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A Mendelian Randomization Study of Cardiovascular Proteins, Immune Cell Traits, and Lifestyle Factors

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ABSTRACT

We aimed to investigate the causal relationship between cardiovascular-related proteins and osteoporosis and to assess the influence of immune cell traits and lifestyle factors such as smoking and alcohol consumption on osteoporosis risk.

A two-sample Mendelian randomization (MR) approach was employed using publicly available genome-wide association study (GWAS) data. Univariable and multivariable MR analyses were conducted using the inverse variance weighted (IVW) method to evaluate causal effects. Additional sensitivity analyses were performed to validate findings.

Three cardiovascular proteins showed significant associations with osteoporosis and pathological fractures: TNF-related apoptosis-inducing ligand receptor 2 (OR=0.10), TNF-related activation-induced cytokine (OR=2.90), and C-C motif chemokine 4 (OR=1.12). Lifestyle factors, including household tobacco smoke exposure, daily smoking quantity, and alcohol consumption, were also significantly associated with increased osteoporosis risk. Immune cell traits were identified as potential mediators in the relationship between cardiovascular proteins and osteoporosis.

This study highlights a novel link between cardiovascular health and osteoporosis, suggesting that specific proteins increase risk, while immune traits mediate this effect and lifestyle factors are independent risk factors. These findings underscore the importance of integrated strategies addressing inflammation and lifestyle in osteoporosis prevention and management.

Keywords: Cardiovascular proteins; Immune cell; Mendelian randomization analysis; Osteoporosis

INTRODUCTION

Osteoporosis, a common skeletal disorder characterized by reduced bone mass and structural deterioration of bone tissue, significantly increases the risk of fractures. Globally, over 200 million people are affected, leading to approximately 9 million fractures annually.[1] Medical costs linked to osteoporosis and related fractures are substantial, and the condition often reduces patients' quality of life.[2] Despite advancements in diagnostic techniques and therapeutic interventions, management remains suboptimal. Many patients are

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either misdiagnosed or receive inadequate treatment.[3] Recent research highlights a critical role for cardiovascular plasma proteins in the development of osteoporosis. Notably, both cardiovascular diseases and osteoporotic disorders share overlapping risk factors, including advanced age, systemic inflammation, and lifestyle behaviors such as smoking and excessive alcohol consumption.[4] The mechanisms by which cardiovascular proteins influence bone health, including the potential mediating role of immune cells, remain unclear.

The immune system constitutes a pivotal element in modulating bone health. Persistent inflammatory states, frequently characterized by heightened concentrations of pro-inflammatory cytokines, have been associated with the process of bone resorption and the pathogenesis of osteoporosis.[5] Immune cells, such as T cells and macrophages, can influence osteoclast and osteoblast activity, thereby affecting bone remodeling and density.[6] The relationship between cardiovascular proteins, immune cells, and osteoporosis is a new area of research. Traditional statistical methods may not accurately establish causality, but Mendelian Randomization can use genetic variants to infer causality more effectively.[7] This investigation utilizes a two-sample Mendelian Randomization (MR) strategy to elucidate the causal relationships between cardiovascular proteins and the risk of osteoporosis. The analysis harnesses data from extensive Genome-Wide Association Studies (GWAS) to inform the empirical framework.

The primary objective of this study is to investigate the causal relationship between cardiovascular plasma proteins and the onset of osteoporosis, with a focus on clarifying the intermediate role of immune cell characteristics. These findings may identify new biomarkers and therapeutic approaches for osteoporosis, addressing a critical clinical need for improved preventive and management strategies against this condition.

MATERIALS AND METHODS

Study Reporting Guidelines and Study Design

This study used two-sample Mendelian randomization and publicly available data to examine the impact of cardiovascular plasma proteins on osteoporosis incidence. It also explored the relationship between cardiovascular proteins and immune cells in osteoporosis development, following the STROBE-MR guidelines for reporting.[8] The study design diagram is shown in Figure 1.

Data Sources

Cardiovascular protein-associated summary statistics from genome-wide association studies (GWAS) were obtained from published research on human cardiovascular diseases.[9] A systematic search using PubMed identifiers (PMIDs) linked to this literature identified 90 relevant cardiovascular proteins, which were compiled into a comprehensive list via the MRC IEU Open database. Subsequent analyses involved procuring and standardizing genetic association data for these proteins using the TwoSampleMR R package (developed by Folkersen et al). These analyses were performed within a cohort of 21 758 individuals of European ancestry.

GWAS data for immune cell characteristics were derived from the scientific literature focused on immune cell biology.[10] The PubMed Identification number of the referenced study was used to search the MRC IEU Open database, yielding a comprehensive list of 731 immune cell features. The TwoSampleMR R package was employed to capture and standardize the summary statistics for the genetic associations of these 731 immune cell characteristics. These data were provided by Valeria Orru and colleagues, based on samples from a European cohort consisting of 3757 individuals. Additionally, GWAS data for osteoporosis were utilized in the study. The GWAS ID for osteoporosis (finn-b-OSTPOPATFRACTURE) was sourced from the MRC IEU OpenGWAS database, with standardized summary association statistics obtained using the TwoSampleMR R package. The study included 156 cases of osteoporosis and 218 007 controls, all of European descent.

In the initial phase of our literature review,[11,12] we identified two correlative risk factors for osteoporosis: tobacco smoking and alcohol consumption. For the analysis of smoking as a risk factor, we conducted a search in the MRC IEU OpenGWAS database using "smoke" as a keyword, which yielded 58 smoking-related traits. The TwoSampleMR R package was utilized to capture and standardize the summary statistics for the genetic associations of these 58 smoking indices. We searched the MRC IEU OpenGWAS database using "alcohol" as a keyword, resulting in the identification of 115 alcohol-related traits. The TwoSampleMR R package was again employed to capture and standardize the summary statistics for the genetic associations of these 115 alcohol-related indicators.

