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Original article

Causal relationship between gut microbiota and childhood obesity: A Mendelian randomization study and case–control study

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SUMMARY

Background: Gut microbiota and obesity are deeply interconnected. However, the causality in the relationship between these factors remains unclear. Therefore, this study aimed to elucidate the genetic relationship between gut microbiota and childhood obesity.
Methods: Genetic summary statistics for the gut microbiota were obtained from the MiBioGen consortium. Genome-wide association studies (GWAS) summary data for childhood obesity were obtained from North American, Australian, and European collaborative genome-wide meta-analyses. Mendelian randomization (MR) analyses were performed using the inverse variance weighting method. 16 children with obesity and 16 without obesity were included for clinical observation, and their weight, body mass index, blood lipid levels, and gut microbiology were assessed. Paired t-test was the primary method of data analysis, and statistical significance was set at $P < 0.05$.
Results: MR identified 16 causal relationships between the gut microbiome and childhood obesity. In the case–control study, we found that five gut microorganisms differed between children with and without obesity, whereas three gut microorganisms changed after weight loss in children with obesity.
Conclusion: Our study provides new insights into the genetic mechanisms underlying gut microbiota and childhood obesity.
Trial registration number: ChiCTR2300072179.
Name of registry: Change of intestinal flora and plasma metabolome in obese children and their weight loss intervention: a randomized controlled tria
URL of registry: https://www.chictr.org.cn/showproj.html.
Date of registration: 2023-06-06.
Date of enrolment of the first participant to the trial: 2023-06-07.

1. Introduction

In children and adolescents younger than 18 years, obesity is defined as body mass index (BMI) ≥ 95th percentile [1]. In recent years, the prevalence of childhood obesity has been increasing; the global age-standardized prevalence of obesity in children and adolescents increased from 0.7% (95% CrI 0.4–1.2) in 1975 to 5.6% (4.8–6.5) in 2016 in girls, and from 0.9% (0.5–1.3) in 1975 to 7.8% (6.7–9.1) in 2016 in boys [2]. Childhood obesity is associated with many adverse health outcomes during childhood and adulthood, including type 2 diabetes mellitus, dyslipidemia, metabolic syndrome, obstructive sleep apnea, and hypertension [3]. As a result, international efforts are underway to explore the factors associated with childhood obesity and its active prevention [4]. Childhood obesity is influenced by a combination of genetics and environment [5]. A genome-wide association meta-analysis identified multiple childhood obesity SNP loci such as rs9568856 and rs9299 [6]. Lifestyle factors such as diet and exercise have been shown to have

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a significant impact on childhood obesity [7]. In addition, the influence of the gut microbiota on childhood obesity is increasingly being emphasized [8]. Although the correlation between obesity and the gut microbiota is well established, the causal relationship remains controversial [9].

Gut microbiota is a key factor in obesity [10] because of its significant impact on the metabolic function of the host [11]. Imbalances in the gut microbiota, such as the genus Clostridium and the species *Eubacterium rectale*, Clostridium coccoides, *Lactobacillus reuteri*, *Akkermansia muciniphila*, and *Staphylococcus aureus*, have been observed in populations with obesity [12]. In addition, gut microbiota metabolites, such as short-chain fatty acids and membrane proteins, may influence host metabolism [13]. With the proposed gut-microbiota–brain axis, fecal microbiota transplants and prebiotic supplements are emerging as new anti-obesity therapies [14]. However, most previous studies were case–control studies in which confounders were difficult to exclude, and these conditions limited the inference of a causal relationship between gut microbiota and obesity.

Mendelian randomization (MR), which uses genetic variation as an instrumental variable (IV), can assess whether the observed associations between risk factors and outcomes are consistent with causal effects [15]. The assignment of genotypes from parent to offspring is randomized so that the association between genetic variation and outcome is not affected by common confounders and the causal sequence is plausible [16]. Thus, MR has been widely used to determine the causal relationship between a variety of diseases and gut microbiota [17–19]. In addition, once the direction of the causal association is confirmed by MR, validation using data obtained from a case–control study can be performed [20].

In this study, we used MR analysis to assess the causal relationship between the gut microbiota and childhood obesity. In addition, we validated this relationship using sequencing data from a case–control study (Fig. 1). Our results will help elucidate the potential genetic relationship between the gut microbiota and childhood obesity to reduce its occurrence.

2. Materials and methods

2.1. Genome-wide association studies (GWAS) summary data

GWAS summary data for the gut microbiota were obtained from the largest genome-wide meta-analysis published to date on gut microbiota composition, conducted by the MiBioGen consortium [21]. This study analyzed genome-wide genotypes and 16S rRNA fecal microbiome data from 18,340 individuals (24 cohorts). The genetic instruments for gut microbiota were acquired from The MRC Integrative Epidemiology Unit (IEU) OpenGWAS data infrastructure, which is a manually curated collection of complete GWAS summary datasets available for download as open-source files or by querying a database of the complete data. Nine phylum-level taxa, 16 class-level taxa, 20 order-level taxa, 37 family level taxa, and 128 genus-level taxa were included in the analyses.

GWAS summary data related to childhood obesity were acquired from a public GWAS dataset [6] that included 5530 cases (BMI ≥95th percentile) and 8318 controls (BMI <50th percentile) of European ancestry. These are the most comprehensive GWAS data available on childhood obesity because of the collaborative meta-analysis of 14 cohort studies. The 14 cohorts included in the study were the Avon Longitudinal Study of Parents and Children, Northern Finland 1966 Birth Cohort, British 1958 Birth Cohort – Type 1 Diabetes Genetics Consortium subset, British 1958 Birth Cohort – Wellcome Trust Case Control Consortium Subset, French Young study PCA adjusted, Lifestyle Immune System Allergy Study, Western Australian Pregnancy Cohort study, Children's Hospital of Philadelphia PCA adjusted, Essen Obesity Study PCA adjusted, Helsinki Birth Cohort Study, Cardiovascular Risk in Young Finns Study, and Copenhagen Study on Asthma in Childhood, CM-GOYA study, and Generation R Study. A full description of the study design, sample characteristics, statistical analyses, and quality control can be obtained from published results [6].

