate dilution curve data of four diversity indices, and the vegan package (Dixon, 2003) was used to draw the dilution curve. Based on the OTU abundance table, the plot_rank_abundance_graph.py script (http://qiime.org/scripts/plot_rank_abundance_graph.html) in the QIIME software package was selected to calculate the rank debt curve index. The data from the specaccum species accumulation curve analysis was calculated and mapped in line with the normalized OTU abundance table.

**Beta diversity analysis**

According to the normalized OTU abundance table, the beta diversity distance was calculated using jackknifed_beta_diversity.py script (http://qiime.org/scripts/jackknifed_beta_diversity.html) in QIIME software. Principal Coordinate Analysis (PCoA) was performed to get principal coordinates and visualize from complex data (Lozupone et al., 2011). A distance matrix of unweighted Unifrac among samples obtained before was transformed to a new set of orthogonal axes, the maximum variation factor is demonstrated by first principal coordinate, the second maximum one by the second principal coordinate, and so on. PCoA analysis was displayed by QIIME2 and ggplot2 package (Ginestet, 2011). Upgma_cluster.py script (http://qiime.org/scripts/umgma_cluster.html) in QIIME software was applied to build the cluster tree of samples using the UPGMA cluster analysis method. Based on the normalized OUT table (otu_table_subsampled), Nonmetric Multidimensional
Table 2 Characteristics of the study population.

| Variables         | Normal weight ($n = 23$) | Obesity ($n = 28$) |
|-------------------|--------------------------|-------------------|
|                   | Mean (SD)                | Mean (SD)         |
| Gender (Boys/Girls) | 11/12                    | 16/12             |
| Age (Years)       | 8.86 (1.61)              | 8.49 (1.48)       |
| BMI (kg/m$^2$)    | 15.32 (1.42)             | 24.44 (2.03)      |

Notes. Asterisks indicate statistical significance (*$P < 0.05$, Wilcoxon rank-sum test).

Scaling (NMDS) analysis was performed with the vegan package and displayed with the ggplot2 package in R software.

**LEfSe and PICRUSt analysis**

The linear discriminant analysis (LDA) effect size (LEfSe) analyses were performed on the website http://huttenhower.sph.harvard.edu/galaxy (Segata et al., 2011). For OTUs with an average abundance in all samples that was greater than 0.1%, abundances were normalized to the sum of the values per sample in 1 million and then subjected to LDA. The cut-off value was the absolute LDA score ($\log_{10}$) >2.0. Functional capacity of gut microbiota was predicted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Galaxy Version 1.0.0) (Langille et al., 2013). The closed reference OTU table was generated from quality control reads in QIIME against the Greengenes reference sequence database. Closed OTU-table drawn by QIIME was compared with KEGG (Kyoto Encyclopedia of Genes and Genomes) database to obtain functional predictions. PICRUSt predictions were categorized as levels 1-3 into KEGG pathways.

**Statistical analysis**

IBM SPSS 23.0 software were applied in all statistical analyses. Depending on the normality of the underlying data drawn from the Shapiro-Wilk test, differences between groups were examined by two-tailed $t$-test or Wilcoxon rank-sum test. Test results at an alpha of $P < 0.05$ were considered statistically significant.

**RESULTS**

**Study population**

In this study, we categorized 28 obese children into the obese group and 23 healthy children into the normal weight group to analyze the two group’s gut microbial composition. The two groups revealed no statistical difference regarding gender ratio and age (Table 2).

**Sequencing data**

A total of 2,349,074 raw sequence reads were obtained from the 51 subjects. After a series of quality filtering, 2,004,646 classifiable 16S rRNA gene sequences were obtained, and the average number of sequences for each individual was 39,307 (ranging from 25,813 to 55,847). All sequences were clustered with representative sequences, and a 97% sequence identity cut-off was used.
The Venn diagram (Fig. 1A) demonstrated the shared and exclusive communities between the groups. The total number of OTUs obtained was 1,213, among which 449 OTUs were shared by both groups, 211 genera were specific to the normal weight group and 104 genera were specific to the obese group, showing a decrease in the gut microbial diversity as the body weight increased.

**Estimation of the alpha diversity and beta diversity**

Rarefaction curves drawn on the observed species and Shannon indices (Figs. 1B and 1C) indicated that although deeper sequencing may reveal rare OTUs, the majority of microbial diversity had been captured. These curves also revealed that the alpha diversity in samples from the normal-weight group was the highest and that in obesity group was the lowest. To confirm its validity, we calculated Chao1 indices by Wilcoxon rank-sum test (Fig. 2), finding that the mean microbial abundance between two groups decreased significantly ($P = 0.006$, Wilcoxon rank-sum test). Shown in the rank-abundance curve, the species
abundance and uniformity of gut microbiota in the obese group were lower than those in the normal weight group (Fig. 1D).

