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ORIGINAL ARTICLE

Association of genetically predicted blood metabolites with osteopenia in individuals over 60 years of age: A Mendelian randomization study

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ABSTRACT

Objectives: This study aims to investigate the causal relationship between genetically predicted blood metabolites and osteoporotic fracture risk in individuals aged over 60 years, focusing on their role in bone metabolism and osteoporosis (OP).

Materials and methods: Using Mendelian randomization (MR), we analyzed 1,400 blood metabolites selected for their involvement in metabolic and inflammatory pathways relevant to bone health. Bone mineral density (BMD) at the femoral neck served as a proxy for fracture risk, with reduced BMD defined as T-score ≤–1.0. Fourteen metabolites were associated with osteopenia, determined by T-score being lower than –1 at the femoral neck. The genome-wide association study (GWAS) data from the European Bioinformatics Institute (GCST005349) included 22,504 cases and 23.7 million SNPs from individuals of European ancestry aged ≥60 years. Genetic associations were evaluated using Inverse Variance Weighted (IVW), MR-Egger, and MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) methods.

Results: After stringent screening, 14 metabolites were significantly associated with OP risk ($p<0.05$, false discovery rate [FDR] <0.2). The AMP-to-alanine ratio (odds ratio [OR]=0.900, 95% confidence interval [CI]: 0.845-0.958) was protective, while tauro-beta-muricholate (OR=0.855), glycosyl-N-stearoyl-sphingosine (OR=1.065), and the mannose-to-glycerol ratio (OR=1.116) increased risk. Sensitivity analyses confirmed robust results without heterogeneity or pleiotropy.

Conclusion: This study identifies blood metabolites as potential causal markers for osteoporotic fracture risk, offering insights for risk assessment and prevention in the elderly.

Keywords: Blood metabolites, bone mineral density, fracture risk, genetic association, Mendelian randomization, osteoporotic fracture, osteoporosis.

Osteoporotic fractures represent a significant public health burden, with over 9 million new cases occurring annually worldwide-approximately one every three seconds.[1-3] These fractures, particularly in the hip, vertebrae, distal radius, and proximal humerus, are associated with high mortality rates, with hip fractures alone leading to a 20% mortality rate within the first year.[4] In China, osteoporosis (OP) affects about 15.7% of the elderly population, and this prevalence continues to rise with aging, contributing to an increasing incidence of osteoporotic fractures.[5] Early screening and the identification of high-risk individuals are essential to reduce fracture incidence and related mortality, highlighting the urgent need for reliable biomarkers to improve diagnosis and intervention strategies for OP.

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Bone mineral density (BMD) is commonly used as a surrogate marker for OP due to its strong association with bone strength and fracture risk, typically measured as area BMD in grams per square centimeter (g/cm^2).[6] While alternative

phenotypes such as bone geometry and strength indices have been explored, BMD remains the most widely studied genetic marker for OP.

Recent studies have pointed to blood metabolites as key regulators of bone density and disease progression.[7] Specific metabolites, such as hydroxyproline, Gly-Gly, and cystine, have been found to differ significantly between individuals with low and normal BMD.[8] These metabolites reflect changes in various biological pathways which are closely tied to bone health.[9,10] However, traditional cross-sectional studies of metabolites and BMD are often confounded by non-genetic factors, making it difficult to establish causal relationships.

To overcome these limitations, Mendelian randomization (MR) has emerged as a powerful tool for inferring causality by using genetic variants as instrumental variables (IVs). This method minimizes the influence of environmental factors and reverse causation, earning the description of nature's randomized-controlled trial.[11,12] The MR has recently been applied to study the relationship between blood metabolites and BMD. To illustrate, studies have identified metabolites linked to BMD and provided insights into the metabolic pathways that influence bone health.[13,14] However, these studies were limited in scope in several ways. First, they focused on a narrower range of metabolites, often examining only a few key metabolites rather than a comprehensive panel. Second, they did not specifically target elderly populations, which are at heightened risk for osteoporotic fractures.

Building on this foundation, in the present study, we aimed to identify novel biomarkers which could be useful in clinical assessments and osteoporotic fracture prevention and to advance our understanding of the mechanisms underlying OP development and management.

MATERIALS AND METHODS

This retrospective study was conducted at Shunyi Hospital, Beijing Traditional Chinese Medicine Hospital, Department of Orthopedics and Traumatology between January 2024 and May 2024. A total of 1,400 blood metabolites were summarized based on relevant literature accessible through the European Bioinformatics Institute (EBI) database.[15] These metabolites were identified and quantified in prior studies using high-throughput techniques, including mass spectrometry and nuclear magnetic resonance spectroscopy, and were selected based on their relevance to BMD and OP. Criteria for inclusion involved specific thresholds of association with BMD or OP and relevance to metabolic pathways related to bone health. More specifically, metabolites were screened based on their statistical significance in association with BMD. We applied a threshold of $p<5\times10^{-8}$ in genome-wide association study (GWAS) data to ensure that the metabolites had strong genetic correlations and relevance to bone health. Additionally, only metabolites which were relevant to bone metabolism and involved in metabolic or inflammatory pathways affecting BMD were included. This relevance was determined by examining the pathways in which the metabolites were implicated, with a focus on those previously associated with bone formation, resorption, and mineralization.

