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From Gut to Heart: Mendelian Randomization Study Reveals the Causal Relationship Between Gut Microbiota and N-Terminal Pro-B-Type Natriuretic Peptide

ABSTRACT

Background: This study aimed to clarify the potential causal relationship between gut microbiota (GM) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) using Mendelian randomization (MR) analysis.

Methods: Genome-wide association study of intestinal flora and NT-proBNP was conducted, and the instrumental variables (IVs) were screened out to assess the causal association between intestinal flora and NT-proBNP. The 2-sample MR analysis was performed using the inverse variance weighted (IVW), MR-Egger, weighted model and simple model methods, respectively, to assess the causal association between intestinal flora and NT-proBNP using the odds ratio. Sensitivity analyses were also performed using the leave-one-out method and MR-Egger intercept test. The MR-PRESSO global test was used to detect horizontal pleiotropy, and Cochran Q was used to detect heterogeneity. Finally, forest plots, scatter plots, and funnel plots of the IVs were generated.

Results: Based on the IVW method, a total of 9 out of 104 GM genera were identified as causally associated with NT-proBNP levels ($P < .05$). The genera *Holdemanella* ($\beta = -0.19$, 95% CI: −0.36 to −0.01, $P = .037$), *Coprococcus* 2 ($\beta = -0.27$, 95% CI: −0.54 to 0.00, $P = .047$), Ruminococcaceae UCG 004 ($\beta = -0.23$, 95% CI: −0.45 to −0.02, $P = .032$), and *Alistipes* ($\beta = -0.29$, 95% CI: −0.55 to −0.03, $P = .031$) were negatively associated with NT-proBNP; genera *Actinomyces* ($\beta = 0.22$, 95% CI: 0.01-0.44, $P = .042$), Lachnospiraceae UCG 008 ($\beta = 0.27$, 95% CI: 0.1-0.44, $P = .002$), *Eubacterium fissicatena* group ($\beta = 0.17$, 95% CI: 0.01-0.32, $P = .033$), *Eubacterium rectale* group ($\beta = 0.40$, 95% CI: 0.13-0.67, $P = .003$), and *Eubacterium ventriosum* group ($\beta = 0.26$, 95% CI: 0.02-0.49, $P = .032$) were positively associated with NT-proBNP. In addition, the results of the sensitivity analysis of the leave-one-out method were stable, there was no horizontal multiplicity in the MR-Egger intercept test and the MR-PRESSO global test, and there was no heterogeneity in the Cochran Q-test.

Conclusions: The study found the causal relationship between GM and NT-proBNP. As a clinical predictor of heart failure, NT-proBNP levels could potentially be modulated through clinical interventions involving GM, further reducing the risk of heart failure.

Keywords: Gut microbiota, NT-proBNP, mendelian randomization, heart failure

INTRODUCTION

N-terminal pro-B-type natriuretic peptide (NT-proBNP), a derivative of the precursor molecule of B-type natriuretic peptide (BNP), serves as a pivotal biomarker for evaluating cardiovascular function and assessing the risk of cardiac disease, particularly heart failure[1] Li-natriuretic peptide-guided therapy improves cardiovascular outcomes of patients with high-risk heart failure and reduced ejection fraction disease.[2] In-hospital 24-hour blood pressure monitoring in patients with decompensated atrial fibrillation (AF) revealed that patients with a dipper blood pressure pattern exhibited a favorable association with the change of NT-proBNP levels.[3] Patients with coronary slow flow phenomenon with scar tissue on cardiac magnetic resonance imaging have higher NT-proBNP levels.[4] In addition, several studies have noted that NT-proBNP contributes to the incidence of coronary

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artery disease by increasing the risk of heart failure disease and is an important assessment indicator of coronary artery disease.[5-7] Despite the fact that diseases caused by heart failure are treated clinically with appropriate measures, including medications, interventions, or cardiac surgery, heart failure continues to threaten humans as a common condition. Therefore, modifying NT-proBNP levels is crucial for preventing and treating heart failure.

The collective microbial community of all microorganisms in the human gut is termed "gut microbiota (GM)."[6] The host provides the environment and nutrients necessary for GM survival, and GM actively participates in various physiological processes within the human body in turn. In recent years, studies have found a close relationship between GM and heart failure. Some researchers have detected identical bacterial DNA in arterial plaques and the intestinal flora of coronary heart disease (CHD) patients, suggesting that GM may be a potential source of bacteria in arterial plaques, and it may be involved in the development of CHD.[7] Although heart failure contributes to cardiovascular diseases such as CHD[8] and myocardial infarction,[9] and GM is associated with heart failure-induced cardiovascular disease,[10] the causality of GM and heart failure remains debated. Modulating or remodeling the GM promises to be a new clinical therapeutic idea for the effective and specific treatment of heart failure.

Mendelian randomization is a statistical method that emphasizes overcoming confounders and reverse causality bias. This approach is based on the fact that IVs are associated with the risk factor or biomarker being studied but not with other potential confounders. Thus, these instrumental variables (IVs) can be considered randomly assigned to individuals, similar to random assignment in an experiment. By adopting this approach, causality can be assessed more reliably without interference from other factors, increasing the credibility of study findings.[11] Although randomized controlled trials (RCTs) are the gold standard for evaluating the safety and efficacy of an intervention, they are challenging to implement because of the complexity of their design, the high cost in terms of time, human and material resources, and ethical constraints. Conceptually, MR is similar to RCTs in that genetic variables are randomly assigned at birth to "case" or "control" groups as IVs and remain constant throughout the life cycle, according to Mendel's second law. Genome-wide association study (GWAS) has played an essential role in complex disease genetics by searching for genotype-phenotype relationships through millions of genetic variations tested on the genomes of many individuals.[12] Single nucleotide polymorphism (SNP) exposure and SNP-outcome associations can be extracted separately from independent GWAS and used in MR analyses of 2 independent samples to generate a single causal estimate. Thus, these enabled us to infer a causal relationship between GM and NT-proBNP using a Mendelian randomization (MR) approach. It is independent of confounders between genetic variation and outcome, and serves as a novel approach to studying causal relationships between GM and NT-proBNP.[13-15] Therefore, the relationship between GM and NT-proBNP was investigated from a Mendelian genetic perspective.