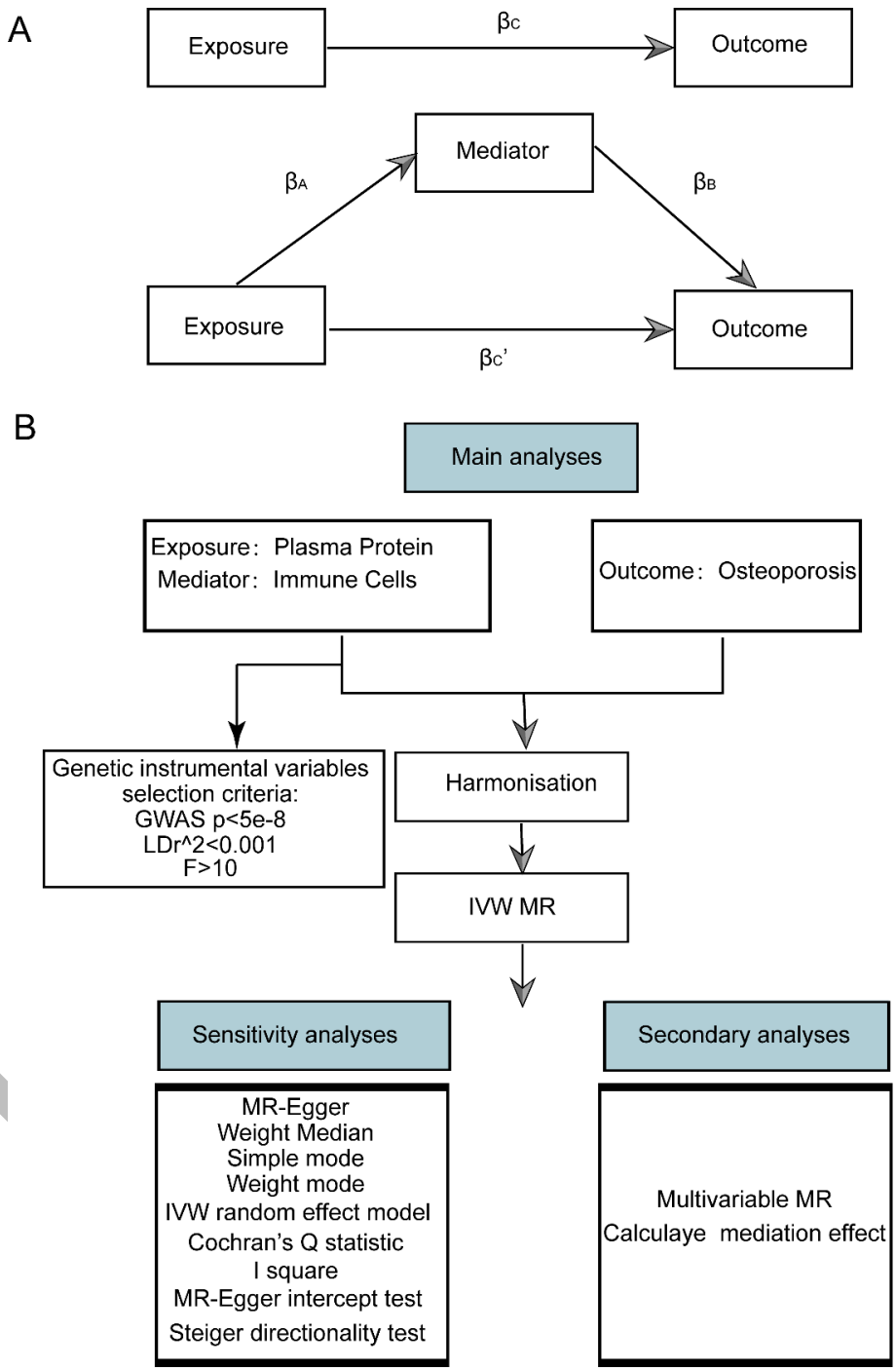

Figure 1. Technology roadmap. A. A representation of multivariate Mendelian randomization and mediation analysis. The basic assumptions of Mendelian randomization analysis are shown in the figure below. (1) There must be a significant relationship between the instrumental variable and the exposure factor, also called the assumption of association; (2) There must be no significant association between the instrumental variable and potential confounders that may influence exposure or outcomes; (3) There is only one path that can affect the outcome: instrumental variable + exposure + outcome. B. Schematic diagram describing the analysis methods in this study. SNP: single nucleotide polymorphism. IVW: independent variable weighting.

Selection of Instrumental Variables

There are three key assumptions to meet for a genetic variation instrumental variable to be valid: association with the exposure factor, independence from potential confounders, and exclusivity in influencing exposure and outcome. The study used a screening criterion of $p<5\times10^{-8}$ for SNPS in the exposure GWAS, excluding SNPS in linkage disequilibrium ($r^2 < 0.001$) and with a physical distance between genes < 10 000 KB. Outcome GWAS data were extracted based on selected SNPs, and F-statistics were calculated to assess instrumental variable bias. F<10 indicated weak instrumental variable bias, which could impact results and should be removed.[13] The formula for calculating the F-statistic is as follows:

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

Note: N represents the sample size, k represents the number of SNPs, R^2 reflects the explanatory power of the instrumental variable for the exposure factor, and a larger F value indicates a stronger association.

R^2 reflects how much the instrumental variables explain the exposure when n is the sample size, k is the number of instrumental variables, and k is the number of instrumental variables used. $R^2=2\times(1-MAF)\times MAF\times\beta^2$, where MAF is the minimum allele frequency and β is the allele effect size.

