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Causal link between gut microbiota and osteoporosis analyzed *via* Mendelian randomization

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Abstract. – OBJECTIVE: Osteoporosis (OP) is closely associated with gut microbiota (GM), yet the nature of their causal relationship remains elusive. Therefore, this study aims to reverse causality between GM and OP by using population cohorts and two-sample MR (TSMR) analysis.

MATERIALS AND METHODS: In this study, we conducted an extensive genome-wide association study (GWAS) using publicly accessible summary statistics data for GM and OP. Employing rigorous criteria ($p < 1*e-5$), we identified independent genetic loci that exhibited significant associations with GM relative abundances as instrumental variables (IVs). A causal evaluation was primarily carried out using the inverse variance-weighted (IVW) method, supplemented by additional analyses such as MR-Egger, weighted median, simple mode, and weighted mode.

RESULTS: We unveiled that increased abundances of the family *Pasteurellaceae*, order Pasteurellales, and genus *Ruminococcaceae UCG004* were linked to an increased risk of OP. Conversely, the family Oxalobacteraceae, unknown family id.1000006161, genus *Lachnospiraceae* NK4A136 group, unknown genus id.1000006162, and order *NB1n* were associated with a reduced risk of OP. To ensure the reliability of our findings, we conducted quality assessments through Cochrane's Q test and a leave-one-out analysis. Furthermore, the stability and consistency of the results were confirmed by the MR-Egger intercept test, Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) global test, and sensitivity analysis ($p > 0.05$). Our study reveals the causal relationships between 211 GM taxa and OP, pinpointing specific GM taxa associated with the risk of OP. This research sheds light on the genetic mechanisms that underlie GM-mediated OP and opens up promising avenues for identifying valuable biomarkers and potential therapeutic targets in future OP research.

CONCLUSIONS: This study establishes a substantial GM-OP link with specific taxa being identified, offering biomarkers for early detection, tailored interventions, and improved patient education. These findings enhance OP diagnosis, prevention, and treatment, promising more effective, individualized care and inspiring future research.

Key Words:
Mendelian randomization, Gut microbiota, Osteoporosis, Causal relationship, Genetic analysis.

Introduction

Osteoporosis (OP) is a systemic bone metabolism disease characterized by decreased bone density, disrupted bone microstructure, and increased risk of fragility fractures. It has become one of the major public health issues worldwide[1]. OP is closely related to the aging of the population and its prevalence is increasing year by year, with females having a four-fold higher incidence than males. According to the World Health Organization (WHO), more than 200 million people worldwide suffer from OP, which causes 8.9

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million fractures annually[2]. In the United States, over 10 million people undergo OP[3]. In Europe, OP is one of the most common bone diseases among the elderly population and causes over 3 million fractures annually[4]. Fragility fractures caused by OP have brought a significant and increasingly growing economic burden to healthcare systems worldwide[5]. The etiology of OP is not yet fully understood, but it may be related to genetic factors, changes in hormone levels, nutrient deficiencies, gut dysbiosis, diseases and drug effects, and unhealthy lifestyles[6,7].

The human gastrointestinal tract is colonized by more than 1,013-14 bacteria, and the gut microbiota (GM) contains approximately 150 times more genes than the human genome. The gut microbial ecosystem is the richest one in the human body. The interaction between the microbial population and the host plays a crucial role in regulating the host's metabolism and immune system and is a key factor that influences health[8]. Gut dysbiosis has been associated with various diseases, including diabetes, obesity, Alzheimer's Disease, rheumatoid arthritis, and multiple sclerosis[9-11]. Depletion of GM might simultaneously affect the gut and the central nervous system through 5-hydroxytryptamine (5-HT), indicating the existence of a gut-brain-bone-axis[12]. In recent years, the study of the relationship between the GM and OP has become a hot topic in scientific research. Investigating the association between GM and bone health, including the gut-bone axis and the role of gut-derived hormones, holds promise for uncovering novel strategies for preventing and treating osteoporosis, particularly in the elderly. This is a field with the potential to improve population health, especially as the aging population grows[13]. GM is a regulator of bone mass, and some scholars[14] have suggested that the inhibitory effect of GM on bone mass is mediated by its impact on the immune system.

Studies[15] have shown that GM regulates bone metabolism through various pathways. GM can affect the production of osteocalcin and cytokines such as interleukins by B cells and Treg cells, and regulate the differentiation of osteoclasts and bone resorption. In addition, GM can also regulate bone metabolism through nucleotide-binding oligomerization domains (NOD), and Wnt/β-catenin signaling pathways[16,17]. Specific inactivation of NOD1 or NOD2 in germ-free (GF) mice did not significantly increase cortical bone mass, while tumor necrosis factor and receptor activator of nuclear factor-kappa B ligand (RANKL) were lower than normal levels, indicating that the regulation of GM on bone mass depends on NOD1 and NOD2 signaling[18]. Leptin, low-density lipoprotein receptor-related protein 5 (LRP5) gene, and transcription factor FOXO1 have also been found to be associated with the regulation of 5-HT by the GM in the anabolic therapy of OP[19].

These studies[18,19] provide some references for relevant human clinical trials. Microbial fermentation of dietary fiber can produce short-chain fatty acids (SCFAs), which can promote the production of serum insulin-like growth factor-1 (IGF-1), thereby regulating bone mass and metabolism[20]. Recent literature have found that gut dysbiosis may alter Th17/Treg immune responses and promote osteogenesis, changing the migration of mononuclear cells and lymphocytes in tissues. Guo et al[21] found that *Lactobacillus rhamnosus* GG (LGG) treatment had a protective effect in an ovariectomized (OVX) rat model. Therefore, we speculate that gut dysbiosis may be one of the reasons leading to the occurrence and aggravation of OP. There may be a causation between them. After all, different study designs may lead to different conclusions, and the human gastrointestinal tract is influenced by various factors, including diet and rest. Therefore, it is necessary to establish research on the relationship between GM and OP.

