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Causal relationship between osteoporosis, bone mineral density, and osteonecrosis: a bidirectional two-sample Mendelian randomization study

Chao Zhang[1], Hao Yu[1], Yulin Miao[1] and Biaofang Wei[2*]

Abstract

Background Osteonecrosis (ON) is a debilitating orthopedic condition characterized by bone cell death due to impaired blood supply, leading to structural changes and disability. Osteoporosis (OP), a systemic skeletal disease, results in reduced bone density and quality, making bones fragile and prone to fractures. Although distinct, OP and ON share several risk factors such as corticosteroid use and smoking. This study aims to investigate the causal relationships between OP, bone mineral density (BMD), and ON using a bidirectional two-sample Mendelian randomization (MR) approach.

Methods This study utilized genome-wide association study (GWAS) data for OP from the FinnGen database, and BMD data for the lumbar spine and femoral neck from the Genetic Factors for Osteoporosis (GEFOS) consortium. ON data were also obtained from the FinnGen database. All participants were of European descent. Genetic instruments were selected based on genome-wide significance, linkage disequilibrium, and strength (F-statistic). Bidirectional MR analysis was performed using inverse-variance weighted (IVW), MR-Egger regression, and weighted median methods to assess causality. Sensitivity analyses, including Cochran's Q test and MR-PRESSO, were conducted to evaluate heterogeneity and pleiotropy.

Results MR analysis demonstrated a positive causal effect of OP on ON using the IVW method, with an odds ratio (OR) of 1.223 (95% CI: 1.026–1.459, $P=0.025$). The weighted median method also confirmed this result with an OR (95% CI) 1.290 (1.021–1.630), $P=0.033$. No significant causal effects were found between BMD (lumbar spine and femoral neck) and ON. Furthermore, ON did not exhibit a causal effect on OP or BMD. Sensitivity analyses confirmed the robustness of the results, showing no evidence of heterogeneity or pleiotropy.

Conclusion This study provides evidence of a unidirectional causal relationship between OP and ON, suggesting that individuals with a genetic predisposition to OP have an increased risk of developing ON. These findings highlight the importance of early OP detection and management to potentially reduce ON incidence. The lack of a significant causal relationship between BMD and ON indicates that factors other than bone density, such as vascular health, may

*Correspondence:
Biaofang Wei
weibiaofang@163.com

Full list of author information is available at the end of the article

play a crucial role in ON development. Future research should explore these mechanisms further to inform clinical interventions.

Keywords Osteonecrosis, Osteoporosis, Bone mineral density, Mendelian randomization, Genetic epidemiology, Causal inference

Background

Osteonecrosis(ON) is a challenging orthopedic condition marked by the disruption of blood flow to the bone, leading to local bone cell death, trabecular necrosis, and structural changes within the bone [1, 2]. It is categorized as either traumatic or non-traumatic [3]. Traumatic ON generally follows an injury that disrupts blood flow [4]. Non-traumatic ON is often linked to corticosteroid use, hemoglobinopathies (e.g. sickle cell anemia), fat embolism, alcoholism, and systemic lupus erythematosus (SLE) [5]. The pathogenesis of ON is not fully understood, and early asymptomatic cases are difficult to diagnose [6]. Evidence points to multiple pathogenic pathways, including intravascular coagulation, mechanical stress, corticosteroid use, and primary cell death [7]. ON leads to significant disability, affecting patients' quality of life and imposing substantial burdens on families and society [8].

Osteoporosis(OP) is a systemic skeletal disease characterized by reduced bone density and quality, making bones fragile and prone to fractures from minor falls or daily activities [9]. Its causes include aging, gender differences (higher prevalence in women), genetic factors, and unhealthy lifestyle habits such as insufficient calcium and vitamin D intake, lack of exercise, smoking, and excessive alcohol consumption [10, 11]. The primary pathological mechanism involves an imbalance between bone resorption and formation, leading to decreased bone mass and trabecular degradation [12].Epidemiological data indicate a high prevalence of OP among the elderly, especially postmenopausal women [13]. Bone mineral density (BMD) is widely used as a proxy phenotype for assessing OP risk, with family studies estimating BMD heritability at 50-70%, and twin studies suggesting heritability as high as 80% [14]. Femoral neck BMD (FN BMD) heritability is approximately 75%, while lumbar spine BMD (LS BMD) heritability is about 83% [15]. OP severely impacts patients' quality of life and imposes significant economic burdens on society and families [16]. Early diagnosis and comprehensive management can effectively prevent fractures and improve bone health [17].Although OP and ON are distinct bone diseases, they share several etiological and risk factors, such as long-term corticosteroid use, alcoholism, and smoking [18]. However, existing studies are mostly cross-sectional or retrospective, limiting their ability to clearly distinguish causality from correlation [19–22].

Mendelian randomization (MR) is an epidemiological approach that utilizes genetic variations as instrumental variables to investigate the causal relationship between exposures and disease outcomes [23].Because genetic variations are randomly distributed among individuals and unaffected by environmental and social behaviors, MR can effectively control for confounding factors [24]. MR infers the causal effect of an exposure on a disease outcome by analyzing genetic markers associated with the exposure. This method has been widely applied in research on cardiovascular diseases, cancer, and metabolic disorders [25–27]. In studying the causal relationships among OP, BMD, and ON, MR can help us better understand their biological mechanisms and provide more reliable evidence to support clinical interventions and preventive strategies.

Materials and methods

Data sources

This study utilized two-sample MR to investigate the causal relationships between OP, BMD, and ON. All data were sourced from publicly available databases, thus no additional ethical approvals were required.