MR Causal Effect Estimation

To evaluate the causal effects of cardiovascular proteins on the incidence of osteoporosis, we employed multiple two-sample Mendelian Randomization (MR) methods, which included the following: the inverse-variance weighted (IVW) approach, the MR-Egger method, the weighted median estimator (weighted median), and the simple and weighted mode methods.[14] According to previous research,[15] the IVW method is slightly superior to other methods under certain conditions, as it is characterized by a regression that does not account for an intercept term and utilizes inverse variance as weights for model fitting. In the absence of pleiotropy, this research primarily used the IVW method for MR analysis, with four other methods as supplements. When pleiotropy was present, the MR-Egger method was used. The Steiger test was used to determine the causal direction.

Sensitivity Analysis

The Cochran Q test showed significant heterogeneity among SNP estimates, leading to the use of the random effects model of IVW to estimate causal effect size. The Cochran Q test can only determine the presence of heterogeneity, not its distribution. The I^2 statistic reflects the level of heterogeneity in the instrumental variable, with values ranging from 0% (no heterogeneity) to over 50% (high heterogeneity). The specific calculation formula is as follows:

$$I^2=\frac{Q-df}{Q}\times100\%$$

The MR-Egger method tests instrumental variables for pleiotropy. A p value below 0.05 for MR-Egger's intercept indicates significant horizontal pleiotropy. Remaining instrumental variables were tested by excluding one SNP at a time to see if it affected the association between cardiovascular proteins and osteoporosis. Differences in MR effect estimates suggest sensitivity to the SNP.

Multivariable MR Analysis-risk Factors

A concept known as multivariate MR (MVMR) is an extension of MR that uses genetic variants associated with multiple potentially related exposures to estimate multiple exposures' effects on a single outcome. When analyzing multivariate MR, we will examine the causal relationship between smoking, drinking, and cardiovascular protein indicators in relation to osteoporosis. This will involve building a multivariate MR model for osteoporosis.

Multivariate MR Analysis and Mediating Effect Estimation

Prior to conducting multivariate Mendelian Randomization (MR) analysis, we performed univariate MR analyses to identify cardiovascular proteins and immune cell characteristic indices with significant causal effects on osteoporosis. Those cardiovascular proteins and immune cell characteristics demonstrating significant causal associations in the univariate analyses were subsequently included as exposures in a subsequent multivariate MR framework. We constructed a multivariable MR model incorporating cardiovascular proteins and immune cell characteristics to investigate their combined effects on osteoporosis. Through the multivariate MR analysis, we obtained estimates of the direct effects of cardiovascular proteins

and immune cell characteristics on the risk of osteoporosis. By comparing these direct effects with the univariate MR estimates of the direct effects of cardiovascular proteins on immune cell characteristics, we were able to calculate the indirect effects of cardiovascular proteins on the pathogenesis of osteoporosis mediated through their influence on immune cell characteristics. This approach allows us to disentangle the direct and indirect pathways by which cardiovascular proteins may contribute to bone loss and the development of osteoporosis. The mediation effect of effect value and standard error is calculated according to the following formula:

$$\beta_M = \beta_A \times \beta_B$$

$$SE_M = \sqrt{(\beta_A \times SE_B)^2 + (\beta_B \times SE_A)^2}$$

The betaM is for the mediation effect of effect value, and SE_m is their corresponding standard error; β_A is the univariate MR effect value of cardiovascular proteins on immune cell characteristics, and SE_A is its corresponding standard error. β_B is the direct effect value of immune cell characteristics on osteoporosis (obtained by multivariate MR), and SE_B is its corresponding standard error.

In conjunction with the causal stepwise regression approach, the significance of the indirect effect is ascertained if both β_A and β_B exhibit statistical significance. In the event that neither β_A nor β_B is significant, the Sobel test is employed to evaluate the significance of the mediated effect coefficient, β_M. If β_M is found to be significant, the indirect effect is deemed significant. Given the presence of a significant indirect effect, if the multivariate MR effect estimate (β_{CVP}) for cardiovascular proteins on osteoporosis is statistically significant, the direct effect (C') is also considered significant, suggesting the potential existence of additional mediators. Conversely, if β_{CVP} is not significant, the direct effect is inferred to be non-significant, and a full mediating effect is postulated. When both indirect and direct effects are significantly present, and if the magnitudes of β_A and β_B differ, the proportion of the effect mediated (cover effect) is calculated using the formula |βA/βB| x 100%. If β_A equals β_B, the proportion of mediation is computed according to the partial mediation effect theory, using the formula (βA/βB) x 100%, which is applicable to the single variable MR analysis focusing on the effect of central cardiovascular protein β_M on osteoporosis (C'). The present discussion is complicated by the mediation effect; hence, this article confines its analysis to scenarios where there is a significant causal relationship between the exposure and outcome, as well as a significant causal association between the exposure and the mediating factors under consideration.

Statistical analysis

Data calculations and statistical analyses were conducted using R programming (version 4.2.2) and the TwoSampleMR package for Mendelian randomization analysis.[16] The Cochran Q test and leave-one-out analysis were used to assess robustness and reliability, with the MR-Egger genetic pleiotropic intercept method for inspection and the Steiger test for causal direction. For the MR analysis of osteoporosis, the evaluation index was the Odds Ratio (OR) and 95% Confidence Interval (95% CI), and all statistical *p* values were for bilateral inspections, statistical tests with a significance level of 0.05.

RESULTS

Instrumental Variable Screening

SNPs with linkage disequilibrium were excluded, and SNPs related to cardiovascular proteins were included as instrumental variables after matching with osteoporosis GWAS data. Table 1 displays only significant MR analysis indicators, with F test statistics greater than 10 indicating strong instrumental variables and limited bias.

