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Advances in the pathogenesis and treatment of osteoporosis: from bench to bedside

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Advances in the pathogenesis and treatment of osteoporosis: from bench to bedside

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Editorial: Advances in the pathogenesis and treatment of osteoporosis: from bench to bedside

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osteoporosis, osteoporotic fracture, pathogenesis, treatment, risk factors

Editorial on the Research Topic
Advances in the pathogenesis and treatment of osteoporosis: from bench to bedside

Osteoporosis is an age- and gender-associated musculoskeletal disorder characterized by compromised bone integrity. With the intensification of global population aging trends, the disease burden of osteoporosis is projected to escalate substantially. This demographic shift necessitates prioritized allocation of scientific and clinical resources toward two critical domains: enhancing fundamental understanding of disease mechanisms through multidisciplinary research and developing innovative therapeutic agents and intervention strategies targeting bone remodeling pathways. The articles in this topic covered the latest advancements in osteoporosis screening, etiology, risk factors, and treatment.

Currently, the relationship between Body Mass Index (BMI) and Bone Mineral Density (BMD) remains controversial. Some studies suggest that a higher BMI may promote bone formation by increasing body weight, thereby enhancing mechanical stimulation on bones (1). However, other research indicates that excessively high BMI may negatively affect bone metabolism due to obesity-related metabolic abnormalities (2, 3). The study by Chen et al. found that fat tissue distribution in different body regions shows significant correlations with BMD in corresponding bone areas. Excessively high regional fat percentages may be detrimental to bone health in both genders. Specifically, trunk fat percentage in females is significantly associated with lumbar spine BMD, while abdominal fat percentage in males shows strong correlations with femoral neck BMD. To promote bone health, males should limit waist circumference and avoid abdominal fat accumulation, whereas females should focus on controlling trunk circumference.

Obstructive sleep apnea syndrome (OSAS) is a sleep disorder characterized by intermittent hypoxia and sleep fragmentation, leading to oxidative stress and systemic inflammation (4). Luo X. et al. analyzed the correlation between OSAS and osteoarthritis (OA) using multivariate logistic regression analysis on 10,641 participants recruited from the National Health and Nutrition Examination Survey (NHANES) dataset. The results indicated that OSAS patients may have a higher prevalence of OA, and

aging also plays a role in this association. After adjusting for covariates, it was found that aging mediates the relationship between OSAS and OA.

Patients with short bowel syndrome (SBS) experience insufficient intestinal absorption capacity due to partial resection of the small intestine (5). Long-term inappropriate parenteral or enteral nutrition can easily lead to complications such as metabolic bone disease (MBD) (Sun et al.). Sun et al. investigated the incidence and risk factors of osteopenia in adult SBS patients through a retrospective longitudinal cohort study. The study included 120 SBS patients, revealing that 76 patients (63.3%) developed osteopenia during the 10-year observation period. Given the high prevalence of metabolic bone disease among SBS patients, early identification and management of skeletal health issues in this population are crucial for preventing MBD.

Among the multifactorial causes of osteoporosis, the gut microbiota has become a focus of research due to its profound impact on bone metabolism (6). Luo Z. et al. conducted a bibliometric analysis of the literature on osteoporosis and the gut microbiota from 2014 to 2024 for the first time and explored the current research status, identifying the forefront and hotspots in this field. The study deeply explored fecal microbiota transplantation or specific dietary interventions as promising approaches for future research, providing references for researchers focused on this field.

Mendelian randomization (MR) is an epidemiological strategy that enhances causal inference by using single nucleotide polymorphisms (SNPs) as unbiased instrumental variables (IVs) (7). In the study by Dong et al., a two-way Mendelian randomization study was conducted to investigate the potential causal relationship between osteoporosis and 91 circulating inflammatory markers (CIMs). Based on a relatively large meta-analysis of genome-wide association studies (GWAS), they found a unidirectional positive causal relationship between CIMs and osteoporosis. Among them, five CIMs (ARTN, CXCL11, IL-18, LIF, and IFNG) showed potential associations with osteoporosis, possessing great value for further research.

Xiang et al. also applied MR methods to explore the causal relationship between Celiac's disease (CeD) and osteoporosis-related traits, while simultaneously examining the mediating role of inflammatory cytokines in the relationship between CeD and osteoporosis. It was found that the genetic susceptibility to CeD increases the risk of osteoporosis and osteoporotic fractures and reduces systemic BMD. Additionally, plasma IL-18 levels seem to play an important role in regulating the relationship between CeD and osteoporosis.

Long-term disuse osteoporosis (DOP) represents a critical health hazard for astronauts during extended spaceflight missions (8). While the regulatory role of long non-coding RNAs (lncRNAs) in bone marrow mesenchymal stem cells (BMSCs) and skeletal disorders has been established, the precise molecular mechanisms through which lncRNAs contribute to DOP pathogenesis remain poorly characterized. In a groundbreaking study, Wei et al. pioneered the construction of a competitive endogenous RNA (ceRNA) network in DOP-affected BMSCs, systematically integrating protein-coding mRNAs with non-coding RNA components (including lncRNAs and miRNAs). Through comprehensive analysis, the research team identified two pivotal hub genes (LAMC1 and LAMA4) and delineated two critical regulatory axes: the JPX/hsa-miR-3619-5p/LAMA4 cascade and the LINC01123/hsa-let-7i-5p/LAMC1 pathway. Notably, three pharmacological compounds—SB-216763, oxymetholone, and flubendazole—were computationally predicted as potential therapeutic agents targeting these molecular pathways.