(1) **OP Data**: Genome-wide association study (GWAS) data for OP were obtained from the FinnGen database (https://www.finngen.fi/en), published in 2021, comprising 212,778 samples and 16,380,452 single nucleotide polymorphisms (SNPs).
(2) **BMD Data**: GWAS data for FN BMD were also from GEFOS, published in 2015, with 32,735 samples and 10,586,900 SNPs.GWAS data for LS BMD were sourced from the Genetic Factors for OP (GEFOS) consortium, published in 2015, including 28,498 samples and 10,582,867 SNPs [28].
(3) **ON Data**: GWAS data for ON were from the FinnGen database (https://www.finngen.fi/en), published in 2021, with a total of 210,179 samples and 16,380,447 SNPs.

The majority of participants were of European descent to minimize potential biases due to population heterogeneity. Detailed information on samples, genotyping, and data processing can be found in the relevant literature (Supplementary File 1, Table S1).

Instrumental variable selection

Following the guidelines for designing MR studies [29], three assumptions must be met for MR analysis: (1) relevance assumption: IVs must be strongly associated with the exposure, (2) independence assumption: IVs must be independent of confounders, (3) exclusion restriction assumption: IVs must influence the outcome only through the exposure (Fig. 1). SNPs were selected as independent genetic predictors based on the following criteria:

(1) A genome-wide significance threshold of $P < 5 \times 10^{-8}$ was applied. If there were insufficient significant SNPs under this threshold, a threshold of $P < 1 \times 10^{-5}$ was used [30].
(2) The Clump function was used to test for linkage disequilibrium (LD) with a threshold of $r^2 < 0.001$ and a distance of 10,000 kb [31].
(3) SNPs related to outcomes were excluded using the LD link database (https://ldlink.nih.gov/?tab=ldtrait, accessed on June 3, 2024) to avoid confounding factors [32, 33].
(4) SNPs with an F-statistic < 10 were excluded to avoid bias from weak instruments [34]. The proportion of exposure variance explained by genetic instruments(R^2) was calculated to quantify the strength of the genetic tools [35]. The F-statistic for each SNP was calculated to assess the strength of the selected instruments.
(5) MR-PRESSO was used to detect outliers and adjust for horizontal pleiotropy. Outliers identified as significantly influencing causal estimates were removed [36].

Statistical analysis

Using the aforementioned data, we employed a bidirectional two-sample MR approach to evaluate the relationships among OP, BMD, and ON. Various methods, including MR-Egger regression, weighted median, inverse-variance weighted (IVW), simple mode, and weighted mode, were used to estimate causal effects. The IVW method was considered the primary analysis when all included SNPs met the valid instrument assumptions, as it provides the most precise estimates in the absence of weak instruments [37].

For sensitivity analysis, Cochran's Q test was used to assess heterogeneity among individual genetic variants [38]. If Cochran's Q test $P < 0.05$, the results indicated heterogeneity, suggesting that the relationship between exposure and outcome was influenced by different ages

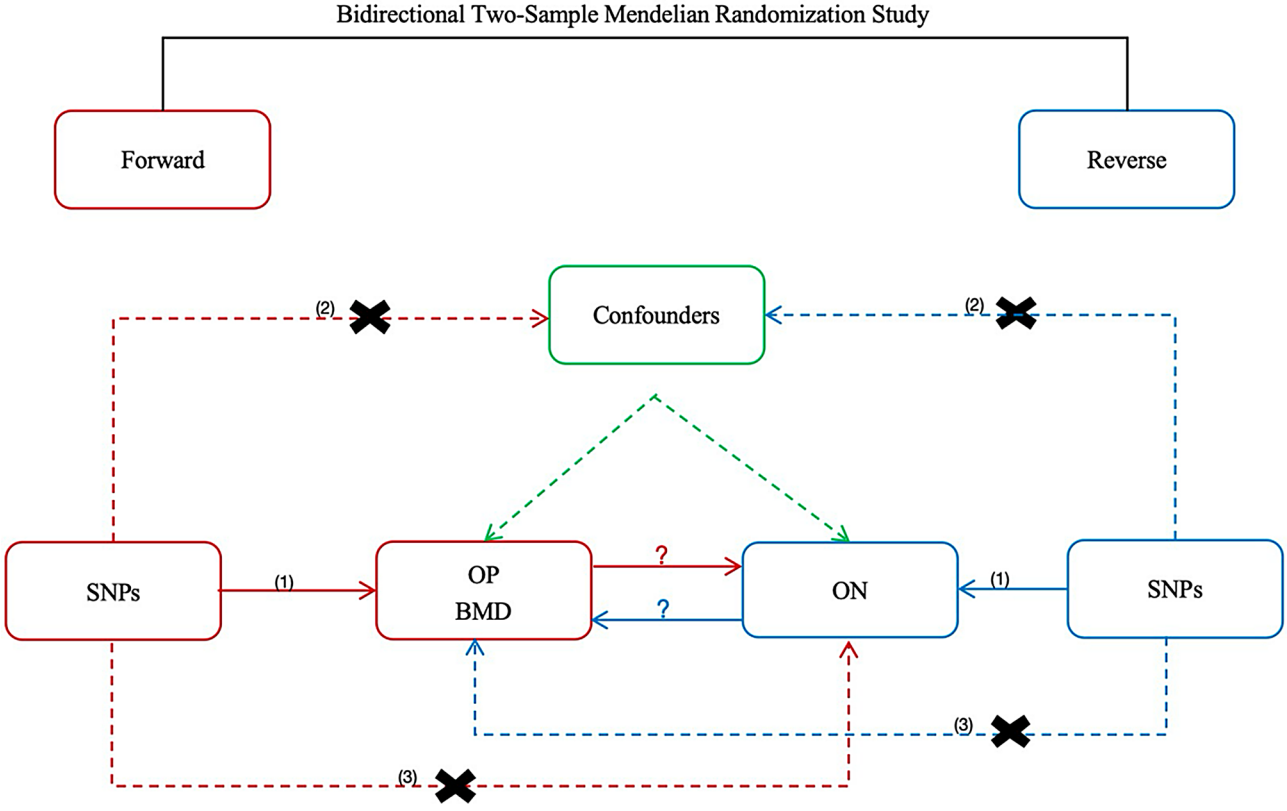

Fig. 1 An overview of this Mendelian randomization study design: (1)Relevance assumption, (2)Independence assumption, (3)Exclusion restriction assumption