HIGHLIGHTS

- Causal link between gut microbiota (GM) and NT-proBNP: Certain bacteria like *Holdemanella* are negatively associated with NT-proBNP, potentially aiding heart failure prevention.
- Potential for GM regulation: Modulating GM could lower NT-proBNP levels and reduce heart failure risk.
- Robust results: Sensitivity analysis confirmed the results' reliability and the validity of the causal relationship.

METHODS

Study Design

Intestinal flora and NT-proBNP were respectively used as the exposure factor and outcome variable, with significantly associated SNPs serving as IVs. Based on publicly available GWAS summary statistics, the causal relationship between 119 bacterial taxa and NT-proBNP was assessed using 2-sample bidirectional MR analysis. This study adhered to 3 crucial assumptions of the MR methodology: (1) there is a significant association between IVs and gut flora; (2) IVs are not associated with any of the gut flora-NT-proBNP confounders; and (3) IVs affect NT-proBNP only through the gut flora, instead of any other pathway.

Data Source

Genetic data on gut flora were obtained from the GWAS meta-analysis published by the largest MiBioGen consortium, which collected 16S rRNA gene sequencing profiles and genotyping data from 18 340 subjects (13 266 of European origin) from 11 countries, spanning Europe and the United States, and analyzed the gut microbiome signature loci.[16] In this study, bacterial taxa were analyzed at the genus level, encompassing 119 bacterial taxa after excluding 15, and a total of 3301 NT-proBNP sources, all of European origin,[17] were included. The NT-proBNP was from a population with a rigorously collected set of demographic data, anthropometric measurements, lifestyle factors, and dietary information. To ensure a clean baseline for outcome assessment, participants with a history of major diseases, recent illnesses, or infections were excluded from the study. The NT-proBNP levels were measured using SomaLogic assays, which employ SOMAmer probes to accurately identify and bind target proteins. This advanced method allows for the detection of proteins even at low concentrations, providing precise and reliable measurements essential for evaluating NT-proBNP levels. The NT-proBNP datasets were chosen primarily from European ancestry populations, consistent with the fact that the GM exposure data, which also predominantly comes from European ancestry. This was done to maintain consistency and reduce confounding variables related to genetic and environmental diversity.

Instrumental Variable

First, SNPs with significant association with GM ($P < 1 \times 10^{-5}$) were selected. Second, SNPs were quality checked according to the following quality control steps to ensure accuracy in establishing a causal relationship between GM and

NT-proBNP: (1) exclusion of SNPs with allelic incongruence between the exposure and outcome samples, such as A/C; (2) removal of palindromic SNPs; (3) setting a coefficient r^2 threshold of 0.001 and a region width of 10 000 kb to exclude the interference of linkage disequilibrium; (4) remove minor allele frequency. The MR-Egger regression was used to assess the average level of pleiotropy of the SNPs as a whole, whereas MR-PRESSO could detect each abnormal SNP and re-analyze the MR analysis by removing the SNPs with level of pleiotropy. For significantly associated SNPs, the F-statistic was used to assess whether the selected genetic variables were weakly instrumental. An *F*-value greater than 10 indicated that the genetic variables did not have weak instrumental bias.

Mendelian Randomization Analysis

In this study, 5 methods were used to verify the causal relationship between GM and NT-proBNP in 119 taxa: MR-random or fixed effects inverse variance weighted (IVW), weighted median estimation (WME), MR-Egger regression, simple mode (SM), and weighted mode (WM). For features containing only 1 IV, the Wald ratio test was used to estimate the association between the identified IV and NT-proBNP. Results with multiple IVs were mainly based on the IVW method, supplemented by the other 4 methods.

Sensitivity Analysis

To ensure the reliability of the causal effect assessment results, sensitivity analysis was conducted in this study. The leave-one-out tests were performed to examine the effect of individual IVs on the overall results. A test for heterogeneity was performed using Cochran's Q statistics to assess whether there were differences between IVs in the 2-sample MR analysis. Q statistics significant at a *P*-value < .05 were considered heterogeneous. Horizontal pleiotropy is indicated if the IV can directly affect the outcome variable without exposure, which violates the exclusivity assumption of MR. This study used the MR-Egger intercept test to examine horizontal pleiotropy. An intercept term approaching 0 with $P > .05$ indicated no horizontal pleiotropy; otherwise, horizontal pleiotropy was present. Additionally, the MR-PRESSO was used as a supplementary method to detect horizontal pleiotropy.

Statistical Analysis

Data were organized and analyzed using R software (version 4.2.0). The "*TwosampleMR*" and "*MR-PRESSO*" R packages were utilized for MR analysis. Evaluate the causal relationship between GM and NT proBNP using IVW, WME, MR-Egger regression, SM, and WM algorithms. Cochran's Q statistical tests were conducted to confirm heterogeneity among the selected IVs, and horizontal gene pleiotropy tests were performed on the MR-Egger algorithm. Perform sensitivity analysis on IV SNPs using the leave-one-out test. Statistical significance was set at $P < .05$.