Mendelian randomization (MR) is a statistical method used in epidemiological inference to uncover causal relationships between exposure and outcomes. It utilizes genetic variation associated with the exposure as a proxy to evaluate the association between the exposure factor and the outcome[22]. Based on the rich harvest of large-scale genome-wide association studies (GWASs) at the genetic and disease levels, MR analysis has been widely applied in various disease studies[23-25]. This study aims to use population cohorts and two-sample MR analysis to reverse causality between GM and OP. The mechanism of using MR to analyze the causal relationship remains unexplored. It is expected that these approaches will further promote the research and development of GM and OP and provide new insights for microbiology and related fields.

Materials and Methods

Research Methodology

This study investigated the causal link between 211 GM taxa and OP using a TSMR anal-

ysis. We adhered to the MR-STROBE guidelines (**Supplementary Table I**)[26] and relied on existing publicly available summary data, eliminating the need for extra ethical approval. The MR analysis must follow three key assumptions: strong association between instrumental variables (IVs) and GM, lack of IVs' association with confounding factors, and IVs' impact solely through the GM pathway referred to in our previous research methods[9]. Detailed methodology is provided in Figure 1.

Genome-Wide Association Study (GWAS) Data Sources

We collected data for this OP study from the UK Biobank (https://gwas.mrcieu.ac.uk/, id: ukb-a-87, accessed on 2 May 2023), a well-established Biobank in the UK with nearly 500,000 participants. This extensive database includes genetic information, as well as comprehensive data on demographics, socioeconomic factors, lifestyle, and health for its participants[27]. We chose a more recent GWAS with a large sample size

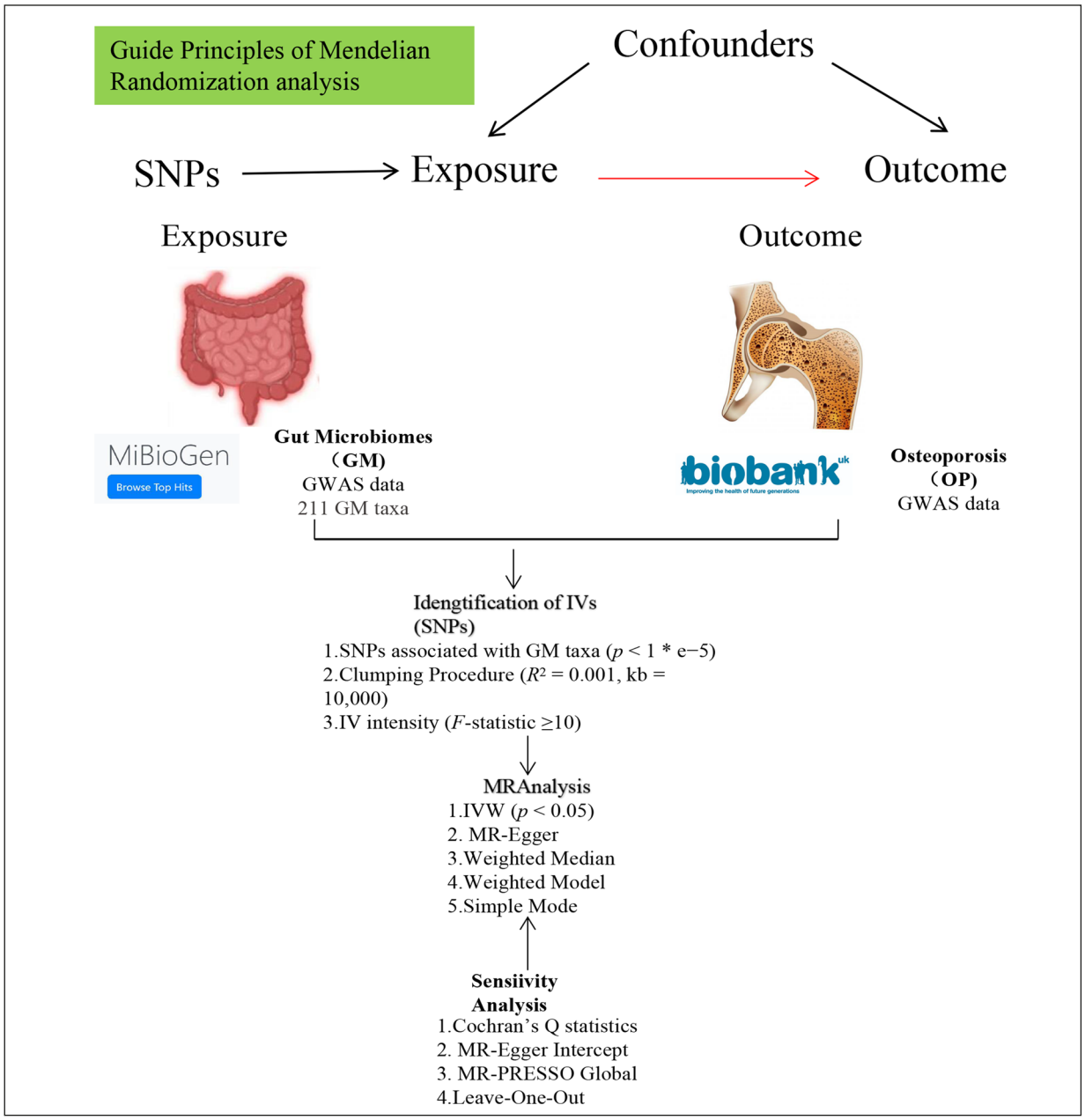

Figure 1. Study flow.

single nucleotide polymorphism (SNPs), and the phenotype "osteoporosis, strict definition" within the UK Biobank on OP cases (5,266) and controls (331,893) identified using the ICD-10 code M81.0 (ID: ukb-a-87).