The alpha diversity indices (the Chao1, observed species, PD whole tree and Shannon index) were used to describe alpha diversity (Fig. 2). Significant differences were found between the normal weight group and the obese group ($P < 0.001$; $P < 0.001$; $P < 0.001$; and $P = 0.008$, respectively, Wilcoxon rank-sum test), showing a higher abundance and diversity in the normal weight group than in the obese group. In addition to the alpha diversity evaluation, the unweighted UniFrac analysis was applied to compare similarities among gut microbial communities (beta diversity). Looking into the bacterial composition profiles, we observed significant differences between the normal weight group and the obese group ($R^2 = 0.054$, $P = 0.001$, adonis analysis). NMDS and PCoA based on the abundance
Figure 3  PCoA and NMDS based on the abundance of OTUs. (A) PCoA and (B) NMDS plots comparing sample distribution between the two groups. Points clustered at the left and the right left represent the gut microbial composition of the obese group and the normal weight group, respectively. The closer the spatial distance of the sample, the more similar the species composition of the sample. (N: normal weight group; F: obese group).

of OTUs revealed differences in the microbial composition (Fig. 3). Specifically, an evident clustering was identified for subjects of normal weight and obesity. The observation revealed significant differences in gut microbial community structure between the normal weight group and the obese group, with two principal component scores accounted respectively for 11.59% and 5.57% of the total variations. Moreover, separation between the two groups was particularly obvious. Representing the intestinal microbial composition, data points for subjects of normal weight clustered at the right and obese ones at the left.

The relative abundance of fecal bacterial communities
Statistics of the OTUs suggested the relative abundance of the bacteria at the categorization of phylum, class, order, family and genus. The results showed that fecal bacterial composition differed between the two groups.

Bacteroidetes was the most predominant phylum, contributing 55.48% and 51.35% of the gut microbiota in the normal weight group and the obese group, respectively, followed by Firmicutes, contributing 37.93% and 36.18%, respectively (Figs. 4A and 4B). Proteobacteria, Fusobacteria, Verrucomicrobia and Actinobacteria constituted the next most dominant phyla. Microbial compositions showed high interindividual variability, among which Bacteroidetes accounted for 21.01-73.78%, and Firmicutes accounted for 18.72-59.49% among all individuals. In regard to phyla, proportion of Bacteroidetes (51.35%) in the obese group was lower than that in the normal weight group (55.48%) ($P = 0.030$, Wilcoxon rank-sum test). No statistical differences were revealed in Firmicutes
Figure 4  Histogram of the community composition of gut microbiota at the phylum level. (A) The ordinate represents the sample, and the abscissa represents the relative abundance. (B) The abscissa represents the group, and the ordinate represents the relative abundance. The figures show species with a relative abundance of 1% or more. All species with a relative abundance of less than 1% and classified as “unclassified” and “unidentified” were classified as “Others”. (N: normal weight group; F: obese group).

Full-size DOI: 10.7717/peerj.8317/fig-4

(P = 0.436, Wilcoxon rank-sum test) and the Firmicutes/Bacteroidetes (P = 0.983, Wilcoxon rank-sum test) between the two groups.

Analysis of the relative abundance of bacterial taxonomic groups showed that the fecal microbiota was dominated by fourteen genera: Bacteroides (mean relative abundance, N, 41.58%; F, 40.06%) (In the following analysis, ”N” is short for “Normal weight” and “F” for “Fat”, namely “Obesity”), Faecalibacterium (N, 7.03%; F, 10.76%), Prevotella (N, 4.72%; F, 6.19%), Oscillospora (N, 4.50%; F, 1.61%), Dialister (N, 4.03%; F, 0.91%), Parabacteroides (N, 4.01%; F, 2.39%), Sutterella (N, 2.68%; F, 5.60%), Roseburia (N, 1.94%; F, 2.32%), Ruminococcus (N, 1.91%; F, 1.13%), Phascolarctobacterium (N, 1.64%; F, 3.43%), Lachnospira (N, 1.55%; F, 3.23%), Escherichia (N, 1.39%; F, 1.32%), Megamonas (N, 0.54%; F, 1.79%), Haemophilus (N, 0.32%; F, 1.96%). Faecalibacterium, Phascolarctobacterium, Lachnospira, Megamonas, and Haemophilus in the obese group were significantly more abundant than those in the normal weight group (P = 0.048, P = 0.018, P < 0.001, P = 0.040 and P = 0.003, respectively, Wilcoxon rank-sum test). As for Oscillospora and Dialister, they took lower proportions in the obese group when compared to the normal weight group (P = 0.002, two-tailed t-test; and P = 0.002, Wilcoxon rank-sum test). For the remaining genera, the gut microbiome sequencing data did not indicate significant differences in the abundance for the obese group compared to the normal weight group.

Phylogenetic and taxonomic profiles of gut microbiota and PICRUSt analysis

To analyze the statistical differences in microbial communities between the normal weight group and the obese group, we compared OTUs with the LEfSe analysis. The histogram reflected the LDA scores that were computed for the features at the OTU level (Fig. 5A). Cladograms of the taxa with LDA values >2.0 were depicted in Fig. 5B.
Figure 5  Different structures of gut microbiota in the two groups. The bar graph and cladogram indicate the taxa that discriminate among the two groups, based on the LEfSe method and linear discriminant analysis (LDA) effect size method. (A) The statistical test was performed using the LDA effect size method. Only taxa with an alpha value of 0.05 and with absolute LDA (log10) scores > 2.0 were considered significant. (B) Cladogram depicting the phylogenetic distribution of microbial lineages associated with the two groups. Each small circle at a different classification level represents a classification at that level, and the diameter of the small circle is proportional to the relative abundance (the levels represent, from the inner to outer rings, genus, family, order, class and phylum). (N: normal weight group; F: obese group).