MR Causal Effect Estimates

Five distinct Mendelian Randomization (MR) models—MR Egger, Weighted Median, Inverse Variance Weighted (IVW), Simple Mode (SM), and Weighted Mode—were applied for the analysis, and the outcomes are depicted in the forest plot (Figure 2). The scatter plot of SNP effect estimates, following screening and excluding instances with fewer than 2 SNPs, is displayed in Figure 3. The scatter plot fitting curves for the 5 models were largely aligned in direction, with most models exhibiting relatively consistent slopes, and the intercept of the IVW model was approximately zero. The results of our selection using the IVW model are detailed in Table 2. The IVW model revealed that cardiovascular protein levels of TNF-related apoptosis-inducing ligand receptor 2 (OR=0.10), TNF-related activation-induced cytokine (OR=2.90), and C-C motif

chemokine 4 (OR=1.12) had significant impacts on the risk of osteoporosis (p<0.05). Additionally, drug-induced osteoporosis was found to have a significant causal relationship with pathological fracture (p<0.05). To ascertain the correctness of the causal direction from cardiovascular proteins to osteoporosis, the Steiger directivity test was conducted (Table 3), which computed the variance explanation rate (r) of SNPs for exposure and outcome, respectively. The results indicated that the SNPs associated with our selected indicators explained a greater proportion of the variance in exposure than in outcome, with the direction confirmed as TRUE, and p<0.05, signifying a statistically significant correct directionality.

Table 1. Screening of instrumental variables for cardiovascular proteins and osteoporosis, and F-test for strength of instrumental variables

Exposure	Number of SNPs	Median of F	Minimum of F	Maximum of F
TNF-related apoptosis-inducing ligand receptor 2 levels	3	32.45	30.14	61.11
TNF-related activation-induced cytokine levels	6	92.12	35.63	248.58
C-C motif chemokine 4 levels	5	62.45	34.15	1135.43

SNP: single nucleotide polymorphism; TNF: tumor necrosis factor.

Figure 2. Forest diagram shows a variety of Mendelian randomization models for cardiovascular proteins and the causal relation analysis result of osteoporosis, the effect evaluation value with the OR and 95% CI, and at the same time shows the model using the instrumental variable number and calculating the beta values and standard error. Abbreviations: SNPs, Single Nucleotide Polymorphisms; OR: Odds Ratio; CI: Confidence Interval.

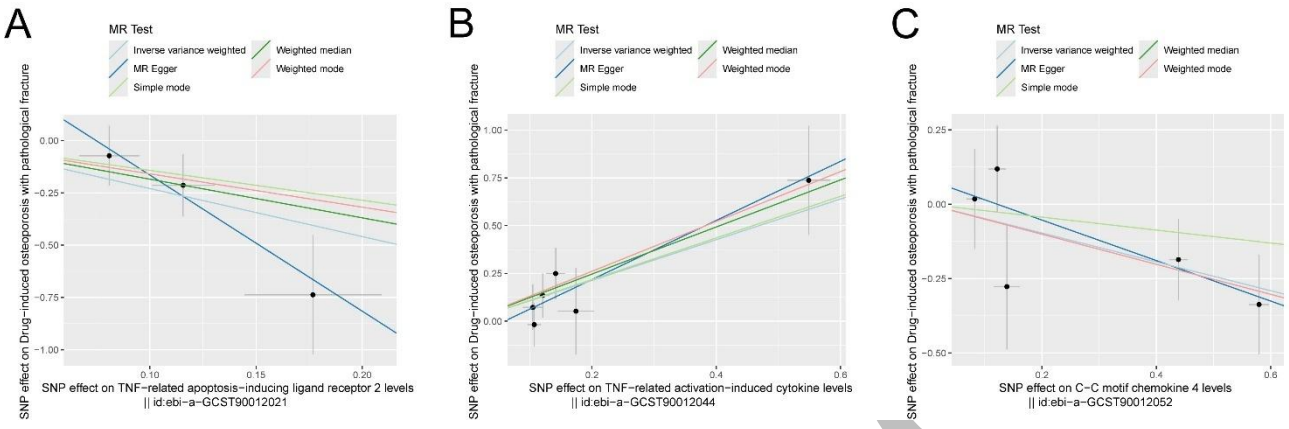

Figure3. Analysis results of multiple models for the Mendelian randomization analysis of cardiovascular proteins and osteoporosis. Estimated effect of different models of Mendelian randomization analysis of cardiovascular proteins on osteoporosis. A–C. TNF-related apoptosis-inducing ligand receptor 2 levels in cardiovascular proteins (A), TNF-related activation-induced cytokine levels (B), and C-C motif chemokine 4 levels (C) of osteoporosis (osteoporosis: drug-induced osteoporosis with pathological fracture) of the different model scatterplot Mendelian randomizations.

Table 2. Mendelian randomization causal effect estimates of cardiovascular proteins on the onset of osteoporosis

Exposure	Outcome	Number of SNPs	Beta	Standard error	OR (95% CI)	*p*
TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	3	2.29	0.88	0.100 (0.017, 0.575)	0.009
TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	6	1.06	0.34	2.903 (1.462, 5.761)	0.002
C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	5	0.48	0.20	0.615 (0.411, 0.922)	0.018

SNP: Single Nucleotide Polymorphism; OR: Odds Ratio; CI: Confidence Interval. OR>1 indicates an increased risk. For instance, for every 1-unit increase in TRANCE protein, the risk of osteoporosis increases by 2.90 times (95% CI, 1.46–5.76).