For the analysis of 16S rRNA gene sequencing profiles and genetic typing data with GM, we utilized data from the latest GWAS summary data provided by the MiBioGen consortium (www.mibiogen.org, accessed on 2 May 2023). This dataset includes 18,340 individuals from 24 European cohorts, spanning 211 taxa and 122,110 variant sites[28]. Our GM study examined 16S rRNA gene regions (V4, V3-V4, V1-V2), using microbiota quantitative trait loci (mbQTL) mapping to spot genetic variations linked to microbial taxa abundance. In summary, our research drew upon the UK Biobank for OP genetic data and the MiBioGen consortium for GM data, allowing us to perform a comprehensive analysis of both aspects.

Instrumental Variables Identification

We aimed to evaluate the association between IVs and GM with a significance threshold set at $p < 1*e{-}5$. The methodology employed can be summarized as follows based on previous studies[29]:

- Relevant Single nucleotide polymorphisms (SNPs) were extracted using the TSMR package in *R* software (version 4.2.0, The *R* Foundation for Statistical Computing, Nashville, TN, USA).
- SNPs were subjected to clustering to mitigate the impact of linkage disequilibrium (LD), with LD status assessed using cumulative explained variance (r^2) and kb values. SNPs meeting the criteria of $r^2 < 0.001$ within a 10,000 kb window were selected.
- Echo SNPs were excluded from the analysis.
- The remaining SNPs were derived from a combination of *p*-value filtering removal of chain imbalances.
- The PhenoScanner database was utilized to retrieve significant results from GWAS data ($p < 1*e{-}5$), while simultaneously eliminating potential confounding factors (http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner, on 2 May 2023)[30].
- The IVs strength was estimated for each GM taxon using the *F*-statistic, calculated as:

$$F = \frac{R^2 \times (N\text{-}1\text{-}k)}{(1\text{-}R^2) \times k}$$

$$R^2 = 2 \times AF \times (1\text{-}MAF) \times \left(\frac{ß}{sd}\right)$$

Here MAF represents the minimum allele frequency, β is derived from the exposed GWAS, sd denotes its variance ($sd = se \times \sqrt{N}$), N signifies the total sample size in the exposed GWAS, and *k* indicates the number of IVs[31].

Statistical Analysis

We used the TSMR method to examine the potential causal link between GM and OP. We primarily employed the random-effects inverse variance weighted (IVW) analysis, along with supplementary methods such as the weighted median, MR-Egger, Robust IVW, and Robust MR-Egger, to ensure robust results and account for outliers. To assess horizontal pleiotropy, we conducted MR-Egger intercept tests and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) tests. Heterogeneity was evaluated using Cochran's Q and I^2 values, with IVs having *p*-values lower than 0.05 considered heterogeneous.

Our rationale for these statistical tests is as follows:

- IVW provides an overall effect estimate by weighted averaging of all IVs, assuming they meet MR assumptions, and it is more powerful without horizontal pleiotropy[32].
- The weighted median method is robust and reduces outlier influence[33].
- MR-Egger handles IVs correlation but demands careful IV selection. It detects and corrects horizontal pleiotropy[34].
- MR-PRESSO methods identify and correct outliers and anomalies, ensuring robust results[35].
- We conducted leave-one-out MR analyses for sensitivity testing[36].

We utilized *R* Studio, with various *R* packages for statistical analysis, setting a significance threshold of $p < 0.001$. These analyses were conducted in January 2023. Funnel and forest plots were generated to detect pleiotropy.

Results

Screening Selection of IVs and Initial MR Analysis Outcomes

A total of 2,551 SNPs were identified as IVs for 211 GM taxa. These IVs were categorized

into five different levels based on their taxonomic classification (Phylum, Class, Order, Family, and Genus). Eight microbial taxa were isolated based on the IVW method, with both a p-value of < 0.05 and an F-statistics > 10 for these positive IVS, indicating no evidence of weak instrument bias. In the heterogeneity test, the p-values for Cochran's Q statistic were all higher than 0.05, indicating the absence of heterogeneity among the SNPs (**Supplementary Table II** presents the confounder factors.).

Subsequently, we employed the fixed-effects IVW model as the primary analytical approach for MR analysis. The distribution of IVs across these levels was as follows: 31 IVs in 2 orders, 46 IVs in 3 families, and 1,372 IVs in 3 genera. It is worth noting that all IVs exhibited a stronger association with exposure compared to the outcome, as indicated by the p-value for exposure being less than that for the outcome (exposure < outcome). The systematic collection of essential information on SNPs is crucial for subsequent analysis. The essential SNP information, such as effect allele, another allele, SE, and p-value were gathered carefully and systematically to ensure accuracy and consistency in the analysis. It provides more detailed information regarding the identified related IVs.