RESULTS

Instrumental Variables Selection

Based on the IVW method, a total of 9 out of 104 GM classes were found to be causally associated with NT-proBNP ($P < .05$). These included 13 SNPs in the genus *Holdemanella*, 9 SNPs in the genus *Coprococcus* 2, 11 SNPs in the genus Ruminococcaceae UCG 004, 8 SNPs in the genus, 8 SNPs in the genus *Actinomyces*, 15 SNPs in the genus *Alistipes*, 12 SNPs in the genus Lachnospiraceae UCG 008, 9 SNPs in the genus *Eubacterium fissicatena* group, 12 SNPs in the genus *Eubacterium rectale* group, and 15 SNPs in the genus *Eubacterium ventriosum* group. All *F* values were greater than 10, indicating a low likelihood of a weak IV bias, consistent with the MR association hypothesis.

Mendelian Randomization Analysis of Gut Microbiota and N-Terminal Pro-B-Type Natriuretic Peptide

As shown in Table 1, the genus *Holdemanella* ($\beta = -0.19$, 95% CI: −0.36 to −0.01, $P = .037$), genus *Coprococcus* 2 ($\beta = -0.27$, 95% CI: −0.54 to 0.00, $P = .047$), genus Ruminococcaceae UCG 004 ($\beta = -0.23$, 95% CI: −0.45 to −0.02, $P = .032$), genus *Alistipes* ($\beta = -0.29$, 95% CI: −0.55 to −0.03, $P = .031$) were negatively associated with NT-proBNP. Conversely, the genus *Actinomyces* ($\beta = 0.22$, 95% CI: 0.01-0.44, $P = .042$), genus Lachnospiraceae UCG 008 ($\beta = 0.27$, 95% CI: 0.1-0.44, $P = .002$), genus *Eubacterium fissicatena* group ($\beta = 0.17$, 95% CI: 0.01-0.32, $P = .033$), genus *Eubacterium rectale* group ($\beta = 0.4$, 95% CI: 0.13-0.67, $P = .003$), and genus *Eubacterium ventriosum* group ($\beta = 0.26$, 95% CI: 0.02-0.49, $P = .032$) were positively associated with NT-proBNP.

Consistent with the above results, the overall direction of causality between GM and NT-proBNP calculated by the 5 methods is shown in Figures 1 and 2. Among them, the genus *Holdemanella*, genus *Coprococcus* 2, genus Ruminococcaceae UCG 004, and genus *Alistipes* appear to exert reduced effects on NT-proBNP. While the genus *Actinomyces*, genus Lachnospiraceae UCG 008, genus *Eubacterium fissicatena* group, genus *Eubacterium rectale* group, and genus *Eubacterium ventriosum* group appear to act as increased factors for proBNP. The influence and range of fluctuation of each SNP in the causal relationship between GM (exposure factor) and NT-proBNP (outcome factor) is illustrated in the figures. The contribution of each SNP to the overall combined effect value of GM (exposure factor) and NT-proBNP (outcome factor) causality is shown in Figure 2.

Sensitivity Analysis Results

The test for heterogeneity of the gut microorganisms of the 9 taxa showed $P > .05$, indicating no heterogeneity. The intercept term of MR-Egger's test was statistically non-significant ($P > .05$), and therefore the assumption of exclusivity was considered to be valid, as well as the causal relationship was robust. As shown in funnel plot (Figure 3), the points represented by individual SNPs are roughly symmetrically distributed, indicating a low likelihood of being affected by potential bias. The results of the leave-one-out method show that there is no big difference between the effect sizes of individual SNPs before and after the exclusion, suggesting that there is no individual SNP that significantly affects the MR estimation results, which indicates that the causal relationship has a certain degree of stability, as shown in Figure 4.

DISCUSSION

Based on MR analysis, a causal association was found between 9 intestinal bacterial species and NT-proBNP.

Table 1. Results of MR analysis of GM and NT-proBNP

Bacterial Taxa (Exposure)	Method	nsSNP	β-Value (95% CI)	P
Holdemanella	MR-Egger	13	−0.22 (−1.04 to 0.61)	.618
Holdemanella	WME	13	−0.20 (−0.44 to 0.04)	.099
Holdemanella	IVW	13	−0.19 (−0.36 to −0.01)	.037
Holdemanella	SM	13	−0.28 (−0.69 to 0.12)	.197
Holdemanella	WM	13	−0.27 (−0.66 to 0.12)	.192
Coprococcus 2	MR-Egger	9	−0.06 (−1.54 to 1.43)	.942
Coprococcus 2	WME	9	−0.16 (−0.51 to 0.19)	.373
Coprococcus 2	IVW	9	−0.27 (−0.54 to 0.00)	.047
Coprococcus 2	SM	9	−0.16 (−0.68 to 0.36)	.562
Coprococcus 2	WM	9	−0.16 (−0.65 to 0.34)	.550
Ruminococcaceae UCG 004	MR-Egger	11	−0.02 (−1.39 to 1.34)	.973
Ruminococcaceae UCG 004	WME	11	−0.21 (−0.50 to 0.08)	.150
Ruminococcaceae UCG 004	IVW	11	−0.23 (−0.45 to −0.02)	.032
Ruminococcaceae UCG 004	SM	11	−0.14 (−0.57 to 0.30)	.550
Ruminococcaceae UCG 004	WM	11	−0.15 (−0.55 to 0.25)	.478
Actinomyces	MR-Egger	8	0.19 (−0.41 to 0.78)	.556
Actinomyces	WME	8	0.19 (−0.09 to 0.47)	.177
Actinomyces	IVW	8	0.22 (0.01 to 0.44)	.042
Actinomyces	SM	8	0.19 (−0.20 to 0.58)	.372
Actinomyces	WM	8	0.19 (−0.18 to 0.56)	.350
Alistipes	MR-Egger	15	−0.08 (−1.38 to 1.22)	.905
Alistipes	WME	15	−0.28 (−0.66 to 0.09)	.134
Alistipes	IVW	15	−0.29 (−0.55 to −0.03)	.031
Alistipes	SM	15	−0.30 (−0.98 to 0.37)	.393
Alistipes	WM	15	−0.26 (−0.93 to 0.41)	.456
Lachnospiraceae UCG 008	MR-Egger	12	0.48 (−0.39 to 1.36)	.305
Lachnospiraceae UCG 008	WME	12	0.23 (0.01 to 0.46)	.037
Lachnospiraceae UCG 008	IVW	12	0.27 (0.10 to 0.44)	.002
Lachnospiraceae UCG 008	SM	12	0.14 (−0.23 to 0.51)	.467
Lachnospiraceae UCG 008	WM	12	0.15 (−0.22 to 0.51)	.441
Eubacterium fissicatena group	MR-Egger	9	0.38 (−0.43 to 1.19)	.388
Eubacterium fissicatena group	WME	9	0.15 (−0.06 to 0.36)	.160
Eubacterium fissicatena group	IVW	9	0.17 (0.01 to 0.32)	.033
Eubacterium fissicatena group	SM	9	0.18 −0.17 to 0.53)	.353
Eubacterium fissicatena group	WM	9	0.15 (−0.18 to 0.48)	.394
Eubacterium rectale group	MR-Egger	12	1.01 (0.23 to 1.78)	.029
Eubacterium rectale group	WME	12	0.26 (−0.12 to 0.64)	.183
Eubacterium rectale group	IVW	12	0.40 (0.13 to 0.67)	.003
Eubacterium rectale group	SM	12	0.21 (−0.39 to 0.82)	.505
Eubacterium rectale group	WM	12	0.23 (−0.37 to 0.83)	.467
Eubacterium ventriosum group	MR-Egger	15	0.55 (−0.47 to 1.58)	.310
Eubacterium ventriosum group	WME	15	0.28 (−0.04 to 0.60)	.083
Eubacterium ventriosum group	IVW	15	0.26 (0.02 to 0.49)	.032
Eubacterium ventriosum group	SM	15	0.32 (−0.25 to 0.89)	.290
Eubacterium ventriosum group	WM	15	0.35 (−0.21 to 0.91)	.237