Bone mineral density at the femoral neck was used as a surrogate phenotype for OP and fracture risk, given its well-established association with fracture incidence in elderly populations. This specific BMD measure was chosen for its predictive value regarding osteoporotic fractures. The BMD GWAS data for this demographic were obtained from the EBI website (GCST005349), encompassing 22,504 cases and over 23.7 million single-nucleotide polymorphisms (SNPs), primarily derived from European populations over 60. By utilizing BMD as an indirect indicator of osteoporotic fracture risk, our MR analysis aims to identify genetic and metabolic markers relevant to osteoporotic fractures prevention in high-risk groups.

Screening of blood metabolites

In this study, we began with an initial pool of 1,400 blood metabolites identified through high-throughput techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy. To screen for potential metabolic indicators affecting BMD, we applied the following criteria:

1. *Statistical significance:* Metabolites were initially screened based on GWAS data, retaining those with significant associations ($p<5\times10^{-8}$) to ensure strong genetic correlations.
2. *Instrumental variable selection:* The SNPs associated with each metabolite were selected as IVs, excluding those that were palindromic or in linkage disequilibrium (LD) (r^2 <0.01 and distance >10,000 kb) to minimize bias. Instrumental variables with

F-statistics <10 were also excluded to avoid weak instrument bias.

3. *MR analysis:* Using MR techniques, we conducted Inverse Variance Weighted (IVW), Mendelian Randomization-Egger (MR-Egger), and MR-Pleiotropy Residual Sum and Outlier (MR-PRESSO) analyses to determine causal effects of each metabolite on BMD, focusing on metabolites meeting both heterogeneity and pleiotropy thresholds. Only metabolites with consistent results across MR methods and without evidence of pleiotropy were retained (Figure 1).

Screening instrumental variables

Single-nucleotide polymorphisms for each metabolite were first plugged and pooled sequentially, and a series of stringent criteria were used in this study to select IVs from the exposed GWAS pooled data. The specific screening steps were as follows: *(i)* SNPs were screened ($p<5\times10^{-8}$) as a threshold; *(ii)* SNPs that were themselves palindromic sequences. palindromic sequences, i.e., the sequence of bases of the SNPs on the deoxyribonucleic acid (DNA), i.e., SNPs with the same base order on the forward and reverse strands of DNA in opposite directions, from which it is impossible to infer whether the strand they are on is the forward or reverse strand, were excluded; *(iii)* LD in SNPs was excluded with the values of $r^2<0.01$ and genetic distance >10,000 kb to screen for independent SNPs, and the LD was based on the European population genome. Linkage disequilibrium was estimated from the Genome Project Reference Panel for European populations; *(iv)* to exclude the presence of weak IV bias, the F-statistic was calculated for each SNPs, and IVs with F<10 were defined as weak IVs and excluded from the MR analysis.[16]

MR analysis process

The metabolite data collated in 1.2 were first analyzed by MR and plotted as hotspots for the MR analysis, and then screened for metabolite data that were relevant for the outcome to be analyzed by MR and plotted as forests.

The 'Two Sample MR package' in R version 4.4.0 software was used for MR analysis, and the 'MRPRESSO' package was used for MR-PRESSO analysis. The specific steps were as follows: *(i)* MR method: In this study, iIVW,[17] MR-Egger regression,[18] weighted median method,[19] simple

