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CLINICAL TRIAL REPORT

# Denosumab for Primary Osteoporosis and Its Impact on Sarcopenia in the Chinese Population: Insights from Clinical Evidence and RANKL Pathway Mendelian Randomization

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**Background:** With the rapid aging of China's population, osteoporosis and sarcopenia have become major public health challenges. Denosumab, a first-line therapy for osteoporosis, may also improve muscle health, a possibility warranting further investigation.

**Objective:** This study evaluated the efficacy of denosumab in treating primary osteoporosis in the Chinese population and explored its potential effects on sarcopenia. A Mendelian randomization (MR) analysis was additionally performed to investigate the causal role of the RANKL pathway in sarcopenia.

**Methods:** This study included two components. In the clinical study, 45 patients with primary osteoporosis received denosumab, of whom 40 completed a 6-month follow-up and 15 completed a 1-year follow-up. Outcomes included bone turnover markers, bone mineral density, muscle strength, and physical performance measures. In the genetic study, two-sample MR was conducted using genome-wide association study (GWAS) summary statistics to assess the causal association between RANKL gene variants and sarcopenia-related traits, including appendicular lean mass and grip strength.

**Results:** Denosumab significantly reduced bone turnover markers and improved muscle function after 6 months, with further gains in bone mineral density and muscle strength observed at 1 year (all $P < 0.05$). Muscle mass showed upward but non-significant trends. MR analysis revealed a significant negative association between RANKL expression and both appendicular lean mass and grip strength, with no evidence of heterogeneity or pleiotropy.

**Conclusion:** Denosumab effectively treats osteoporosis and improves muscle function in Chinese patients. Genetic evidence supports a causal role of the RANKL pathway in sarcopenia, indicating that RANKL overexpression may contribute to its development. By integrating clinical and genetic evidence, our findings suggest that denosumab may represent a promising therapeutic option for patients with concurrent osteoporosis and sarcopenia.

**Keywords:** osteoporosis, sarcopenia, denosumab, RANKL gene, Mendelian randomization

## Introduction

According to the 2020 National Census by the National Bureau of Statistics of China, the population aged 60 years and older was nearly 270 million (18.7% of the total population), with over 190 million individuals aged 65 years and older (13.5%).[1] As China transitions into an aging society, degenerative conditions such as osteoporosis and sarcopenia have emerged as major health threats among the elderly. The Epidemiological Survey of Osteoporosis in China (2018) reported that over 90 million individuals aged 50 years and above were affected by osteoporosis, with a prevalence of

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19.2% in those over 50 and 32% in those over 65.[2] The prevalence in women was significantly higher than in men. Due to shared genetic determinants, endocrine regulatory pathways, and signaling mechanisms, osteoporosis and sarcopenia are closely interrelated and frequently coexist, thereby jointly increasing the risk of falls and fractures.[3,4] Fragility fractures, particularly hip fractures resulting from osteoporosis, exert a profound impact on patients' health and quality of life, while imposing a substantial burden on healthcare systems.[5] In recent years, denosumab, a novel targeted anti-osteoporotic agent, has been widely adopted in clinical practice.[6] However, evidence regarding the effects of denosumab on sarcopenia in Chinese patients with osteoporosis remains scarce, with limited systematic clinical studies and published data. Moreover, the pathophysiological mechanisms underlying sarcopenia and the potential role of denosumab in mitigating or treating sarcopenia remain unclear. Therefore, investigating the impact of denosumab on sarcopenia in the context of osteoporosis is currently a research priority.

Mendelian randomization (MR) is a powerful tool in epidemiological research, employing genetic variants as instrumental variables to assess causal relationships between risk factors and disease outcomes.[7–10] A key advantage of MR is its ability to mitigate confounding factors, which often hinder causal inference in observational studies. Because genetic variants are randomly allocated during meiosis and fixed at conception, MR mimics the design of a randomized controlled trial.[11,12] As such, MR analyses can reduce the influence of confounding, reverse causation, as well as feasibility limitations commonly found in traditional observational and interventional studies.[13] However, to date, no MR study has specifically investigated the potential causal association between the RANKL gene and sarcopenia.

Taken together, this study aims to evaluate the efficacy of denosumab in treating primary osteoporosis, as well as its potential impact on muscle-related parameters in the Chinese population. Furthermore, we apply Mendelian randomization analysis to explore the possible mechanisms underlying sarcopenia progression and assess the feasibility of denosumab as a therapeutic option for sarcopenia. The findings are expected to provide an evidence-based therapeutic strategy for managing osteoporosis with comorbid sarcopenia and contribute to the theoretical foundation for the prevention and management of this chronic degenerative condition in China.

# Materials and Methods

## Study Design

This study consisted of two primary components. The first was a single-center, prospective, self-controlled clinical trial. The second component involved a Mendelian randomization (MR) analysis.

## Ethical Approval and Registration

The study protocol was approved by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Approval No. [2023] Ethics Review (0906–01)). The study was conducted in accordance with the principles outlined in the Declaration of Helsinki (2008). All participants were thoroughly informed of the study objectives, and written informed consent was obtained from each individual. Confidentiality and data protection were rigorously upheld to safeguard participants' rights.

The study has been registered on ClinicalTrials.gov and is publicly accessible.

**Protocol ID**: UHCT230847

**ClinicalTrials.gov ID**: NCT06154707

## Clinical Study

### Study Population and Inclusion/Exclusion Criteria

According to the World Health Organization (WHO),[14] the diagnosis of osteoporosis is established using dual-energy X-ray absorptiometry (DXA), with a T-score of $\leq -2.5$ at the lumbar spine (L1–L4) indicating osteoporosis. Participants were recruited from the Geriatric Hospital Affiliated with Wuhan University of Science and Technology.