IVW, inverse variance weighted; MR-Egger, Mendelian randomization-Egger; nsSNP, non-synonymous single nucleotide polymorphism; SM, simple mode; WM, weighted mode; WME, weighted median estimation.

Among them, genera *Holdemanella*, *Coprococcus* 2, Ruminococcaceae UCG 004, and *Alistipes* were reduced factors for NT-proBNP; while genera *Actinomyces*, Lachnospiraceae UCG 008, *Eubacterium fissicatena* group, *Eubacterium rectale* group, and *Eubacterium ventriosum* group were increased factors for NT-proBNP. Several studies

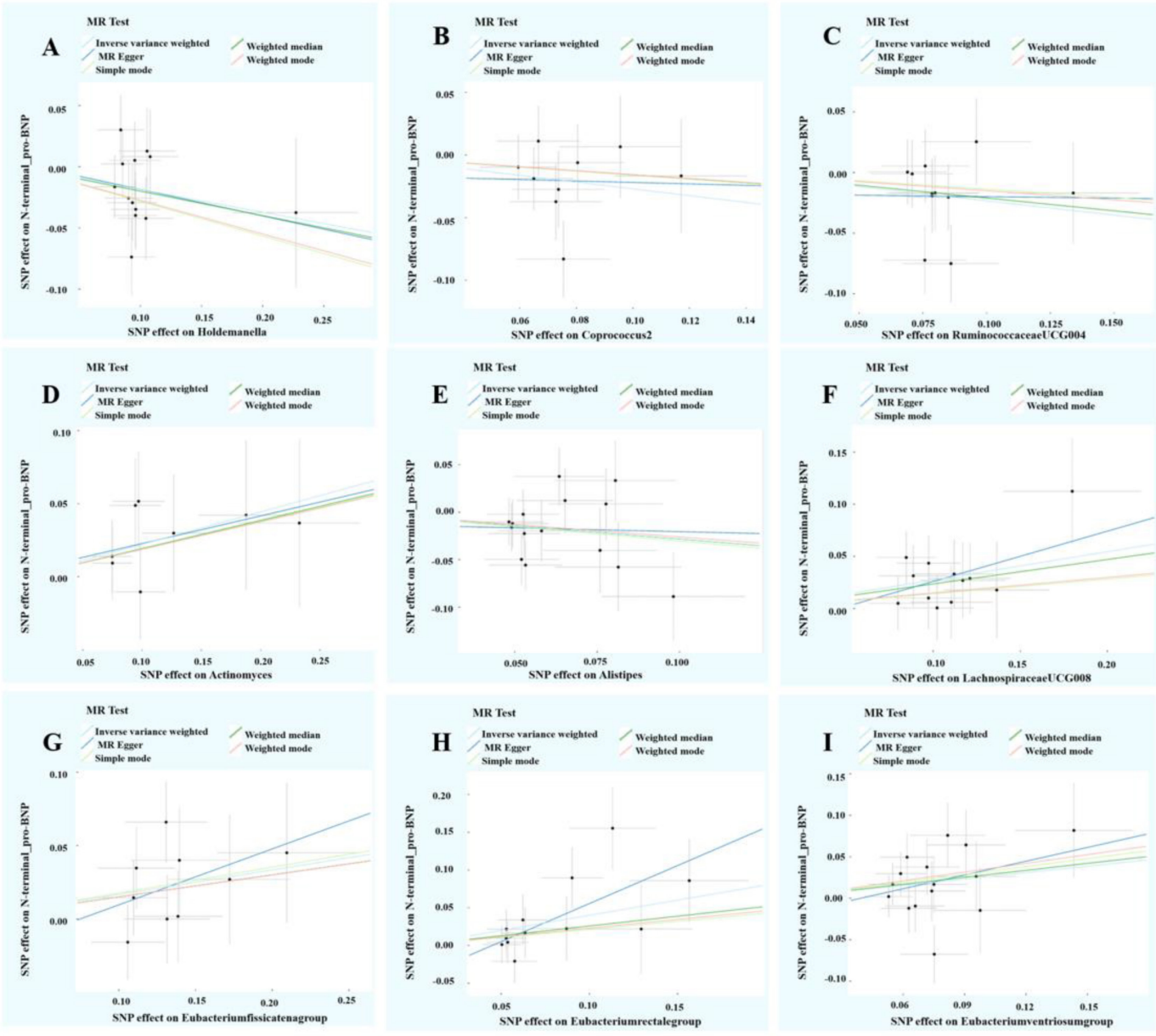

Figure 1. Scatter plot of MR analysis results of GM versus NT-proBNP. A, *Holdemanella*; **B,** *Coprococcus* **2; C, Ruminococcaceae UCG 004; D,** *Actinomyces*; **E,** *Alistipes*; **F, Lachnospiraceae UCG 008; G,** *Eubacterium fissicatena* **group; H,** *Eubacterium rectale* **group; I,** *Eubacterium ventriosum* **group.**

have noted that increased NT-proBNP promotes heart failure, which further exacerbates cardiovascular disease.[18,19] Therefore, regulating the GM levels mentioned above is beneficial in improving outcomes of cardiovascular diseases such as heart failure caused by NT-proBNP.