Table 3. Mendelian randomization analysis of cardiovascular proteins for osteoporosis Steiger directionality test

Exposure	Outcome	SNP r2 exposure	SNP r2 outcome	Correct causal direction
TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	0.005	4.15×10^{-5}	TRUE
TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	0.029	5.46×10^{-5}	TRUE
C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.082	3.79×10^{-5}	TRUE

SNP: single nucleotide polymorphism; r^2: variance explained rat.

Sensitivity Analysis

Heterogeneity of the significant results was tested using the Cochran Q test and I^2 statistic (Table 4). The results showed that the heterogeneity of the MR results of osteoporosis was not significant (Cochran Q *p* value>0.05), and the heterogeneity ratio was low (I^2<50%). Indicators of the instrumental variable funnel diagram are shown in Figure 4. The figure only presents the results for SNP numbers greater than 2. According to the causal correlation effect, the scatter distribution in the IVW model is symmetrical on each side, indicating that the result does not suggest potential bias. Indicators with fewer than 3 SNPs could not be subjected to the subsequent horizontal pleiotropy test and one-by-one elimination test. MR-Egger regression was used to test the horizontal pleiotropy of instrumental variables, as shown in Figure 5. The statistical hypothesis test *p* values of the intercept terms for each index were greater than 0.05, and the intercepts were close to 0, indicating that the causal inference in this study was not affected by horizontal pleiotropy (Table 5). The sensitivity analysis showed that removing each SNP did not significantly change the effect estimate, indicating stable results

Table 4. Mendelian randomization analysis heterogeneity test for cardiovascular proteins on osteoporosis

Exposure	Outcome	Q	Q_df	Q_pval	I^2 (%)
TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	2.088	2	0.35	4.25
TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	2.634	5	0.75	0
C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	2.748	4	0.60	0

Q: Cochran Q test statistic; Q_df: Q test degree of freedom; Q_pval: P value of Q test; The I^2 statistic reflects the proportion of the heterogeneous part of the instrumental variable in the total variation.

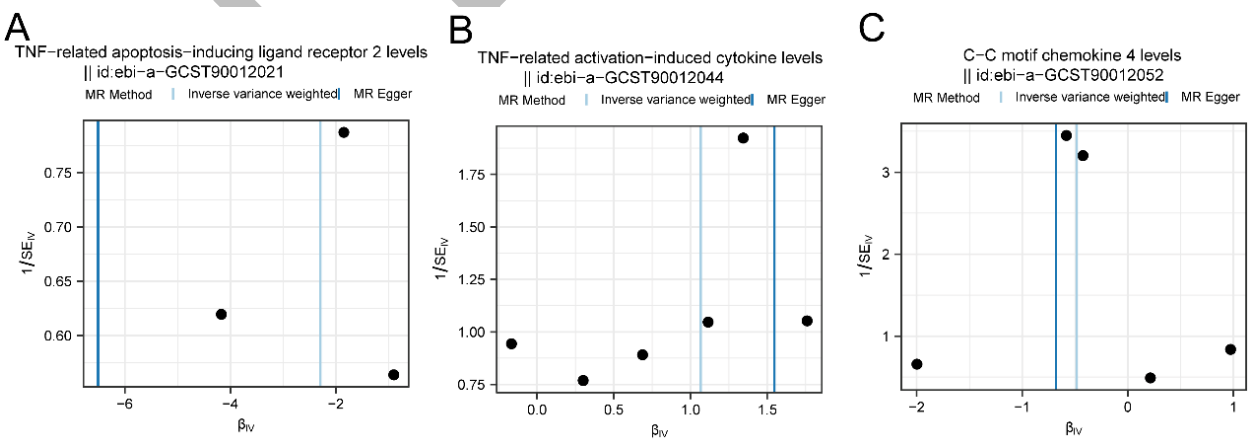

Figure 4. Funnel plot of heterogeneity test for Mendelian randomization analysis of cardiovascular proteins on osteoporosis. A–C. TNF-related apoptosis-inducing ligand receptor 2 levels in cardiovascular proteins (A), TNF-related activation-induced cytokine levels (B), and the effect of C-C motif chemokine 4 levels (C) on osteoporosis (osteoporosis: drug-induced osteoporosis with pathological fracture) funnel plot of Mendelian randomization different models heterogeneity test.

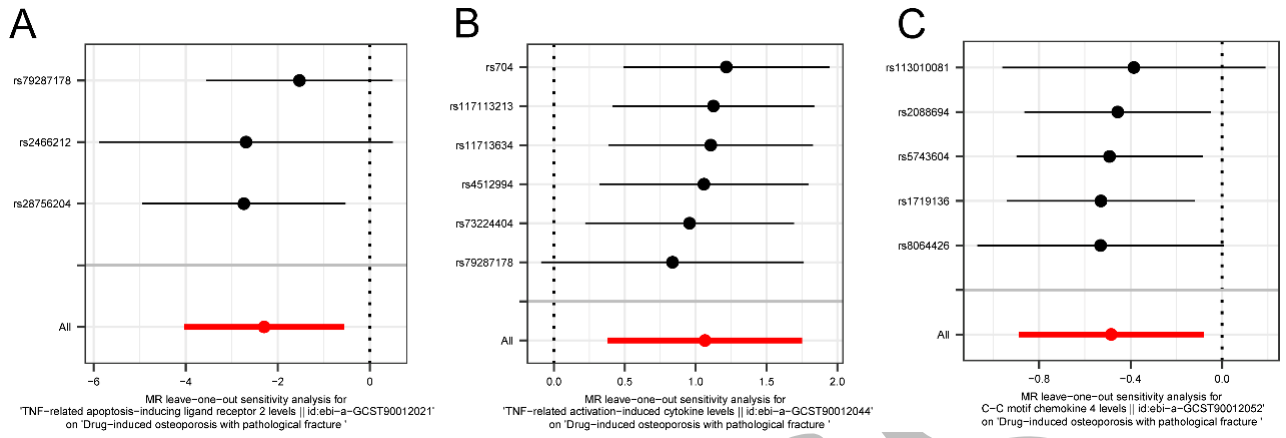

Figure 5. Mendelian randomization analysis of cardiovascular proteins on osteoporosis one-by-one exclusion test forest plot. A–C. TNF-related apoptosis-inducing ligand receptor 2 levels in cardiovascular proteins (A), TNF-related activation-induced cytokine levels (B), and C-C motif chemokine 4 levels (C) in osteoporosis:Drug-induced osteoporosis with pathological fracture Mendelian randomization elimination-by-elimination-test forest plot.