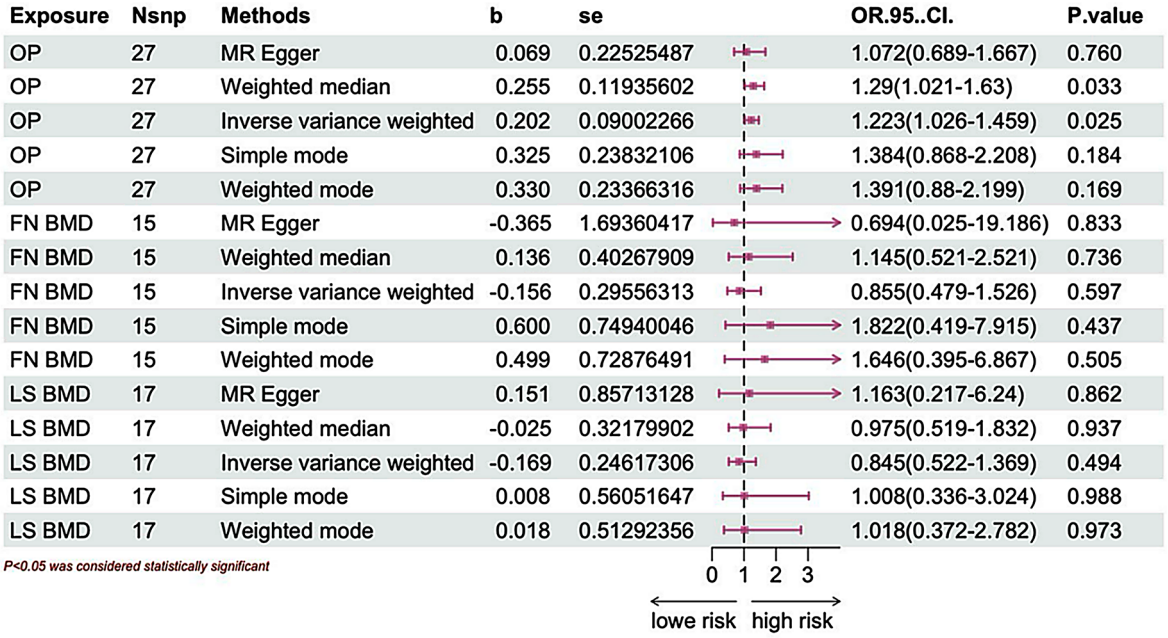

Fig. 2 Forward Mendelian Randomization Analysis Results. Abbreviation: OP, Osteoporosis; FN BMD, Femoral neck bone mineral density; LS BMD, Lumbar spine bone mineral density; SNP, single nucleotide polymorphism

and genders. MR-PRESSO and MR Egger-intercept tests were also used to examine horizontal pleiotropy [39]. If $P<0.05$, it indicates the presence of horizontal pleiotropy, meaning the selected instrumental variables significantly influence the outcome through pathways other than the exposure, contradicting assumptions 2 and 3 in Fig. 1. If $P>0.05$, it suggests that the exposure does not exert a significant influence on the outcome variable through pathways other than the exposure itself. All analyses were performed using the TwoSampleMR and MR PRESSO packages in R software version 4.1.2.

Results

After excluding SNPs with incompatible alleles, Supplementary File 1 provides details on all independent SNPs linked to exposure. In our analysis, the F statistics for the instrumental variables related to exposure were all above 10, suggesting a minimal risk of bias from weak instrumental variables.

Forward Mendelian randomization analysis

Causal effect of OP on ON

A total of 27 SNPs were selected as instrumental variables for OP. MR results showed consistent directions across IVW, MR-Egger, weighted median, simple mode, and weighted mode methods. The IVW method indicated an odds ratio (OR) of 1.223 (95% CI: 1.026–1.459, $P=0.025$), suggesting a positive association between OP and ON. The weighted median method also supported this finding, with an OR of 1.290 (95% CI: 1.021–1.630, $P=0.033$) (Fig. 2). Heterogeneity tests showed no evidence of heterogeneity (IVW Cochran's Q = 19.185, $P=0.829$; MR-Egger Cochran's Q = 18.776, $P=0.808$) (Table 1). Pleiotropy analysis indicated no evidence of horizontal pleiotropy (MR-PRESSO global test $P=0.845$; MR-Egger intercept $P=0.529$) (Table 2). The visualization of Mendelian randomization showed stable results, further validating the reliability of our study findings (Figs. 3, 4, 5 and 6).