Osteoporosis increases the risk of fragility fractures, especially of the lumbar and femoral fractures (9). Early detection of osteoporosis is crucial. Yoshida et al. created a dataset using bone mineral density and bilateral chest X-rays of 1,624 patients aged ≥20 years and proposed a new method using a deep learning model with anterior and lateral chest radiographs (CXR) inputs for early detection of osteoporosis. These results suggest that bidirectional CXR can improve the accuracy of BMD estimation and osteoporosis screening compared to single-view CXR and has the potential to serve as a criterion for clinical decision-making.

In the past few decades, percutaneous vertebroplasty (PVP) and percutaneous kyphoplasty (PKP) have been widely used to treat osteoporotic vertebral compression fractures (OVCF) due to their rapid pain relief and functional improvement effects (10). Understanding and effectively intervening with factors related to secondary fractures after discharge is crucial for improving patients' quality of life and avoiding future fractures. Yang et al. conducted a prospective analysis of OVCF patients who underwent PVP or PKP and further analyzed the risk factors for new vertebral fractures after treatment. It was found that for osteoporotic fracture patients undergoing PKP/PVP surgery, older age, poor blood glucose control, lower BMD, lower 25-OH-D3 levels, weaker paraspinal muscles, and higher fat infiltration were more prone to new vertebral fractures. On the other hand, maintaining regular physical activity and adhering to osteoporosis treatment can help prevent new vertebral fractures.

Osteoporosis and the fractures it causes remain a significant health challenge in the global aging society (11). However, the research compiled in this Research Topic systematically elucidates the "multi-factor, multi-target" pathogenesis of osteoporosis and precise intervention strategies by integrating multi-omics technologies, translational medical models, and clinical data. These groundbreaking discoveries not only promote the translation of basic research into clinical practice but also provide a new theoretical framework and practical guidance for developing individualized and staged precision prevention and treatment.

Author contributions

WX: Writing – original draft. PA: Writing – review & editing. MB: Writing – review & editing. EA: Writing – review & editing. DL: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A decade of insight: bibliometric analysis of gut microbiota's role in osteoporosis (2014–2024)

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Purpose: Osteoporosis represents a profound challenge to public health, underscoring the critical need to dissect its complex etiology and identify viable targets for intervention. Within this context, the gut microbiota has emerged as a focal point of research due to its profound influence on bone metabolism. Despite this growing interest, the literature has yet to see a bibliometric study addressing the gut microbiota's contribution to both the development and management of osteoporosis. This study aims to fill this gap through an exhaustive bibliometric analysis. Our objective is to uncover current research hotspots, delineate key themes, and identify future research trends. In doing so, we hope to provide direction for future studies and the development of innovative treatment methods.

Methods: Relevant publications in this field were retrieved from the Web of Science Core Collection database. We used VOSviewer, CiteSpace, an online analysis platform and the R package "Bibliometrix" for bibliometric analysis.

Results: A total of 529 publications (including 351 articles and 178 reviews) from 61 countries, 881 institutions, were included in this study. China leads in publication volume and boast the highest cumulative citation. Shanghai Jiao Tong University and Southern Medical University are the leading research institutions in this field. Nutrients contributed the largest number of articles, and *J Bone Miner Res* is the most co-cited journal. Of the 3,166 scholars who participated in the study, Ohlsson C had the largest number of articles. Li YJ is the most co-cited author. "Probiotics" and "inflammation" are the keywords in the research.

Conclusion: This is the first bibliometric analysis of gut microbiota in osteoporosis. We explored current research status in recent years and identified frontiers and hot spots in this research field. We investigate the impact of gut microbiome dysregulation and its associated inflammation on OP progression, a topic that has garnered international research interest in recent years. Additionally, our study delves into the potential of fecal microbiota

transplantation or specific dietary interventions as promising avenues for future research, which can provide reference for the researchers who focus on this research filed.

KEYWORDS

gut microbiota, osteoporosis, bibliometric analysis, CiteSpace, VOSviewer, R package "Bibliometrix"

1 Introduction

Osteoporosis is a systemic skeletal disease characterized by an overall reduction in bone mass and deterioration of bone microarchitecture, leading to decreased bone strength and a significantly increased risk of fractures (1). Osteoporosis presents a major health concern, especially among the elderly and postmenopausal women. The International Osteoporosis Foundation (IOF) estimates that over two hundred million people suffer from osteoporosis, which leads to a high number of fractures annually (2).

Critical to understanding osteoporosis is the recognition of the bone remodeling unit as the functional entity, within which the coupling of bone formation to resorption is tightly controlled. Disruption in this coupling in osteoporosis is mediated by a multifaceted interplay of biomolecules including, but not limited to cytokines (such as RANKL, OPG, and interleukins), growth factors (e.g., TGF-β and IGF-1), and hormones (including estrogen, testosterone, and parathyroid hormone) (3). These entities not only regulate the proliferation, differentiation, and activity of osteoblasts and osteoclasts but also modulate the local bone microenvironment and systemic bone metabolism.

Recent years have brought notable progress in osteoporosis treatments, including bisphosphonates, Selective Estrogen Receptor Modulators (SERMs), calcitonin, and parathyroid hormone (PTH) and its analogs, along with the newer Sclerostin inhibitors (4, 5). Focused on slowing bone resorption or boosting bone formation to increase bone density and lower fracture risks, these medications typically necessitate prolonged use and can entail side effects, with limited success in preventing or reversing bone loss (4). Additionally, not all patients experience identical therapeutic outcomes from these drugs, indicating considerable variations in individual responses.