Table 5. Mendelian randomization analysis level pleiotropy test for cardiovascular proteins on osteoporosis

Exposure	Outcome	MR-Egger intercept	Standard error	*p*
TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	0.48	0.35	0.404
TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	0.09	0.10	0.441
C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.08	0.12	0.560

Multivariate MR Analysis-Risk Factors

We used Mendelian randomization to analyze smoking and alcohol indicators from the MRC IEU OpenGWAS database and their relationship to osteoporosis. Significant causal relationships were found between smoking indicators (exposure to tobacco smoke at home, number of cigarettes smoked daily) and alcohol indicators (alcohol drinker status) with osteoporosis (Table 6). The Steiger directional test was employed to ascertain the correctness of the causal direction from significant factors such as smoking and alcohol consumption to osteoporosis (Table 7). This test calculated the variance explanation rate (r) of SNPs for exposure and outcome, respectively. The findings indicated that the SNPs associated with our selected indicators explained a greater proportion of the variance in exposure compared to outcome, with the direction confirmed as TRUE, thus establishing the correctness of the causal direction. A multivariate MR analysis of osteoporosis was conducted, with significant factors such as smoking and alcohol, along with significant cardiovascular protein indices, as exposures to assess the direct effect of cardiovascular proteins on osteoporosis (Table 8). In Model 1, which adjusted for the indirect effect of exposure to tobacco smoke at home, the results revealed that levels of TNF-related activation-induced cytokine in cardiovascular proteins significantly impacted osteoporosis. Model 2, which adjusted for the indirect effect of the number of cigarettes currently smoked daily among current smokers, showed that both TNF-related activation-induced cytokine levels and C-C motif chemokine 4 levels in blood proteins had a significant influence on osteoporosis. After adjusting for the indirect effect of alcohol drinker status in Model 3, no significant effect of cardiovascular proteins on osteoporosis was observed.

Multivariate MR Analysis-mediator

Univariate MR analysis was conducted on cardiovascular proteins and immune cell characteristics, identifying 68 significant causal associations. Steiger directivity tests confirmed no reverse causality. Taking

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these significant results separately as exposure for multivariate MR analysis of osteoporosis, we obtained a total of 68 meaningful multivariate MR models. The results showed that the relationship between immune cell characteristics and osteoporosis was significant in 5 models (p<0.05) (Table 9) and was not significant in the remaining models (p>0.05).

The Mediation Effect Analysis

The models with a significant causal relationship between mediating factors and outcomes in the multivariate MR analysis were evaluated for mediating effect. The remaining models were tested by the Sobel test to determine whether the mediating effect was significant, and the significant models were evaluated for the mediating effect. According to the results of multivariate analysis of MR, the immune cells in only 5 model characteristic indexes of osteoporosis showed a significant effect (p<0.05), while the Sobel test results show that the residual mediation effects were not significant (p>0.05). Therefore, we only discuss the possible mediating effects in these 5 models. As there was a significant causal relationship between cardiovascular proteins and osteoporosis in all 5 models (p<0.05), all 5 models may constitute a partial mediating effect.

According to the results of the univariate analysis, the direct effects of MR cardiovascular proteins on the characteristic index values of immune cells were obtained. Additionally, the multivariate MR analysis yielded the characteristic indexes of immune cells with respect to the direct effect values on osteoporosis. The influence of cardiovascular proteins on the pathogenesis of osteoporosis, mediated by the characteristic indexes of immune cells, was calculated, and the results are shown in Table 10. The results indicate that, in Model 1, the relationship between the indirect effect and the direct effect (E–O amount) is positive, and the partial mediation effect accounts for 3.16%. In Models 2, 3, 4, and 5, the indirect effect and the direct effect (E–O) have opposite signs, indicating a suppression (cover) effect. Specifically, the suppression effect accounts for 33.64% in Model 2, 24.37% in Model 3, 28.02% in Model 4, and 27.55% in Model 5.

Table 6. Smoking (smoke), drinking (alcohol), and osteoporosis Mendelian randomization causal effect estimates

Exposure	Outcome	Number of SNPs	Beta	Standard error	OR (95% CI)	*p*
Exposure to tobacco smoke at home	Drug-induced osteoporosis with pathological fracture	4	1.63	0.66	0.19 (0.05, 0.72)	0.014
Number of cigarettes currently smoked daily (current cigarette smokers)	Drug-induced osteoporosis with pathological fracture	2	3.79	1.67	0.02 (0.0008, 0.59)	0.023
Alcohol drinker status	Drug-induced osteoporosis with pathological fracture	5	0.37	0.15	0.68 (0.50, 0.93)	0.016

SNP: Single Nucleotide Polymorphism; β: The Effect Coefficients in Mendelian Randomization Analysis; OR: Odds Ratio; CI: Confidence Interval.