Table 1 Heterogeneity for forward Mendelian randomization analysis

Exposure	Outcome	IVW		MR egger	
		Cochran's Q	Q-*P* value	Cochran's Q	Q-*P* value
OP	ON	19.185	0.829	18.776	0.808
FN BMD	ON	15.292	0.358	15.274	0.291
LS BMD	ON	8.024	0.948	7.873	0.929

Abbreviation OP, Osteoporosis; ON, Osteonecrosis; FN BMD, Femoral neck bone mineral density; LS BMD, Lumbar spine bone mineral density

Table 2 Pleiotropy for forward Mendelian randomization analysis

Exposure	Outcome	MR-PRESSO Global test-*p*	MR egger—intercept	Intercept-*P* value
OP	ON	0.845	0.026	0.529
FN BMD	ON	0.375	0.013	0.902
LS BMD	ON	0.952	−0.023	0.703

Abbreviation OP, Osteoporosis; ON, Osteonecrosis; FN BMD, Femoral neck bone mineral density; LS BMD, Lumbar spine bone mineral density

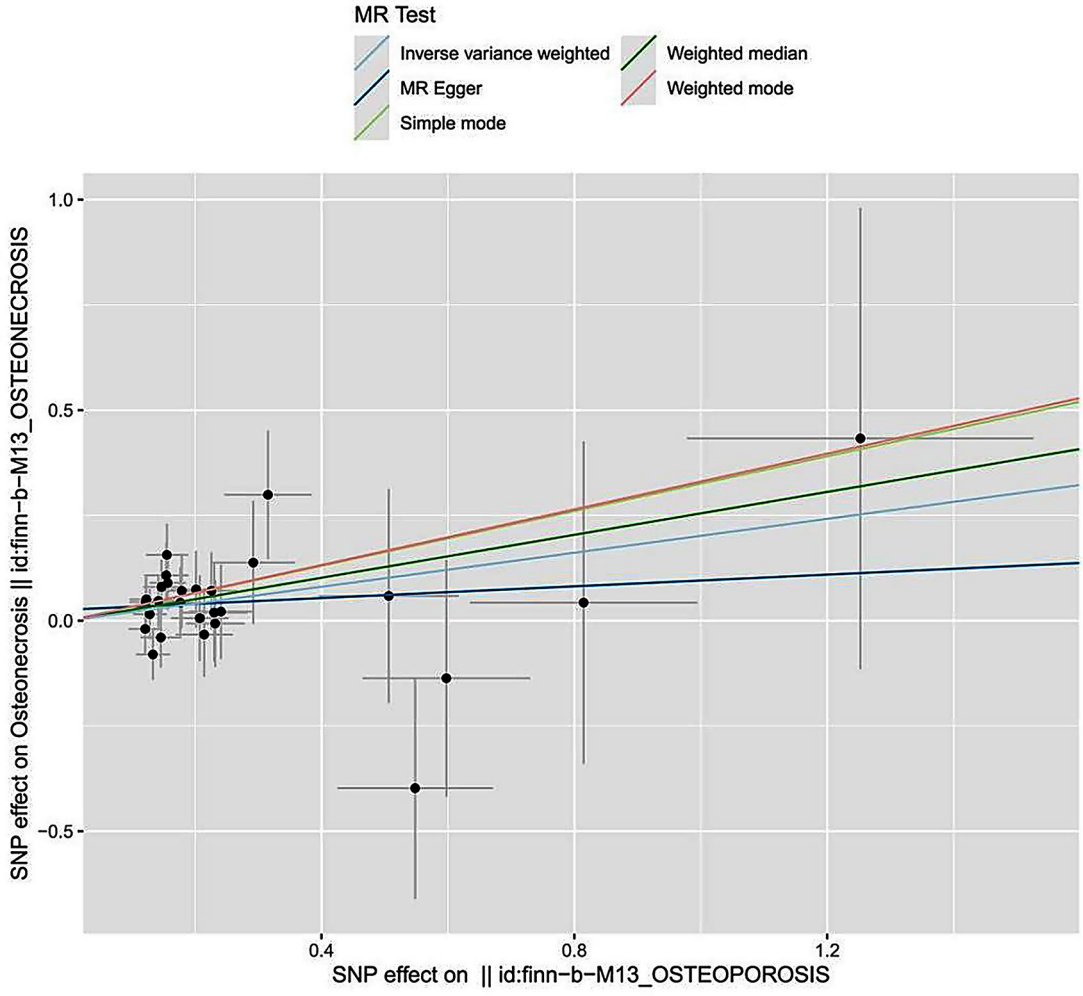

Fig. 3 The scatter plot of the impact of osteoporosis on osteonecrosis

Causal effect of BMD on ON

For FN BMD, 15 SNPs were selected as instrumental variables. MR results indicated no significant association between FN BMD and ON (IVW OR = 0.855, 95% CI: 0.479–1.526, $P = 0.597$) (Fig. 2). Heterogeneity tests showed no evidence of heterogeneity (IVW Cochran's Q = 15.292, $P = 0.358$; MR-Egger Cochran's Q = 15.274, $P = 0.291$) (Table 1). Pleiotropy analysis indicated no evidence of horizontal pleiotropy (MR-PRESSO global test $P = 0.375$; MR-Egger intercept $P = 0.902$) (Table 2). The charts from Mendelian randomization displayed stable results, further supporting the conclusions of our research (Supplementary File 2; Figs. S1-S4).

For LS BMD, 17 SNPs were selected as instrumental variables. MR results showed no significant association between LS BMD and ON (IVW OR = 0.855, 95% CI: 0.522–1.369, $P = 0.494$) (Fig. 2). Heterogeneity tests showed no evidence of heterogeneity (IVW Cochran's Q = 8.024, $P = 0.948$; MR-Egger Cochran's Q = 7.873, $P = 0.929$) (Table 1). Pleiotropy analysis indicated no evidence of horizontal pleiotropy (MR-PRESSO global test $P = 0.857$; MR-Egger intercept $P = 0.559$) (Table 2). By visualizing Mendelian randomization, we obtained stable results, further confirming the reliability of our study results (Supplementary File 2; Figs. S1-S4).