Upon analyzing the fecal samples, a total of 55 species of bacteria were abundant in the normal weight group and 45 species were abundant in the obese group. As shown in Fig. 5A, the relative abundance of taxonomic groups showing an LDA score greater than $10^4$ was summed for the normal weight group (*Oscillospira* and *Bacteroides uniformis*) and the obese group (*Proteobacteria*, *Prevotella copri*, *Alcaligenacea*, *Sutterella*, Betaproteobacteria, and Burkholderiales). Multiple genera were found in significantly high abundances in the normal weight group. These included *Oscillospira*, *Ruminococcus*, *Prevotella*, *Adlercreutzia*, *Sporobacter*, *Bifidobacterium*, *Clostridium*, *Desulfovibrio*, *Bilophila*, *Christensenella*, *Alistipes*, *Anaerotruncus*, *Eubacterium*, *Holdemania*, *Oxalobacter*, *Defluviitalea*, and *Collinsella*.

The genera that were enriched in the obese group were *Turicibacter*, *Campylobacter*, *Actinobacillus*, *Aggregatibacter*, SMB53, *Rothia*, *Granulicatella*, *Streptococcus*, *Veillonella*, *Megamonas*, *Fusobacterium*, *Phascolarctobacterium*, *Haemophilus*, and *Lachnospira*, and *Sutterella*.

Cladograms (Fig. 5B) were generated from the LEfSe analysis, which showed the most differentially abundant taxa enriched in microbiota with green for the normal weight group and red for the obese group. The diameter of each circle is proportional to its abundance. The obese group showed a significant decrease in the phylum Actinobacteria, phylum Tenericutes, class Deltaproteobacteria, class Erysipelotrichia and some taxonomic groups.
belonging to the order Bacteroidales, such as family Paraprevotellaceae, Barnesiellaceae, S24-7, and Rikenellaceae and a greater abundance of the phylum Proteobacteria, phylum Fusobacteria and class Bacilli when compared with the normal weight group (Fig. 5B).

To study the changes of gut microbial function in obese children, we adopted PICRUSt. Based on KEGG database, PICRUSt revealed a total of six biological metabolism pathways at Level 1 pathways: metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases. Among them, metabolism, genetic information processing and environmental information processing dominated, accounting for 46.89%-51.41%, 18.33%-22.12% and 10.22%-14.87%, respectively. These six pathways in the obese group were lower than those in the normal weight group. Meanwhile, the secondary function of the predicted gene was analyzed, finding it consisted of 39 sub-functions including membrane transport, carbohydrate metabolism, amino acid metabolism, replication and repair, energy metabolism, translation, cellular processes and signaling. Within the 39 predicted functional categories at KEGG pathway hierarchy level 2, except immune system diseases, neurodegenerative diseases and signal transduction, the remaining 36 sub-functions within the predicted gene all decreased in obese children.

DISCUSSION

Gut microbiota plays is essential to regulate energy metabolism and fat storage and is closely associated with the occurrence and development of obesity (MetaHIT Consortium, 2011; Collins et al., 2015; Musso, Gambino & Cassader, 2010; Walters, Xu & Knight, 2014). Studies have confirmed that the composition of gut microbiota changes and the microbial diversity decreases in obese people and obese rats (Petriz et al., 2014). In the present study we found alterations in gut microbiota composition in the obese and normal-weight Chinese children. Rarefaction curves and rank-abundance curve suggested the abundance and diversity of gut microbiota in obese children were lower than those in normal weight children and. Alpha diversity metrics generated a more accurate verification. Alpha diversity is an index reflecting the variety of microbial species in stool samples. A higher alpha diversity indicates higher abundance in one sample (Liu et al., 2017). In this study, the alpha diversity indices, including Chao1, observed species, PD whole tree and Shannon index, revealed that the gut microbial composition of children in different BMI categories was statistically significant. The abundance and diversity of gut microbiota in obese children were significantly lower than those in normal weight children, which is consistent with existing research (Le Chatelier et al., 2013; Gao et al., 2017; Menni et al., 2017; Scheithauer et al., 2016). Such differences are attributed to the excessive intake of high fat foods by obese children (Tilg & Moschen, 2014). A high-fat diet can reduce the expression of the intestinal epithelial tight junction proteins occludin and ZO-1, affect the integrity of the intestinal epithelium, cause changes in intestinal permeability and increase the level of circulating lipopolysaccharide, which contribute to increase the incidence of obesity (Cani et al., 2008). Beta diversity is an index reflecting the heterogeneity of gut microbiota between samples in each group. A higher beta diversity is indicative of larger compositional
differences in the gut microbiota between samples in a certain group (Liu et al., 2017). We used unweighted UniFrac analysis to compare the similarity and heterogeneity among gut microbial communities and identified an apparent clustering pattern in the normal weight group and the obese group. The normal weight group and the obese group were distinct from each other in terms of gut microbiota composition, it showed that the structure of gut microbiota changed significantly with body weight, and these changes may be associated with the occurrence and development of obesity.