Table 7. Steiger directivity test for Mendelian randomization analysis of smoke, alcohol, and osteoporosis

Exposure	Outcome	SNP r2 exposure	SNP r2 outcome	Correct causal direction
Number of cigarettes currently smoked daily (current cigarette smokers)	Drug-induced osteoporosis with pathological fracture	0.004	2.46×10^{-5}	TRUE
Exposure to tobacco smoke at home	Drug-induced osteoporosis with pathological fracture	0.023	3.78×10^{-5}	TRUE
Alcohol drinker status	Drug-induced osteoporosis with pathological fracture	0.086	3.60×10^{-5}	TRUE

SNP: Single Nucleotide Polymorphism; β: The effect coefficients in multivariate Mendelian randomization analysis.

Table 8. Smoking (smoke), drinking (alcohol), and cardiovascular protein effect on the onset of osteoporosis of multivariate Mendelian randomization analysis results

Model	Exposure	Outcome	Beta	Standard error	*p*
Model 1	TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	0.07	0.28	0.80
	TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	1.00	0.29	0.001
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.49	0.19	0.008
	Exposure to tobacco smoke at home	Drug-induced osteoporosis with pathological fracture	0.25	0.71	0.724
Model 2	TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	0.20	0.31	0.528
	TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	0.82	0.33	0.014
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.45	0.28	0.108
	Number of cigarettes currently smoked daily (current cigarette smokers)	Drug-induced osteoporosis with pathological fracture	0.41	0.27	0.136
Model 3	TNF-related apoptosis-inducing ligand receptor 2 levels	Drug-induced osteoporosis with pathological fracture	0.18	0.33	0.586
	TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	0.48	0.43	0.260
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.37	0.29	0.208
	Alcohol drinker status	Drug-induced osteoporosis with pathological fracture	0.06	0.05	0.242

SNP: Single Nucleotide Polymorphism; β: The effect coefficients in multivariate Mendelian randomization analysis.

Table 9. Results of multivariate Mendelian randomization analysis of the effect of cardiovascular proteins and immune cell characteristics on the incidence of osteoporosis

Model	Exposure	Outcome	Beta	Standard error	*p*
Model 1	Basophil Absolute Count	Drug-induced osteoporosis with pathological fracture	0.18	0.07	0.011
	TNF-related activation-induced cytokine levels	Drug-induced osteoporosis with pathological fracture	1.05	0.24	0
Model 2	Terminally Differentiated $CD4^{-}CD8^{-}$ T cell Absolute Count	Drug-induced osteoporosis with pathological fracture	0.84	0.40	0.035
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.58	0.17	0.001
Model 3	BAFF-R on $CD20^{-}$ B cell	Drug-induced osteoporosis with pathological fracture	0.97	0.45	0.030
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.51	0.26	0.048
Model 4	CD62L on $CD62L^{+}$ Dendritic Cell	Drug-induced osteoporosis with pathological fracture	1.08	0.41	0.008
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.59	0.14	0
Model 5	CD45RA on Terminally Differentiated $CD8^{+}$ T cell	Drug-induced osteoporosis with pathological fracture	0.98	0.46	0.034
	C-C motif chemokine 4 levels	Drug-induced osteoporosis with pathological fracture	0.57	0.18	0.001

SNP: Single Nucleotide Polymorphism; β: The effect coefficients in multivariate Mendelian randomization analysis.

Table 10. Evaluation of Mendelian randomization mediating effect of cardiovascular protein-mediated immune cell characteristics on the incidence of osteoporosis

Model	Exposure	Mediator	Outcomes	Total effect (95% CI)	Effect E-M (95% CI)	Effect M-O (95% CI)	Effect E-O (95% CI)	Mediation effect (95% CI)	Proportion of mediating effect
Model 1	TNF-related activation-induced cytokine levels	Basophil Absolute Count	Drug-induced osteoporosis with pathological fracture	1.06 (0.38, 1.75)	0.185 (0.35, 0.01)	0.18 (0.32, 0.04)	1.05 (0.58, 1.53)	0.03 (0.001, 0.07)	3.16%
Model 2	C-C motif chemokine 4 levels	Terminally Differentiated $CD4^{-}CD8^{-}$ T cell Absolute Count	Drug-induced osteoporosis with pathological fracture	0.48 (−0.88, −0.08)	0.23 (−0.35, −0.10)	0.84 (−1.63, −0.05)	0.58 (−0.92, −0.24)	0.19 (−0.01, 0.40)	33.64%
Model 3	C-C motif chemokine 4 levels	BAFF-R on $CD20^{-}$ B cell	Drug-induced osteoporosis with pathological fracture	0.48 (−0.88, −0.08)	0.12 (−0.23, −0.001)	0.97 (−1.86, −0.08)	0.51 (−1.02, −0.01)	0.12 (0.03, 0.28)	24.37%
Model 4	C-C motif chemokine 4 levels	CD62L on $CD62L^{+}$ Dendritic Cell	Drug-induced osteoporosis with pathological fracture	0.48 (−0.88, −0.08)	0.15 (−0.28, −0.02)	1.08 (1.81, 0.28)	0.59 (0.88, 0.31)	0.16 (−0.01, 0.35)	28.02%
Model 5	C-C motif chemokine 4 levels	CD45RA on Terminally Differentiated $CD8^{+}$ T cell	Drug-induced osteoporosis with pathological fracture	0.48 (−0.88, −0.08)	0.16 (−0.31, −0.01)	0.98 (1.89, 0.07)	0.57 (−0.94, −0.21)	0.15 (0.05, 0.37)	27.55%

CI: confidence interval.

In Model 1, immune cells (basophils) mediated 3.16% of the effect, indicating that cardiovascular proteins can partially affect bone health through immune pathways.