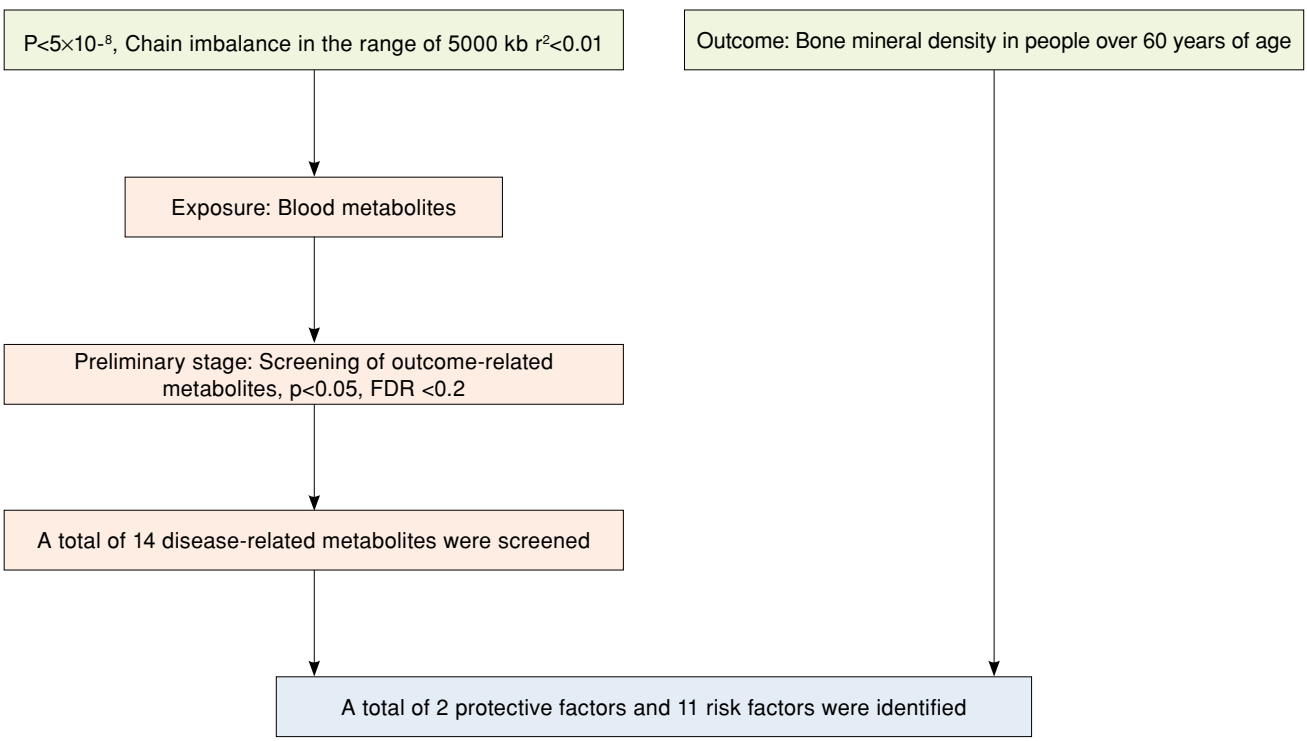

FIGURE 1. Screening process of blood metabolites associated with bone mineral density in individuals over 60 years of age.
FDR: False discovery rate.

and weighted models were used for the analysis, and if there was no horizontal multivariate validity between SNPs, the results of the IVW analysis were used as the main results.[20] *(ii)* Statistical heterogeneity: Cochran Q test was applied to assess whether there was statistical heterogeneity among SNPs, with p≤0.05 suggesting the existence of statistical heterogeneity.[21] *(iii)* Horizontal pleiotropy: The horizontal pleiotropy of SNP was analyzed using the intercept term of MR-Egger regression, MR-PRESSO test and funnel plot; if the intercept term was not statistically significant compared with 0, it indicated that there was no horizontal pleiotropy in the SNP; the results of the MR-PRESSO test showed that the p>0.05 suggests that there is no horizontal pleiotropy in the SNP; and the results of the funnel plot analysis showed that the SNP left-right distribution is basically

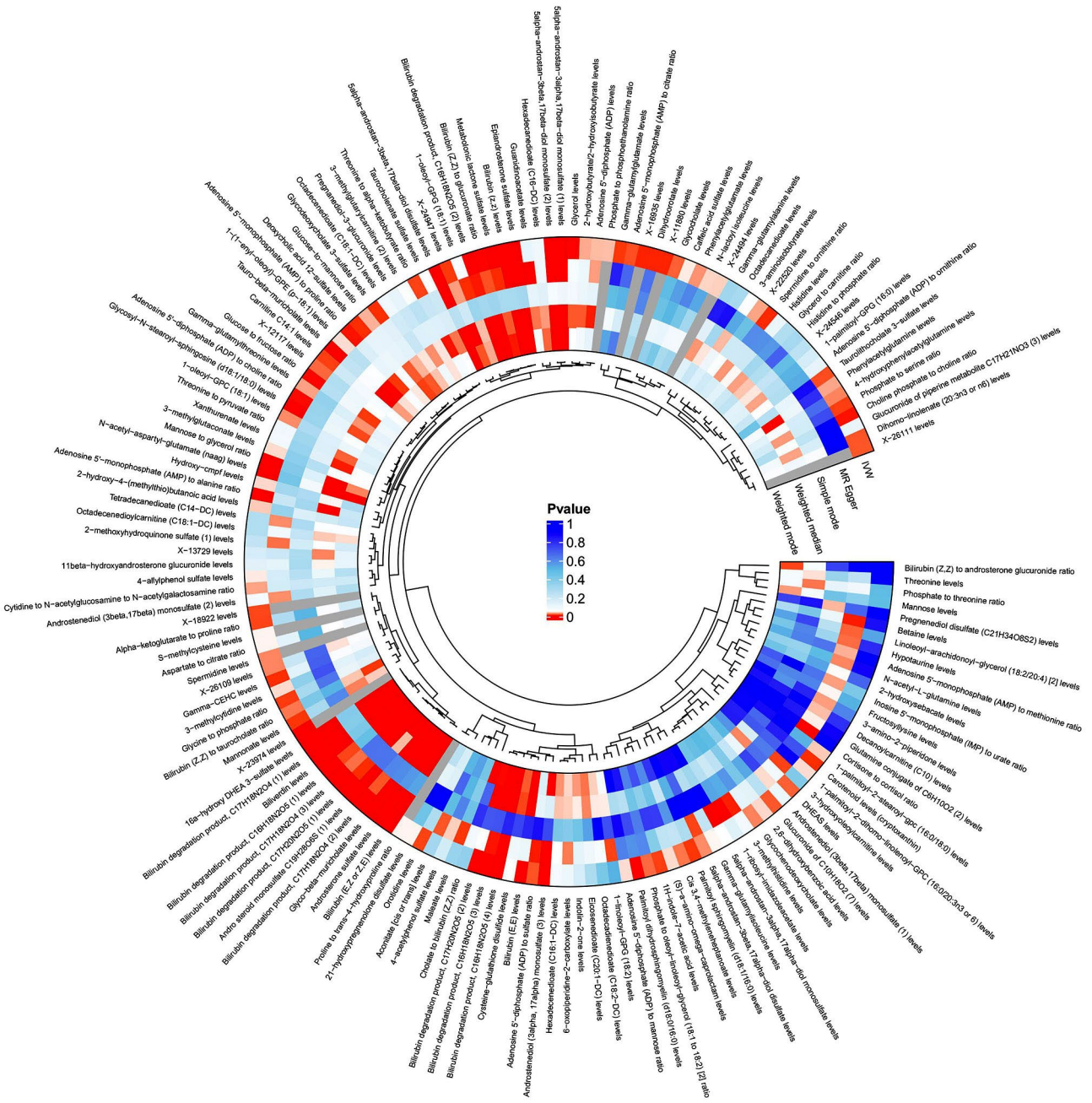

FIGURE 2. Preliminary Mendelian randomization results in a circular heat map.