Inclusion criteria: Individuals with a DXA-confirmed diagnosis of primary osteoporosis, defined as a lumbar spine (L1–L4) T-score $\leq -2.5$, were eligible for inclusion.

Exclusion criteria: Participants were excluded if they (1) had secondary osteoporosis or pathological fractures; (2) had enrolled in other clinical trials within the previous 3 months or received other first-line anti-osteoporotic treatments within the past 6 months; or (3) had severe chronic diseases (eg, diabetes mellitus), organ failure, or were otherwise deemed unsuitable for participation by the investigators.

### Intervention and Outcomes

Eligible participants received subcutaneous injections of denosumab (Mailsu®, Mabwell Bioscience) at a dose of 60 mg every 6 months. All participants were also supplemented with calcium and vitamin D (Caltrate®, one tablet daily). Bone mineral density was assessed using DXA, and muscle-related parameters were evaluated using bioelectrical impedance analysis (BIA).

Primary outcomes included bone mineral density (BMD), bone turnover markers, and muscle function parameters (dominant handgrip strength, 6-m walk gait speed, 5 times sit-up time, and Timed Up and Go [TUG]), as well as muscle mass indicators (upper arm muscle circumference, skeletal muscle mass index [SMI], and appendicular skeletal muscle mass index [ASMI]).

Secondary outcomes included serum calcium and 25-hydroxyvitamin D (25-OH-VD) levels.

## Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM, U.S.A). Continuous variables with normal distribution and homogeneity of variance were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared using paired t-tests. Categorical variables were reported as counts and percentages and analyzed using the chi-square test. A two-sided p-value of $< 0.05$ was considered statistically significant.

## Mendelian Randomization Study

### Methods and Design

A two-sample Mendelian randomization (MR) analysis was conducted using summary-level data from genome-wide association studies (GWAS). Given the current lack of a comprehensive and independent GWAS dataset specifically targeting sarcopenia, surrogate phenotypes strongly associated with sarcopenia—appendicular lean mass (ALM) and dominant (right) handgrip strength—were employed. ALM is considered a key indicator of skeletal muscle mass in older adults,[15] while dominant handgrip strength is widely accepted as a reliable measure of muscle strength.[16] By assessing the causal relationship between RANKL gene expression and these sarcopenia-related phenotypes, we sought to infer a potential genetic association between RANKL and the risk of sarcopenia. Sensitivity analyses were conducted to assess the robustness and consistency of the findings (Figure 1).

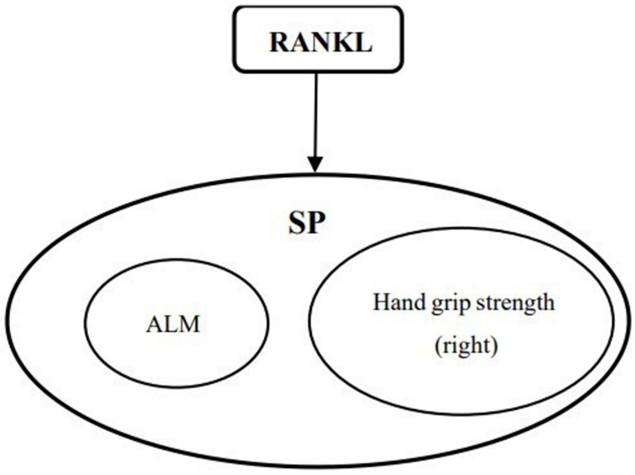


**Figure 1** Mendelian Randomization Study of RANKL and Sarcopenia-Related Traits.


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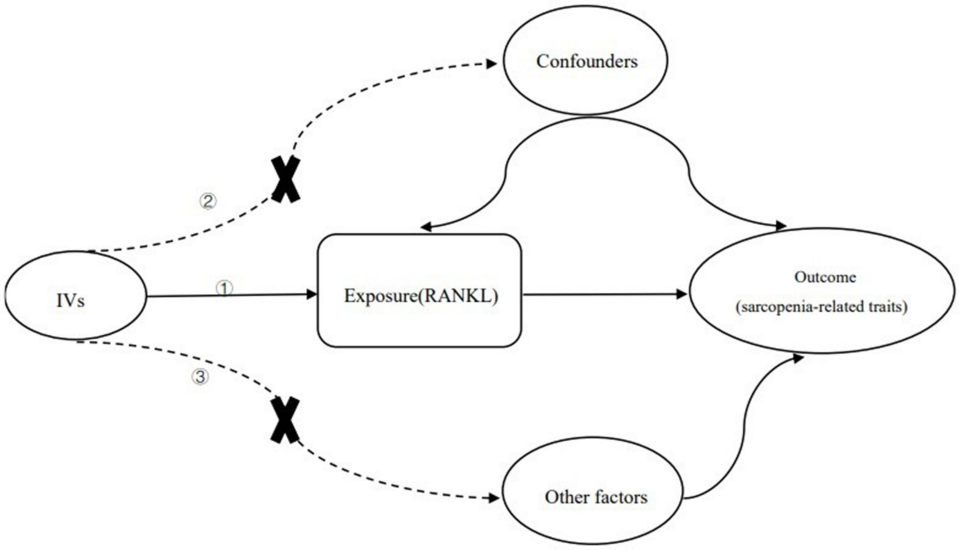


**Figure 2** Model of the two-sample Mendelian Randomization analysis ① Relevance – the instrumental variables (IVs) must be strongly associated with the exposure of interest.② Independence – the IVs must be independent of confounders that could influence the exposure-outcome relationship.③ Exclusion Restriction – the IVs must affect the outcome exclusively through the exposure and not through alternative pathways.