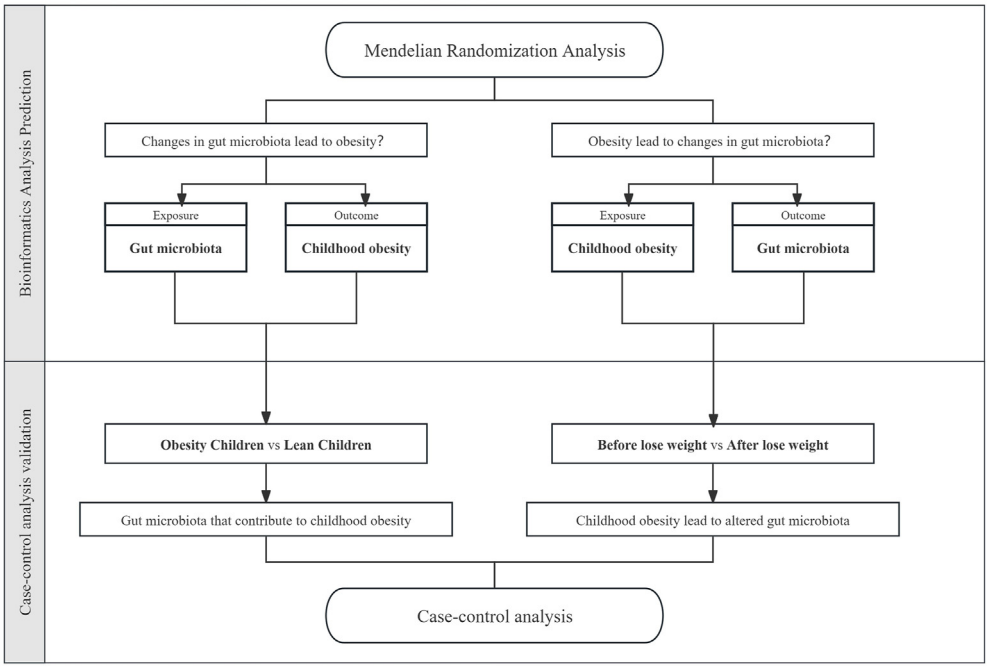

Fig. 1. Study design.

2.2. Selection of instrumental variables (IVs)

The IVs used in MR analysis should meet three conditions: 1) they are correlated with exposure, 2) they are not associated with confounding factors, and 3) they are not related to the outcome directly but through the exposure [22]. The IVs used in this study met the above conditions and are listed in the Supplementary Table S1. The variables for the gut flora met the genome-wide significance threshold of $P < 5 \times 10^{-5}$, and the variables for childhood obesity met $P < 5 \times 10^{-8}$. The parameters kb = 10,000 and $r^2 = 0.01$ were used to remove the linkage disequilibrium between variables. F-statistics were computed to estimate whether weak instrument bias was observed and to improve the power of the selected instrumental variables. The F-statistics for all IVs were above the threshold of 10 [23].

2.3. Two-sample MR analysis

Two-sample MR analysis was used to evaluate the causal relationship between gut microbiota and childhood obesity. The SNPs used as IVs were within a distance of 10,000 kb and $r^2 > 0.001$. A two-sample MR package (version 0.5.6) was used to analyze the MR data [15]. Five models were used in the MR analysis: 1) the inverse variance-weighted (IVW) model, 2) the weighted median estimator, 3) the MR-Egger regression method, 4) the simple mode, and 5) the weighted mode. The IVW model was used as the primary method to evaluate the causal relationship between gut microbiota and childhood obesity. The significance level was set at $P < 0.05$. Gut microbiota and childhood obesity SNPs were further assessed using statistical analyses, including Cochran's Q test, pleiotropy test, and leave-one-out sensitivity test. If the pleiotropy test suggested the presence of pleiotropy ($P < 0.05$), the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method was used to filter potential outliers and assist in correcting them [24]. Finally, leave-one-out sensitivity analysis was performed to evaluate whether a single SNP provided significant results.

2.4. Study population

This study was approved by the Chinese Clinical Trial Registry (ChiCTR2300072179). All studies involving human participants or human tissue must be in accordance with the principles set out in the Declaration of Helsinki. This study recruited total of 40 children, including 20 children who aspired to lose weight and 20 children whose weight was within the normal range for their gender and age. The inclusion criteria were age between 9 and 12 years, no history of liver- or thyroid-related diseases, and no history of congenital diseases or genetic defects. In addition, children who have already reached puberty are excluded. The children were matched with corresponding controls without obesity based on age and sex at baseline. After written informed consent was obtained from all the participants and their families, the children underwent their first physical examination and laboratory tests. Children with obesity received a 3-month weight loss intervention that included calorie restriction and increased activity levels. Three children with obesity opted out because they could not adhere to the diet. Seven children completed the weight loss intervention and underwent a second physical and laboratory examination (Fig. 2).

2.5. Clinical physical and laboratory examinations

Physical measurements of the participants were completed in the early morning fasting state. The participants' height and weight were measured, BMI was calculated, and abdominal circumference (AC) was measured around the abdomen at the level of the umbilicus. The participants were asked to refrain from overeating or excessive hunger the day before the laboratory tests. Fasting serum was used to test for lipids including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Participants' feces were collected and stored immediately at −80 °C for further analyses. In this study, the Illumina NovaSeq/HiSeq high-throughput sequencing platform was used, and the whole-genome birdshot (WGS) strategy was adopted. The total DNA of the macro-genome of the extracted colonies or the cDNA double strand of the macro-transcriptome synthesized using mRNA as a template was randomly interrupted into short fragments, and libraries with inserts of appropriate length were constructed, which were then subjected to the paired-end (PE) method. The remaining values were expressed as the mean ± standard deviation. Statistical analyses were performed using SPSS version 24.0.

The student-t test was used to detect differences between the two groups when the data were normally distributed and satisfied the variance chi-square test; the Welch t' test was used to detect differences between the two groups when the data were normally distributed but did not satisfy the variance chi-square test; and the Mann–Whitney nonparametric test was used to detect differences between the two groups when the data did not satisfy the normal distribution. Statistical significance was set at $P < 0.05$.

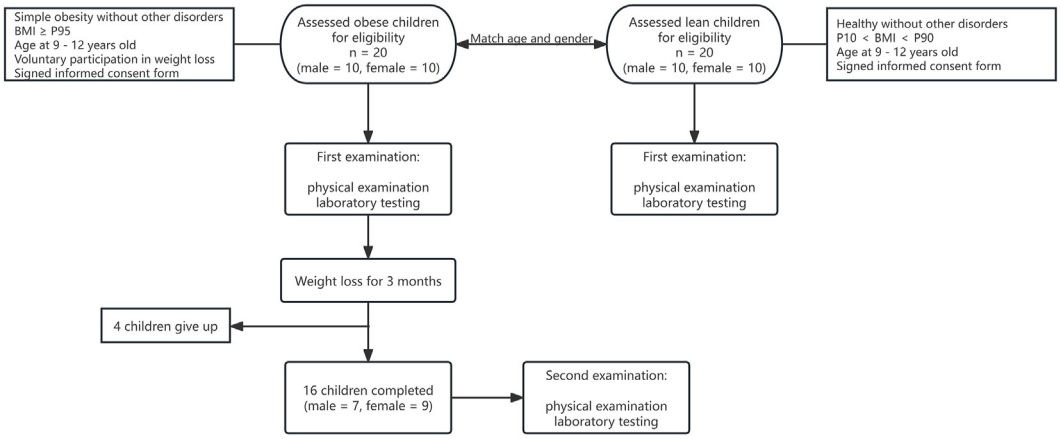

Fig. 2. Trial flowchart. BMI: body mass index.