In the multifactorial etiology of osteoporosis, recent research has zeroed in on the gut-bone axis, illuminating how gut microbiota can influence bone metabolism through immune modulation, metabolic product generation, and nutrient absorption, thereby revealing a potential avenue for regulating bone health (6–8). Short-chain fatty acids (SCFAs) such as butyrate and propionate, produced in the gut, are proven to directly engage in bone remodeling by fostering osteoblast proliferation and differentiation and curbing osteoclast formation (9, 10). Moreover, gut microbiota also indirectly modulates osteoporosis development by impacting the host's immune system (11), notably through adjusting T cell subset balances and the host's inflammatory state (12).

Although there has been significant progress in understanding the role of the gut microbiota in osteoporosis, current research still lacks a systematic understanding of the relationship between gut microbial diversity and specific bone pathological states, as well as the precise mechanisms by which microbes regulate bone health.

Bibliometrics is a quantitative research method aimed at systematically analyzing the distribution, growth, and developmental trends of academic literature. Utilizing bibliometrics, scholars can gain a clearer perception of a field's current research landscape, prominent research areas, and prospective research directions.

Regrettably, there are currently no bibliometric studies that have explored the relationship between the gut microbiota and osteoporosis. Given the context, this article employs bibliometric analysis to holistically assess existing research on the relationship between the gut microbiota and osteoporosis, aiming to identify research trends, key findings, existing gaps, and potential future directions.

2 Materials and methods

2.1 Search strategy

We selected the Web of Science Core Collection (WoSCC) database to conduct a literature search on 01/03/2024. The search terms is as follows: #1: TS = (osteoporosis OR osteopenia OR osteoporotic OR bone loss* OR Low bone mass OR low bone density), #2: TS = ("gut microbiota" OR "intestinal microbiota" OR "fecal microbiota" OR "gastrointestinal microbiota" OR "gut microbiome" OR "intestinal microbiome" OR "fecal microbiome" OR "gastrointestinal microbiome" OR "intestinal bacteria" OR "gut bacteria" OR "fecal bacteria" OR "gastrointestinal bacteria" OR "intestinal flora" OR "gut flora" OR "fecal flora" OR "gastrointestinal flora" OR "gut microflora" OR "intestinal microflora" OR "fecal microflora" OR "gastrointestinal microflora"), final = #1 AND #2. LA = (English), and the type of documents was set to "articles" and "review.". The publication period was specified as 01/01/2014 to 30/03/2024. Following the initial retrieval, we screened the titles and abstracts to confirm the eligibility of the articles based on predefined inclusion and

Abbreviations: SERMs, selective estrogen receptor modulators; PTH, parathyroid hormone; FMT, fecal microbiota transplantation; RANKL, receptor activator for nuclear factor-κ B ligand; OPG, osteoclastogenesis inhibitory factor; WoSCC, Web of Science Core Collection; JCR, Journal Citation Reports; TLS, total link strength; IF, impact factor; LC, local citation; PY, published year.