In the gut of healthy humans, intestinal microorganisms can be divided into six phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia (Eckburg et al., 2005; Lozupone et al., 2012), among which Firmicutes and Bacteroidetes accounted for more than 90% (Mariat et al., 2009). We also found that the top 6 phyla of gut microbiota in children were Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Verrucomicrobia and Actinobacteria, among which Firmicutes and Bacteroidetes accounted for approximately 90%. Research regarding the changes in Bacteroidetes and Firmicutes in gut microbiota is not settled. Some studies found that Bacteroidetes in obese children decreased while Firmicutes increased (Rahat-Rozenbloom et al., 2014; Turnbaugh et al., 2008; Patrone et al., 2016), while others revealed that the number of both Bacteroidetes and Firmicutes in obese children increased (Ismail et al., 2011). This study found that compared with the normal weight group, the number of Bacteroidetes in the obese group was significantly reduced, which was consistent with the results of Ley et al. (Krajmalnik-Brown et al., 2012; Ley et al., 2006; Turnbaugh et al., 2008). In the healthy gut, Bacteroidetes plays an essential role in degraded plant polysaccharides that cannot be absorbed in the human body and participating in the nutrition metabolism of the human body together with other bacteria. A long-term high-fat diet will reduce the number of Bacteroidetes, affect the absorption of polysaccharides and proteins, and hence result in obesity (Murphy et al., 2010). In contrast, this study found that the number of Firmicutes did not change significantly. Finucane et al. also found no difference between obese versus lean individuals in their relative abundance of Firmicutes (Finucane et al., 2014). This can be explained by the differences in ethnic groups, diet and lifestyle of the subjects (De Filippo et al., 2010; Zhang et al., 2013; Matsuyama et al., 2019; Wu et al., 2011; Bai, Hu & Briner, 2019). Additionally, the ratio of Firmicutes to Bacteroidetes (F/B) is often regarded as a marker of obesity in related studies; especially the F/B value in obese animals is higher than normal weight, but this result does not apply to the study of the human body (Finucane et al., 2014; Ley et al., 2005). This study found that the F/B value was significantly different among different individuals, indicating that not all obese individuals had significantly increased F/B values, and the F/B ratio between the two groups had no statistical difference, which confirmed other relevant reports (Karlsson et al., 2012; Schwiertz et al., 2010). However, studies on the gut microbiota of obese children in the Antwerp region of Belgium and the Kazak region of Xinjiang found that the F/B ratio increased (Bervoets et al., 2013; Xu et al., 2012). To sum up, currently, there are ambiguities in the relationship between the changes in the F/B ratio and the incidence of obesity. Research conclusions on this aspect are not consistent all over the world, mainly because the effect of gut microbiota on obesity is far more complicated than the imbalance or
interaction of two microbial phyla. Therefore, whether it is appropriate to simply use the F/B ratio as a marker of obesity remains to be discussed. More detailed research is needed to evaluate the relationship between the F/B ratio and obesity.

To investigate the relationship between gut microbiome functions and obesity, we predicted the potential metagenomes with PICRUSt. The inferred gene families were annotated and combined with level 1 to level 3 pathways. After analyzing the predicted gene copy number of gut microbiota in different groups, we found that the relative abundance of gut microbiota in obese children changed, and their functions also changed correspondingly. The overall trend of predicted gene copy number in the Level 1 to Level 3 was lower than that in the normal weight group. Unfortunately, these changes were not statistically significant. A larger sample size will help to carry out in-depth research on this aspect.

To further study the difference in gut microbiota in children with different body weights, LEfSe analysis based on the OUT level was conducted to screen key biomarker species. At the genus level, significant differences existed in the composition of some microorganisms between the normal weight group and the obese group. LDA difference analysis of intestinal microflora in the two groups was conducted to compare strains with LDA scores greater than 2. The results showed that the genera *Oscillospira*, *Ruminococcus*, *Prevotella*, *Adlercreutzia*, *Sporobacter*, *Bifidobacterium*, *Clostridium*, *Desulfovibrio*, *Bilophila*, *Christensenella*, *Alistipes*, *Anaerotruncus*, *Eubacterium*, *Holdemania*, *Oxalobacter*, *Defluviitalea*, and *Collinsella* were significantly higher in the gut microbiome of the normal weight group. The genera *Turicibacter*, *Campylobacter*, *Actinobacillus*, *Aggregatibacter*, *SMB53*, *Rothia*, *Granulicatella*, *Streptococcus*, *Veillonella*, *Megamonas*, *Fusobacterium*, *Phascolarctobacterium*, *Haemophilus*, *Lachnospira*, and *Sutterella* were enriched in the obese group. A recent study from Canada discovered that the abundance of *Oscillospira*, which is closely related to the decline of childhood obesity, increased in the infant’s gut microbiota three months after birth or when the expectant mother was in contact with pet animals (Tun et al., 2017). Sclerenchyma contains a large number of bacteria with fermentation functions, such as *Ruminococcus*, which can decompose food fibers that cannot be digested by the human body into absorbable short-chain fatty acids (acetic acid, propionic acid, butyric acid, lactic acid, etc.) and increase energy intake through intestinal absorption (Turnbaugh et al., 2006). Moreover, the short-chain fatty acids produced by fermentation can also act on the G protein-coupled receptors 41 and 43 to promote intestinal endocrine cells (L cells and I cells) to synthesize and secrete peptide YY, PYY and glucagon-like peptide-1 (GLP-1), slow intestinal peristalsis and promote sugar-induced insulin secretion, eventually leading to energy concentration and fat accumulation (Duca, Sakar & Covasa, 2013). Cani et al. (2007) showed that a high-fat diet led to a decrease in the number of *Eubacterium*, *Clostridium* and *Bifidobacterium* and simultaneously caused inflammation, obesity and insulin resistance. Hao et al. analyzed the bacterial content in feces of obese people in China. The results showed that compared with normal weight people, the number of *Bifidobacterium* in obese people had a decreasing trend (Zuo et al., 2011). Recent studies have reached the same conclusion (Gao et al., 2017). Compared with the control group, Wang et al. found that in a high-fat diet group, the weights of SD rats increased with the
numbers of *Lactobacillus* and *Bifidobacterium* in the intestinal tract dropped significantly, while the numbers of *Bacteroides* and *Clostridium* showed an upward trend, especially *Clostridium* (Wang et al., 2013; Bao et al., 2012). *Clostridium* can upregulate the expression of glucose transporter 2 in the jejunal mucosa and the expression of lipoyltransferase in the ileal mucosa, which can lead to an increase in the absorption of glucose and fats (Woting et al., 2014). Some studies also showed that *Clostridium* was negatively correlated with fasting blood glucose, HbA1c and insulin levels (Karlsson et al., 2013b). Gao et al. documented that the abundance of *Fusobacterium* was significantly higher in obese people (Gao et al., 2017). A study in 2014 showed that compared with plant foods (e.g., fruits and vegetables), high-fat animal foods (e.g., meat, eggs and milk) had a greater impact on gut microbiota. In the intestinal tracts of people that eat animal foods, bacteria that were resistant to cholate, such as *Bilophila*, grew significantly (David et al., 2014). *Sutterella* was significantly high in the obese group in a study of the relationship of gut microbiota and diarrhea, autism and eczema (Finegold, 2011; Lv et al., 2017). Some studies have noted that *Streptococcus* is related to Crohn’s disease (CD), and *Streptococcus* is of great importance in the inflammatory mucosal region of CD patients (Fyderek et al., 2009). *Veillonella* was able to decompose glucose and lactic acid into short-chain fatty acids (Burger-van Paassen et al., 2009). However, these short-chain fatty acids cannot synthesize mucoprotein but can lead to the increased permeability of intestinal mucosa and facilitate the formation of inflammation (Brown et al., 2011). Some researchers indicted that the species of *Lachnospira* might be associated with type 2 diabetes (Kameyama & Itoii, 2014). However, there are few reports on the relationship between *Bilophila*, *Streptococcus*, *Veillonella*, *Lachnospira* and obesity, which warrants further research. In brief, our findings provide further support for the fact that the increase and decrease of these key biomarker species are closely associated with obesity. Our findings may also suggest potentially hazardous microorganisms. In the future, studies carried on larger sample size will be conducive to illustrate the function of gut microbiome composition in influencing the BMI.