The Impact of GM on OP From a MR Analysis

Using five MR research methods, including IVW, we causally identified eight microbial taxa associated with OP. Results suggest that family *Pasteurellaceae* [odds ratio (OR) = 1.002, p = 0.030], order *Pasteurellales* (OR = 1.002, p = 0.030), and genus *Ruminococcaceae UCG004* (OR = 1.003, p = 0.040) may increase OP risk. Conversely, family *Oxalobacteraceae* (OR = 0.998, p = 0.040), unknown family id.1000006161 (OR = 0.998, p = 0.015), genus *Lachnospiraceae NK4A136* group (OR = 0.996, p = 0.003), unknown genus id.1000006162 (OR = 0.998, p = 0.015), and order *NB1n* (OR = 0.998, p = 0.015) are negatively associated with OP, indicating reduced risk (Figure 2, Table I, **Supplementary Table III**).

Quality Control Methods

Scatter plots can assist in identifying outliers or data points that deviate from the norm. As depicted in Figure 3, it is evident that the directions calculated for the family *Lachnospiraceae NK4A136* group, family *Oxalobacteraceae*, and genus *Ruminococcaceae UCG004* are consistent with the expected values. Moreover, the funnel plot also eliminates the potential influence of outliers. Due to the superior statistical power of the IVW method, our findings primarily rely on IVW estimation. No evidence of horizontal pleiotropy was found in the results.

Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) can automatically identify and remove outliers and then recalculate the causal effects. We used MR-PRESSO to further validate the statistical results of the causal relationship between GM and OP. Our results demonstrate that both the MR-Egger intercept test and the MR-PRESSO global test indicate a low possibility of horizontal pleiotropy (p > 0.05). Additionally, the leave-one-out analysis further confirms the robustness of the data (Figure 4). This demonstrates that, in the absence of heterogeneity and pleiotropy, the results from the IVW method are reliable (See sensitivity analysis in Table II).

Discussion

To the best of our knowledge, this is the first study to explore the causal relationship between the relative abundance of 211 gut microbiota species and OP using gut microbiota genome-wide association study (GWAS) data from public databases and OP GWAS data. Using TSMR analysis, we assessed the potential causal relationship between transgenic populations and OP from a host genetic perspective, confirming their impact on OP susceptibility. Our findings discovered the causal relationship between GM and OP at the genetic level. Specifically, two families, one order, and two genera were found to be associated with low OP risk, while one family, one order, and one genus were associated with high OP risk. These findings may contribute to the future discovery of novel biomarkers in OP. These results may contribute to the future discovery of novel biomarkers for OP and provide new insights for intervention and treatment approaches by targeting specific GM populations to address ecological imbalances in OP. There is evidence to suggest that GM may have an impact on OP by modulating bone metabolism[26]. Microbial products such as SCFA can stimulate bone formation while inhibiting bone resorption[37]. Additionally, GM can influence bone metabolism through mechanisms such as increasing cell proliferation and differen-