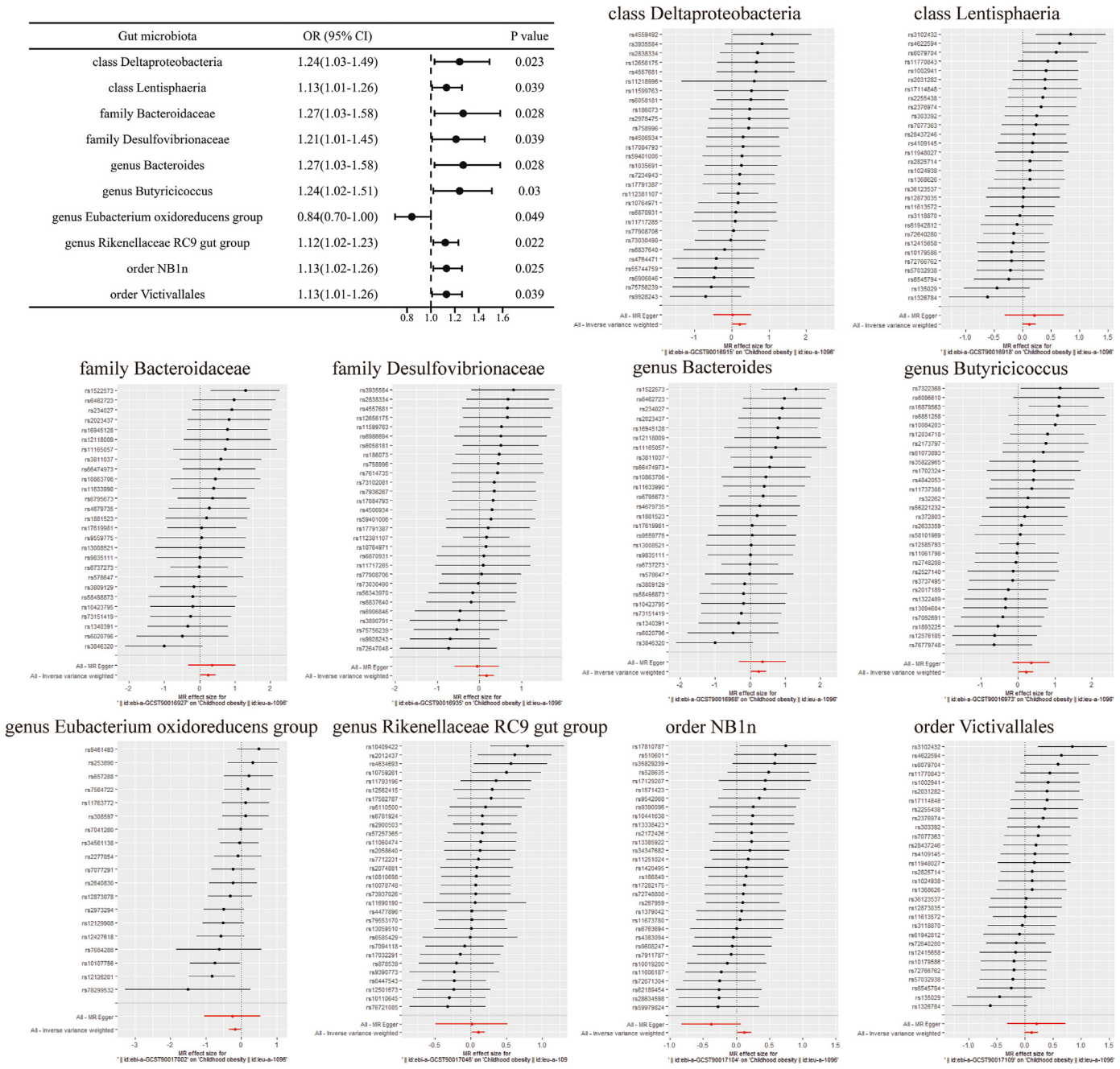

Fig. 3. Forest plot of the association between gut microbiota and childhood obesity.

3. Results

3.1. Two-sample Mendelian randomization of gut microbiota (exposure) on childhood obesity (outcome)

An MR study identified causal relationships between the gut microbiota and childhood obesity. Ten gut microbiota showed causal relationships with childhood obesity, including class Deltaproteobacteria (OR = 1.24, 95 % CI: 1.03–1.49; P = 0.023), class Lentisphaeria (OR = 1.13, 95 % CI: 1.01–1.26; P = 0.039), family Bacteroidaceae (OR = 1.27, 95 % CI: 1.03–1.58; P = 0.028), family Desulfovibrionaceae (OR = 1.21 95 % CI: 1.01–1.45; P = 0.039), genus Bacteroides (OR = 1.24, 95 % CI: 1.03–1.49; P = 0.023), genus Butyricicoccus (OR = 1.24 95 % CI: 1.02–1.51; P = 0.030), genus *Eubacterium oxidoreducens* (OR = 0.84, 95 % CI: 0.70–1.00; P = 0.030), genus Rikenellaceae RC9 gut group (OR = 1.12, 95 % CI: 1.02–1.23; P = 0.022), order NB1n (OR = 1.13, 95 % CI: 1.02–1.26; P = 0.025), and order Victivallales (OR = 1.13, 95 % CI: 1.01–1.26; P = 0.039) (Fig. 3, Supplementary Table S2).