A valid MR analysis must satisfy three core assumptions: (1) Relevance – the instrumental variables (IVs) must be strongly associated with the exposure of interest; (2) Independence – the IVs must be independent of confounders that could influence the exposure-outcome relationship; and (3) Exclusion Restriction – the IVs must affect the outcome exclusively through the exposure and not through alternative pathways (Figure 2).[17]

## Data Sources

The data used in this study were derived primarily from the UK Biobank and accessed through the IEU OpenGWAS platform (https://gwas.mrcieu.ac.uk/). Specifically, the exposure dataset comprised 3,301 individuals with RANKL gene-related genotypic data. The outcome datasets included 450,243 samples for appendicular lean mass and 461,089 samples for right handgrip strength. Detailed descriptions of the datasets and summary statistics are provided in Table 1.

## Instrumental Variables

To satisfy the first core assumption of Mendelian randomization (MR)—that instrumental variables (IVs) must be robustly associated with the exposure—single nucleotide polymorphisms (SNPs) significantly associated with the RANKL gene at the genome-wide level ($P < 5\times10^{-8}$) were selected. To ensure a sufficient number of valid SNPs, the linkage disequilibrium (LD) threshold was set at $r^2 < 0.9$ with a 10,000 kb window.[18] To meet the second assumption—that IVs must be independent of potential confounders—pleiotropy was assessed using the MR-Egger intercept, and the Phenoscanner database (http://www.phenoscanner.medschl.cam.ac.uk/) was queried to exclude SNPs associated with known confounders. Cochran's Q test was employed to evaluate heterogeneity, and SNPs with significant heterogeneity were excluded. To evaluate instrument strength and rule out weak instrument bias, the F-statistic was calculated for each SNP,[19] with values above 10 indicating sufficient strength. All selected SNPs had F-statistics greater than 10, confirming the absence of weak instrument bias.[20]

**Table 1** Summary of the GWAS Included in the Study

| Variables | ID | Sample Sizes | SNPs | Population | Sex | Year |
|---|---|---|---|---|---|---|
| RANKL | Prot-a-3054 | 3301 | 10,534,735 | European | Males and Females | 2018 |
| ALM | Ebi-a-GCST90000025 | 450,243 | 18,071,518 | European | NA | 2020 |
| Hand grip strength (right) | ukb-b-10215 | 461,089 | 9,851,867 | European | Males and Females | 2018 |

## Mendelian Randomization Analysis

Mendelian randomization (MR) analyses were conducted to evaluate the potential causal effects of RANKL gene expression on appendicular lean mass (ALM) and right-hand grip strength. For the ALM analysis, the inverse variance weighting (IVW), weighted median, and MR-Egger methods were employed to ensure robustness and consistency. In the analysis of right-hand grip strength, the IVW method served as the primary analytical approach due to its high statistical power and consistency under valid assumptions.[21] Causal estimates were expressed as beta coefficients, with corresponding odds ratios (ORs) and 95% confidence intervals (CIs) calculated. A P-value < 0.05 was considered statistically significant. Heterogeneity was assessed using Cochran's Q statistic under both IVW and MR-Egger models. Horizontal pleiotropy was evaluated via the MR-Egger intercept test to ensure the validity of instrumental variables.

# Results

## Clinical Study Results

In the clinical trial component of this study, 45 patients were enrolled based on predefined inclusion and exclusion criteria. Five patients were lost to follow-up, resulting in 40 participants completing six months of treatment and follow-up, of whom 15 completed the full one-year treatment course. Baseline characteristics are presented in Table 2. After six months of denosumab treatment, significant reductions were observed in bone turnover markers compared to baseline: t-PINP (16.83±13.03 ng/mL; 95% CI: 33.89 to 45.71; P < 0.001), N-terminal mid-fragment osteocalcin (15.66±4.57 ng/mL; 95% CI: 6.64 to 14.48; P < 0.001), and β-CTx (0.14±0.05 ng/mL; 95% CI: 0.15 to 0.26; P < 0.001). Improvements in muscle function were observed in 6-m walk gait speed (1.01±0.16 m/s; 95% CI:–0.20 to–0.06; P < 0.001), 5 times sit-up time (12.14±2.98 s; 95% CI: 1.22 to 5.30; P = 0.020), and TUG (9.48±2.33 s; 95% CI: 0.38 to 2.64; P = 0.010), as shown in Table 3.

At the one-year mark, lumbar spine bone mineral density showed significant improvement (T-score:–2.61±0.83; 95% CI:–0.87 to–0.16; P = 0.007). Muscle function indicators also demonstrated significant gains: dominant hand grip strength (24.39±3.10 kg; 95% CI:–3.54 to–1.07; P = 0.001), 6-m walk gait speed (1.15±0.11 m/s; 95% CI:–0.30 to–

**Table 2** Basic Information of Two Groups of Follow-Up Patients

| | Six-Month Follow-Up | 1-Year Follow-Up |
|---|---|---|
| N | 40 | 15 |
| Gender | Females: 40 Males: 0 | Females: 15 Males: 0 |
| Age | 64.3±7.63 | 60.93±7.86 |
| BMI | 23.21±3.07 | 22.95±3.40 |
| Smoking | 0 | 0 |
| Drinking | 0 | 0 |