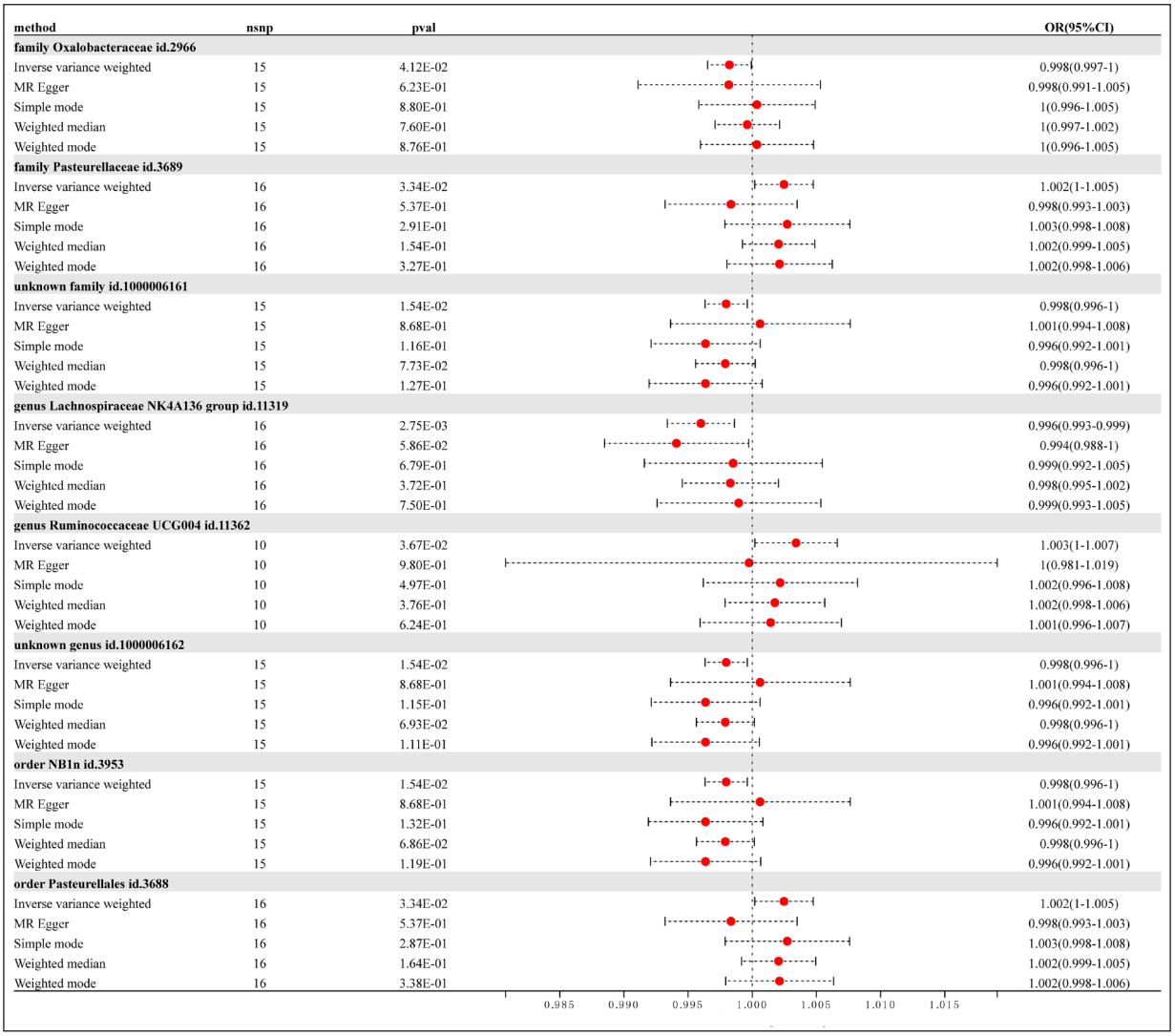

Figure 2. Forest plots depicting the results of five MR methods (Inverse variance-weighted , MR Egger, weighted median, simple mode and weighted mode) to analyze the causal relationship between GM and OP.

tiation, regulating the immune and nervous systems, and affecting hormone levels[13,38]. Molecular products produced by intestinal bacteria can have both beneficial and harmful effects. It is known that they can influence endocrine cells, the enteric nervous system, intestinal permeability, and the immune system in the gut. The composition of GM undergoes changes as individuals age, with particularly significant variations observed in individuals aged 65 and above, which coincides with a period of high incidence of OP[39]. GM serves as a potential source of antigens for the host immune system. However, the relationship between GM and OP is not yet fully understood. Recent studies, such as the work conducted by Yu et al[40], have indicated that GM can promote the expansion of bone marrow T cells and increase tumor necrosis factor-alpha (TNF-α) production, suggesting that intestinal T cells could be used as targets for bone loss-associated sex steroid deficient mice. GM composition may also be involved in regulating bone mass reduction in postmenopausal women. Furthermore, the use of various types of probiotics has shown promise in preventing bone loss caused by ovariectomy[41]. Metabolic cofactor patterns can ameliorate bone loss by modulating gut-bone axis participants, such as probiotics, which may reverse GM-induced inflammation, enhance bone density, and improve bone quality. Probiotics can regulate the

Table I. Causal analysis of MR results for GM *vs.* OP ($p < 1 \times 10^{-5}$).

Classification		No. of SNP	MR analysis method	SE	*p*-value	OR (95% CI)	*F*
Family	*Oxalobacteraceae*	15	Inverse variance weighted	0.0008	0.0412	0.998 (0.997-1)	97.426
			MR Egger	0.0036	0.6228	0.998 (0.991-1.005)	
			Simple mode	0.0023	0.8795	1 (0.996-1.005)	
			Weighted median	0.0013	0.7602	1 (0.997-1.002)	
			Weighted mode	0.0022	0.8762	1 (0.996-1.005)	
	Pasteurellaceae	16	Inverse variance weighted	0.0012	0.0334	1.002 (1-1.005)	61.793
			MR Egger	0.0026	0.5365	0.998 (0.993-1.003)	
			Simple mode	0.0025	0.2909	1.003 (0.998-1.008)	
			Weighted median	0.0014	0.1544	1.002 (0.999-1.005)	
			Weighted mode	0.0021	0.3274	1.002 (0.998-1.006)	
	unknown family id.1000006161	15	Inverse variance weighted	0.0008	0.0154	0.998 (0.996-1)	92.765
			MR Egger	0.0036	0.8683	1.001 (0.994-1.008)	
			Simple mode	0.0022	0.1158	0.996 (0.992-1.001)	
			Weighted median	0.0012	0.0773	0.998 (0.996-1)	
			Weighted mode	0.0023	0.1275	0.996 (0.992-1.001)	
Genus	*Lachnospiraceae NK4A136 group*	16	Inverse variance weighted	0.0013	0.0028	0.996 (0.993-0.999)	34.490
			MR Egger	0.0029	0.0586	0.994 (0.988-1)	
			Simple mode	0.0035	0.6786	0.999 (0.992-1.005)	
			Weighted median	0.0019	0.3720	0.998 (0.995-1.002)	
			Weighted mode	0.0033	0.7501	0.999 (0.993-1.005)	
	Ruminococcaceae UCG004	10	Inverse variance weighted	0.0016	0.0367	1.003 (1-1.007)	47.311
			MR Egger	0.0098	0.9802	1 (0.9817-1.019)	
			Simple mode	0.0031	0.4968	1.002 (0.996-1.008)	
			Weighted median	0.0020	0.3763	1.002 (0.998-1.006)	
			Weighted mode	0.0028	0.6241	1.001 (0.996-1.007)	
	unknown genus id.1000006162	15	Inverse variance weighted	0.0008	0.0154	0.998 (0.996-1)	92.765
			MR Egger	0.0036	0.8683	1.001 (0.994-1.008)	
			Simple mode	0.0022	0.1149	0.996 (0.992-1.001)	
			Weighted median	0.0012	0.0693	0.998 (0.996-1)	
			Weighted mode	0.0022	0.1110	0.996 (0.992-1.001)	
Order	*NB1n*	15	Inverse variance weighted	0.0008	0.0154	0.998 (0.996-1)	92.765
			MR Egger	0.0036	0.8683	1.001 (0.994-1.008)	
			Simple mode	0.0023	0.1322	0.996 (0.992-1.001)	
			Weighted median	0.0011	0.0686	0.998 (0.996-1)	
			Weighted mode	0.0022	0.1191	0.996 (0.992-1.001)	
	Pasteurellales	16	Inverse variance weighted	0.0012	0.0334	1.002 (1-1.005)	61.793
			MR Egger	0.0026	0.5365	0.998 (0.993-1.003)	
			Simple mode	0.0025	0.2874	1.003 (0.998-1.008)	
			Weighted median	0.0015	0.1641	1.002 (0.999-1.005)	
			Weighted mode	0.0021	0.3383	1.002 (0.998-1.006)	

Mendelian randomization (MR), gut microbiota (GM), osteoporosis (OP), single nucleotide polymorphism (SNP).