It is known that BNP is expressed by the myocardium in response to elevated atrial wall pressure, both BNP as well as NT-proBNP have become important biomarkers of cardiovascular disease.[20] Quantification of NT-proBNP has been shown to predict the progression of coronary artery disease. One previous study had found different doses of azithromycin affected the abundance and grouping of GM in rat models of heart failure, and the degree of myocardial damage, GM variations, and clinical manifestations of heart failure were found to be consistent with each other.[21] Another study noted that hypertension could result in the imbalance of GM, while chronic hypertension could lead to dysregulation of fecal microbial ecology and elevated levels of the biomarker NT-proBNP.[22] Although the above studies indicate that gut flora may be associated with NT-proBNP-induced heart failure disease, no studies have directly demonstrated a causal relationship between GM and NT-proBNP.

In the present study, it was found that the genus *Holdemanella*, genus *Coprococcus* 2, genus Ruminococcaceae UCG 004, and genus *Alistipes* were reduced factors for NT-proBNP. Consistent with our

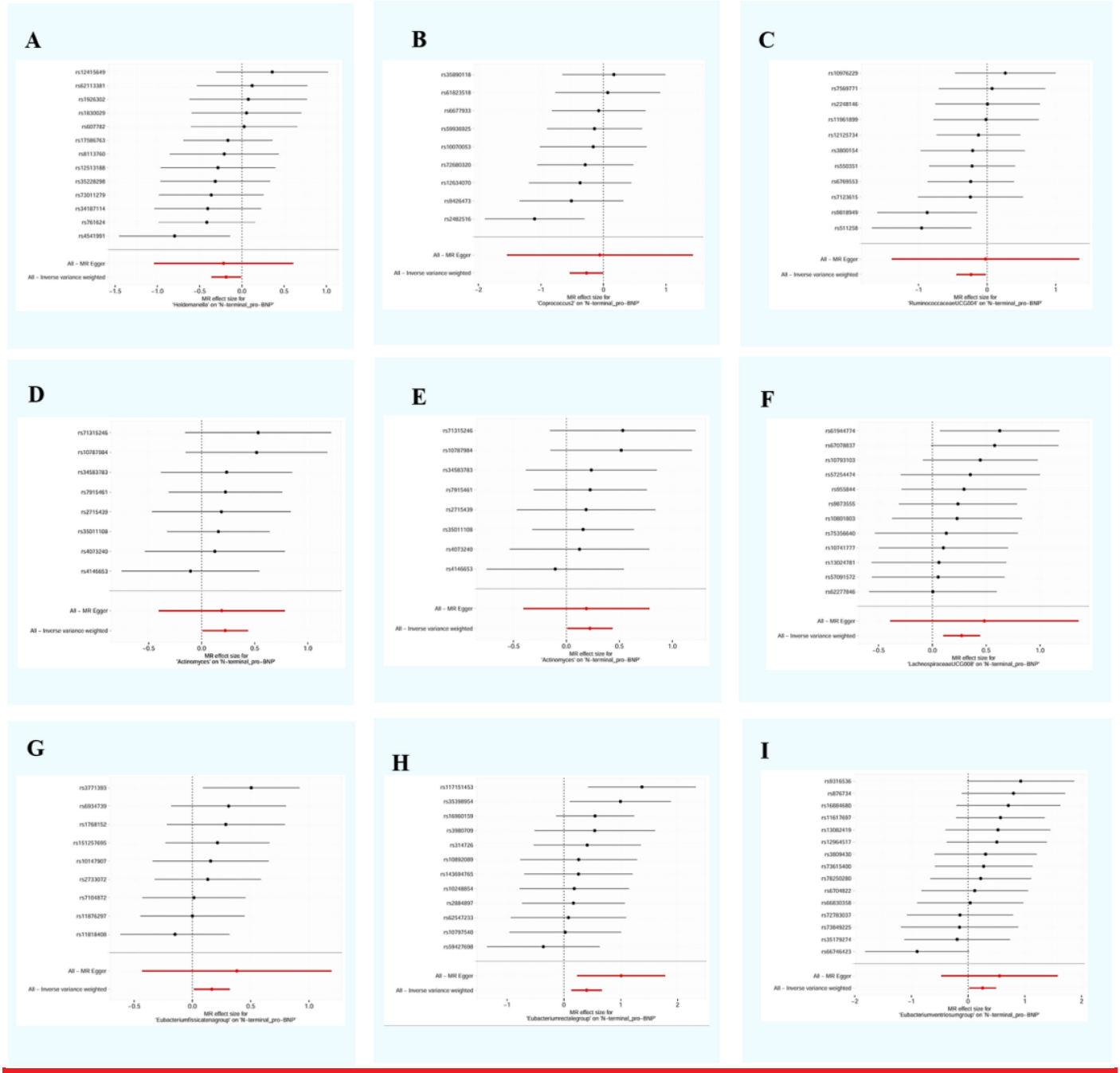

Figure 2. Forest plot of MR analysis results of GM versus NT-proBNP. A, *Holdemanella*; **B,** *Coprococcus* **2; C, Ruminococcaceae UCG 004; D,** *Actinomyces*; **E,** *Alistipes*; **F, Lachnospiraceae UCG 008; G,** *Eubacterium fissicatena* **group; H,** *Eubacterium rectale* **group; I,** *Eubacterium ventriosum* **group.**