**Table 3** Baseline-Follow-Up Results After Six Months

| | Base Line | Six-Month Follow-Up | t (39) | d | 95% CI | P |
|---|---|---|---|---|---|---|
| t-P1NP (ng/mL) | 56.63±22.09 | 16.83±13.03 | 13.62 | 2.16 | 33.89 to 45.71 | 0.000 |
| VD (25-OH) (ng/mL) | 17.91±4.00 | 23.77±4.27 | −6.39 | −1.01 | −7.72 to −4.01 | 0.000 |
| N-MID (ng/mL) | 26.21±11.12 | 15.66±4.57 | 5.45 | 0.86 | 6.64 to 14.48 | 0.000 |
| β-CTx (ng/mL) | 0.34±0.19 | 0.14±0.05 | 7.02 | 1.11 | 0.15 to 0.26 | 0.000 |
| PTH (ng/L) | 44.38±23.78 | 44.00±22.65 | 0.08 | 0.01 | −8.83 to 9.59 | 0.934 |
| Calcium (mmol/L) | 2.34±0.12 | 2.37±0.12 | −1.02 | −0.16 | −0.07 to 0.02 | 0.315 |
| Grip strength (kg) | 20.01±4.86 | 21.43±3.48 | −1.88 | −0.30 | −2.96 to 0.11 | 0.068 |
| 6-m walk gait speed (m/s) | 0.88±0.16 | 1.01±0.16 | −3.84 | −0.61 | −0.20 to −0.06 | 0.000 |
| 5 times sit-up time (s) | 15.40±6.02 | 12.14±2.98 | 3.24 | 0.51 | 1.22 to 5.30 | 0.02 |
| TUG (s) | 10.99±2.78 | 9.48±2.33 | 2.70 | 0.43 | 0.38 to 2.64 | 0.010 |


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**Table 4** Baseline-1 Year Follow-Up Results

| | Base Line | 1-Year Follow-Up | t (14) | d | 95% CI | P |
|---|---|---|---|---|---|---|
| DXA spine | −3.12±0.49 | −2.61±0.83 | −3.12 | −0.81 | −0.87 to −0.16 | 0.007 |
| VD (25-OH) (ng/mL) | 17.56±3.57 | 26.12±4.33 | −6.74 | −1.74 | −11.30 to −5.84 | 0.000 |
| Calcium (mmol/L) | 2.41±0.08 | 2.49±0.15 | −1.76 | −0.45 | −0.17 to −0.02 | 0.101 |
| Grip strength (kg) | 22.08±3.41 | 24.39±3.10 | −4.01 | −1.04 | −3.54 to −1.07 | 0.001 |
| 6-m walk gait speed (m/s) | 0.95±0.16 | 1.15±0.11 | −4.34 | −1.12 | −0.30 to −0.10 | 0.001 |
| 5 times sit-up time (s) | 13.54±2.82 | 10.71±2.83 | 2.92 | 0.75 | 0.75 to 4.91 | 0.011 |
| TUG (s) | 9.75±1.55 | 8.17±0.93 | 3.33 | 0.86 | 0.56 to 2.61 | 0.005 |
| Upper arm circumference (muscle) (cm) | 22.85±1.14 | 22.99±1.10 | −0.42 | −0.11 | −0.81 to 0.54 | 0.679 |
| SMI (kg/m$^2$) | 7.93±0.69 | 8.13±0.71 | −0.93 | −0.24 | −0.66 to 0.26 | 0.366 |
| ASMI (kg/m$^2$) | 6.02±0.52 | 6.18±0.50 | −1.27 | −0.33 | −0.43 to 0.11 | 0.225 |

0.10; P = 0.001), 5 times sit-up time (10.71±2.83 s; 95% CI: 0.75 to 4.91; P = 0.011), and TUG (8.17±0.93 s; 95% CI: 0.56 to 2.61; P = 0.005), as detailed in Table 4. However, improvements in muscle mass indicators—including upper arm muscle circumference, skeletal muscle mass index (SMI), and appendicular skeletal muscle mass index (ASMI)—did not reach statistical significance (P > 0.05). Throughout the treatment period, all participants received adequate calcium and vitamin D supplementation, maintaining serum levels within or above the normal range.

## Mendelian Randomization Study Results

### Selection of Instrumental Variables and Assessment of Weak Instrument Bias

Following correlation analysis and linkage disequilibrium (LD) pruning, a total of 16 and 14 single nucleotide polymorphisms (SNPs) strongly associated with the RANKL gene were identified as instrumental variables for assessing causal relationships with appendicular lean mass (ALM) and right-hand grip strength, respectively. All selected SNPs had F-statistics exceeding 10, indicating the absence of weak instrument bias and supporting the robustness of the selected instruments. Two-sample Mendelian randomization analyses were subsequently conducted to evaluate the causal effect of each SNP on ALM and right-hand grip strength. The corresponding results are presented in Figure 3a and Figure 3b.

### Impact of RANKL Gene on Appendicular Lean Mass (ALM)

Mendelian randomization analysis using the inverse variance-weighted (IVW) method revealed a significant negative association between the RANKL gene and appendicular lean mass (ALM) (Beta = −0.014; OR = 0.986, 95% CI: 0.979–0.992; P = 2.03 $\times 10^{-5}$). The weighted median method yielded consistent results (Beta = −0.017; OR = 0.983, 95% CI: 0.975–0.991; P = 2.88 $\times 10^{-5}$). However, the MR-Egger regression did not yield statistically significant results, suggesting potential limitations in precision with that method (Figure 4a). To evaluate the robustness and reliability of these findings, heterogeneity was assessed using both IVW and MR-Egger approaches, while horizontal pleiotropy was tested using the Egger intercept. No evidence of heterogeneity or horizontal pleiotropy was observed (P > 0.05), indicating the validity of the instrumental variables and the stability of the results (Table 5).