Reverse Mendelian randomization analysis

Causal effect of ON on OP and BMD

A total of 11 SNPs were selected as instrumental variables to investigate the causal effect of ON on OP. MR results using the IVW method showed an OR of 1.036 (95% CI: 0.984–1.091, $P = 0.181$), suggesting no causal relationship between ON and OP. For the causal effect of ON on FN BMD and LS BMD, 10 SNPs were selected as instrumental variables. The MR results using the IVW method for FN BMD showed an OR of 1.001 (95% CI: 0.985–1.018, $P = 0.432$). For LS BMD, the MR results using the IVW method showed an OR of 0.998 (95% CI: 0.980–1.017, $P = 0.852$), indicating no causal relationship between ON and BMD (Fig. 7). Cochran's Q test showed

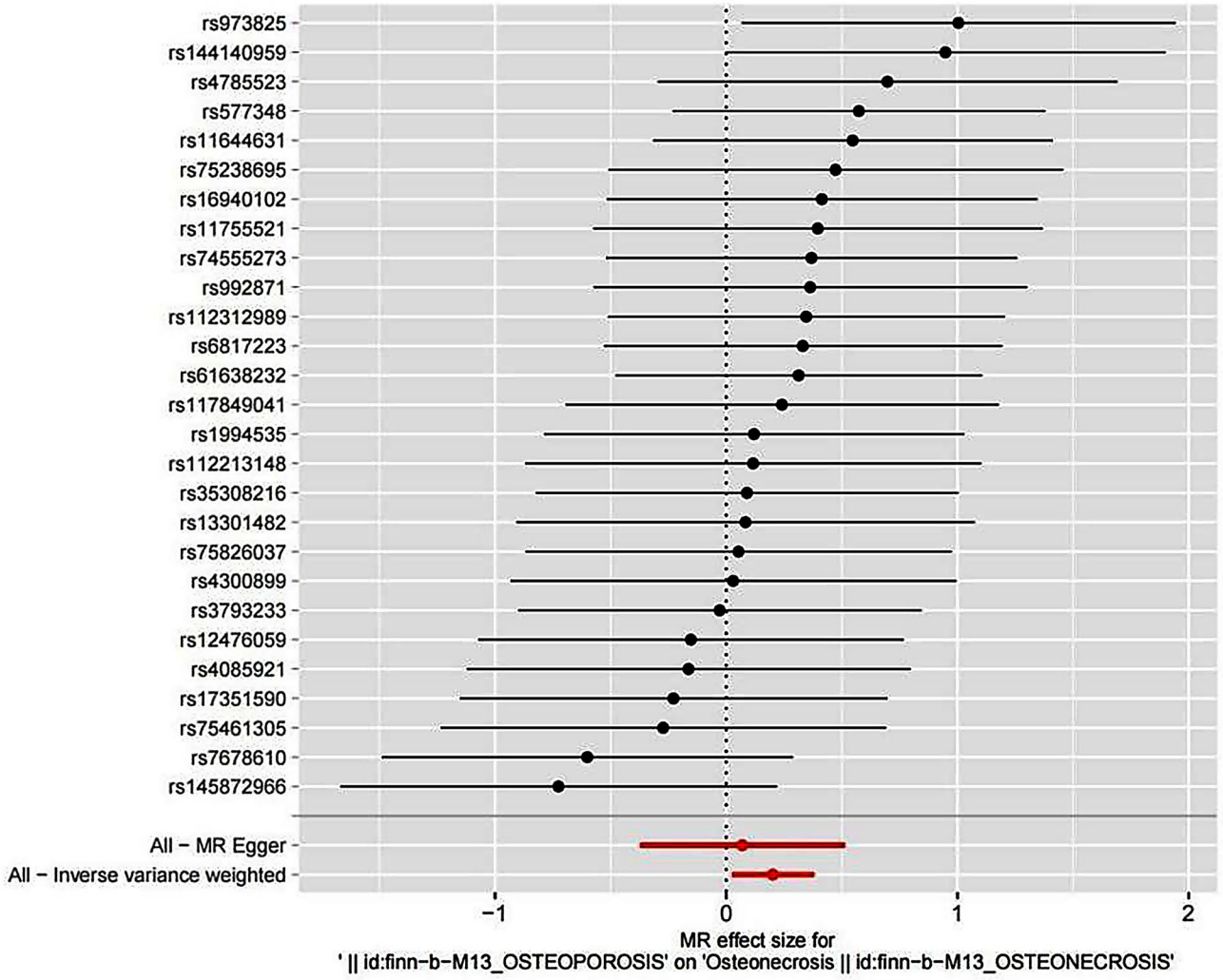

Fig. 4 The forest plot plot of the impact of osteoporosis on osteonecrosis

no evidence of heterogeneity, and both the MR-PRESSO and MR Egger-intercept tests showed no evidence of pleiotropy (Supplementary File 2; Table S1-S2).The stable results shown in the Mendelian randomization visualization further corroborate the reliability of our study findings (Supplementary File 2; Figs. S5-S8).

Discussion

This study utilized MR to explore the causal relationships between OP, BMD and ON. Findings from this MR analysis support a unidirectional causal relationship between OP and ON. The data suggest that individuals with genetic predisposition to OP have a higher risk of developing ON, independent of BMD. The link between OP and ON has clinical implications, highlighting the importance of early detection and management of OP to potentially reduce the incidence of ON. OP treatments, such as bisphosphonates, might not only improve bone density but also reduce ON risk [40]. This aligns with previous research indicating that improved bone health through pharmacological and lifestyle interventions could mitigate ON progression [41]. Interestingly, our analysis did not find a significant causal relationship between BMD and ON. This suggests that factors contributing to OP might influence ON risk through mechanisms other than bone density reduction. It is possible that the microstructural changes in bone or systemic factors like vascular health play a more critical role in ON development. The study also examined the reverse causality, investigating whether ON influences OP or BMD. The results showed no significant causal effect, indicating that ON does not contribute to the development of OP or alterations in BMD. This helps clarify the directionality of the relationship, emphasizing that managing OP could be more beneficial in preventing ON rather than vice versa.