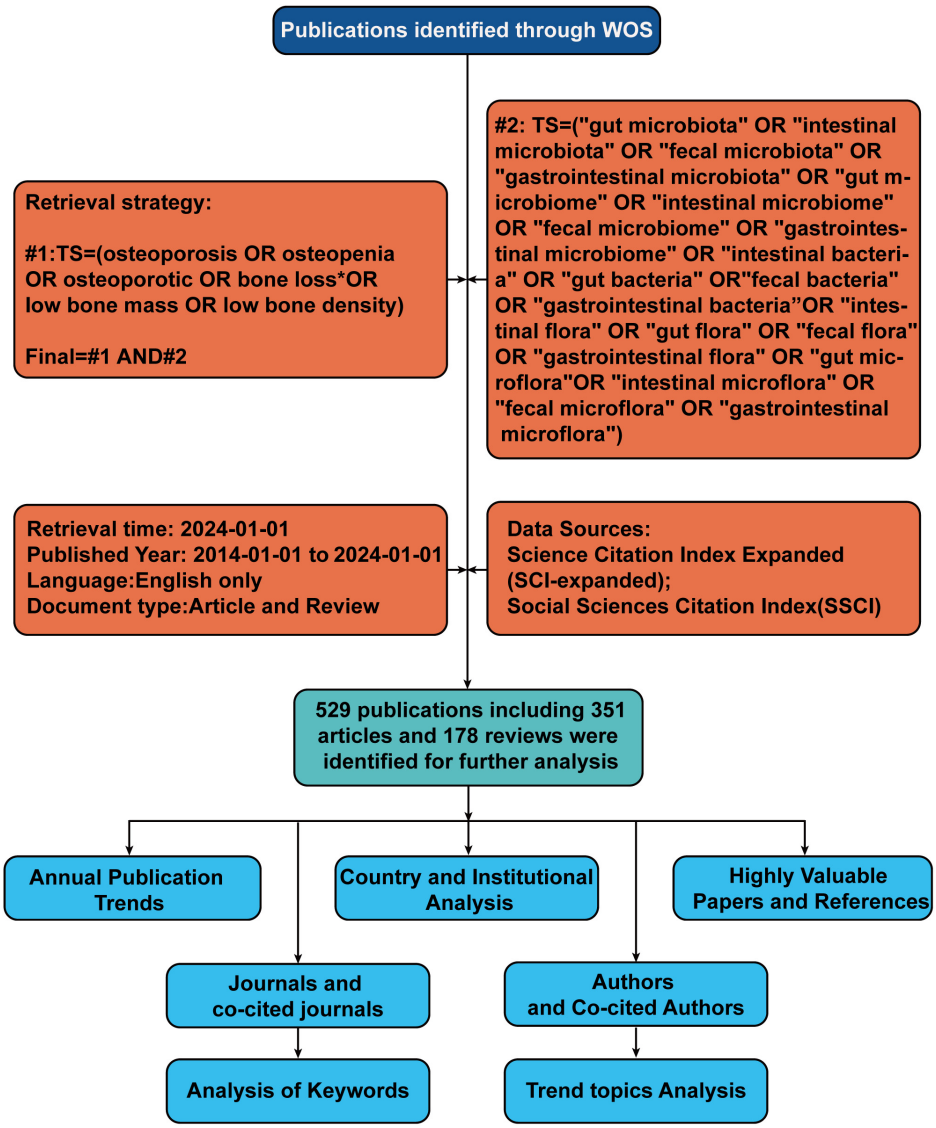

FIGURE 1
Flowchart of literature identification and analysis process. TS, topic; WoS, Web of Science.

exclusion criteria. The flowchart of the screening process is shown in Figure 1.

2.2 Data analysis

Leveraging the capabilities of VOSviewer (version 1.6.18), a bibliometric analysis software of substantial renown (13), we facilitated the generation of visualizations representing cooperative, co-citation, and co-occurrence networks. The analyses conducted in this study utilizing VOSviewer encompassed co-occurrence analysis of keywords, nations, journals and co-cited journals, authors, and co-cited authors, as well as institutions. In the label view used for these visualizations, the colors of the nodes represent different clusters or groups of items (such as institutions, authors, or journals) that are more closely related to each other within the same cluster than to those in other clusters (14).

We also engaged CiteSpace (version 6.1. R1), an alternative software for bibliometric analysis and visualization (15), devised by Professor Chen Meichao of Drexel University. With the assistance of CiteSpace, we established dual map overlay visualizations of journals pertinent to this inquiry and pinpointed references and keywords exhibiting high citation bursts.

We used the R package "Bibliometrix" (version 3.2.1)[1] to illustrate the annual publication volume trends in this research field and the publication output of various countries, which not only showcases the academic output of these countries but also elucidates the state of international collaboration among them. Subsequently, we created trend graphs of cumulative publication volumes for the top 10 institutions and journals, which further revealed the influence of major institutions and journals within the

1 https://www.bibliometrix.org

field. Moreover, we conducted a detailed analysis of trend topics using the "Bibliometrix" package.

2.3 Procedures for analysis

Full records and cited references of the retrieved articles were downloaded from the WoSCC database and saved as.txt format for below analysis.

2.3.1 R package "Bibliometrix"

In utilizing the "Bibliometrix" package in R Studio for bibliometric analysis, the process begins with executing the biblioshiny () function to upload data via a web interface. For collaborative mapping among countries, set the parameters to a minimum of three connections and an edge size of 2.1 (Figure 3A). In the visualization of the corresponding author's countries, set the number of countries to 20 (Figure 3C). The analysis then focuses on the top 14 institutions by publication volume, outlined in Figure 4B, and extends to the top 10 journals, depicted in Figure 5C. Lastly, to discern trending topics, adjust settings for a word frequency threshold of five and select three significant words per year, enabling an insightful delineation of research trends (Figure 8A).

2.3.2 VOSviewer

In the visualization of country cooperation relationships, we set a threshold of a minimum of 5 publications, resulting in 21 countries (out of 61) meeting the criteria (Figure 3B). In the analysis of institutional cooperation networks, out of 881 institutions, 45 had a publication count of at least 5 (Figure 4A). For the journal cooperation network, setting a threshold of at least 3 publications identified 37 journals (out of a total of 298) that qualified (Figure 5A). The co-cited journal network visualization used a minimum of 30 citations as a threshold, with 266 journals (out of 4,464) meeting the standard (Figure 5B). In the visualization of the author and co-cited author collaboration networks, we set thresholds of a minimum of 3 publications per author and 30 citations per author, respectively. The findings show that among 3,166 authors, only 25 satisfied the publication threshold (Figure 6A), while among 21,680 co-cited authors, 78 met the citation threshold (Figure 6B). For keyword co-occurrence analysis, a threshold of at least 5 co-occurrences was set, with 230 keywords (from a total of 2,606) meeting the standard (Figure 8B), and an overlay visualization of keywords was conducted, see Figure 8C.

2.3.3 CiteSpace

During our analysis with CiteSpace software, we applied the following selection criteria: G-index set to 25; Link Retaining Factor (LRF) at 3.0; Look Back Year (LBY) of 5 years; and the percentage of marked nodes at 1.0%.

For the burst analysis of references (Figure 7A) and the strong burst analysis of keywords (Figure 7B), we configured a specific detection model: $f(x) = \alpha e^{-\alpha x}$, $\alpha_1/\alpha_0 = 0.2$, $\alpha_i/\alpha_{i-1} = 0/2$; The Number of States = 2; $\gamma = 0.2$; Minimum Duration = 2.

3 Results

3.1 Annual publication trends

Considering the yearly increase in publication numbers, the entire period can be divided into two phases: Phase I (2014–2019), and Phase II (2020–2024). As shown in Figure 2, the number of publications in Phase I was relatively low, with an average annual publication count of about 20.6, representing the initial stage of research on gut microbiota- osteoporosis. Entering Phase II, the number of publications began to increase significantly, with an average annual publication count of 81, marking a substantial rise compared to Phase I. This trend demonstrates the growing recognition among scholars of the significance of gut microbiota in osteoporosis.