symmetrical, suggesting that there is no obvious horizontal polyvalence of SNP. *(iv)* Sensitivity analysis: the leave-one-out method was used to assess the effect of individual SNPs on the results of IVW analysis; if there was no significant change in the results of IVW analysis after the exclusion of a single SNP, it was suggested that the SNP did not have a significant effect on the results of IVW analysis.[22]

RESULTS

Preliminary MR analysis results

A total of 1,400 blood metabolites were included for preliminary MR analysis after excluding weak IVs. Following this, we excluded metabolites that were not statistically significant by any of the five methods, resulting in the final set of 283 metabolites for further analysis. In addition to individual metabolites, we also examined specific metabolite ratios, such as the AMP-to-alanine ratio, mannose-to-glycerol ratio, and bilirubin-to-glucose ratio, which were of particular interest due to their potential involvement in metabolic and inflammatory pathways related to bone health. The MR results for these metabolites and ratios were plotted on a heat map (Figure 2), showing the associations between metabolite levels and BMD.

MR analysis of disease-related metabolites

Among the remaining 283 metabolites, 14 disease-associated metabolites were screened by the IVW method with p-values <0.05 and false discovery rate (FDR) <0.2 (Table I). The MR analysis was performed on these 14 metabolites, which included individual metabolites as well as significant metabolite ratios. The results indicated that higher levels of the AMP-to-alanine ratio (odds ratio [OR]=0.900, 95% confidence interval [CI]: 0.845-0.958) were associated with a protective effect against reduced BMD, suggesting that this ratio may play a role in preserving bone health. Other

TABLE I
Disease-related metabolite screening results

Exposure ID	Metabolite name	SNPs	β	SE	IVW *p*	OR	95%CI	FDR
GCST90199719	Tauro-beta-muricholate levels	3	–0.157	0.041	0.000	0.855	0.789~0.927	0.025
GCST90199846	Glycosyl-N-stearoyl-sphingosine (d18:1/18:0) levels	34	0.063	0.022	0.004	1.065	1.020~1.111	0.182
GCST90199858	5alpha-androstan-3 alpha,17 beta-diol monosulfate (1) levels	21	0.091	0.020	0.000	1.095	1.054~1.138	0.002
GCST90200257	Metabolonic lactone sulfate levels	30	0.052	0.017	0.003	1.053	1.018~1.090	0.145
GCST90200264	Bilirubin degradation product, C17H20N2O5 (2) levels	23	0.063	0.021	0.003	1.065	1.022~1.109	0.145
GCST90200269	Bilirubin degradation product, C17H20N2O5 (1) levels	23	0.067	0.021	0.001	1.070	1.027~1.114	0.086
GCST90200275	Bilirubin degradation product, C16H18N2O5 (2) levels	28	0.050	0.017	0.004	1.052	1.017~1.088	0.177
GCST90200684	N-acetyl-aspartyl-glutamate (NAAG) levels	18	0.062	0.020	0.002	1.064	1.023~1.106	0.137
GCST90200686	Bilirubin (E,E) levels	27	0.053	0.018	0.002	1.055	1.019~1.092	0.137
GCST90200687	Bilirubin (E,Z or Z,E) levels	18	0.081	0.016	0.000	1.085	1.051~1.119	0.000
GCST90200703	Bilirubin degradation product, C17H18N2O4 (3) levels	23	0.055	0.016	0.000	1.056	1.024~1.089	0.069
GCST90200706	Biliverdin levels	29	0.052	0.016	0.001	1.053	1.021~1.086	0.082
GCST90200741	Adenosine 5'-monophosphate (AMP) to alanine ratio	23	–0.105	0.032	0.001	0.900	0.845~0.958	0.082
GCST90200920	Mannose to glycerol ratio	22	0.109	0.032	0.001	1.116	1.048~1.187	0.069

SNPs: Single-nucleotide polymorphisms; SE: Standard error; IVW *p*: Inverse variance weighted *p* value; OR: Odds ratio; CI: Confidence interval; FDRX False discovery rate.

metabolites, including tauro-beta-muricholate (OR=0.855, 95% CI: 0.789-0.927) and several risk factors such as glycosyl-N-stearoyl-sphingosine (OR=1.065, 95% CI: 1.020-1.111), 5α-androstan-3α, 17β-diol monosulfate (OR=1.095, 95% CI: 1.054-1.138), and the mannose-to-glycerol ratio (OR=1.116, 95% CI: 1.048-1.187) were found to influence BMD, either positively or negatively, depending on their association with bone metabolism pathways (Figure 3).