findings, Wang et al. found that the structure of several GM, including *Holdemanella*, was associated with response to statins. Therefore the authors hypothesized that manipulating the composition of the GM may be a clinical treatment for controlling lipids in patients with coronary artery disease . However, no clinical studies have shown that regulating the genus *Holdemanella* can alleviate diseases such as heart failure caused by NT-proBNP. Reducing *Alistipes* promoted lipid reduction and delayed ventricular remodeling after myocardial infarction.[23] A randomized controlled study found that differential gut microbiome profiling could be used to identify patients with AF and that reduced *Alistipes* abundance was detected in patients with AF.[24] Additionally, the genus *Coprococcus* 2 was identified as a protective factor against atherosclerosis in another MR study,[25] and the genus Ruminococcaceae UCG 004 was also found to be a protective factor for AF flutter.[26] In summary, both GM and NT-proBNP are closely associated with cardiovascular diseases, and the genus *Holdemanella*, genus *Coprococcus* 2, genus Ruminococcaceae UCG 004, as well

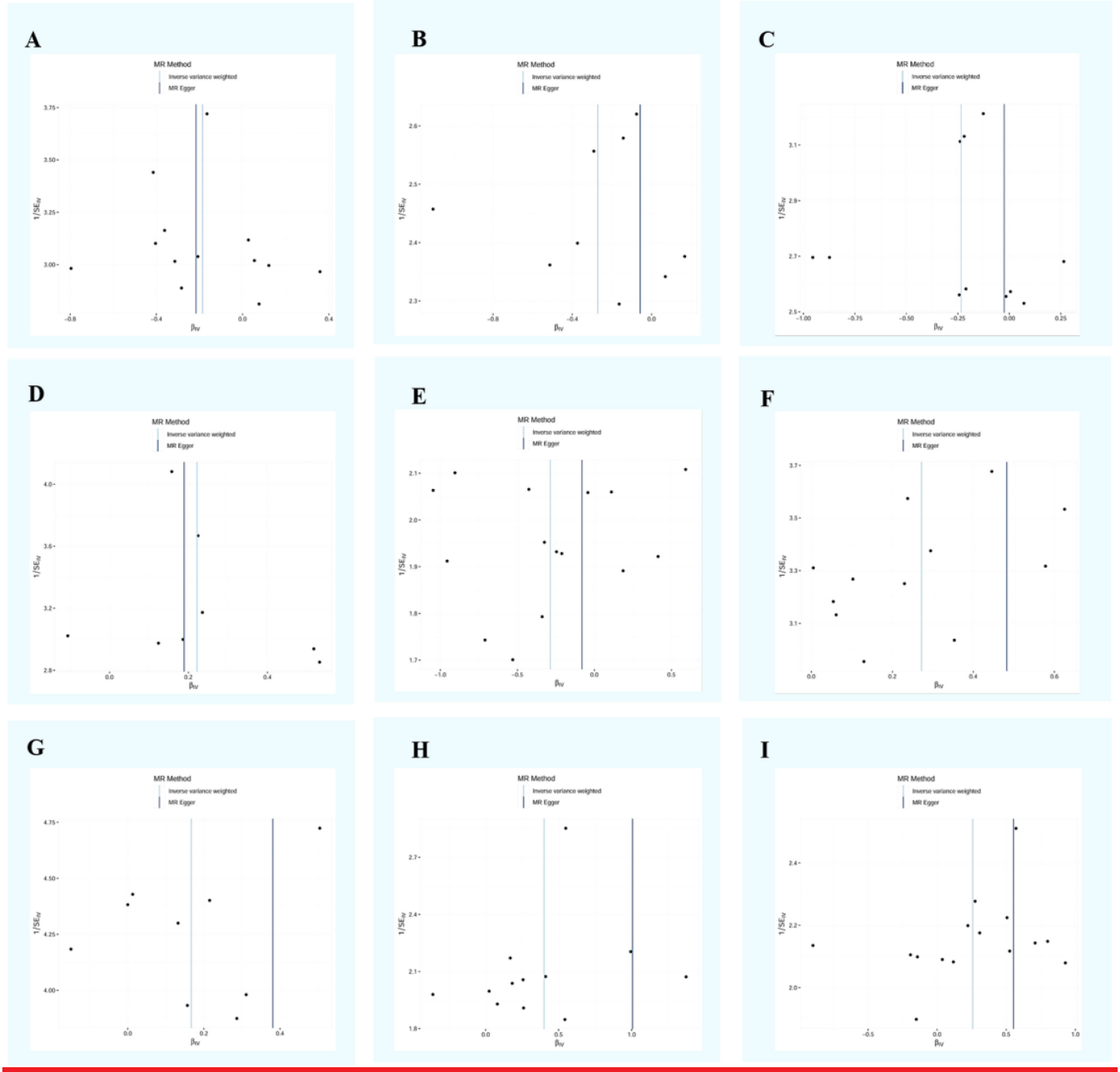

Figure 3. Funnel plot of the results of MR analysis of GM versus NT-proBNP. A, *Holdemanella*; **B,** *Coprococcus* **2; C, Ruminococcaceae UCG 004; D,** *Actinomyces*; **E,** *Alistipes*; **F, Lachnospiraceae UCG 008; G,** *Eubacterium fissicatena* **group; H,** *Eubacterium rectale* **group; I,** *Eubacterium ventriosum* **group.**