3.2 Country and institutional analysis

A total of 881 institutions, and 61 nations have contributed to this collective body of literature. As depicted in the geographical network map of Figure 3A, the top 10 contributors hail from diverse regions encompassing Asia, Europe, and North America. Table 1 discloses that a predominant portion of publications originates from China (253) and USA (111), collectively accounting for a commanding 68.8% of the total global publications. Hot on their heels are South Korea ($N = 28$, 5.29%), Italy ($N = 27$, 5.10%), and Japan ($N = 27$, 5.10%).

Further, Table 1 accentuates that the publications from China boast the highest cumulative citation frequency (3,883), followed distantly by those from USA (3,537), Italy (769), and Japan (757). In Figure 3B, lines are utilized to represent the frequency of international academic collaboration, with the node's size signifying each country's publication tally. The visual representation in Figure 3B underscores a thriving international research collaboration landscape, with China, the United States, and Japan engaging in dynamic cooperative efforts. A close collaborative synergy between South Korea and Canada is also discernible.

Although China leads in publication volume by a significant margin compared to other countries, the proportion of its multi-country publications (MCP) is relatively low in comparison to its domestic research output, as shown in Figure 3C. This suggests that in this field of research, there is a notable lack of academic collaboration between the China and other countries.

We employed VOSviewer to conduct a visual analysis of the 881 institutions incorporated in this study, as portrayed in Figure 4A. Figure 4A offers a graphical delineation of the inter-institutional collaboration network, accentuating the strong ties that exist among a diverse array of institutions. As suggested by Figure 4A's visualization, considerable collaboration is apparent between Shanghai Jiao Tong University and Zhejiang University, as well as between Southern Medical University and Central South University. However, it is pivotal to note that these collaborations principally take place within each institution's home country, revealing a striking paucity of vibrant academic collaborations between institutions from disparate nations.

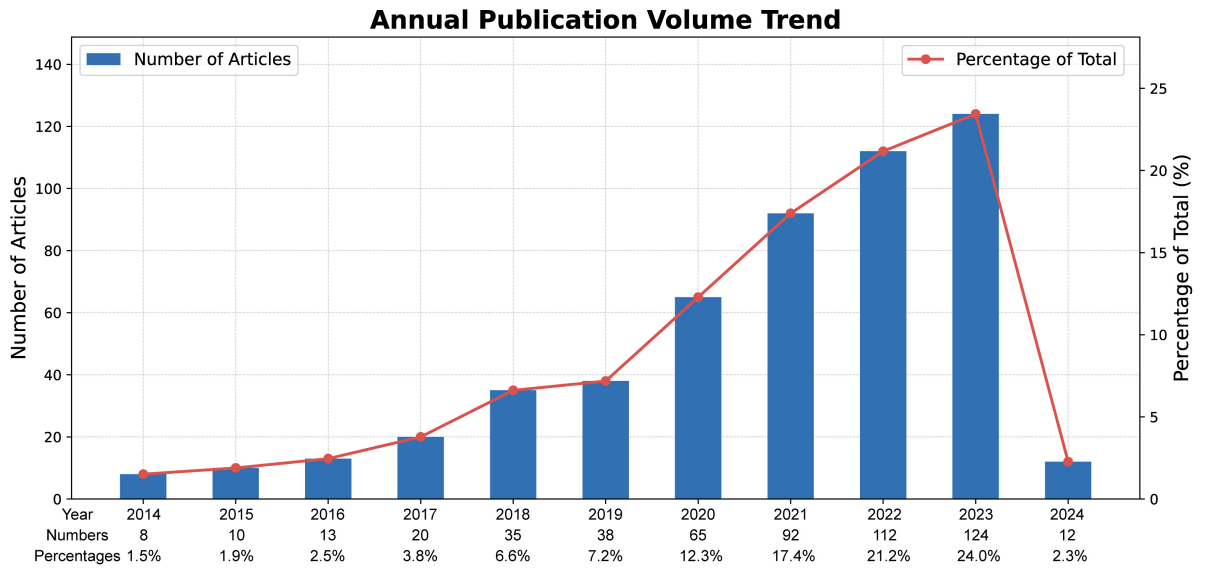

FIGURE 2
Annual outputs of publications regarding gut microbiota in osteoporosis field.

Figure 4B, alongside Table 1, ranks the top institutions by their contributions to the literature. Shanghai Jiao Tong University leads the cadre with 37 publications, closely followed by Southern Medical University and Central South University with 33 and 32 publications, respectively. Notably, the disparity in publication volume across these institutions does not emerge as significant.

Figure 4B showcases a promising incline in the annual publication output from the top 14 institutions in recent years. Nanjing University of Chinese Medicine blazed the trail by being the first to contribute to this field, but its cumulative document count appears to have plateaued starting from 2017. Conversely, although Shanghai Jiao Tong University joined the field later, it has witnessed a swift escalation in publication output beginning from 2019.

3.3 Journals and co-cited journals

Harnessing the capabilities of VOSviewer, we curated a visual representation of journals and co-cited journals within this research realm. Our dataset includes a total of 246 journals, and we incorporated the top 33 journals with a minimum publication count of 4 (Figure 5A); the size of the nodes represents the publication volume of each journal. Figure 5B unfurls a network map of co-cited journals, featuring those commanding a minimum of 25 citations. As explicated in Figure 5B, 312 co-cited journals were displayed, reflecting the aggregate link strength. The five most frequently co-cited journals, exhibiting the most formidable total link strength (TLS), comprised: *J Bone Miner Res* (TLS = 85,399), *Nutrients* (TLS = 58,480), *Nature* (TLS = 57,100), *PLoS one* (TLS = 55,021), and *Bone* (TLS = 54,481) (refer to Table 2).