Sensitivity analysis

Heterogeneity test and multivariate test of MR-Egger intercept were performed on the data, and the results showed that there was no heterogeneity and multivariate test for all metabolite data (Table II). The results of the funnel plot analysis showed that the left-right distribution of SNPs that were highly correlated with BMD was basically symmetrical (Figure 4). In the leave-one-out method of analysis,

Exposure	NSNP	Method	*p*	OR (95% CI)
Tauro−beta−muricholate levels	3	Weighted median	**<0.001**	0.851 (0.804 to 0.900)
Tauro−beta−muricholate levels	3	Inverse variance weighted	**<0.001**	0.855 (0.789 to 0.927)
Tauro−beta−muricholate levels	3	Weighted mode	**0.032**	0.845 (0.796 to 0.897)
Glycosyl−N−stearoyl−sphingosine (d18:1/18:0) levels	34	Weighted median	0.071	1.060 (0.995 to 1.129)
Glycosyl−N−stearoyl−sphingosine (d18:1/18:0) levels	34	Inverse variance weighted	**0.004**	1.065 (1.020 to 1.111)
Glycosyl−N−stearoyl−sphingosine (d18:1/18:0) levels	34	Weighted mode	0.119	1.064 (0.986 to 1.147)
5alpha−androstan−3alpha,17beta−diol monosulfate (1) levels	21	Weighted median	**<0.001**	1.120 (1.080 to 1.161)
5alpha−androstan−3alpha,17beta−diol monosulfate (1) levels	21	Inverse variance weighted	**<0.001**	1.095 (1.054 to 1.138)
5alpha−androstan−3alpha,17beta−diol monosulfate (1) levels	21	Weighted mode	**<0.001**	1.119 (1.075 to 1.164)
Metabolonic lactone sulfate levels	30	Weighted median	**0.022**	1.063 (1.009 to 1.119)
Metabolonic lactone sulfate levels	30	Inverse variance weighted	**0.003**	1.053 (1.018 to 1.090)
Metabolonic lactone sulfate levels	30	Weighted mode	**0.009**	1.074 (1.022 to 1.129)
Bilirubin degradation product, C17H20N2O5 (2) levels	23	Weighted median	**<0.001**	1.089 (1.036 to 1.145)
Bilirubin degradation product, C17H20N2O5 (2) levels	23	Inverse variance weighted	**0.003**	1.065 (1.022 to 1.109)
Bilirubin degradation product, C17H20N2O5 (2) levels	23	Weighted mode	**0.004**	1.091 (1.035 to 1.151)
Bilirubin degradation product, C17H20N2O5 (1) levels	23	Weighted median	**<0.001**	1.100 (1.045 to 1.159)
Bilirubin degradation product, C17H20N2O5 (1) levels	23	Inverse variance weighted	**0.001**	1.070 (1.027 to 1.114)
Bilirubin degradation product, C17H20N2O5 (1) levels	23	Weighted mode	**0.003**	1.097 (1.040 to 1.158)
Bilirubin degradation product, C16H18N2O5 (2) levels	28	Weighted median	**<0.001**	1.068 (1.028 to 1.109)
Bilirubin degradation product, C16H18N2O5 (2) levels	28	Inverse variance weighted	**0.004**	1.052 (1.017 to 1.088)
Bilirubin degradation product, C16H18N2O5 (2) levels	28	Weighted mode	**0.002**	1.068 (1.029 to 1.109)
N−acetyl−aspartyl−glutamate (naag) levels	18	Weighted median	**<0.001**	1.057 (1.023 to 1.091)
N−acetyl−aspartyl−glutamate (naag) levels	18	Inverse variance weighted	**0.002**	1.064 (1.023 to 1.106)
N−acetyl−aspartyl−glutamate (naag) levels	18	Weighted mode	**0.008**	1.056 (1.019 to 1.094)
Bilirubin (E,E) levels	27	Weighted median	**<0.001**	1.059 (1.025 to 1.095)
Bilirubin (E,E) levels	27	Inverse variance weighted	**0.002**	1.055 (1.019 to 1.092)
Bilirubin (E,E) levels	27	Weighted mode	**0.003**	1.058 (1.023 to 1.094)
Bilirubin (E,Z or Z,E) levels	18	Weighted median	**<0.001**	1.091 (1.052 to 1.131)
Bilirubin (E,Z or Z,E) levels	18	Inverse variance weighted	**<0.001**	1.085 (1.051 to 1.119)
Bilirubin (E,Z or Z,E) levels	18	Weighted mode	**<0.001**	1.091 (1.052 to 1.132)
Bilirubin degradation product, C17H18N2O4 (3) levels	23	Weighted median	**<0.001**	1.061 (1.025 to 1.097)
Bilirubin degradation product, C17H18N2O4 (3) levels	23	Inverse variance weighted	**<0.001**	1.056 (1.024 to 1.089)
Bilirubin degradation product, C17H18N2O4 (3) levels	23	Weighted mode	**0.003**	1.062 (1.025 to 1.100)
Biliverdin levels	29	Weighted median	**<0.001**	1.062 (1.027 to 1.098)
Biliverdin levels	29	Inverse variance weighted	**<0.001**	1.053 (1.021 to 1.086)
Biliverdin levels	29	Weighted mode	**0.002**	1.063 (1.026 to 1.102)
Adenosine 5'−monophosphate (AMP) to alanine ratio	23	Weighted median	**0.007**	0.883 (0.806 to 0.966)
Adenosine 5'−monophosphate (AMP) to alanine ratio	23	Inverse variance weighted	**<0.001**	0.900 (0.845 to 0.958)
Adenosine 5'−monophosphate (AMP) to alanine ratio	23	Weighted mode	0.226	0.895 (0.752 to 1.065)
Mannose to glycerol ratio	22	Weighted median	**0.004**	1.150 (1.047 to 1.264)
Mannose to glycerol ratio	22	Inverse variance weighted	**<0.001**	1.116 (1.048 to 1.187)
Mannose to glycerol ratio	22	Weighted mode	**0.020**	1.144 (1.030 to 1.271)

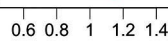

FIGURE 3. Results of MR analysis of disease-related metabolites.
MR: Mendelian randomization; NSNP: Number of single-nucleotide polymorphisms; OR: Odds ratio; CI: Confidence interval.