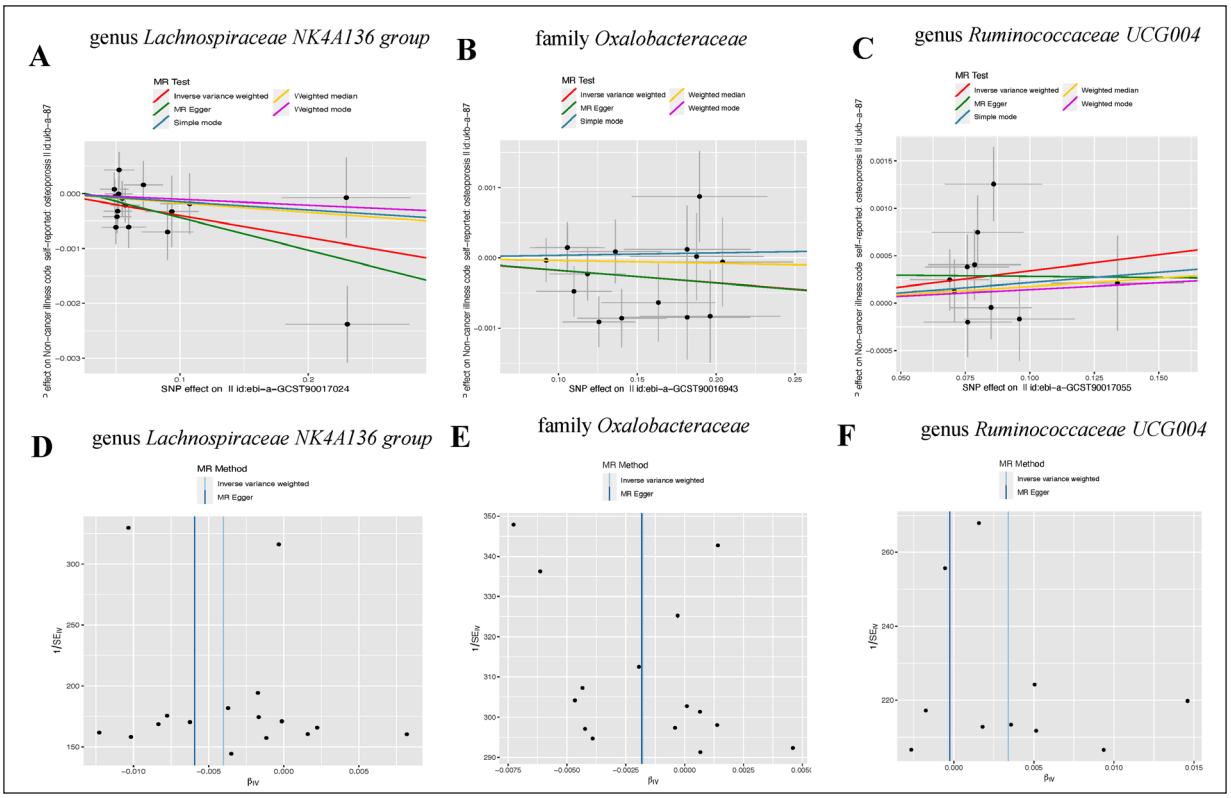

Figure 3. Scatter (**A-C**) and funnel (**D-F**) plots of the causal relationship between GM and OP. In scatter plots, the different lines represent various statistical methods as follows: The red line represents the Inverse variance-weighted method. The green line represents the MR Egger method. The blue line represents the Simple mode. The yellow line represents the weighted median. The purple line represents the weighted mode. In funnel plots, the different lines represent various statistical methods as follows: the deep blue line represents the Inverse variance-weighted method, while the light blue line represents the MR Egger method.

immune activity, including Insulin-like growth factor-1 (IGF-1), TNF-α, and interleukin 1-B (IL-1β) while stimulating IL-7 and interferon-gamma (IFN-γ)[42].

Pasteurellaceae is a Gram-negative bacterial family, including *Haemophilus influenzae*, causing respiratory infections in humans, and pathogenic to animals. It is relevant in bone research, particularly in osteomyelitis[43]. Research[44] has found a causal relationship between microbial communities and bone development in twins, including *Pasteurellaceae* and *Lachnospiraceae*, and identified specific bacterial groups that regulate changes in bone mass. Research[45,46] indicates that *Pasteurellaceae* may promote bone resorption by stimulating osteoclast function. Bacterial components stimulate the formation and activation of osteoclasts, leading to the destruction of bone tissue. Infection with *Pasteurellaceae* triggers an inflammatory response, disrupting normal bone metabolism and increasing the risk of bone loss. Certain bacterial metabolites induce apoptosis in bone cells and interfere with normal cellular signaling pathways[45,46].

Ruminococcaceae is a family of intestinal bacteria that belongs to the anaerobic group and is predominantly found in the intestines of humans and animals[47]. There is currently ongoing debate regarding the relationship between *Ruminococcaceae* and bone health.