All gut microbiota identified as significantly associated with childhood obesity were further analyzed using Cochran's Q test (Supplementary Table S3), pleiotropy test (Supplementary Table S4), and leave-one-out sensitivity analysis. The test results were used to determine the optimal MR method. In the absence of heterogeneity and pleiotropy, the estimated IVW results were preferentially used; thus, this method was used most frequently in this study. When the data exhibited heterogeneity but no pleiotropy, a weighted median or random-effect IVW was used. Although

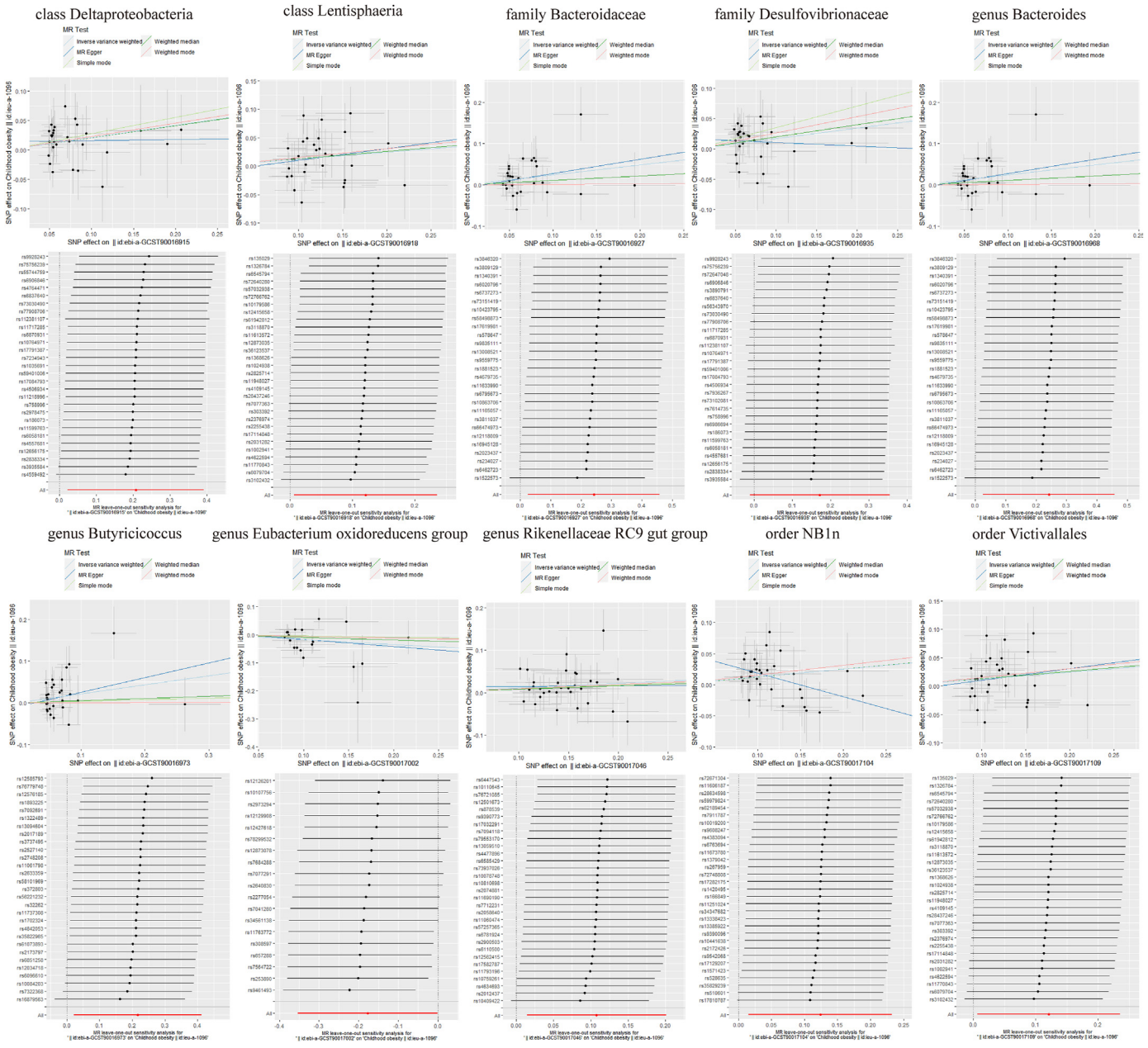

Fig. 4. Scatter and leave-one-out sensitivity analysis of the association between gut microbiota and childhood obesity.

a few results were heterogeneous, the direction of the effect obtained using the other methods was consistent with the IVW results. If the pleiotropy test suggested that the result was multiefficacious, the MR-Egger method was used. The leave-one-out sensitivity test identified nine SNPs that were significantly associated with and causally relevant to childhood obesity and should be considered reliable (Fig. 4).

3.2. Reverse Mendelian randomization analysis of childhood obesity (exposure) on gut microbiota (outcome)

Based on the results of reverse MR analysis, there was a suggestive association between childhood obesity and six gut microbiota, including genus Barnesiella (OR = 0.93, b = −0.08; P = 0.035), genus Clostridium sensustricto1 (OR = 0.94, b = −0.06; P = 0.040), genus Marvinbryantia (OR = 0.92 b = −0.09; P = 0.046), genus Oscillospira (OR = 0.07, b = 1.08; P = 0.039), genus Romboutsia (OR = 0.94, b = −0.07; P = 0.028), and genus Turicibacter (OR = 0.90, b = −0.11; P = 0.005) (Fig. 5, Supplementary Table S2). The results of the sensitivity analysis are shown in Fig. 6.

3.3. Differential gut microbiota in children with obesity and controls

To validate the intestinal flora that contributes to childhood obesity, we selected 16 children with obesity and matched them with 16 control children based on sex and age. Table 1 shows a comparison of the baseline demographics and blood lipid levels of all participants. We further examined the macrogenes of the gut microbiota in the feces of these children and identified five gut microbiota that showed significant differences between the children with and without obesity (Fig. 7). Our results show that the