as genus *Alistipes* have been identified as increased factors linked to NT-proBNP. Therefore, modulating these genera of bacteria may help alleviate NT-proBNP-induced cardiovascular diseases. The GM can be regulated by diet and fecal transplants. Although the above GM has not been used clinically, the findings provide a research basis for preventing diseases such as heart failure by intervening in GM. It was found that the genera Actinomyces, Lachnospiraceae UCG 008, Eubacterium fissicatena group, Eubacterium rectale group, and Eubacterium ventriosum group were increased factors for NT-proBNP. The genera *Actinomycetes* and *Eubacterium rectale* group have also been associated with coronary artery disease as well as adverse cardiovascular events.[27,28] The genera *Actinomyces* was orally colonized in mice and was beneficial in alleviating pneumonia in mice.[29] The results illustrate that the hearts and central nervous systems of the colonized mice did not show any abnormalities, and it is speculated that colonizing Actinomyces genera may be a feasible strategy for preventing and controlling heart failure and other diseases. One previous study observed

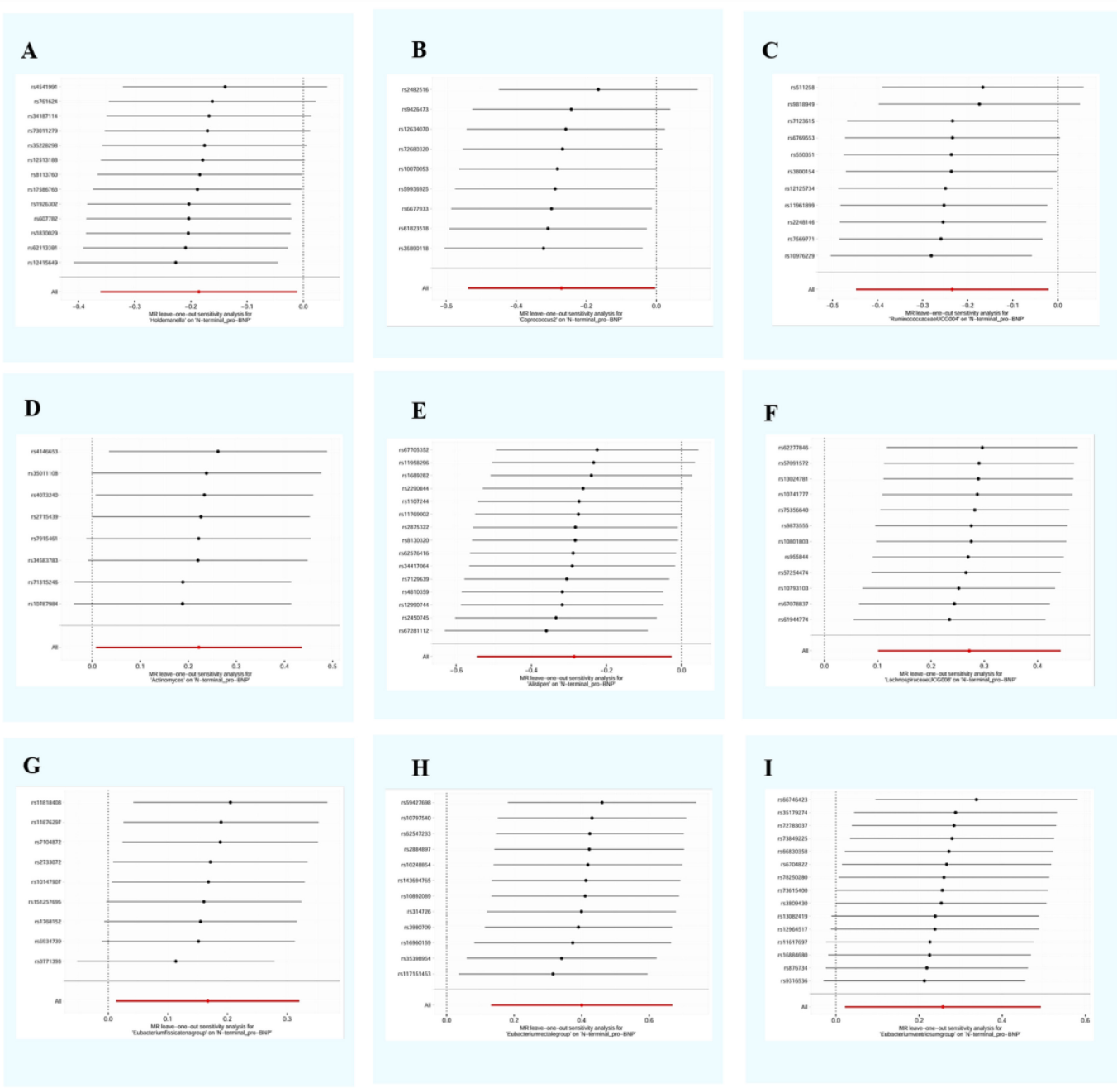

Figure 4. Plots of leave-one-out analysis of the results of MR analysis of GM versus NT-proBNP. A, *Holdemanella*; **B,** *Coprococcus* **2; C, Ruminococcaceae UCG 004; D,** *Actinomyces*; **E,** *Alistipes*; **F, Lachnospiraceae UCG 008; G,** *Eubacterium fissicatena* **group; H,** *Eubacterium rectale* **group; I,** *Eubacterium ventriosum* **group.**

significantly higher levels of *Eubacterium rectale* group in the gut flora of individuals in hypertensive group compared to the control group.[30] *Eubacterium ventriosum* group and NT-proBNP were found to be correlated and associated with myocardial infarction in a RCT.[31] Although no clinical trials have confirmed definitive associations between the aforementioned GM and NT-proBNP, based on the previous evidence, it can be hypothesized that an imbalance in GM may lead to NT-proBNP abnormalities, further exacebating cardiovascular diseases.