### Impact of RANKL Gene on Right Hand Grip Strength

The inverse variance-weighted (IVW) method was primarily employed to evaluate the causal relationship between the RANKL gene and right hand grip strength. The analysis revealed a significant negative association (Beta = −0.006; OR = 0.994, 95% CI: 0.989–1.000; P = 0.050) (Figure 4b). Heterogeneity was assessed using both IVW and MR-Egger methods, while horizontal pleiotropy was examined via the Egger intercept. No evidence of heterogeneity or horizontal pleiotropy was identified (P > 0.05), supporting the reliability and validity of the instrumental variables used (Table 3–4).

## Sensitivity Analysis

A leave-one-out sensitivity analysis was conducted in both Mendelian randomization (MR) analyses to assess the influence of individual single nucleotide polymorphisms (SNPs) on the overall causal estimates. Systematic removal

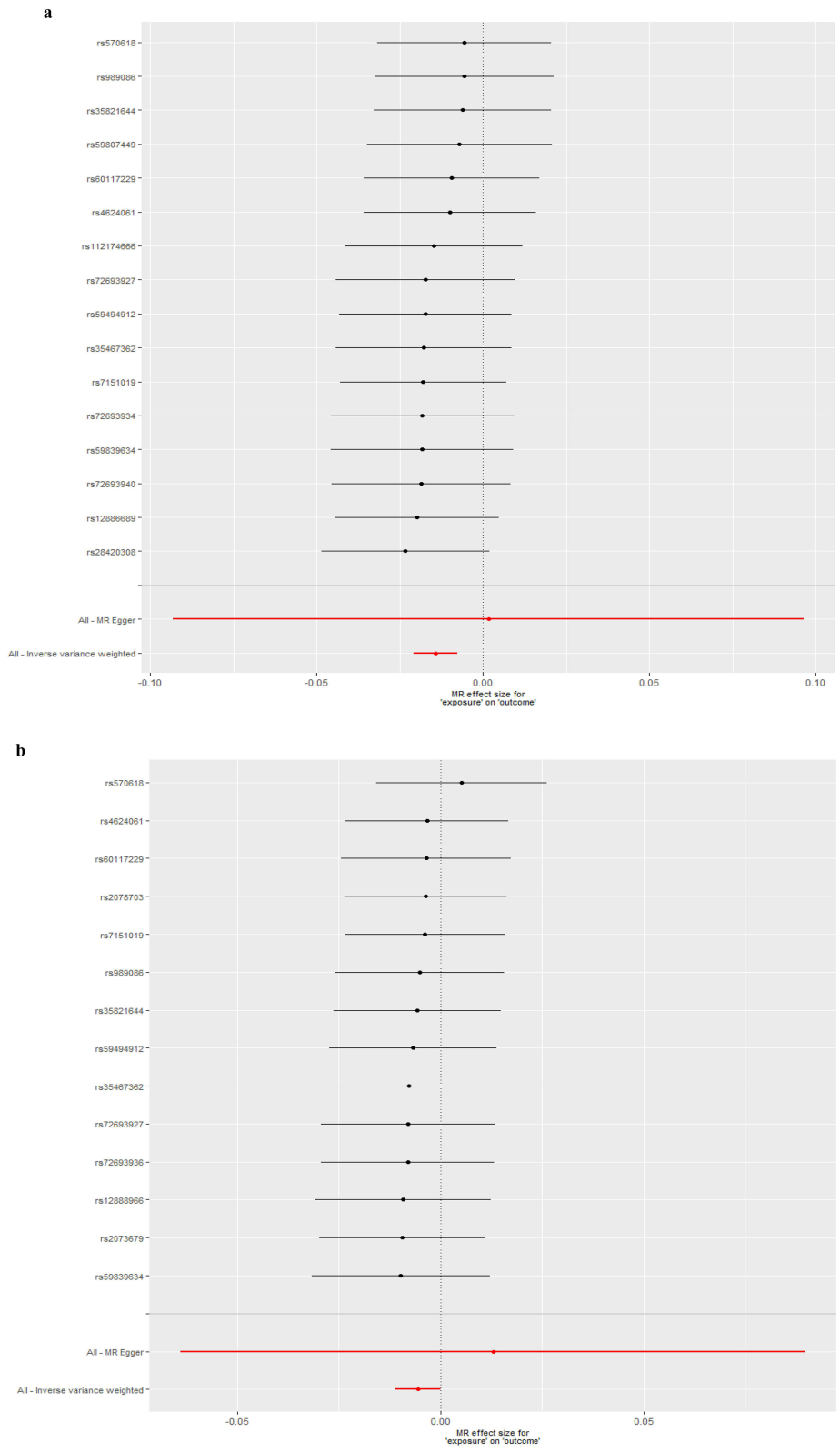


**Figure 3** (**a**) Forest plot of the two-sample Mendelian Randomization analysis (RANKL-ALM) (**b**) Forest plot of the two-sample Mendelian Randomization analysis (RANKL-Right hand grip strength).