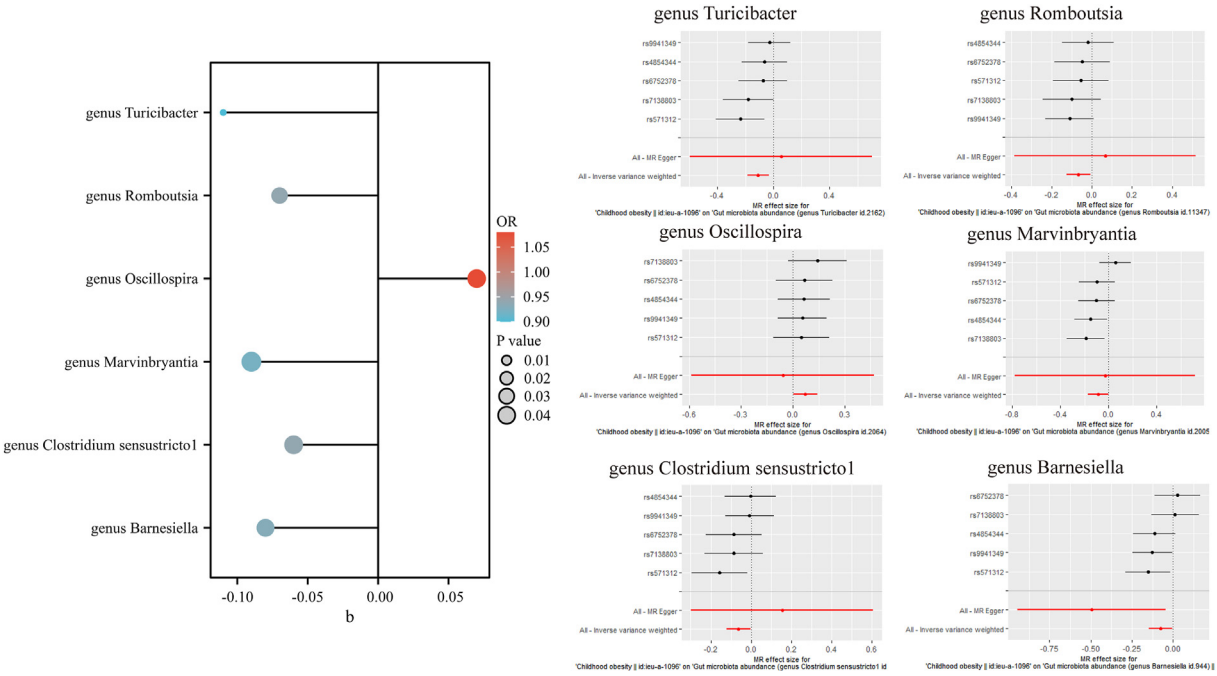

Fig. 5. Forest plot of the association between childhood obesity and gut microbiota.

class Deltaproteobacteria, family Bacteroidaceae, family Desulfovibrionaceae, genus Bacteroides, and genus Butyricoccus were more abundant in children with obesity than control children.

3.4. Differential gut microbiota before and after weight loss in children with obesity

To validate the changes in gut flora caused by obesity, we examined the gut flora macrogenes before and after weight loss in these children. Table 2 shows the changes in physical examination and blood lipid levels before and after weight loss. We further examined the macrogenes of the gut microbiota in the feces of these children and identified three gut microbiota that showed significant differences between the children with and without obesity (Fig. 8). Our study showed that the abundance of genus Clostridium sensustricto1, genus Romboutsia, and Turicibacter was elevated after weight loss in children with obesity.

4. Discussion

Using MR analysis, we identified nine gut microorganisms that contributed to an increased risk of childhood obesity through MR analysis and one gut microorganism that contributed to a reduced risk of childhood obesity. Furthermore, we used inverse MR and found that childhood obesity resulted in a decreased abundance of five gut microbes and an increased abundance of one gut microbe. All IVs were identified using the PhenoScanner and PheWAS databases to avoid confounding effects. After confirming the causal relationship between the gut microbiome and childhood obesity using MR, we validated the results in a case–control study. We verified the five gut microorganisms with elevated abundances in children with obesity, which included the class Deltaproteobacteria, family Bacteroidaceae, family Desulfovibrionaceae, genus Bacteroides, and genus Butyricicoccus, which is consistent with the MR analysis. Validation using the case–control study showed that weight loss increased the abundance of genus Clostridium sensustricto1, genus Romboutsia, and Turicibacter, which was consistent with the results of the MR analysis.

The gut microbiota has a symbiotic relationship with their human hosts, and Bacteroidetes and Firmicutes are predominantly detected in fecal samples from healthy humans [25]. Changes in the gut microbial abundance shape the unique characteristics of microbial communities and may influence human health and disease [26]. The gut microbiota produce a variety of substances through digestion, including short-chain fatty acids [27], and support adipogenesis [28], and hormones production [29]. Thus, obesity-associated microbiota contributes to the development of obesity by altering host energy metabolic homeostasis, insulin resistance, inflammation, and central appetite via the microbiota-gut–brain axis [30].

The class Deltaproteobacteria includes Desulfovibrio, Desulfobacter, Desulfococcus, Desulfonema, and Desulfuromonas. Studies have shown that Deltaproteobacteria are more abundant in obese mice [31] and are strongly associated with obesity-induced nonalcoholic fatty liver disease [32]. Our study demonstrated that the class Deltaproteobacteria may be associated with a higher risk of childhood obesity and is higher in children with obesity than control children, but does not change significantly after weight loss, suggesting that class Deltaproteobacteria contributes to the onset of obesity and is not a consequence of obesity.

Bacteroidaceae are non-pathogenic dominant bacteria in the human gut, and studies have shown a modest positive correlation between Bacteroidaceae and BMI [33]. The increased abundance of Bacteroidaceae in individuals with obesity suggests a reduction in other microbial species [34], and reduced gut microbiota populations have been associated with elevated levels of pro-inflammatory markers and insulin resistance [35]. Bacteroidaceae were also significantly higher in children with obesity than control children in our study, and MR analysis predictions suggested that Bacteroidaceae increased the risk of childhood obesity.

Butyricoccus secretes short-chain fatty acids, antimicrobial peptides, and *Clostridium butyricum*, which is the dominant intestinal flora in humans. Some studies have shown that Butyricoccus