Local citations, deduced from the reference list, afford insight into their localized impact, whereas total citations mirror wider interest across various disciplines. Within this ranking, *J Bone Miner Res* commandeered the list with 1,096 citations, followed by *PLoS One* with 727 citations, and *Nature* with 725 citations (as illustrated in Table 2). This clearly indicates a high proportion of high-caliber publications within these journals. Clearly, these journals are high-quality international publications that provide support for gut microbiota-osteoporosis research.

Figure 5C sketches the annual outputs of the top 10 journals spanning from 2014 to 2024. The publication volume in *Nutrients* has experienced a steep ascent in recent years. Conversely, the publication growth in *Journal of Agricultural and Food Chemistry* has been relatively placid. Table 2 catalogs the top 10 most productive and co-cited journals incorporated in this inquiry. *Nutrients* (impact factor = 6.71, 2024) surfaced as the preeminent publisher, boasting 29 publications. Further, there were 16 publications in *Frontiers in Endocrinology* (IF = 6.05, 2024), 12 publications in *Frontiers in Cellular and Infection Microbiology* (IF = 6.07, 2024) and *Calcified Tissue International* (IF = 4.20, 2023). Eight of the top 10 journals fell under the Q1 JCR region.

In CiteSpace, the dual-map overlay technique provides researchers with a macroscopic view of the cross-disciplinary interactions and citation relationships among scholarly articles. This visualization is composed of two parts: one represents the disciplines of the citing articles, and the other represents the disciplines of the cited articles, each displayed on the left and right maps, respectively. The labels on these maps identify the journals or research areas involved. The paths from citing to cited articles reveal the flow of knowledge between disciplines. The color and thickness of these paths indicate the citation intensity and the time frame, assisting researchers in quickly identifying academic trends and impacts over different periods. Thus, the dual-map overlay not only serves as a bridge for communication between various disciplines but also highlights key areas in academic dissemination, providing a powerful tool for exploring trends, predicting hot research topics, and fostering potential interdisciplinary collaborations (16, 17).