TABLE II
Results of heterogeneity and horizontal polytropy tests

		heterogeneity test		Multivariate validity test	
Exposure ID	Metabolite name	Q value	*p*	Intercept	*p*
GCST90199719	Tauro-beta-muricholate levels	2.532	0.111	0.059	0.502
GCST90199846	Glycosyl-N-stearoyl-sphingosine (d18:1/18:0) levels	32.720	0.431	0.001	0.882
GCST90199858	5alpha-androstan-3 alpha, 17 beta-diol monosulfate (1) levels	27.502	0.093	–0.010	0.115
GCST90200257	Metabolonic lactone sulfate levels	28.257	0.450	–0.005	0.272
GCST90200264	Bilirubin degradation product, C17H20N2O5 (2) levels	14.082	0.866	–0.002	0.679
GCST90200269	Bilirubin degradation product, C17H20N2O5 (1) levels	20.806	0.470	–0.006	0.313
GCST90200275	Bilirubin degradation product, C16H18N2O5 (2) levels	28.445	0.336	–0.004	0.303
GCST90200684	N-acetyl-aspartyl-glutamate (naag) levels	25.553	0.061	0.013	0.150
GCST90200686	Bilirubin (E,E) levels	35.423	0.080	0.003	0.538
GCST90200687	Bilirubin (E,Z or Z,E) levels	16.113	0.445	–0.004	0.409
GCST90200703	Bilirubin degradation product, C17H18N2O4 (3) levels	20.623	0.482	0.001	0.848
GCST90200706	Biliverdin levels	26.149	0.510	–0.007	0.082
GCST90200741	Adenosine 5'-monophosphate (AMP) to alanine ratio	22.983	0.345	–0.000	0.996
GCST90200920	Mannose to glycerol ratio	16.935	0.657	0.005	0.574

we sequentially rejected one SNP and analyzed the data with the remaining SNPs, and the results showed that there was no SNPs that had a great influence on the resultant effect (Figure 5).

DISCUSSION

In the present study, we investigated the causal relationship between blood metabolites and BMD using MR and a comprehensive panel of 1,400 metabolites. Compared to previous studies, which typically analyzed fewer metabolites-around 486-and focused less on elderly populations, our study provides a broader and more targeted perspective.[23,24] In their study, Moayyeri et al.[25] identified four sulfated adrenal androgens (e.g., androsterone sulfate) causally linked to BMD via CYP3A5 and SULT2A1 genes, highlighting steroid metabolism in bone health. In contrast, our study revealed novel associations with energy-related ratios (e.g., AMP-to-alanine) and lipid metabolites (e.g., glycosyl-N-stearoyl-sphingosine), potentially due to our expanded metabolite panel and focus on elderly populations. Similarly, Chen et al.[26] conducted a metabolome-wide MR analysis, but did not stratify by age; their findings on amino acid metabolism partially align with our observations on alanine and mannose-glycerol ratios, though discrepancies may arise from differences in cohort characteristics. Another MR study reported associations between blood metabolites and OP, yet their metabolite selection was narrower (481 metabolites) and lacked elderly-specific analysis.[27] These comparisons underscore that while prior studies emphasized steroid or amino acid pathways, our inclusion of 1,400 metabolites and elderly-focused design uncovered novel energy-lipid interplay in OP rist.

By emphasizing the metabolic factors influencing BMD, particularly in individuals over 60 years old, we offer novel insights into OP and fracture risk in aging populations. This broader metabolite panel, combined with MR, offers a more comprehensive view of the complex metabolic pathways that affect bone health and OP risk.

Our findings emphasize the role of specific metabolites and metabolite ratios in bone health. To illustrate, the AMP-to-alanine ratio was identified as a protective factor for reduced BMD (OR=0.900, 95% CI: 0.845-0.958), highlighting the importance of energy metabolism in bone preservation. This novel finding suggests that the regulation of energy balance, as reflected in the AMP-to-alanine ratio, could play a crucial role in maintaining bone density. In this context, our study is the first to suggest this relationship, providing new evidence that energy metabolism is a key factor in bone health, particularly in the aging population, where metabolic changes often accelerate bone loss.