DISCUSSION

This investigation tackles the pivotal query of the impact of cardiovascular plasma proteins on the occurrence of osteoporosis and their potential mediation via immune cells within the disease's pathophysiological pathways. The principal objective was to delineate the causal effects of cardiovascular proteins on osteoporosis through the application of two-sample MR techniques. The study harnessed publicly accessible GWAS data to pinpoint valid instrumental variables and conducted a suite of sensitivity analyses to corroborate the validity of the results. Furthermore, the research implemented multivariable MR frameworks to evaluate the direct and indirect impacts of tobacco smoking, alcohol consumption, and cardiovascular proteins on osteoporosis, uncovering significant causal associations. These discoveries provide novel perspectives on the interplay between cardiovascular and skeletal health, potentially pinpointing innovative avenues for the prevention and therapeutic intervention of osteoporosis.

The causal relationship between cardiovascular proteins and osteoporosis, as revealed in this study, arises from the intricate interplay among cardiovascular health, immune responses, and bone metabolic processes. Our research identifies several key cardiovascular proteins—including TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2), TNF-related activation-induced cytokine (TRANCE), and C-C motif chemokine 4 (CCL4)—that demonstrate significant causal associations with osteoporosis onset. These findings suggest that these proteins play critical roles in modulating bone metabolism through pathways linked to inflammation and apoptosis. This aligns with existing literature, particularly the work by Azeez (2023), who detailed shared risk factors and inflammatory mechanisms underlying both osteoporosis and cardiovascular diseases, further supporting our observation of overlapping etiological pathways.[18]

In the context of our investigation, we found that TRAIL-R2 levels were inversely related to the incidence of drug-induced osteoporosis and pathological fractures. This finding is congruent with earlier studies indicating that TRAIL-R2 can modulate osteoclastogenesis, thereby impacting the equilibrium between bone resorption and formation.[19] Additionally, the Inverse Variance Weighted (IVW) model revealed that the SNPs associated with TRAIL-R2 accounted for a significant proportion of the variance in exposure, which strengthens the evidence for the proposed causal direction. TRANCE levels were found to be positively associated with the risk of osteoporosis. TRANCE, also recognized as RANKL, is a critical mediator in the differentiation and activation of osteoclasts, which is essential for the process of bone resorption.[20] The pronounced effects observed in both the IVW and multivariable MR analyses, coupled with the results of the Steiger directionality test, underscore the validity and robustness of this association. CCL4 levels exhibited a significant positive correlation with osteoporosis. CCL4 is known to regulate immune responses and has been linked to the recruitment of osteoclast precursors, thereby influencing bone metabolic processes.[21] The coherent results across different MR methodologies provide further validation of CCL4's role in the pathogenesis of osteoporosis. The present study's exploration into the characteristics of immune cells uncovered that 5 distinct immune cell traits bore significant causal relationships with osteoporosis, thereby emphasizing the pivotal role of immune system regulation in maintaining bone health. Our research has identified specific immune cell characteristics, such as the absolute count of basophils, which was found to influence osteoporosis via TRANCE levels ($p<0.05$). This suggests that basophils may play a role in modulating inflammatory responses that impact bone health.

The absolute count of terminally differentiated $CD4^{-}CD8^{-}$ T cells was identified as a mediator in the relationship between cardiovascular proteins and osteoporosis, as influenced by CCL4 levels ($p<0.05$). The mediation analysis revealed that in Model 1, immune cell traits mediated 3.16% of the effect of cardiovascular proteins on osteoporosis, underscoring the complex interplay between the cardiovascular and immune systems in the context of bone health. The masking effects observed in other models indicate that the relationship between these proteins and bone health is not linear and may involve intricate feedback loops. Previous studies have documented that immune cells, such as T-cells and macrophages, are known to secrete cytokines and growth factors that influence bone resorption and formation, thereby indirectly linking immune function to bone health.[22,23] Our novel findings propose a potential mechanistic connection between immune function and bone health, an area that has been less investigated in the existing literature.

Our study further emphasized the significant role of lifestyle factors in influencing osteoporosis risk. Prolonged exposure to secondhand smoke was strongly correlated with an elevated risk of osteoporosis, and the daily smoking volume emerged as a key determinant. The detrimental effects of smoking on bone health are well-established, including delayed bone healing and heightened fracture susceptibility, largely due to its adverse impact on bone mineral density and turnover. Additionally, alcohol consumption status was identified as a critical contributor to osteoporosis. Chronic alcohol use has been shown to impair bone formation while accelerating bone resorption, leading to reduced bone mass and weakened structural integrity.[25] The significant associations of TRANCE and CCL4 levels with osteoporosis were maintained even after adjusting for the confounding effects of smoking and alcohol consumption. This indicates that these proteins have a direct influence on bone health that is independent of lifestyle factors. The resilience of these associations within multivariable models reinforces the potential of these proteins as robust biomarkers and therapeutic targets for the prevention and treatment of osteoporosis.

Our study elucidates the complex interplay between cardiovascular proteins, immune cell characteristics, and lifestyle factors in the etiology of osteoporosis. These results pave the way for future research to explore these biomarkers as potential therapeutic targets or diagnostic tools.

STATEMENT OF ETHICS

This study utilized publicly available genome-wide association study (GWAS) data, including cardiovascular protein-related GWAS data from Folkersen et al,[9] immune cell trait GWAS data from Orru et al[10], and osteoporosis GWAS data (finn-b-OSTPOPATFRACTURE) from the MRC IEU OpenGWAS database. All original datasets used in this research have obtained ethical approval from the respective institutional review boards (IRBs) or ethics committees of their source institutions and have complied with the principles of the Declaration of Helsinki and relevant human research ethics regulations.

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Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

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

AI ASSISTANCE DISCLOSURE

Not applicable.

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