Research indicates[48] that there is a correlation between OP and the β-diversity, taxonomy, and functional composition of the GM. The abundance of *Ruminococcaceae* is negatively correlated with the presence of OP. Additionally, another study[49] arrived at a similar conclusion, indicating that OP affects the composition of the GM. Treatment with parathyroid hormone can regulate microbial metabolic function, increase the abundance of various GMs, including *Ruminococcaceae*, and thereby reduce bone loss. Interestingly, contrary to these researches, a study on human

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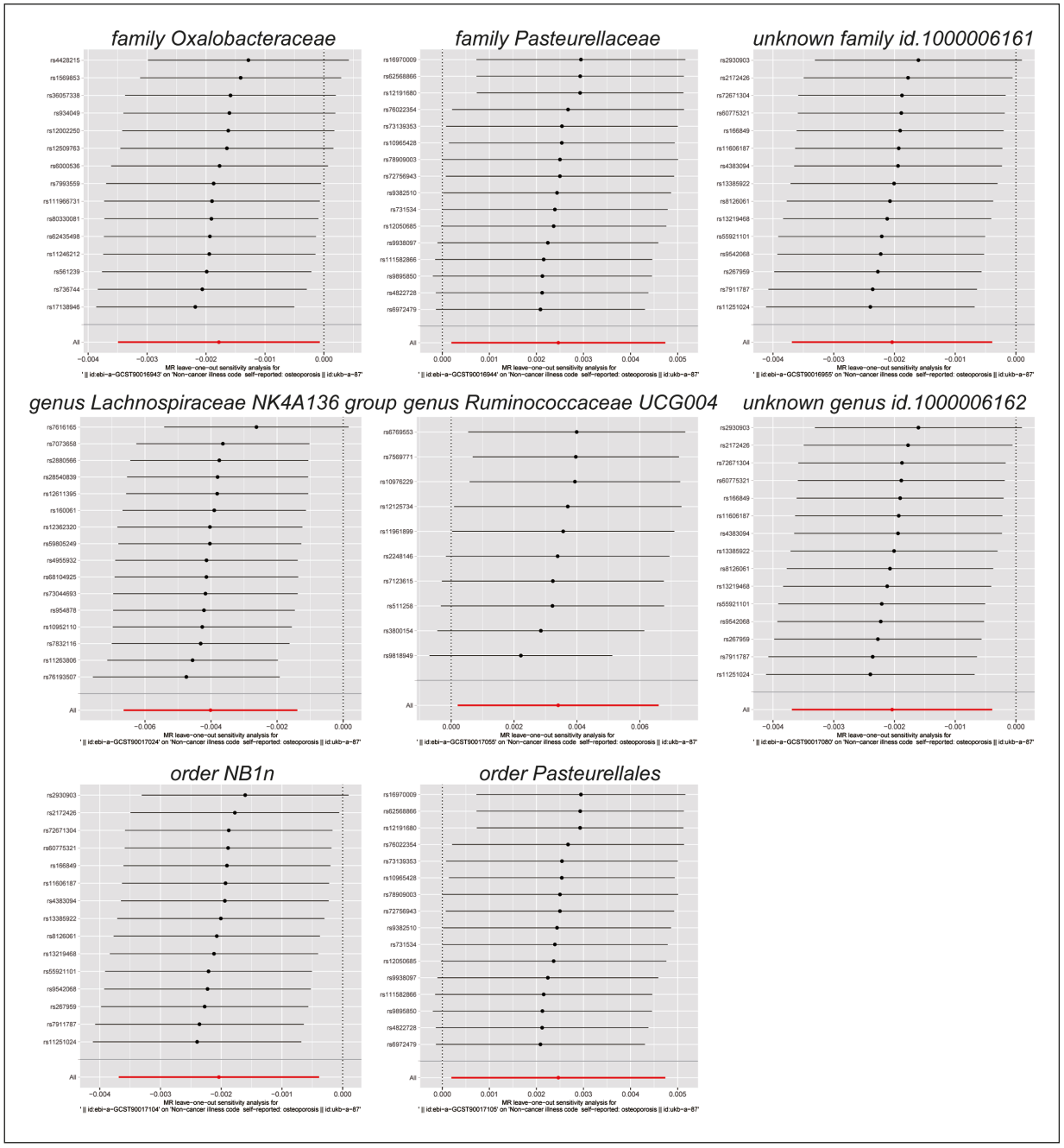

Figure 4. Leave-one-out analysis of GM on OP. Mendelian randomization (MR), gut microbiota (GM), osteoporosis (OP).

immunodeficiency virus (HIV)-infected women found that women with lower bone mineral density (BMD) had a higher relative abundance of *Ruminococcus*, which differed significantly from women with normal BMD[50]. Furthermore, another study using fecal 16S rRNA gene sequencing diversity analysis found a significant increase in the abundance of the genera *Blautia*, *Eubacterium*, and *Ruminococcaceae* family in the OP group compared to the control group. Population differences and dissimilar experimental design methods may have contributed to the opposite results[51]. The results of the latter two studies[52,53] collectively indicate that *Ruminococcus* may be one of the biomarkers promoting bone loss, which is consistent with our research findings. Further functional studies are needed in the future to validate this result. The mechanisms by which the

Table II. Sensitivity analysis of MR results for GM *vs*. OP.