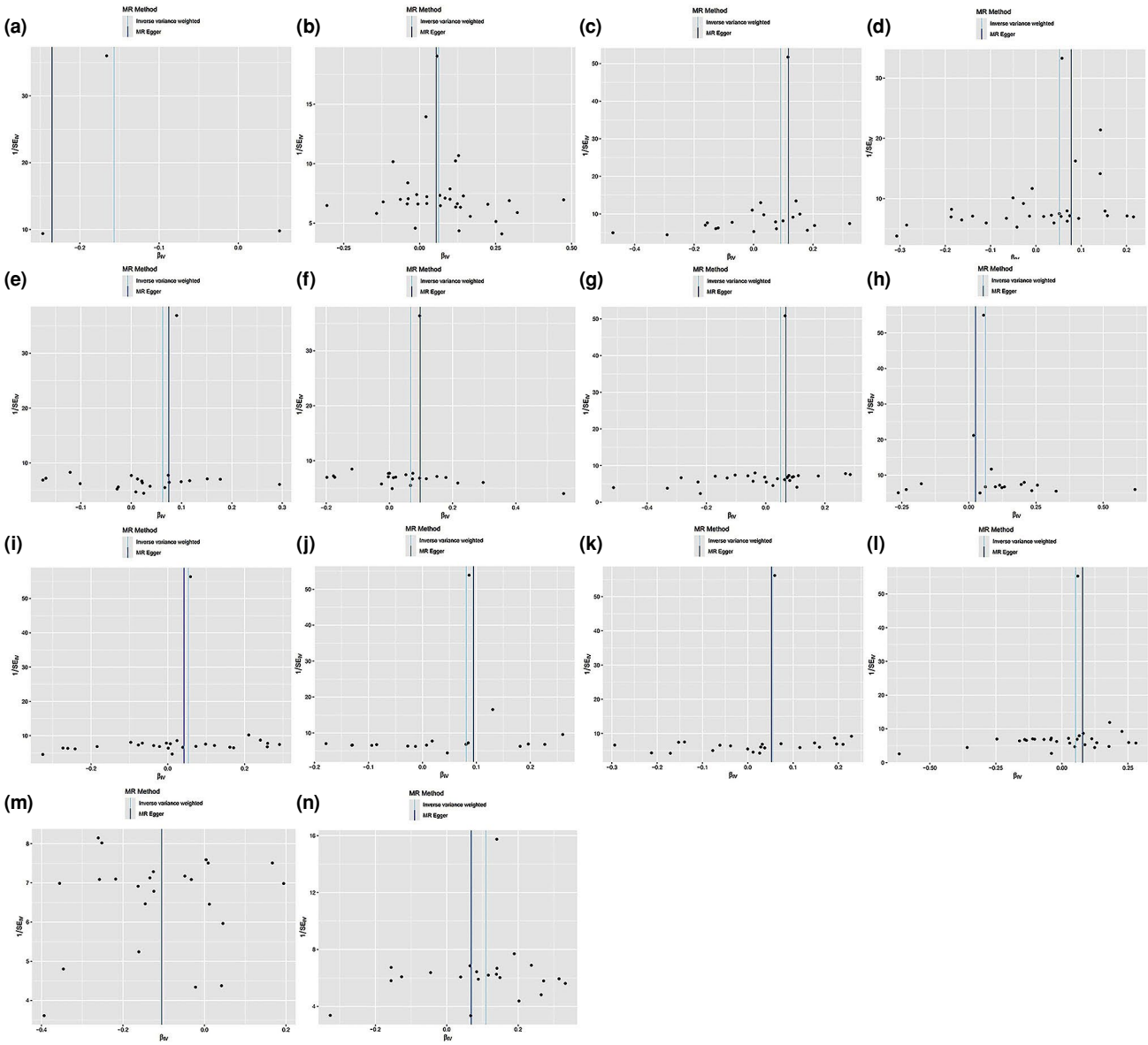

FIGURE 4. Funnel plot results. **(a)** GCST90199719, **(b)** GCST90199846, **(c)** GCST90199858, **(d)** GCST90200257, **(e)** GCST90200264, **(f)** GCST90200269, **(g)** GCST90200275, **(h)** GCST90200684, **(i)** gcst90200686, **(j)** gcst90200687, **(k)** gcst90200703, **(l)** gcst90200706, **(m)** gcst90200741, **(n)** gcst90200920.

Additionally, metabolites such as glycosyl-N-stearoyl-sphingosine and the mannose-to-glycerol ratio were associated with higher risks of reduced BMD, indicating their potential involvement in lipid metabolism and inflammation pathways. These findings build upon earlier studies that have shown how lipid metabolism disruptions and inflammation can contribute to bone resorption and OP.[28-30] The mannose-to-glycerol ratio may reflect the broader impact of inflammation and altered metabolism in the context of aging, further confirming the complex role that metabolic dysregulation plays in OP risk.[31-34]

Several metabolites identified in this study, including bilirubin degradation products (such as bilirubin [E,E], bilirubin [E,Z or Z,E]) and tauro-beta-muricholate, were linked to oxidative stress and inflammatory responses-critical factors in bone metabolism. Bilirubin, a byproduct of hemoglobin breakdown, has previously been shown to protect against bone loss by reducing oxidative damage and bone turnover.[35-37] Our results