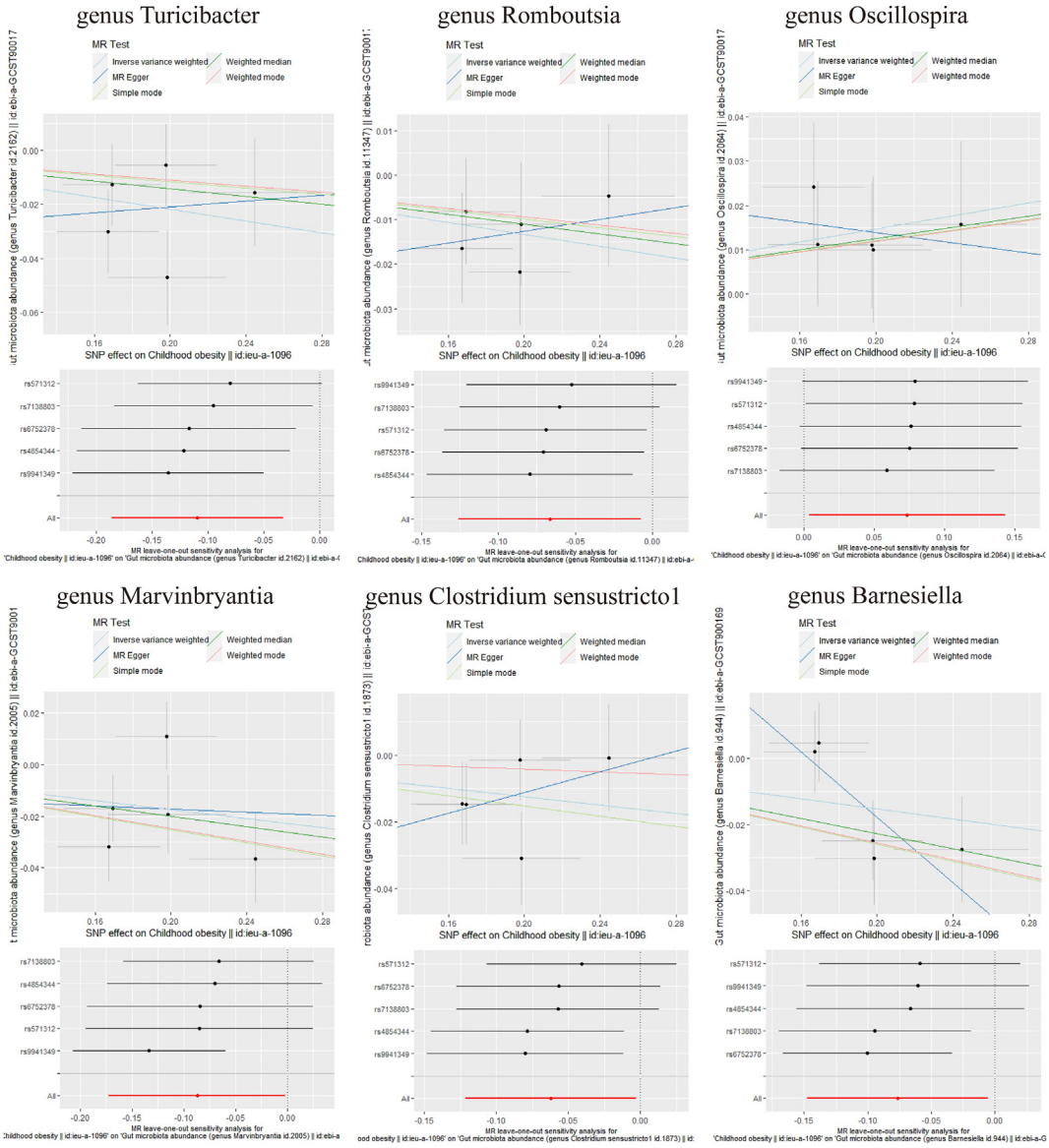

Fig. 6. Scatter and leave-one-out sensitivity analysis of the association between childhood obesity and gut microbiota.

Table 1
Demographic and blood lipids feature of participants in the case–control study.

characteristics	Obese	Lean	p[a]	
n	16	16		
Age	10.694 ± 1.0188	10.738 ± 1.0138	0.904	
Weight	62.056 ± 14.407	28.297 ± 6.7959	<0.001	***
BMI	28.894 ± 3.053	16.119 ± 1.4432	<0.001	***
AC	85.444 ± 12.514	59.069 ± 4.6969	<0.001	***
TC	4.71 (4.4425, 5.33)	4.37 (3.82, 4.45)	0.006	**
TG	1.53 (1.4575, 1.83)	1.12 (0.855, 1.44)	<0.001	***
HDL	1.125 (0.94, 1.205)	2.01 (1.7475, 2.4)	<0.001	***
LDL	2.77 (2.4475, 2.9625)	2.24 (2.14, 2.3925)	0.043	*
VLDL	0.6 (0.5225, 0.665)	0.58 (0.45, 0.61)	0.508	

Normally distributed data: Mean ± SD. Non-normally distributed data: Median (P25, P75). AC: abdominal circumference. TC: total cholesterol. TG: triglyceride. HDL: high density lipoprotein. LDL: low-density lipoprotein. VLDL: very low-density lipoprotein. [a] unpaired t test. *P < 0.05, **P < 0.01, ***P < 0.001.

was negatively correlated with BMI and lipid levels [36]. Exogenous supplementation with Amuc_1100 [37] or calebin A [38] increased the relative abundance of Butyricoccus and exerted anti-obesity effects. However, other studies have shown that Butyricoccus was positively correlated with the HOMA-IR index in individuals with obesity [39]. Inulin supplementation reduced Butyricoccus abundance and improved insulin sensitivity in hum-ob mice [40]. The reason for this discrepancy may be that the gut microbiota and fecal short-chain fatty acids vary by type of obesity and country of origin. Our findings support the latter hypothesis that Butyricoccus increases the risk of developing obesity.

A study on gut microbes in children with obesity showed that *Clostridium leptum* and *Eubacterium hallii* were associated with adipose tissue storage, whereas *Clostridium difficile* and the Staphylococcus genus were correlated with low BMI [41]. Similarly, our findings suggested a decrease in the abundance of the genus Clostridium sensustricto1 in children with obesity after weight loss, which was validated by MR analysis and case–control studies.

Romboutsia is more abundant in the healthy gut and plays an important role in maintaining gut health [42]. Increasing Romboutsia abundance is beneficial for reducing high-fat diet (HFD)-

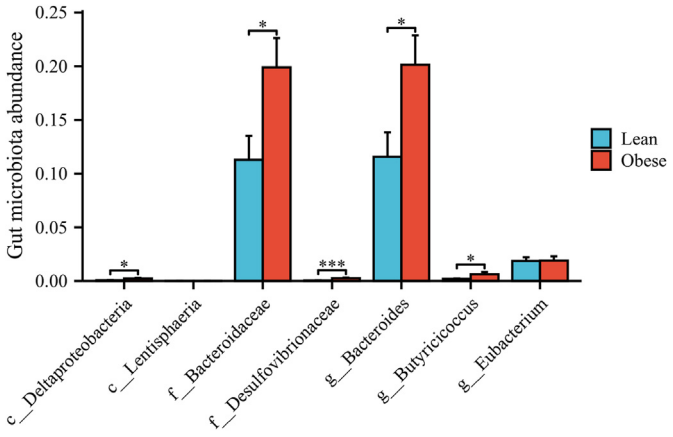

Fig. 7. Unpaired t-tests for participants' gut microbiota.

Table 2
Physical exam and blood lipids before and after weight loss in obese children.

characteristics	Before weight loss	After weight loss	p[a]	
n	16	16		
Weight	62.056 ± 14.407	57.481 ± 13.016	<0.001	***
BMI	28.894 ± 3.053	27.049 ± 2.8992	<0.001	***
AC	85.444 ± 12.514	80.656 ± 11.034	<0.001	***
TC	4.8225 ± 0.69996	3.6956 ± 0.64079	<0.001	***
TG	1.5875 ± 0.36084	1.0194 ± 0.47508	<0.001	***
HDL	1.1225 ± 0.23173	1.18 ± 0.26967	0.409	
LDL	2.6581 ± 0.45625	2.17 ± 0.39727	0.002	**
VLDL	0.615 ± 0.20258	0.39125 ± 0.21577	0.002	**

Normally distributed data: Mean ± SD. Non-normally distributed data: Median (P25, P75). AC: abdominal circumference. TC: total cholesterol. TG: triglyceride. HDL: high density lipoprotein. LDL: low-density lipoprotein. VLDL: very low-density lipoprotein. [a] paired t test. *P < 0.05, **P < 0.01, ***P < 0.001.