Several limitations in our study should be considered. It was conducted in cross-section and could not provide evidence of a causal effect between the gut microbiota and obesity. With the small sample sizes, we cannot rule out the possibility of chances in our findings. Thus, validation on a larger sample size is needed. High-throughput sequencing technology requires high quality samples and the DNA concentration of whole genome in some sample bacteria cannot reach amplification requirements. Subjects selected for this study only represent the gut microbial composition of children in one specific region. In spite of the inclusion and exclusion criteria, the results were still influenced by the factors of subjects themselves, the individual differences among the subjects were apparent. Gut microbial samples in this study were all from one certain point in time, but it is currently considered that a long-term observation may be more valuable to study the dynamically changing gut microbiota.

**CONCLUSIONS**

The gut microbiota composition of obese children is quite different from that of normal weight people, and intestinal dysbiosis has a close relationship with the occurrence and
development of obesity. However, there are still many inconsistencies at present, which requires a large number of studies to verify the causality between specific intestinal bacterial species and obesity, and the relevant mechanism needs to be explored more deeply and accurately. On the other hand, as far as existing studies are concerned, an overwhelming majority of experimental results are obtained from animal experiments, but there are few studies on the mechanism and flora in the human body. Both gut microbiota and obesity are affected by a variety of factors. It will be of far-reaching significance to expound on their acting mechanism and incorporate multiple variables into long-term clinical studies in a reasonable way. Upon diagnosis, gut microbiota is a therapeutic target of obesity. The regulation of the composition of gut microbiota or the regulation of the production of gut microbiota metabolites, including the transplant of host bacteria, the use of antibiotics, biological bacterial agents, food and drug treatment, will bring new ideas to obesity treatment, but we still need a large number of medical studies to support them.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**
This work was supported by the National Natural Science Foundation of China (No. 81472940). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**
The following grant information was disclosed by the authors:
National Natural Science Foundation of China: 81472940.

**Competing Interests**
The authors declare there are no competing interests.

**Author Contributions**
- Xiaowei Chen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Haixiang Sun performed the experiments, analyzed the data, prepared figures and/or tables, approved the final draft.
- Fei Jiang and Yan Shen performed the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Xin Li and Xueju Hu performed the experiments, prepared figures and/or tables, approved the final draft.
- Xiaobing Shen and Pingmin Wei conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

**Human Ethics**
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):
The study was approved by the ethics committee of Zhongda Hospital, Southeast University (2017ZDSYLL109-P01).

Data Availability
The following information was supplied regarding data availability: The raw data is available in the Supplementary Files.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8317#supplemental-information.

REFERENCES

Asnicar F, Weingart G, Tickle TL, Huttenhower C, Segata N. 2015. Compact graphical representation of phylogenetic data and metadata with GraPhlAn. PeerJ 3:e1029 DOI 10.7717/peerj.1029.

Bai J, Hu Y, Briner DW. 2019. Composition of gut microbiota and its association with body mass index and lifestyle factors in a cohort of 7–18 years old children from the American Gut Project. Pediatric Obesity 14:e12480 DOI 10.1111/ijpo.12480.

Bao Y, Wang ZL, Zhang Y, Zhang JC, Wang LF, Dong XM, Su F, Yao GQ, Wang SQ, Zhang HP. 2012. Effect of Lactobacillus plantarum P-8 on lipid metabolism in hyperlipidemic rat model. European Journal of Lipid Science and Technology 114:1230–1236 DOI 10.1002/ejlt.201100393.

Bervoets L, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C, Goossens H, Desager KN, Vankerckhoven V. 2013. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. Gut Pathogens 5(1):10 DOI 10.1186/1757-4749-5-10.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120 DOI 10.1093/bioinformatics/btu170.

Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, Casella G, Drew JC, Ilonen J, Knip M, Hyoty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW. 2011. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLOS ONE 6:e25792 DOI 10.1371/journal.pone.0025792.

Burger-van Paassen N, Vincent A, Puiman PJ, Van der Sluis M, Bouma J, Boehm G, Van Goudoever JB, Van Seuningen I, Renes IB. 2009. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. Biochemical Journal 420:211–219 DOI 10.1042/BJ20082222.

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761–1772 DOI 10.2337/db06-1491.
Cani PD, Bibiloni R, Knauf C, Neyrinck AM, Neyrinck AM, Delzenne NM, Burcelin R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57:1470–1481 DOI 10.2337/db07-1403.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JJ, Hutten JG, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pitter M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336 DOI 10.1038/nmeth.f.303.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America 108:4516–4522 DOI 10.1073/pnas.1000080107.

Chao A, Chazdon RL, Colwell RK, Shen TJ. 2010. Abundance-based similarity indices and their estimation when there are unseen species in samples. Biometrics 62:361–371.

Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, Mccarrell DM, Bandela AM, Cardenas E, Garrity GM, Tiedje JM. 2007. The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acids Research 35:D169–D172 DOI 10.1093/nar/gkl889.

Collins KH, Paul HA, Reimer RA, Seerattan RA, Hart DA, Herzog W. 2015. Relationship between inflammation, the gut microbiota, and metabolic osteoarthritis development: studies in a rat model. Osteoarthritis and Cartilage 23:1989–1998 DOI 10.1016/j.joca.2015.03.014.

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559–563 DOI 10.1038/nature12820.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America 107:14691–14696 DOI 10.1073/pnas.1005963107.

Dietrich P, Hellerbrand C. 2014. Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. Best Practice & Research Clinical Gastroenterology 28:637–653 DOI 10.1016/j.bpg.2014.07.008.

Dixon P. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 14:927–930 DOI 10.1111/j.1654-1103.2003.tb02228.x.

Duca FA, Sakar Y, Covasa M. 2013. The modulatory role of high fat feeding on gastrointestinal signals in obesity. Journal of Nutritional Biochemistry 24:1663–1677 DOI 10.1016/j.jnutbio.2013.05.005.
Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. 2005. Diversity of the human intestinal microbial flora. Science 308:1635–1638 DOI 10.1126/science.1110591.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461 DOI 10.1093/bioinformatics/btq461.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200 DOI 10.1093/bioinformatics/btr381.

Farni K, Shoham DA, Cao GC, Luke AH, Layden J, Cooper RS, Dugas LR. 2014. Physical activity and pre-diabetes—an unacknowledged mid-life crisis: findings from NHANES 2003–2006. PeerJ 2:e499 DOI 10.7717/peerj.499.

Finegold SM. 2011. State of the art; microbiology in health and disease. Intestinal bacterial flora in autism. Anaerobe 17:367–368 DOI 10.1016/j.anaerobe.2011.03.007.

Finucane MM, Sharpton TJ, Laurent TJ, Pollard KS. 2014. A taxonomic signature of obesity in the microbiome? Getting to the guts of the matter. PLOS ONE 9:e84689 DOI 10.1371/journal.pone.0084689.

Fyderek K, Strus M, Kowalska-Duplaga K, Gosiewski T, Wedrychowicz A, Jedynak-Wasowicz U, Sladek M, Pieczarkowski S, Adamski P, Kochan P, Heczko PB. 2009. Mucosal bacterial microflora and mucus layer thickness in adolescents with inflammatory bowel disease. World Journal of Gastroenterology 15:5287–5294 DOI 10.3748/wjg.15.5287.

Gao RY, Zhu CL, Li H, Yin MM, Pan C, Huang LS, Kong C, Wang XC, Zhang Y, Qu S. 2017. Dysbiosis signatures of gut microbiota along the sequence from healthy, young patients to those with overweight and obesity. Obesity 26:351–361.

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. 2006. Metagenomic analysis of the human distal gut microbiome. Science 312:1355–1359 DOI 10.1126/science.1124234.

Ginestet C. 2011. ggplot2: elegant graphics for data analysis. Journal of the Royal Statistical Society Series A-Statistics in Society 174:245 DOI 10.1111/j.1467-985X.2010.00676_9.x.

Global Burden of Metabolic Risk Factors for Chronic Disease Collaboration. 2014. Metabolic mediators of the effects of body-mass index, overweight, and obesity on coronary heart disease and stroke: a pooled analysis of 97 prospective cohorts with 1.8 million participants. The Lancet 383:970–983 DOI 10.1016/S0140-6736(13)61836-X.

Guyton K, Alverdy JC. 2017. The gut microbiota and gastrointestinal surgery. Nature Reviews Gastroenterology & Hepatology 14:43–54 DOI 10.1038/nrgastro.2016.139.

Han JC, Lawlor DA, Kimm SYS. 2010. Childhood obesity. Lancet 375:1737–1748 DOI 10.1016/S0140-6736(10)60171-7.

Hollister EB, Riegle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, Raza S, Doddapaneni HV, Metcalf GA, Muzny DM, Gibbs RA, Petrosino JF, Shulman RJ, Versalovic J. 2015. Structure and function of the healthy pre-adolescent pediatric gut microbiome. Microbiome 3:36 DOI 10.1186/s40168-015-0101-x.
Ismail NA, Ragab SH, Elbaky AAbd, Shoeib ARS, Alhosary Y, Fekry D. 2011. Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Archives of Medical Science* 7:501–507.