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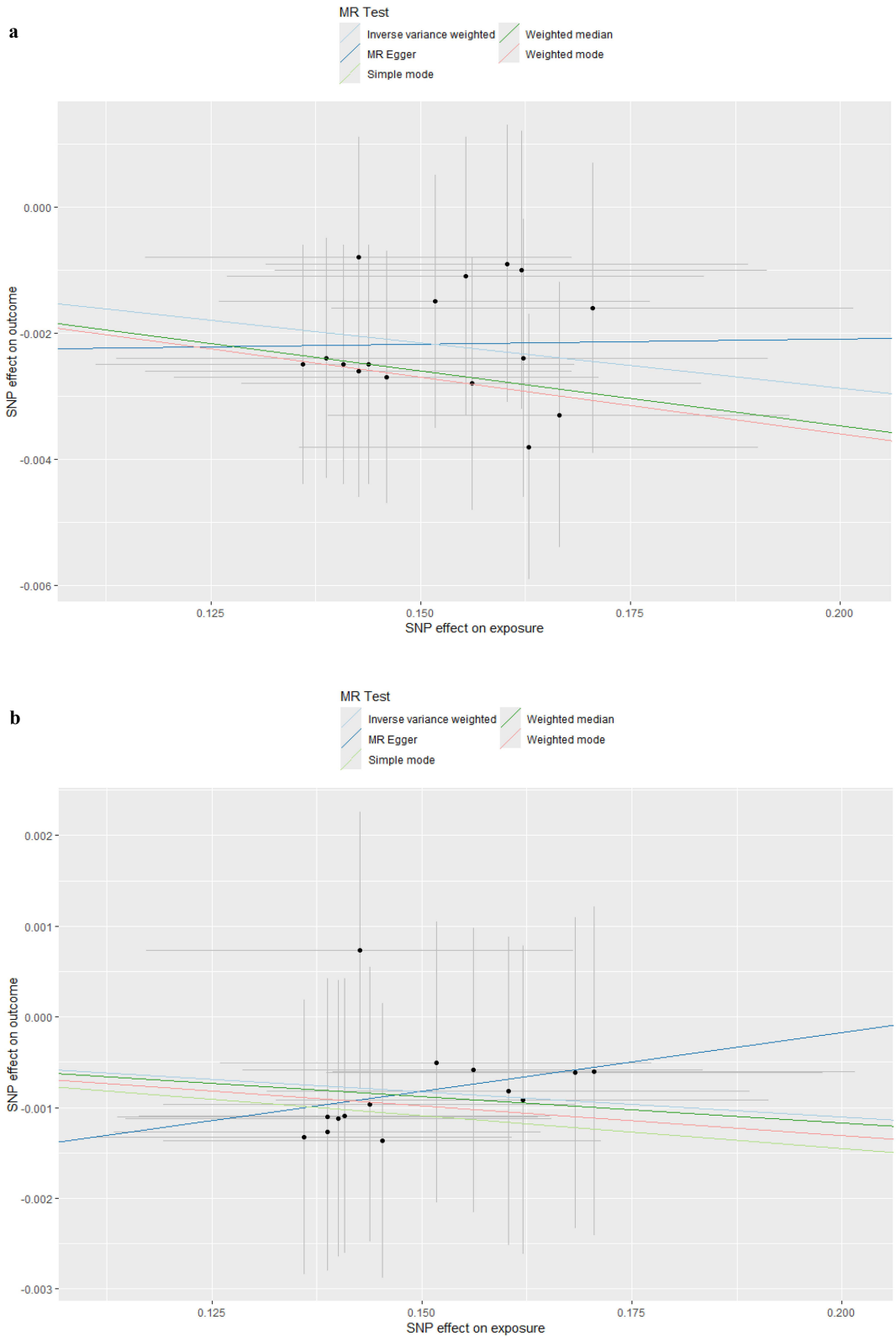


**Figure 4** (**a**) Scatter plot of the two-sample Mendelian Randomization analysis (RANKL-ALM) (**b**) Scatter plot of the two-sample Mendelian Randomization analysis (RANKL-Right hand grip strength).

**Table 5** Mendelian Randomization Estimates for RANKL on Sarcopenia-Related Traits with All Selected Ivs

| Exposures | Outcomes | No. of IVs | Heterogeneity Test | Pleiotropy Test | MR Results | | | |
|---|---|---|---|---|---|---|---|---|
| | | | IVW/MR-Egger | Egger Intercept | Method | Beta | OR/95% CI | P |
| RANKL | ALM | 16 | 1.000/0.999 | 0.745 | IVW | −0.014 | 0.986(0.979–0.992) | 2.026E-05 |
| | | | | | Weighted median | −0.017 | 0.983(0.975–0.991) | 2.875E-05 |
| | | | | | MR-Egger | 0.002 | 1.002(0.911–1.101) | 0.973 |
| RANKL | Hand grip strength(right) | 14 | 1.000/1.000 | 0.645 | IVW | −0.006 | 0.994/ (0.989–1.000) | 0.05 |

of individual SNPs followed by repeated MR analyses revealed no significant changes in the estimated causal relationships. These findings suggest that the observed effects are not driven by any single genetic variant, thereby supporting the robustness of the MR results (Figure 5a and Figure 5b).

## Discussion

This study initially evaluated the potential effects of denosumab treatment on muscle-related parameters in Chinese patients with osteoporosis through clinical observation, proposing that denosumab may improve both muscle mass and function. To further test this hypothesis, a Mendelian randomization (MR) analysis was conducted to explore the underlying mechanisms involved in the development and progression of sarcopenia. These findings provide theoretical support for the use of denosumab in enhancing muscle function and offer valuable insights for future research into therapeutic strategies targeting sarcopenia and related muscular disorders.