	GM exposure	No. of SNP	MR Egger interpreter	Steiger approach	MR-PRESSO global test	Cochran 'Q
			p-value	*p*-value	*p*-value	*p*-value
Family	*Oxalobacteraceae*	15	0.989	4.79E-67	0.364	0.349
	Pasteurellaceae	16	0.106	4.50E-77	0.151	0.135
	unknown family id.1000006161	15	0.460	7.02E-66	0.566	0.554
Genus	*Lachnospiraceae NK4A136 group*	16	0.463	4.70E-68	0.329	0.365
	Ruminococcaceae UCG004	10	0.714	9.52E-44	0.219	0.2110
	unknown genus i d.1000006162	15	0.460	7.02E-66	0.577	0.554
Order	*NB1n*	15	0.460	7.02E-66	0.583	0.554
	Pasteurellales	16	0.106	4.50E-77	0.152	0.135

Mendelian randomization (MR), gut microbiota (GM), osteoporosis (OP), single nucleotide polymorphism (SNP), Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO).

Ruminococcaceae family regulates bone metabolism may be related to the fermentation of dietary fibers, which produce SCFAs. These SCFAs promote the activation of osteoblasts while inhibiting the activity of osteoclasts, thereby exerting a positive regulatory effect on bone metabolism. Furthermore, the synthesis of nutrients such as vitamin K by the *Ruminococcaceae* family may have a beneficial impact on bone metabolism[52,53].

Research suggests that a high-fiber diet is associated with a reduction in the levels of *Oxalobacteraceae*[54]. On the other hand, a fiber-rich diet helps reduce the risk of OP. Therefore, we speculate that *Oxalobacteraceae* may regulate bone health through diet pattern[55].

Lachnospiraceae is an important member of the GM. They have the ability to degrade cellulose and other complex polysaccharides in the diet, producing beneficial SCFAs such as propionic acid, butyric acid, and acetic acid. These SCFAs serve as crucial energy sources for intestinal epithelial cells and also play a regulatory role in the immune system[56].

GM dysbiosis has been found to be associated with the development of arthritis. Research[57] has discovered that a high-fiber diet can effectively mitigate arthritis and bone erosion while simultaneously increasing T-cell and IL-10 levels. Moreover, this dietary intervention exerts its impact by inducing changes in the gut microbiota, including the modulation of the *Lachnospiraceae NK4A136 group*, ultimately resulting in elevated levels of SCFAs. In an OVX rat model experiment, it was found that the abundance of *Lachnospiraceae bacterium 10 1* and *Lachnospiraceae bacterium A4* significantly increased[58]. Another study[59] revealed that *Lachnospiraceae* were more abundant in individuals with low BMD, and it was positively correlated with BMD and T-score. This phenomenon may be related to pathways associated with lipopolysaccharide (LPS) biosynthesis. Gushudan (GSD), a traditional Chinese herbal formula for the treatment of OP, was studied[60] regarding its pharmacological mechanisms on bone metabolism. It was found that GSD can increase the abundance of bacteria *g_Lachnospiraceae_nk4a136_group*, and lactate-producing bacteria *Lactobacillus*. Therefore, *Lachnospiraceae* might have bone-protective effects, which is consistent with the conclusions of our study.

This study has some highlights: we first delved into the intricate causal relationship between GM and OP using a robust combination of GWAS data and MR analysis. By employing rigorous statistical methodologies, including MR-Egger and sensitivity tests, we uncovered specific GM taxa that either increase or decrease the risk of OP, thereby pinpointing precise genetic mechanisms involved in GM-mediated OP.

Limitations

Firstly, this study only utilized GWAS data from European populations for TSMR analysis, and further research is needed in other populations. Secondly, the quantity of GM abundance

data included in this study is limited. Although the GWAS dataset used is one of the largest and most up-to-date population cohorts, additional GWAS data on GM are required to comprehensively explore the causal relationship between GM and OP. Thirdly, although TSMR is an effective method for causal relationship analysis, future animal experiments are necessary to further validate the potential causal relationship between GM and OP. Lastly, the relationship between GM and OP is not solely a single causal relationship, and the investigation of the etiology and pathogenesis of OP should be approached from multiple perspectives.

Conclusions

In summary, we have confirmed the causal effect of GM on OP through TSMR. We discovered that the family *Pasteurellaceae*, order *Pasteurellales*, genus *Ruminococcaceae UCG004,* family *Oxalobacteraceae*, and genus *Lachnospiraceae NK4A136 group* were causally associated with OP. These strains may serve as potential novel biomarkers. Furthermore, those GMs, such as the *Oxalobacteraceae* family and genus *Lachnospiraceae NK4A136 group* negatively correlated with OP may hold promising prospects for the prevention and treatment of OP. This study provides insights into the genetic level of treatment and prevention of OP.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Availability of Data and Materials

All data used in this study are available in publicly available datasets. The GM data can be found here: MiBioGen consortium (www.mibiogen.org, accessed on 2 May 2023), and the OP data can be found here: UKB consortium (https: // gwas.mrcieu.ac.uk/, id: ukb-a-87, accessed on 2 May 2023).

Ethics Approval and Informed Consent

All data used in our study were based on existing publicly available summary data; no additional ethical approval was required.

Authors' Contribution

Huiqiong Zeng, Wei Liu, and Ye Zhang are responsible for conception and design. All authors are responsible for data curation. All authors are responsible for investigation. All authors are responsible for methodology. Huiqiong Zeng, Guan Li, and Ye Zhang are responsible for formal analysis. Kaixia Zhou, Aidong Li, and Ye Zhang are responsible for project administration. Aidong Li, Guan Li, and Kaixia Zhou are responsible for data analysis. All authors are responsible for resources. Guan Li, Aidong Li, and Ye Zhang are responsible for supervision. Huiqiong Zeng, and Ye Zhang are responsible for the writing-original draft. Huiqiong Zeng, and Wei Liu are responsible for the writing-review and editing. Approval of final manuscript: all authors.

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