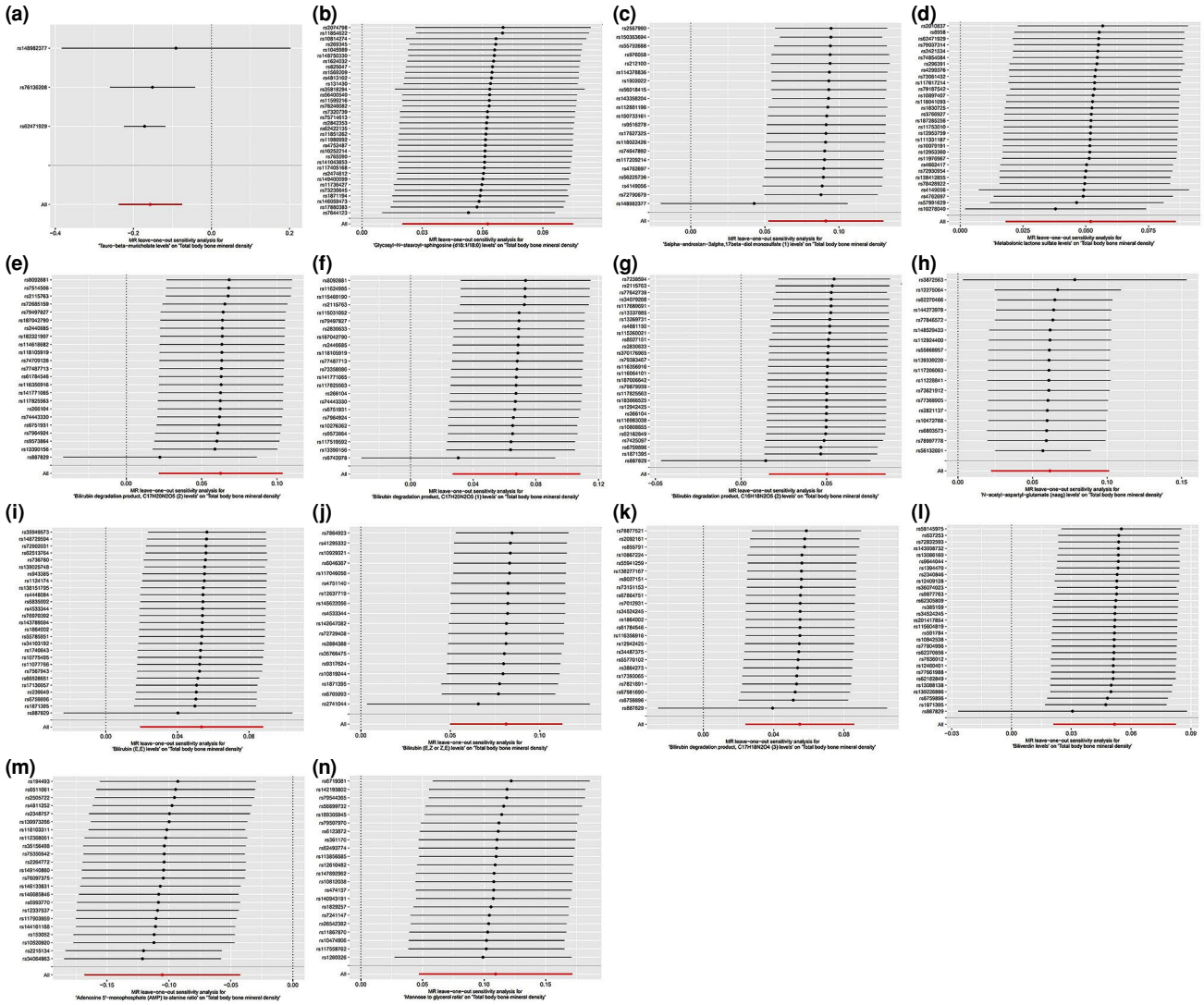

FIGURE 5. Results of leave-one-out method. **(a)** GCST90199719, **(b)** GCST90199846, **(c)** GCST90199858, **(d)** GCST90200257, **(e)** GCST90200264, **(f)** GCST90200269, **(g)** GCST90200275, **(h)** GCST90200684, **(i)** gcst90200686, **(j)** gcst90200687, **(k)** gcst90200703, **(l)** gcst90200706, **(m)** gcst90200741, **(n)** gcst90200920.

support this notion, suggesting that higher serum bilirubin levels may act as a protective marker against bone loss. However, the exact mechanisms by which bilirubin and related metabolites influence bone metabolism require further investigation, particularly in relation to their roles in oxidative stress and inflammation. This highlights an exciting avenue for future research, as these metabolites could potentially be targeted to prevent OP.

Furthermore, our study emphasizes the significance of TUG1 (taurine upregulated gene 1), an lncRNA involved in regulating taurine levels and osteoblast differentiation. Changes in TUG1 levels, influenced by taurine content, have been linked to bone formation processes, which align with our findings regarding tauro-beta-muricholate.[38-41] Also, TUG1 has been shown to promote osteoblast differentiation, suggesting that taurine and bile acid metabolism may directly influence bone remodeling. Our study extends this understanding by linking taurine and bile acid conjugates to bone health, providing new insights into the metabolic regulation of bone formation and resorption.

The novelty of our study lies in its application of MR to analyze a broad range of metabolites, enabling a more robust and causally interpretable assessment of the relationship between metabolism and BMD. Unlike observational studies, which

are often confounded by reverse causation and environmental factors, MR minimizes such biases and provides stronger evidence for causal links between metabolites and BMD. By identifying novel metabolites and metabolite ratios that influence bone health, our study lays the groundwork for future studies that can explore these metabolic markers as potential biomarkers for OP risk and progression.

Nonetheless, there are some limitations to this study to be acknowledged. First, the GWAS data used in this study were primarily derived from European populations, which may limit the generalizability of our findings to other ethnic groups. It is crucial to validate these results in diverse populations to confirm their applicability. Second, while we have identified several metabolites associated with BMD, the underlying biological mechanisms for some of these metabolites remain unclear. To illustrate, the role of glycosyl-N-stearoyl-sphingosine in bone health needs to be further explored, as its connection to lipid metabolism and OP risk is still not fully understood. Further research is required to elucidate these pathways and assess whether these metabolites can be targeted for therapeutic interventions in OP prevention and management.

In conclusion, the present MR study identifies key blood metabolites-including the AMP-to-alanine ratio (protective) and mannose-to-glycerol ratio (risk)-as causal biomarkers for reduced BMD in individuals aged over 60 years. These findings highlight energy and lipid metabolism as critical pathways in age-related bone loss, offering actionable targets for early screening and personalized interventions. While our results advance understanding of metabolic drivers in OP, validation in diverse populations and mechanistic studies are essential to translate these biomarkers into clinical practice.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conceived of the study: Z.B; Participated in its design and data analysis and statistics: L.G., Z.B.; Helped to draft the manuscript: L.G. All authors read and approved the final manuscript.

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