Kameyama K, Itoii K. 2014. Intestinal colonization by a Lachnospiraceae bacterium contributes to the development of diabetes in obese mice. *Microbes and Environments* 29:427–430 DOI 10.1264/jsme2.ME14054.

Karlsson CLJ, Onnerfalt J, Xu J, Molin G, Ahrne S, Thorngren-Jerneck K. 2012. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* 20:2257–2261 DOI 10.1038/oby.2012.110.

Karlsson FH, Nookaew I, Nielsen J. 2014. Metagenomic data utilization and analysis (MEDUSA) and construction of a global gut microbial gene catalogue. *PLOS Computational Biology* 10:e1003706 DOI 10.1371/journal.pcbi.1003706.

Karlsson F, Tremaroli V, Nielsen J, Backhed F. 2013a. Assessing the human gut microbiota in metabolic diseases. *Diabetes* 62:3341–3349 DOI 10.2337/db13-0844.

Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B, Nielsen J, Backhed F. 2013b. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 498:99–103 DOI 10.1038/nature12198.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30:3059–3066 DOI 10.1093/nar/gkf436.

Kontis V, Mathers CD, Rehm J, Stevens GA, Shield KD, Bonita R, Riley LM, Poznyak V, Beaglehole R, Ezzati M. 2014. Contribution of six risk factors to achieving the 25×25 non-communicable disease mortality reduction target: a modelling study. *Lancet* 384:427–437 DOI 10.1016/S0140-6736(14)60616-4.

Krajmalnik-Brown R, Ilhan ZE, Kang DW, DiBaise JK. 2012. Effects of gut microbes on nutrient absorption and energy regulation. *Nutrition in Clinical Practice* 27:201–214 DOI 10.1177/0884533611436116.

Kuczynski J, Stombaugh J, Walters WA, Gonzalez A, Caporaso JG, Knight R. 2011. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. *Current Protocols in Bioinformatics* Chapter 10:Unit10.7.

Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31:814–821 DOI 10.1038/nbt.2676.

Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N, Peterson DA, Stappenbeck TS, Hsieh CS. 2011. Peripheral education of the immune system by colonic commensal microbiota. *Nature* 478:250–U142 DOI 10.1038/nature10434.

Le Chatelier E, Nielsen T, Qin JJ, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li JH, Burgdorf K, Grarup N, Journiensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal E, Brunak S, Clement K, Dore J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, De Vos WM, Zunker JD, Raes J, Hansen T, Bork P, Wang J, Ehrlich SD, Pedersen O. 2013.
Richness of human gut microbiome correlates with metabolic markers. *Nature* 500:541–546 DOI 10.1038/nature12506.

Leung C, Rivera L, Furness JB, Angus PW. 2016. The role of the gut microbiota in NAFLD. *Nature Reviews Gastroenterology & Hepatology* 13:412–425 DOI 10.1038/nrgastro.2016.85.

Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. 2005. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America* 102:11070–11075 DOI 10.1073/pnas.0504978102.

Ley RE, Turnbaugh PJ, Klein S, Gordon JI. 2006. Microbial ecology: human gut microbes associated with obesity. *Nature* 444:1022–1023 DOI 10.1038/nrgastro.2016.85.

Li H, Zong X, Cheng Y, Mi J. 2010. Body mass index cut-offs for overweight and obesity in Chinese children and adolescents aged 2-18 years. *Zhonghua liuxingbingxue zazhi* 31:616–620.

Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, Shi J, Zhao S, Liu W, Wang X, Xia H, Liu Z, Cui B, Liang P, Xi L, Jin J, Ying X, Wang X, Zhao X, Li W, Jia H, Lan Z, Li F, Wang R, Sun Y, Yang M, Shen Y, Jie Z, Li J, Chen X, Zhong H, Xie H, Zhang Y, Gu W, Deng X, Shen B, Xu X, Yang H, Xu G, Bi Y, Lai S, Wang J, Qi L, Madsen I, Wang J, Ning G, Kristiansen K, Wang W. 2017. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nature Medicine* 23:859–868 DOI 10.1038/nm.4358.

Lozupone C, Lladser ME, Dan K, Stombaugh J, Knight RJJ. 2011. UniFrac: an effective distance metric for microbial community comparison. *ISME Journal* 5:169–172 DOI 10.1038/ismej.2010.133.

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability and resilience of the human gut microbiota. *Nature* 489:220–230 DOI 10.1038/nature11550.

Luke A, Bovet P, Plange-Rhule J, Forrester TE, Lambert EV, Schoeller DA, Dugas LR, Durazo-Arvizu RA, Shoham DA, Cao G, Brage S, Ekelund U, Cooper RS. 2014. A mixed ecologic-cohort comparison of physical activity & weight among young adults from five populations of African origin. *BMC Public Health* 14:397 DOI 10.1186/1471-2458-14-397.

Lv WJ, Liu C, Ye CX, Sun JQ, Tan XW, Zhang C, Qu Q, Shi DY, Guo SN. 2017. Structural modulation of gut microbiota during alleviation of antibiotic-associated diarrhea with herbal formula. *International Journal of Biological Macromolecules* 105:1622–1629 DOI 10.1016/j.ijbiomac.2017.02.060.

Lynch SV, Pedersen O. 2016. The human intestinal microbiome in health and disease. *New England Journal of Medicine* 375:2369–2379 DOI 10.1056/NEJMra1600266.

Magoc T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27:2957–2963 DOI 10.1093/bioinformatics/btr507.