The findings of this study align with some previous research while contradicting others, highlighting the complexity of the relationship between OP, BMD, and

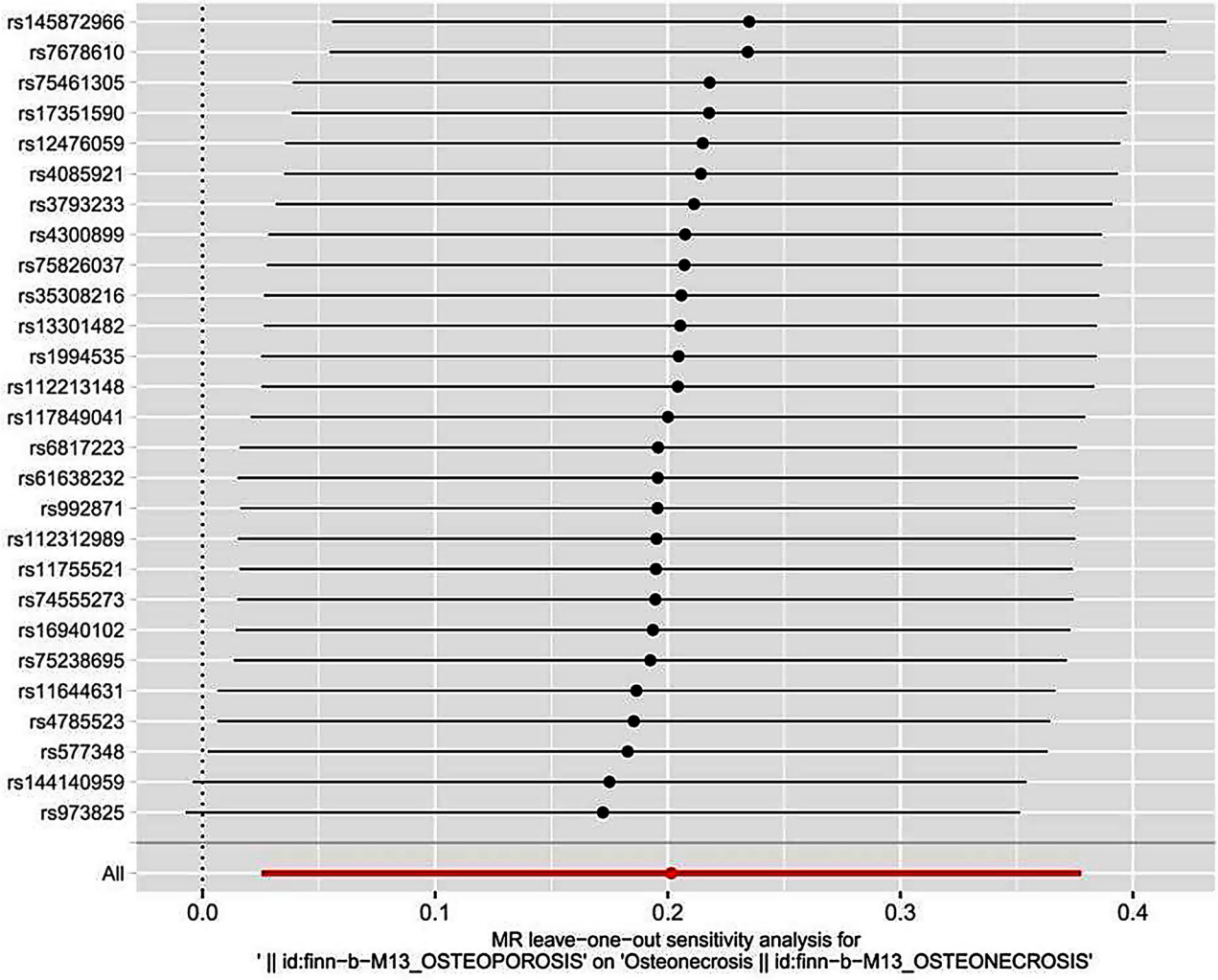

Fig. 5 The leave-one-out plot plot of the impact of osteoporosis on osteonecrosis

ON. Multiple cross-sectional and retrospective studies have shown an association between low bone mass and non-traumatic ON. For example, Gangji et al. [19] studied BMD in 243 patients with ON of the femoral head (ONFH) and 399 age- and sex-matched healthy controls, finding an association between non-traumatic ONFH and low bone density. Another study by Marissa et al. [20] on 50 patients with spontaneous osteonecrosis of the knee (SONK) found a higher rate of OP in SONK patients compared to unaffected individuals, suggesting that bone density assessment might be a useful adjunct in evaluating underlying diseases in SONK patients. However, some studies have proposed different views on the relationship between OP and ON. For instance, Nelson et al. [21] found that only 5 out of 32 patients with spontaneous ON of the knee had OP, suggesting that OP is not the primary cause of the disease in most patients. Shoji et al. [22] evaluated bone density within necrotic lesions of pre-collapse osteonecrosis of the femoral head using CT Hounsfield units and did not show a decrease in bone density in necrotic lesions before collapse. These conflicting findings indicate significant controversy regarding the relationship between OP and ON, necessitating further research to clarify the causal links. This study employs the MR method, reducing confounding factors and biases inherent in observational studies, providing strong evidence for the causal role of OP in ON.