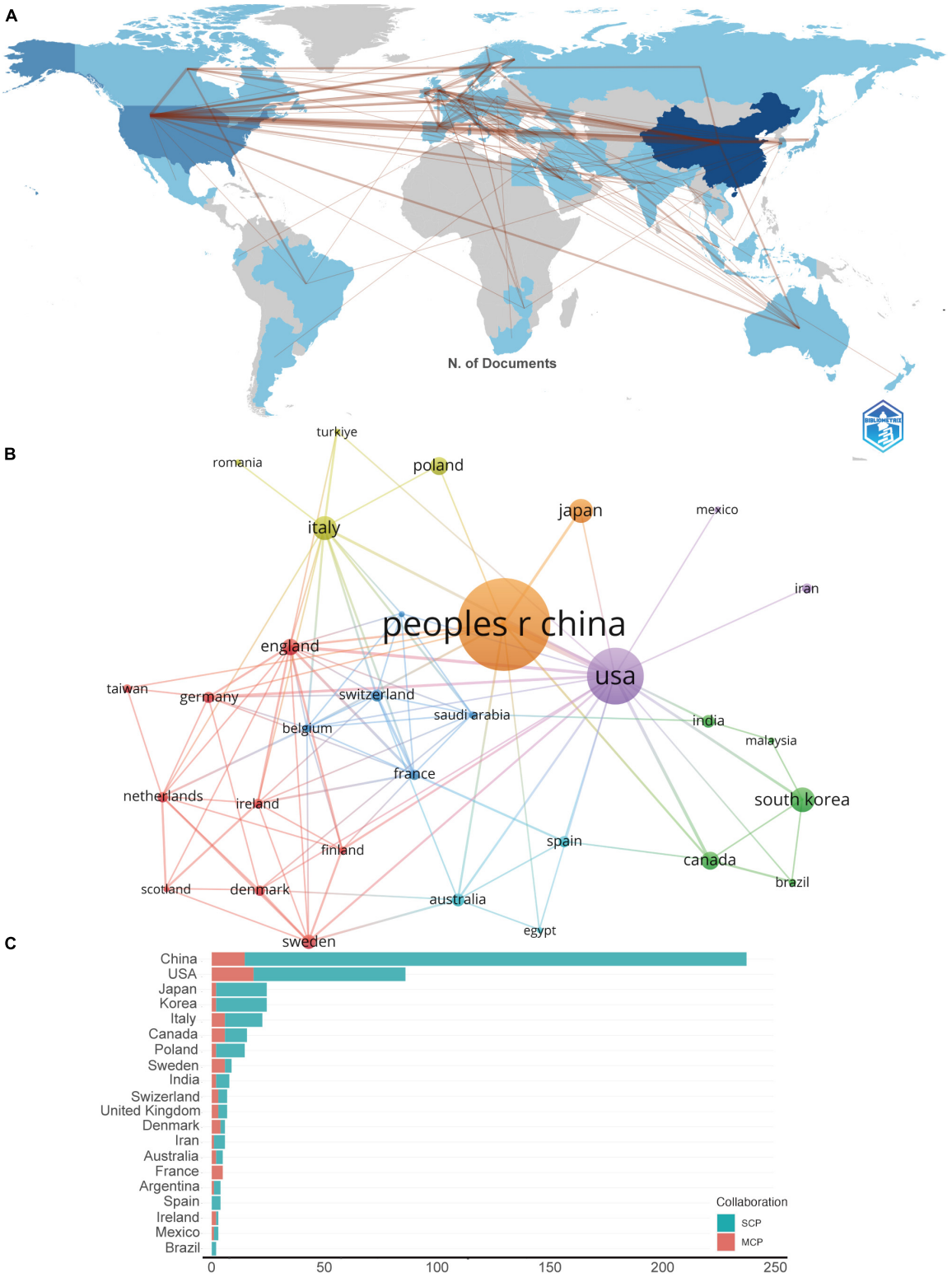

FIGURE 3
(A) The geographical network map. **(B)** The overlay visualization map of country co-authorship analysis conducted by VOSviewer. **(C)** TOP 20 corresponding author's countries that produced the largest number of literature. SCP, single country publications; MCP, multiple country publications.

Utilizing CiteSpace, we crafted a dual map overlay of journals pertaining to the role of gut microbiota in osteoporosis, as illustrated in Figure 5D. Clusters residing on the left of the orange line designate citing journals, whereas the cluster to the right of the orange trajectory signifies co-cited journals. The principal path (the yellow, orange, and one green paths) reveals that articles emanating

TABLE 1 Top 10 countries and institutions on research of gut microbiota in osteoporosis field.

Rank	Country	Articles	Citations	Rank	Institution	Counts
1	China	253	3,883	1	Shanghai Jiao Tong University	37
2	USA	111	3,537	2	Southern Medical University	33
3	South Korea	28	573	3	Central South University	32
4	Italy	27	769	4	University of Gothenburg	27
5	Japan	27	757	5	Nanjing University Chinese Medicine	26
6	Canada	17	350	6	Sichuan University	25
7	Poland	17	222	7	Cornell University	19
8	England	15	621	8	Jinan University	17
9	Sweden	11	391	9	Hong Kong Polytechnic University	16
10	Australia	10	256	10	Zhejiang University	15

from the realms of molecular/biology/genetics are primarily cited by researchers engaged in veterinary/animal/science journals, molecular/biology/immunology journals and medicine/medical/clinical journals. Furthermore, the other green path indicates that articles originating from the spheres of health/nursing and medicine are mainly cited by researchers involved in medicine and clinical journals. The outcomes from the dual-map overlay of journals may suggest that the current gut microbiota – osteoporosis research is zeroed in on molecular medical and clinical aspects.

3.4 Authors and co-cited authors

In the exploration of gut microbiota in osteoporosis, 3,166 researchers participated. The top 10 contributors collectively produced 79 publications, representing approximately 14.9% of the total output within this field (Table 3). Ohlsson C and Hernandez CJ emerged as the most productive authors, with 9 publications (Table 3). The H-index, a metric designed to quantify a scholar's impact who has authored H papers each garnering at least H citations, was employed to appraise the influence of the scientific investigations. As evidenced in Table 3, Li YJ distinguished himself as the author boasting the highest H-index, followed by Hernandez CJ et al. Anchored in the understanding of the brain-gut-bone axis and its effects on bone metabolism, Li YJ's highest impact factor paper (published in *Crit Rev Food Sci Nutr*, IF = 11.20) probes into the potential influence of probiotics and prebiotics on osteoporosis (18). This extensive exploration touches on various facets, including the regulation of gut metabolites, the integrity of the intestinal epithelial barrier, and the critical roles played by neuromodulation, immune regulation, and endocrine regulation. By doing so, the article shines a light on a promising and innovative approach toward the prevention and treatment strategies of osteoporosis in the future.

VOSviewer provides a visualization of the interconnections among authors, as exhibited in Figure 6A. There exists a profound collaboration between Xiao HH and Wong MS as well as Chan CO. Similarly, a dynamic partnership is observed between Yao, Xin-Sheng and Yao, Zhi-Hong. Co-citation analysis scrutinizes the association among items based on their co-citation frequency. Deploying VOSviewer, a totality of 153 authors, each with a minimum citation count of 20, were evaluated, as delineated in Figure 6B.

As expounded in Table 3, Li YJ emerges as the most frequently co-cited author (co-citation = 205), succeeded by Ohlsson C (co-citation = 197), and Yan J (co-citation = 180). Out of the 17,853 co-cited authors, six scholars received more than 100 co-citations. Ohlsson C's most cited article reviews the impact of the gut microbiota on bone mass (19). The research indicates that the gut microbiota regulates bone mass by modulating the host immune system. Moreover, dietary and environmental factors can influence the composition of the gut microbiota, thus affecting bone metabolism. The authors put forward the idea that the gut microbiota could serve as a novel therapeutic target for treating osteoporosis. This groundbreaking work is the first to propose that the gut microbiota could be an innovative approach toward managing osteoporosis and preventing fractures.

3.5 Hotspots investigation

3.5.1 Highly valuable papers

To evaluate the publications' influence on osteoporosis research, we evaluated local citations. Table 4 lists the top 10 co-cited documents on gut microbiota studies in osteoporosis. The most frequently cited paper, "Sex steroid deficiency–associated bone loss is microbiota dependent and prevented by probiotics," amassed 143 citations (20). This article investigates the relationship between the gut microbiota, sex steroid deficiency-induced bone loss, and the potential therapeutic role of probiotics. The research shows that sex steroid deficiency results in increased gut permeability, expanded Th17 cells, and elevated levels of osteoclastogenic cytokines in the small intestine and bone marrow. These changes were not observed in germ-free mice, suggesting the necessity of gut microbiota-induced alterations in intestinal permeability and inflammatory responses for sex steroid deficiency-induced trabecular bone loss.