Mariat D, Firmesse O, Levenez F, Guimaraes VD, Sokol H, More J, Corthier G, Furet JP. 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiology* 9:123 DOI 10.1186/1471-2180-9-123.
Matsuyama M, Morrison M, Cao KL, Pruilh S, Davies PSW, Wall C, Lovell A, Hill RJ. 2019. Dietary intake influences gut microbiota development of healthy Australian children from the age of one to two years. *Scientific Reports* **9**:12476 DOI 10.1038/s41598-019-48658-4.

Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. 2017. Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. *International Journal of Obesity* **41**:1099–1105 DOI 10.1038/ijo.2017.66.

MetaHIT Consortium. 2011. Enterotypes of the human gut microbiome. *Nature* **473**:174–180 DOI 10.1038/nature09944.

Murphy EF, Cotter PD, Healy S, Marques TM, O’Sullivan O, Fouhy F, Clarke SF, O’Toole PW, Quigley EM, Stanton C, Ross PR, O’Doherty RM, Shanahan F. 2010. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut* **59**:1635–1642 DOI 10.1136/gut.2010.215665.

Musso G, Gambino R, Cassader M. 2010. Obesity, diabetes, and gut microbiota the hygiene hypothesis expanded? *Diabetes Care* **33**:2277–2284 DOI 10.2337/dc10-0556.

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. 2012. Host-gut microbiota metabolic interactions. *Science* **336**:1262–1267 DOI 10.1126/science.1223813.

Ondov BD, Bergman NH, Phillippy AM. 2011. Interactive metagenomic visualization in a web browser. *BMC Bioinformatics* **12**:385 DOI 10.1186/1471-2105-12-385.

Patrone V, Vajana E, Minuti A, Callegari ML, Federico A, Loguercio C, Dallio M, Tolone S, Docimo L, Morelli L. 2016. Postoperative changes in fecal bacterial communities and fermentation products in obese patients undergoing biliointestinal bypass. *Frontiers in Microbiology* **7**:200.

Petriz BA, Castro AP, Almeida JA, Gomes CP, Fernandes GR, Kruger RH, Pereira RW, Franco OL. 2014. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMCGenomics* **15**:511 DOI 10.1186/1471-2164-15-511.

Pettitt DJ, Talton J, Dabelea D, Divers J, Imperatore G, Lawrence JM, Liese AD, Linder B, Mayer-Davis EJ, Pihoker C. 2014. Prevalence of diabetes in US Youth in 2009: the SEARCH for diabetes in youth study. *Diabetes Care* **37**:402–408 DOI 10.2337/dc13-1838.

Popkin BM, Adair LS, Ng SW. 2012. Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition Reviews* **70**:3–21 DOI 10.1111/j.1753-4887.2011.00456.x.

Price MN, Dehal PS, Arkin AP. 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution* **26**:1641–1650 DOI 10.1093/molbev/msp077.

Prospective Studies Collaboration. 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* **373**:1083–1096 DOI 10.1016/S0140-6736(09)60318-4.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng
H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Meta HITC, Bork P, Ehrlich SD, Wang J. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65 DOI 10.1038/nature08821.

R Core Team. 2013. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rahat-Rozenbloom S, Fernandes J, Gloor GB, Wolever TMS. 2014. Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. International Journal of Obesity 38:1525–1531 DOI 10.1038/ijo.2014.46.

Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlf Bauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Treuren WVAn, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. 2013. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341:1079–U49.

Sandhu KV, Sherwin E, Schellekens H, Stanton C, Dinan TG, Cryan JF. 2017. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. Translational Research 179:223–244 DOI 10.1016/j.trsl.2016.10.002.

Saydah S, Bullard KM, Cheng YL, Ali MK, Gregg EW, Geiss L, Imperatore G. 2014. Trends in cardiovascular disease risk factors by obesity level in adults in the United States, NHANES 1999–2010. Obesity 22:1888–1895 DOI 10.1002/oby.20761.

Scheithauer TP, Dallinga-Thie GM, De Vos WM, Nieuwdorp M, Van Raalte DH. 2016. Causality of small and large intestinal microbiota in weight regulation and insulin resistance. Molecular Metabolism 5:759–770 DOI 10.1016/j.molmet.2016.06.002.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75:7537–7541 DOI 10.1128/AEM.01541-09.

Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD. 2010. Microbiota and SCFA in lean and overweight healthy subjects. Obesity 18:190–195 DOI 10.1038/oby.2009.167.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. Genome Biology 12(6):R60 DOI 10.1186/gb-2011-12-6-r60.

Sonnenburg ED, Sonnenburg JL. 2014. Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. Cell Metabolism 20:779–786 DOI 10.1016/j.cmet.2014.07.003.

Tang WW, Hazen SL. 2016. Dietary metabolism, gut microbiota and acute heart failure. Heart 102:813–814 DOI 10.1136/heartjnl-2016-309268.
Chen et al. (2020), PeerJ, DOI 10.7717/peerj.8317
Zhang J, Zheng Y, Guo Z, Qiao J, Gesudu Q, Sun Z, Huo D, Huang W, Huo Q, Kwok L, Zhang H. 2013. The diversity of intestinal microbiota of Mongolians living in Inner Mongolia, China. *Benef Microbes* 4:319–328 DOI 10.3920/BM2013.0028.

Zuo HJ, Xie ZM, Zhang WW, Li YR, Wang W, Ding XB, Pei XF. 2011. Gut bacteria alteration in obese people and its relationship with gene polymorphism. *World Journal of Gastroenterology* 17:1076–1081 DOI 10.3748/wjg.v17.i8.1076.