The NT-proBNP is an essential biomarker for diagnosing heart failure in clinical practice. The level of NT-proBNP increases with the severity of heart failure, and chronic heart failure patients often have intestinal dysfunction.[32] The GM can regulate and maintain intestinal and overall host health by releasing secretions. However, there is currently no direct evidence to suggest the mechanism by which GM regulates NT pro-BNP in the research findings. Studies have shown that GM products short-chain fatty acid (SCFA) can promote intestinal fermentation processes, which is beneficial

for the prognosis of heart failure patients.[33] Whereas in the results, genus *Holdemanella*, genus *Coprococcus* 2, genus Ruminococcaceae UCG 004, genus *Actinomyces*, genus Lachnospiraceae UCG 008, genus *Eubacterium fissicatena* group, genus *Eubacterium rectale* group, and genus *Eubacterium ventriosum* group all produce secreted SCFA. Therefore, it is hypothesized that these GMs can regulate the intestinal microenvironment through the production of secreted SCFA, thus further regulating NT-proBNP-induced heart failure and other diseases. In addition, *Actinomyces* metabolites include vitamin B12 and lactic acid. Patients with heart failure are often deficient in vitamin B12.[34] Arterial blood lactate concentration is positively correlated with NT-proBNP in patients with acute heart failure and both levels are abnormal[35] It is hypothesized that *Actinomyces* can regulate diseases such as heart failure caused by NT-proBNP through multiple pathways. The metabolites of *Eubacterium fissicatena* group also include secondary bile, and the primary and secondary bile acid pathways of GM are involved in cardiovascular and cerebrovascular diseases.[36] Elevated levels of NT-proBNP in people without manifestations of heart failure suggest a potential risk of cardiovascular diseases, such as CHD, hypertension, etc. They can be used for early screening and cardiovascular disease risk stratification.[37] The SCFA are involved in human blood pressure homeostasis.[38] In addition, specific bile acids regulate host lipid metabolism and glucose/insulin metabolism.[39] Therefore, it is hypothesized that GM regulates cardiovascular diseases caused by NT-proBNP. Although no study has shown the mechanism of action of the 9 GMs and NT-proBNP, it is speculated that these GMs may regulate the level of NT-proBNP through their own metabolites, which may be further involved in the progression of heart failure and other diseases. Of course, the above is only a simple speculation of the mechanism, and the specific mechanism of action needs to be further verified by experiments.

Although it has been suggested that the GM would be a potential therapeutic target for heart failure as it plays a key role in regulating host physiology and metabolism and in the development of heart failure. However, the discovery of 9 GMs with a causal relationship with heart failure has not been applied in the clinical setting. Only studies have used genera *Actinomyces* to mitigate disease progression by oral colonization in mice.[29] Dietary interventions, probiotic therapy, fecal microbiota transplantation, and antibiotics are all ways to regulate GM. As research continues, the prevention and treatment of disease through dietary modification and oral colonization will be one of the directions for clinical disease treatment.[40]

This study used large-scale GWAS data and MR methods to analyze the causal relationship between 104 GM classes and NT-proBNP. The present study, due to its large sample size, helped to reduce sampling error and was able to help exclude the effect of confounding factors, resulting in a more accurate assessment of the causal relationship between GM and NT-proBNP. In addition, the present study conducted several sensitivity analyses to provide comprehensive quality control on aspects such as multiplicity and heterogeneity of MR analyses, and thus, the conclusions obtained are robust.

The present study provides the most comprehensive assessment of the causal relationship between GM and NT-proBNP based on MR methods, but this study has some limitations. First, due to the limited current understanding of the etiology and mechanisms of abnormal NT-proBNP levels in humans, if genetic tools influence NT-proBNP through confounding variables other than exposure variables, it may introduce observational bias, leading to inaccurate causal estimates. Based on these potential confounding variables, an inherent limitation of the MR method, the results will also be validated using RCTs in the future. Second, there may be gene-environment interactions in the effect of SNPs on exposure factors, implying that SNPs may have a nonlinear impact on the risk of NT-proBNP abnormalities; however, MR analyses can only assess linear associations and cannot evaluate the effect of extremes of exposure factors on disease. In addition, the results of the MR analyses in this study may not be generalizable to populations outside of European ancestry or populations residing in different geographic regions, as genetic heterogeneity and environmental factors vary between areas. This data was not analyzed for specific stratification by gender, age, dietary habits, etc. Finally, complex physiological mechanisms are involved in causing NT-proBNP abnormalities, and the MR analyses performed in this study may not fully capture this causal relationship. Therefore, if GM is to be regulated as a clinical measure to combat diseases such as heart failure caused by NT-proBNP abnormalities, it must be refined by completing higher-quality MR analyses or RCTs. In addition, this study could not point out the specific mechanisms by which GM exerts its effects on NT-proBNP, and further studies are needed to reveal the pathophysiological mechanisms underlying these causal relationships.

CONCLUSION

In summary, this study explored the causal relationship between GM and NT-proBNP applying MR analysis. Of note, this study indicates that regulating gut flora may potentially prevent and control NT-proBNP abnormalities, thereby reducing the risk of NT-proBNP-related heart failure and other diseases, which lays the foundation for further exploring the mechanism linking GM with heart failure and other diseases. It is also acknowledged that the study's focus on populations of European ancestry might limit the generalizability of the findings to other populations.

Ethics Committee Approval: This study was approved by the Ethics Committee of Tangshan Fengnan District Hospital (Approval no.: 20240426; Date: April 26, 2024).

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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