From the perspective of bone metabolism, our research results can be better understood. OP is characterized by reduced bone mass and deterioration of bone microarchitecture, leading to increased bone resorption and decreased bone formation. These changes result in a significant reduction in bone strength, increasing the risk of fractures and microtraumas, which can subsequently cause ON [42]. Furthermore, both OP and ON involve disruptions in bone metabolism and blood supply, potentially influencing and promoting each other [43]. OP, through its comprehensive impact on bone metabolism,

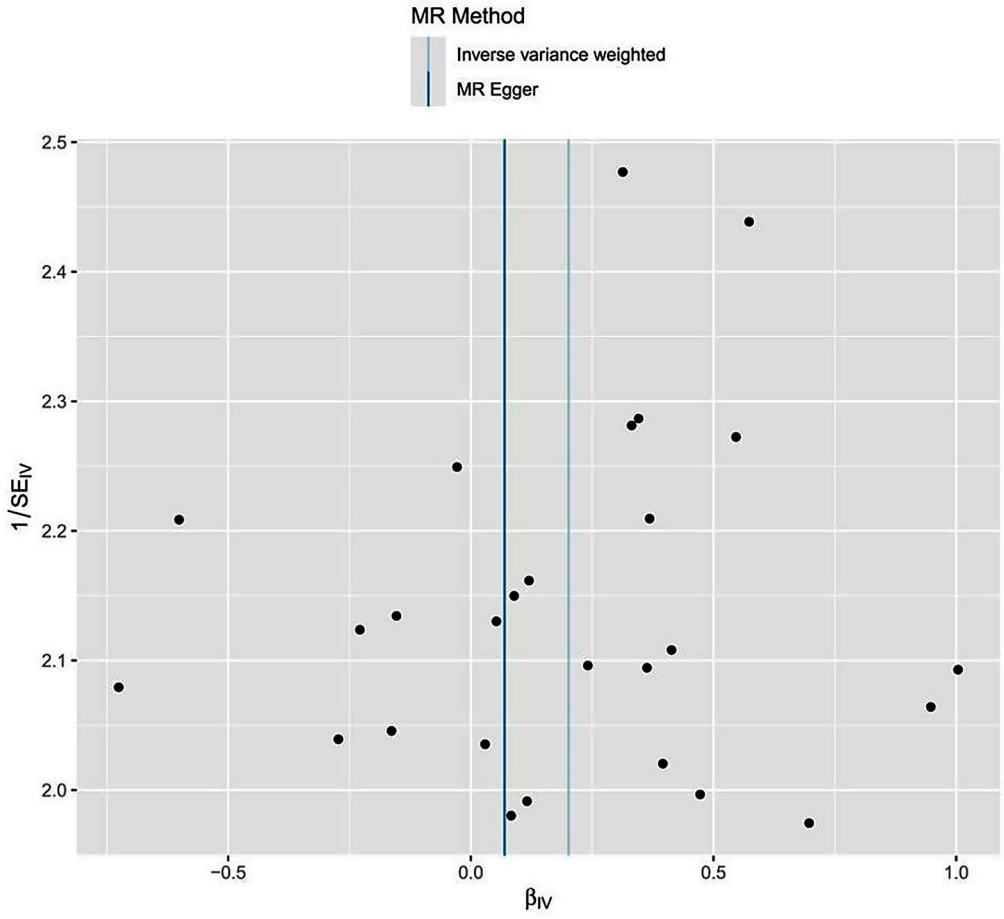

Fig. 6 The funnel plot of the impact of osteoporosis on osteonecrosis

Exposure	Nsnp	Outcome	Methods	b	se	OR(95% CI)	P-value
ON	11	OP	MR Egger	0.081	0.051	1.084(0.981-1.198)	0.147
	11	OP	Weighted median	0.042	0.036	1.043(0.971-1.12)	0.251
	11	OP	Inverse variance weighted	0.035	0.026	1.036(0.984-1.091)	0.181
	11	OP	Simple mode	0.045	0.064	1.046(0.923-1.185)	0.494
	11	OP	Weighted mode	0.046	0.058	1.047(0.934-1.174)	0.447
ON	10	FN BMD	MR Egger	-0.013	0.016	0.987(0.957-1.018)	0.432
	10	FN BMD	Weighted median	0.004	0.011	1.005(0.984-1.026)	0.672
	10	FN BMD	Inverse variance weighted	0.001	0.009	1.001(0.985-1.018)	0.879
	10	FN BMD	Simple mode	0.008	0.015	1.008(0.978-1.038)	0.63
	10	FN BMD	Weighted mode	0.004	0.013	1.004(0.979-1.029)	0.777
ON	10	LS BMD	MR Egger	-0.015	0.017	0.986(0.953-1.019)	0.418
	10	LS BMD	Weighted median	0.007	0.013	1.007(0.982-1.033)	0.579
	10	LS BMD	Inverse variance weighted	-0.002	0.009	0.998(0.98-1.017)	0.852
	10	LS BMD	Simple mode	0.012	0.02	1.012(0.974-1.053)	0.55
	10	LS BMD	Weighted mode	0.013	0.016	1.013(0.981-1.045)	0.451