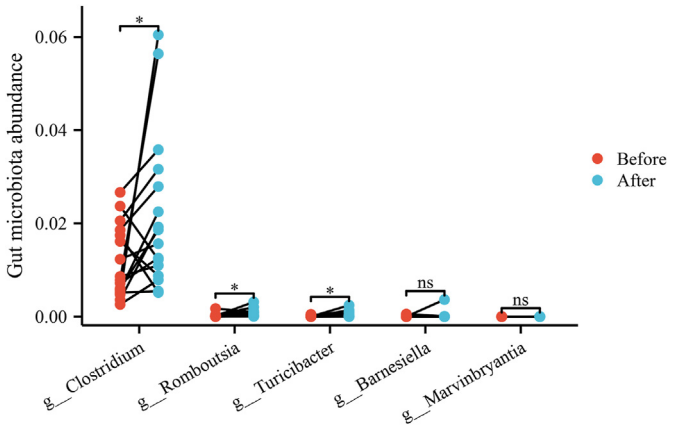

Fig. 8. Paired t-tests for gut microbiota before and after weight loss in obese children. Before: gut microbiota before weight loss in obese children. After: gut microbiota after weight loss in obese children.

induced obesity [43]. Exogenous supplementation with substances such as chlorogenic acid [44] and oral hydroxysafflor yellow A [45] ameliorated HFD-induced obesity in mice by increasing the abundance of Romboutsia. Our findings suggest that Romboutsia enrichment is elevated after weight loss in children with obesity, indicating that Romboutsia may contribute to weight loss.

Turicibacter belongs to the order Bifidobacterium and is a common intestinal probiotic. Turicibacter abundance is significantly reduced in individuals with obesity [46] and even in their offspring [47]. Prolonged HFD feeding leads to obesity and insulin resistance in mice, accompanied by a reduced relative abundance of Turicibacter and Anaeroplasma [48]. The consumption of pinto beans improved HFD-induced obesity and insulin resistance by increasing the abundance of Ruminococcus, Turicibacter, and Lactobacillus sp [49]. Our study also observed improvements in BMI, abdominal circumference, and lipid levels after weight loss in children with obesity, accompanied by an increase in Turicibacter abundance.

Recent studies have shown that gut flora is influenced by gender and hormone levels. A study [50] of the gut microbiota of 39 men and 36 postmenopausal women showed that after correcting for dietary habits, age and BMI, there were still differences in the composition of the gut microbiota between men and women. Among them, men had the higher presence of Veillonella and Methanobrevibacter genera, while the abundance of Bilophila was higher in women. Puberty and menopause are periods of dramatic changes in hormone levels in women. A study [51] showed that menopausal women have a higher ratio of stercobacteria/bacteroidetes, higher relative abundance of Lachnospira and Roseburia, and lower abundance of the genera Prevotella, Parabacteroides and Bilophila. However, changes in gut microbiota around puberty have not been reported. None of the participants included in this study had reached puberty. There was no significant difference in the abundance of gut microbiota detected between boys and girls in this study. Therefore, more studies on gut flora and puberty are needed.

To the best of our knowledge, this is the first application of MR analysis to explore the causal relationship between the gut microbiome and childhood obesity and to validate the findings using a case–control study. The strength of this study lies in the fact that confounding factors could be avoided by exploring the causal relationship between the gut microbiome and childhood obesity using MR analysis. The conclusions of our study were confirmed by robust sensitivity analyses. In addition, MR results were shown to be clinically informative in a case–control study. However, our study has some limitations. First, the selection of SNPs as IVs may be affected by potential horizontal pleiotropy. Genetic inheritance, lifestyle, and environmental factors can alter the gut microbiome, resulting in small differences in the IVs. The current study could not determine whether all IVs were associated with confounding factors. Second, the participants selected for the GWAS summary statistics in this study were from European populations; therefore, extrapolation of the study findings to other ethnic populations may be limited, even though we partially validated the results in Asian populations. Finally, because children with obesity who voluntarily participate in a weight loss interventions are difficult to recruit on a large scale, our case–control sample size was not large enough, which may have resulted in false-positive results. Therefore, the gut microbes that were identified in the MR analysis but did not show differences in the case–control analysis should be investigated in future studies.

5. Conclusions

Based on the GWAS summary data for gut microbiota and childhood obesity, MR evaluations revealed that 16 gut microbiotas were causally associated with childhood obesity, including the genera Bacteroides, Butyricicoccus, Clostridium, Romboutsia, and Turicibacter. These results provide a novel understanding of the genetic mechanisms underlying the relationship between the gut microbiota and childhood obesity.

Author contributions

Author Mengnan Lu and Ruoyang Feng collected and processed the data and wrote the article. Meng Li and Lujie Liu collected

serum and fecal samples. Chunyan Yin and Yanfeng Xiao designed the study.

Funding

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Institutional review board statement

This study was approved by the China Clinical Trial Registry (ChiCTR2300072179).

Informed consent statement

Informed consent statement was not applicable to our study as publicly available data were used for all analyses. Informed consent was obtained from all participants involved in the original study.

Data availability statement

The datasets analyzed during the current study are available from the UK biobank (http://geneatlas.roslin.ed.ac.uk/) (fields: 20002), IEU OpenGWAS data infrastructure (https://gwas.mrcieu.ac.uk/).

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We are indebted to all the individuals who participated in and helped with our research.

Abbreviation List

AC	Abdominal Circumference
BMI	Body Mass Index
GWAS	Genome-wide Association Studies
HDL	High-density Lipoprotein
HFD	High-fat Diet
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IEU	Integrative Epidemiology Unit
IV	Instrumental Variable
LDL	Low-density Lipoprotein
MR	Mendelian Randomization
OR	Odds Ratio
PCA	Principal Component Analysis
PE	Paired-end
SNP	Single Nucleotide Polymorphism
TC	Total Cholesterol
TG	Triglycerides
VLDL	Very Low-density Lipoprotein
WGS	Whole-genome Birdshot

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2024.05.012.