In the clinical component of the study, patients receiving denosumab therapy for osteoporosis were followed longitudinally. In addition to evaluating its efficacy in increasing bone mineral density (BMD), particular emphasis was placed on the drug's potential impact on muscle mass and function. The results demonstrated that denosumab not only significantly improved BMD but also substantially enhanced muscle function, aligning with the findings of Yasser El Miedany et al.[22] After six months of treatment, significant improvements were observed in muscle-related functional indicators, including 6-m walk gait speed (P = 0.000), 5 times sit-up time (P = 0.02), and TUG (P = 0.01). After one year, in addition to a further significant increase in BMD (P = 0.007), improvements in dominant handgrip strength (P = 0.001), 6-m walk gait speed (P = 0.001), 5 times sit-up time (P = 0.011), and TUG (P = 0.005) became more pronounced. Moreover, improvements in lower limb function (6-m walk gait speed, 5 times sit-up time, and TUG) were observed earlier and were more substantial than those in upper limb strength (dominant handgrip strength). Significant lower limb enhancements were evident by 6 months, while improvements in upper limb strength became apparent after one year. However, no statistically significant changes in muscle mass indicators—such as upper arm circumference (muscle-specific), skeletal muscle mass index (SMI), and appendicular skeletal muscle mass index (ASMI)—were detected during the follow-up period. This lack of change is likely attributable to the relatively short observation duration and the inherently slower muscle metabolism observed in elderly individuals.

In the Mendelian randomization analysis, we systematically investigated the causal relationship between RANKL gene expression and sarcopenia-related traits, specifically appendicular lean mass (ALM) and right-hand grip strength. Our findings demonstrated a significant negative association between RANKL expression and both ALM and grip strength, suggesting that upregulation of the RANKL gene may contribute to reductions in muscle mass and strength. This implies a potential positive causal role of RANKL in the onset and progression of sarcopenia, whereby its overexpression increases susceptibility to the condition. Although the pathogenesis of sarcopenia remains incompletely understood, accumulating evidence suggests that it may be mediated by factors such as chronic inflammation, insulin resistance, and intramuscular fat infiltration. Previous studies by Miedany Y.E. and Gaafary M.E.,[22] as well as Bonnet

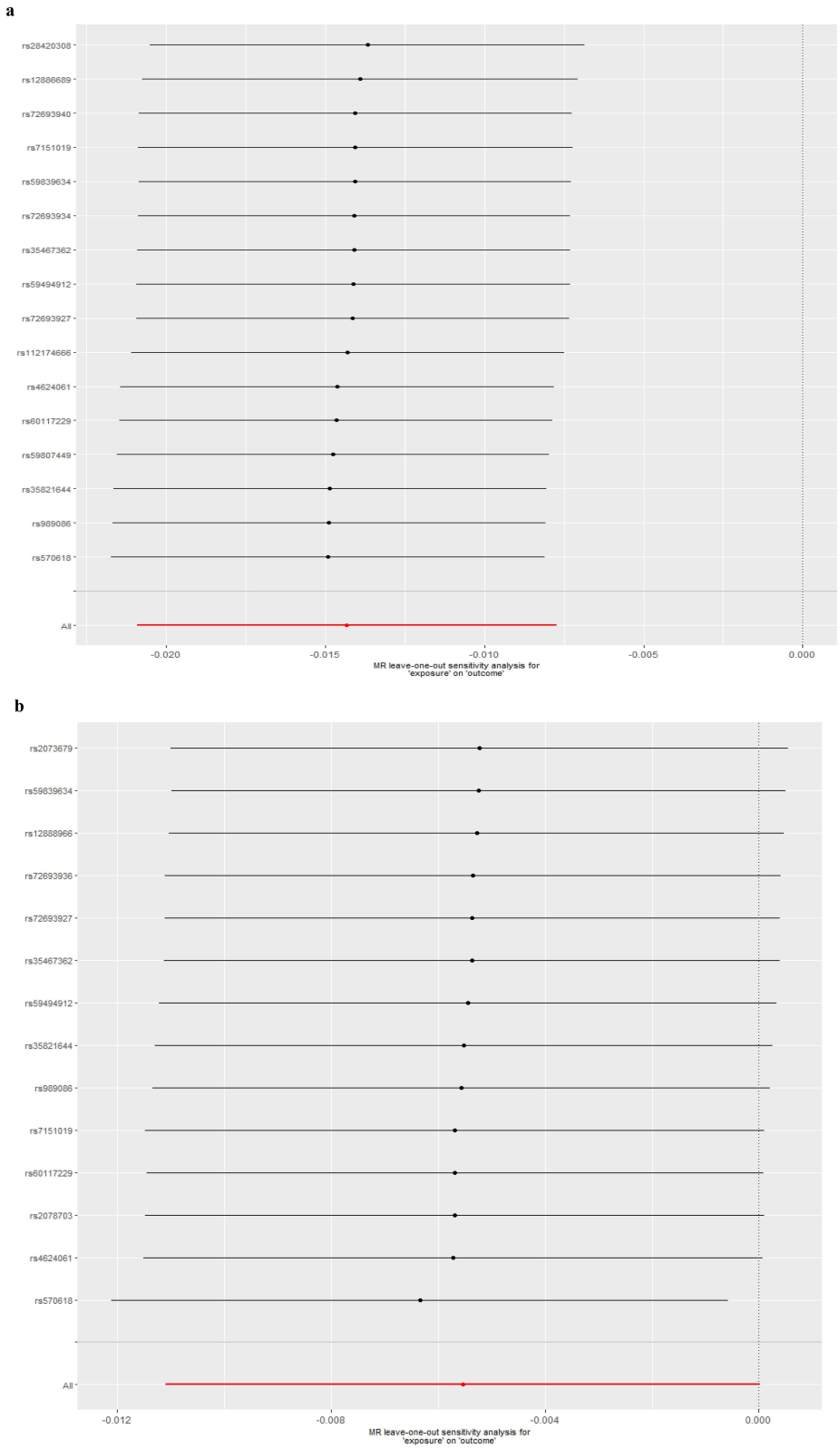


**Figure 5** (**a**) Result of "leave-one-out"sensitivity analysis (RANKL-ALM) (**b**) Result of "leave-one-out"sensitivity analysis (RANKL-Right hand grip strength).