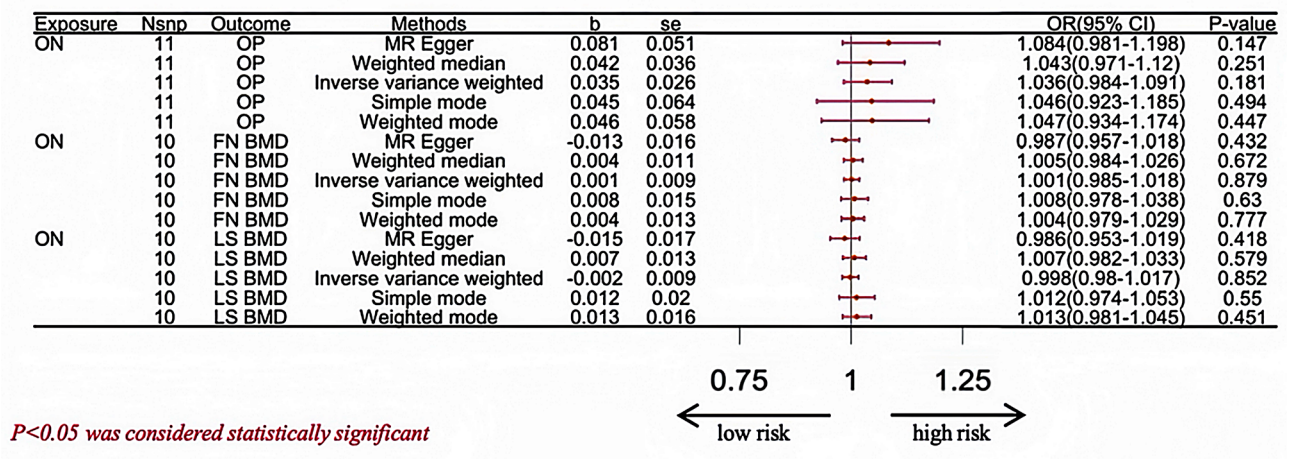

Fig. 7 Reverse Mendelian randomization results. Abbreviation: OP, Osteoporosis; ON, Osteonecrosis; FN BMD, Femoral neck bone mineral density; LS BMD, Lumbar spine bone mineral density

especially by increasing bone resorption and decreasing bone formation, leads to significant deterioration in bone structure and function, thereby raising the risk of ON. Consequently, OP patients are more prone to developing ON, particularly when using glucocorticoids that affect bone metabolism for extended periods. BMD primarily reflects the mineral content of bone, which, while an important indicator of bone strength, is not the sole factor [44]. High BMD does not necessarily equate to good bone quality and microarchitecture [45]. In some cases,

individuals may have normal BMD but abnormal bone microstructure, still posing a risk of bone microtrauma and interrupted blood supply, potentially leading to ON [46]. Additionally, BMD measurements can be influenced by various factors, including measurement techniques, individual metabolic status, and genetic factors, which may obscure the potential association between BMD and ON [47]. This finding suggests that preventing and treating ON should focus on the comprehensive regulation of bone metabolism, not just increasing BMD. It also emphasizes the need for a more holistic assessment and intervention for bone health in OP patients to reduce the risk of ON.

Through reverse Mendelian randomization analysis, we found no causal relationship between ON and either OP or BMD. ON is primarily caused by interrupted blood supply to the bone, leading to bone cell death and tissue breakdown, typically triggered by external factors such as trauma, long-term use of corticosteroids, or alcohol abuse, rather than the inherent quality or density of the bone [48]. OP and BMD mainly reflect the long-term state of bone metabolism, contrasting sharply with the acute and localized nature of ON [49]. A single occurrence of ON does not significantly impact the overall development of OP, which explains the lack of significant causal relationships observed in the reverse analysis. Furthermore, the selection and efficacy of instrumental variables may also affect the detection of reverse causality [50]. In reverse Mendelian randomization analysis, the instrumental variables used for ON may not be as effective as those used for OP or BMD. Additionally, ON's complex genetic basis makes it difficult to find efficient and specific instrumental variables to accurately reflect the risk of ON. Therefore, the insufficiency of instrumental variables may lead to the inability to detect significant causal relationships in reverse analysis. This finding further underscores the unique pathological characteristics and external triggers of ON, rather than intrinsic bone conditions.

Although the MR approach has advantages in inferring causal relationships, this study has some limitations. First, the study population is limited to individuals of European ancestry, which may restrict the generalizability of the results to other populations. Genetic differences between populations might affect the associations between OP, BMD, and ON, necessitating similar studies in different racial groups. Second, while the MR method helps reduce confounding factors, it is not entirely free of bias. Pleiotropy, where genetic variants affect multiple traits, can impact the validity of the instrumental variables used. Although we used MR-PRESSO and MR-Egger intercept tests to detect and adjust for horizontal pleiotropy, residual confounding cannot be completely ruled out. Third, the study relies on publicly available GWAS summary statistics, which may introduce variability due to differences in study design, phenotyping methods, and sample sizes. Additionally, using different GWAS datasets to study OP, BMD, and ON might lead to inconsistent results. Despite these limitations, the study provides valuable insights into the complex relationship between OP, BMD, and ON. By utilizing MR, the research offers a robust approach to disentangle causality from correlation, providing stronger evidence for clinical recommendations. Future studies should continue to explore the underlying mechanisms linking OP to ON, potentially investigating the role of vascular health, bone microarchitecture, and other systemic factors.

Conclusion

This MR study demonstrated a unidirectional causal relationship between OP and ON but found no significant causal relationships between BMD and ON. The results support the hypothesis that OP contributes to the development of ON, but BMD does not independently affect ON risk. These findings suggest that OP management may help reduce ON incidence, emphasizing the importance of early OP diagnosis and treatment, offering new directions for clinical strategies and further research into the biological pathways involved.

Abbreviations

ON	Osteonecrosis
OP	Osteoporosis
BMD	Bone mineral density
FN BMD	Femoral neck bone mineral density
LS BMD	Lumbar spine bone mineral density
MR	Mendelian randomization
GWAS	Genome-wide association study
SNP	Single nucleotide polymorphisms
LD	Linkage disequilibrium
IVW	Inverse-variance weighted
OR	Odds ratio
SONK	Spontaneous osteonecrosis of the knee

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

Z C was responsible for writing the original draft, reviewing and editing the manuscript, conceptualizing the study, curating data, conducting investigations, developing the methodology, managing the project, validating results, visualizing data, providing resources, and utilizing software tools. Y H contributed to writing the original draft, conceptualizing the study, curating data, conducting investigations, developing the methodology, managing the project, and visualizing data. M Y L was involved in writing the original draft, curating data, conducting investigations, developing the methodology,

supervising the project, managing the project, and validating results. W B F was responsible for reviewing and editing the manuscript, supervising the study, and managing the project. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Guangzhou University of Chinese Medicine, Guangzhou, China
[2]Linyi People's Hospital, Linyi, China

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