References

[1] Mapping local patterns of childhood overweight and wasting in low- and middle-income countries between 2000 and 2017. Nat Med 2020;26(5):750–9.
[2] Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet 2017;390(10113):2627–42.
[3] Daniels SR, et al. Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. Circulation 2005;111(15):1999–2012.
[4] Peterson CM, et al. Tri-ponderal mass index vs body mass index in estimating body fat during adolescence. JAMA Pediatr 2017;171(7):629–36.
[5] Steinsbekk S, et al. Polygenic risk, appetite traits, and weight gain in middle childhood: a longitudinal study. JAMA Pediatr 2016;170(2):e154472.
[6] Bradfield JP, et al. A genome-wide association meta-analysis identifies new childhood obesity loci. Nat Genet 2012;44(5):526–31.
[7] Ring-Dimitriou S, et al. Salto - study protocol and rationale of a community-oriented obesity prevention Program in the kindergarten. Obes Facts 2018;11(3):234–46.
[8] Stanislawski MA, et al. Pre-pregnancy weight, gestational weight gain, and the gut microbiota of mothers and their infants. Microbiome 2017;5(1):113.
[9] Harley IT, Karp CL. Obesity and the gut microbiome: striving for causality. Mol Metabol 2012;1(1–2):21–31.
[10] Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489(7415):242–9.
[11] Ussar S, et al. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. Cell Metabol 2015;22(3):516–30.
[12] Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: metabolism and perspective in obesity. Gut Microb 2018;9(4):308–25.
[13] Cuevas-Sierra A, et al. Diet, gut microbiota, and obesity: links with host genetics and epigenetics and potential applications. Adv Nutr 2019;10(suppl_1):S17–s30.
[14] Asadi A, et al. Obesity and gut-microbiota-brain axis: a narrative review. J Clin Lab Anal 2022;36(5):e24420.
[15] Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. Int J Epidemiol 2017;46(6):1734–9.
[16] Warrington NM, et al. Estimating direct and indirect genetic effects on offspring phenotypes using genome-wide summary results data. Nat Commun 2021;12(1):5420.
[17] Li P, et al. Association between gut microbiota and preeclampsia-eclampsia: a two-sample Mendelian randomization study. BMC Med 2022;20(1):443.
[18] Long Y, et al. Causal relationship between gut microbiota and cancers: a two-sample Mendelian randomisation study. BMC Med 2023;21(1):66.
[19] Liu X, et al. Mendelian randomization analyses support causal relationships between blood metabolites and the gut microbiome. Nat Genet 2022;54(1):52–61.
[20] Ma J, et al. Association of gut microbiome and primary liver cancer: a two-sample Mendelian randomization and case-control study. Liver Int 2023;43(1):221–33.
[21] Kurilshikov A, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet 2021;53(2):156–65.
[22] Zou XL, et al. Childhood obesity and risk of stroke: a mendelian randomisation analysis. Front Genet 2021;12:727475.
[23] Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol 2011;40(3):740–52.
[24] Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44(2):512–25.
[25] Ursell LK, et al. The intestinal metabolome: an intersection between microbiota and host. Gastroenterology 2014;146(6):1470–6.
[26] Hollister EB, Gao C, Versalovic J. Compositional and functional features of the gastrointestinal microbiome and their effects on human health. Gastroenterology 2014;146(6):1449–58.
[27] Al-Lahham SH, et al. Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. Biochim Biophys Acta 2010;1801(11):1175–83.
[28] Muccioli GG, et al. The endocannabinoid system links gut microbiota to adipogenesis. Mol Syst Biol 2010;6:392.
[29] Neuman H, et al. Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS Microbiol Rev 2015;39(4):509–21.
[30] Torres-Fuentes C, et al. The microbiota-gut-brain axis in obesity. Lancet Gastroenterol Hepatol 2017;2(10):747–56.
[31] Pindjakova J, et al. Gut dysbiosis and adaptive Immune response in diet-induced obesity vs. Systemic inflammation. Front Microbiol 2017;8:1157.
[32] Lin YC, et al. Pathogenic effects of Desulfovibrio in the gut on fatty liver in diet-induced obese mice and children with obesity. J Gastroenterol 2022;57(11):913–25.
[33] Yassour M, et al. Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. Genome Med 2016;8(1):17.
[34] Aron-Wisnewsky J, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. Gut 2019;68(1):70–82.
[35] Crusell MKW, et al. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. Microbiome 2018;6(1):89.

[36] Zeng Q, et al. Discrepant gut microbiota markers for the classification of obesity-related metabolic abnormalities. Sci Rep 2019;9(1):13424.
[37] Song Z, et al. Amuc attenuates high-fat diet-induced metabolic disorders linked to the regulation of fatty acid metabolism, bile acid metabolism, and the gut microbiota in mice. Int J Biol Macromol 2023;242(Pt 2):124650.
[38] Lee PS, et al. Calebin-A prevents HFD-induced obesity in mice by promoting thermogenesis and modulating gut microbiota. J Tradit Complement Med 2023;13(2):119–27.
[39] Atzeni A, et al. Taxonomic and functional fecal microbiota signatures associated with insulin resistance in non-diabetic subjects with overweight/obesity within the frame of the PREDIMED-plus study. Front Endocrinol 2022;13:804455.
[40] Rodriguez J, et al. Discovery of the gut microbial signature driving the efficacy of prebiotic intervention in obese patients. Gut 2020;69(11):1975–87.
[41] Indiani C, et al. Childhood obesity and firmicutes/bacteroidetes ratio in the gut microbiota: a systematic review. Child Obes 2018;14(8):501–9.
[42] Ma J, et al. Comparing the bacterial community in the gastrointestinal tracts between growth-retarded and normal yaks on the qinghai-Tibetan plateau. Front Microbiol 2020;11:600516.
[43] Zhu L, et al. Faecal microbiota transplantation-mediated jejunal microbiota changes halt high-fat diet-induced obesity in mice via retarding intestinal fat absorption. Microb Biotechnol 2022;15(1):337–52.
[44] Ye X, et al. Chlorogenic acid-induced gut microbiota improves metabolic endotoxemia. Front Endocrinol 2021;12:762691.
[45] Liu J, et al. Oral hydroxysafflor yellow A reduces obesity in mice by modulating the gut microbiota and serum metabolism. Pharmacol Res 2018;134:40–50.
[46] Jiao N, et al. Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. Physiol Genom 2018;50(4):244–54.
[47] Gilley SP, et al. Associations between maternal obesity and offspring gut microbiome in the first year of life. Pediatr Obes 2022;17(9):e12921.
[48] Velázquez KT, et al. Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. World J Hepatol 2019;11(8):619–37.
[49] Zhao Q, et al. Adzuki bean alleviates obesity and insulin resistance induced by a high-fat diet and modulates gut microbiota in mice. Nutrients 2021;13(9).
[50] Haro C, et al. Intestinal microbiota is influenced by gender and body mass index. PLoS One 2016;11(5):e0154090.
[51] Santos-Marcos JA, et al. Influence of gender and menopausal status on gut microbiota. Maturitas 2018;116:43–53.