N. and Bourgoin L.,[23] reported that denosumab treatment in osteoporotic patients led to significant improvements in sarcopenia-related indicators, including appendicular muscle mass and grip strength—benefits not consistently observed with other first-line anti-osteoporotic therapies such as bisphosphonates. Furthermore, preclinical studies in murine models of muscle atrophy have shown that denosumab can increase muscle mass and strength. As a highly specific, high-affinity monoclonal antibody against RANKL, denosumab inhibits the interaction between RANKL and its receptor RANK, thereby suppressing osteoclastogenesis and bone resorption. Given this mechanism, it is plausible to hypothesize that denosumab may also ameliorate sarcopenia by disrupting the RANK–RANKL signaling axis. This raises a compelling question: could RANKL overexpression directly contribute to the pathogenesis of sarcopenia? To date, no basic experimental studies have directly validated this hypothesis. Therefore, in our study, we first evaluated the clinical effects of denosumab on muscle-related outcomes during osteoporosis treatment, followed by a Mendelian randomization approach to genetically assess and interpret these clinical observations. Collectively, our findings are both robust and encouraging, providing novel insights into the potential dual role of RANKL in bone and muscle regulation.

By integrating clinical evidence with Mendelian randomization analysis, this study demonstrates that denosumab not only improves bone mineral density but also significantly enhances muscle function in patients with osteoporosis. A key strength of this study lies in its focus on a Chinese population, distinguishing it from prior research predominantly conducted in Western cohorts. This enhances the generalizability of our findings to Chinese and potentially broader Asian populations. Furthermore, the innovative use of Mendelian randomization to explore the genetic mechanisms underlying denosumab's effects on muscle function—and to elucidate the role of RANKL in the pathogenesis of sarcopenia—adds a novel dimension to the existing body of literature.

Despite these strengths, several limitations warrant consideration. In the clinical component, all participants receiving denosumab were concurrently supplemented with adequate calcium and vitamin D, in accordance with standard treatment protocols. However, previous studies have indicated that vitamin D alone may exert beneficial effects on muscle function. To address this potential confounding factor, we established a control group receiving only calcium and vitamin D. Unfortunately, due to the ongoing follow-up, data from this control group were not yet available for analysis. We plan to include these data in future analyses to more comprehensively assess the isolated effects of denosumab. In the Mendelian randomization analysis, all summary statistics were derived from the UK Biobank and limited to individuals of European ancestry. This introduces concerns about the translatability of the genetic findings to Chinese or other non-European populations. Moreover, to ensure an adequate number of single nucleotide polymorphisms (SNPs) for robust analysis, we adopted a relatively lenient linkage disequilibrium clumping threshold ($r^2 < 0.9$). While this approach increased statistical power, it may have introduced multicollinearity among selected instruments, potentially biasing causal estimates. Finally, inherent to the MR design is the inability to perform subgroup analyses (eg, by age or sex), limiting the exploration of potential effect modifiers across diverse subpopulations.

## Conclusion

Denosumab not only effectively treats osteoporosis in Chinese patients but also exerts beneficial effects on muscle health. These findings suggest that denosumab may serve as a preferred therapeutic option for individuals with coexisting osteoporosis and sarcopenia. Moreover, the observed negative association between RANKL gene expression and muscle-related parameters implies that overexpression of RANKL may contribute to the development and progression of sarcopenia, highlighting the potential role of the RANK–RANKL signaling pathway in its pathogenesis.

As a first-line anti-osteoporotic agent, denosumab warrants further investigation through multicenter, prospective, and long-term clinical studies across diverse ethnic and geographic populations to confirm its therapeutic potential for sarcopenia. The dual benefit of denosumab in improving both bone density and muscle function, as demonstrated in our study, merits continued exploration and validation. Importantly, our research team is currently conducting a randomized controlled trial in China to assess the effects of denosumab on sarcopenia-related outcomes in patients with osteoporosis. This study has been prospectively registered on ClinicalTrials.gov (ID: NCT06154707) and is expected to provide further insights into the therapeutic value of denosumab for the Chinese population. Additionally, these findings may inspire the development of novel therapeutic approaches targeting the RANK–RANKL pathway. Next-generation RANKL antagonists with greater specificity or potency could represent promising candidates for the

treatment of sarcopenia. However, further mechanistic and translational studies are essential to establish a solid biological foundation for such strategies.

## Data Sharing Statement

All authors agree to share individual deidentified participant data in this article and research, we can share the experimental result data from clinical research as well as the data obtained from the GWAS database, and we do not have any other research documents to provide. The data for this study can be obtained by contacting the first author of this article (Shaotian Li, Email: 1398998676@qq.com). After this article is formally accepted and published, the data will be permanently accessible. The data results in this study are publicly available and can be used.

## Ethics Approval and Consent to Participate

The same as Section 2, Materials and Methods.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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