Moreover, references which garner widespread citation over time within a particular subject are identified as references with

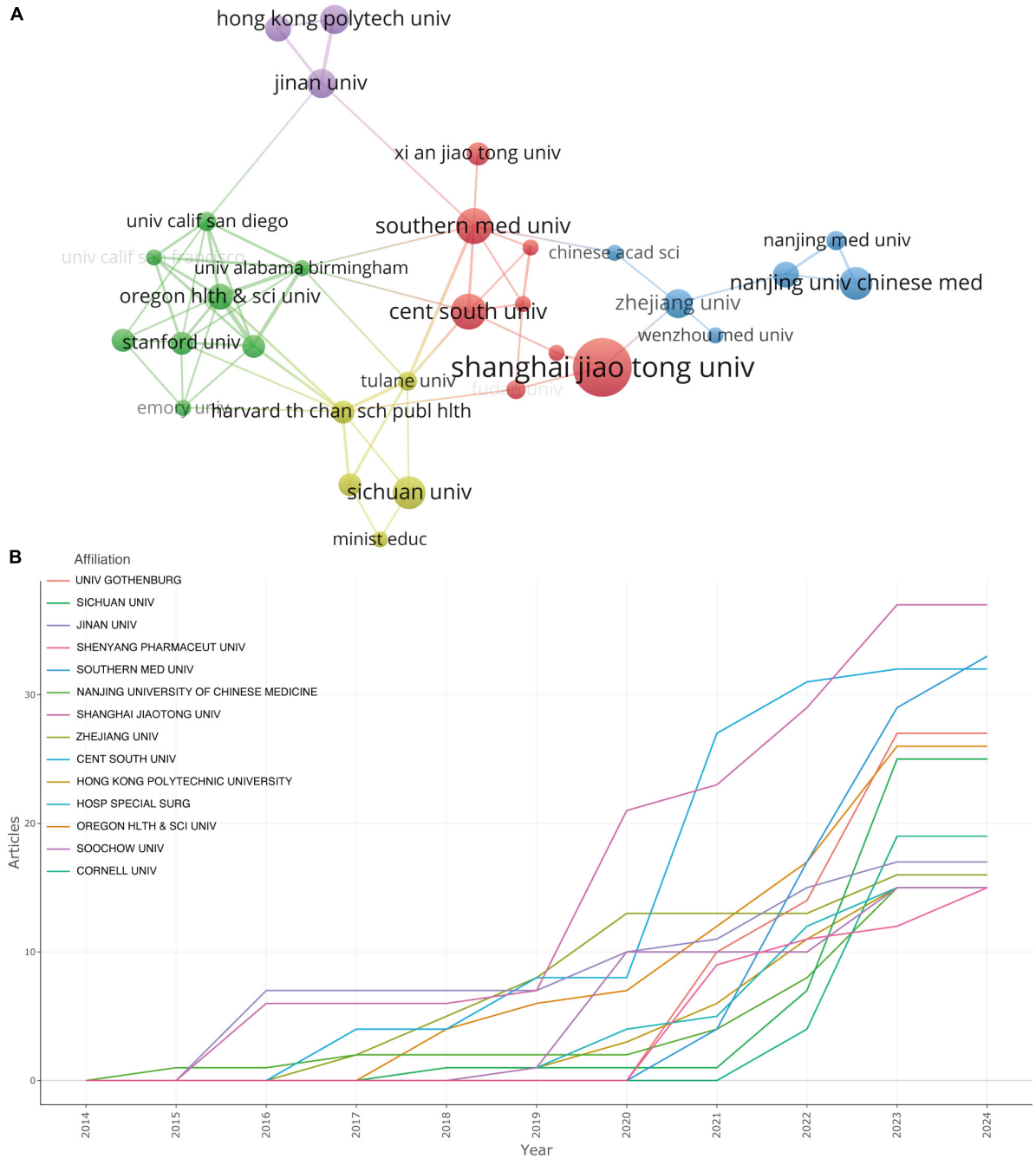

FIGURE 4
(A) The visualization of institutions cooperation networks based on VOSviewer. **(B)** Top 14 institutions' production over time.

citation bursts. Serving as a valuable metric, these burst citations highlight references that have captured academic interest within a specified field during a certain timeframe. In this investigation, CiteSpace pinpointed the top 25 references bearing the most significant citation bursts, displayed in Figure 7A. Among these, Li JY's article, which was mentioned above as the most frequently cited paper (20), held the highest rank (strength = 20.07). Ranking secondly, the research article by Britton et al., titled "Probiotic *L. reuteri* Treatment Prevents Bone Loss in a Menopausal Ovariectomized Mouse Model," published in the *Journal of Cellular Physiology*, investigates the effects of the probiotic *Lactobacillus reuteri* ATCC PTA 6475 on bone health in a model of menopause-induced osteoporosis. The study concludes that *L. reuteri* treatment suppresses bone resorption and loss associated with estrogen deficiency, suggesting that it may be a cost-effective approach to mitigate post-menopausal bone loss. This underscores the importance of gut microbiota in bone health and the potential of probiotics as a therapeutic strategy for osteoporosis (21).

R package "Bibliometrix" identified the top 10 most co-cited references, which are exhibited in Table 5. The two most-cited references are the same as those mentioned above, written by Li JY and Britton RA, respectively, and will not be reiterated here.

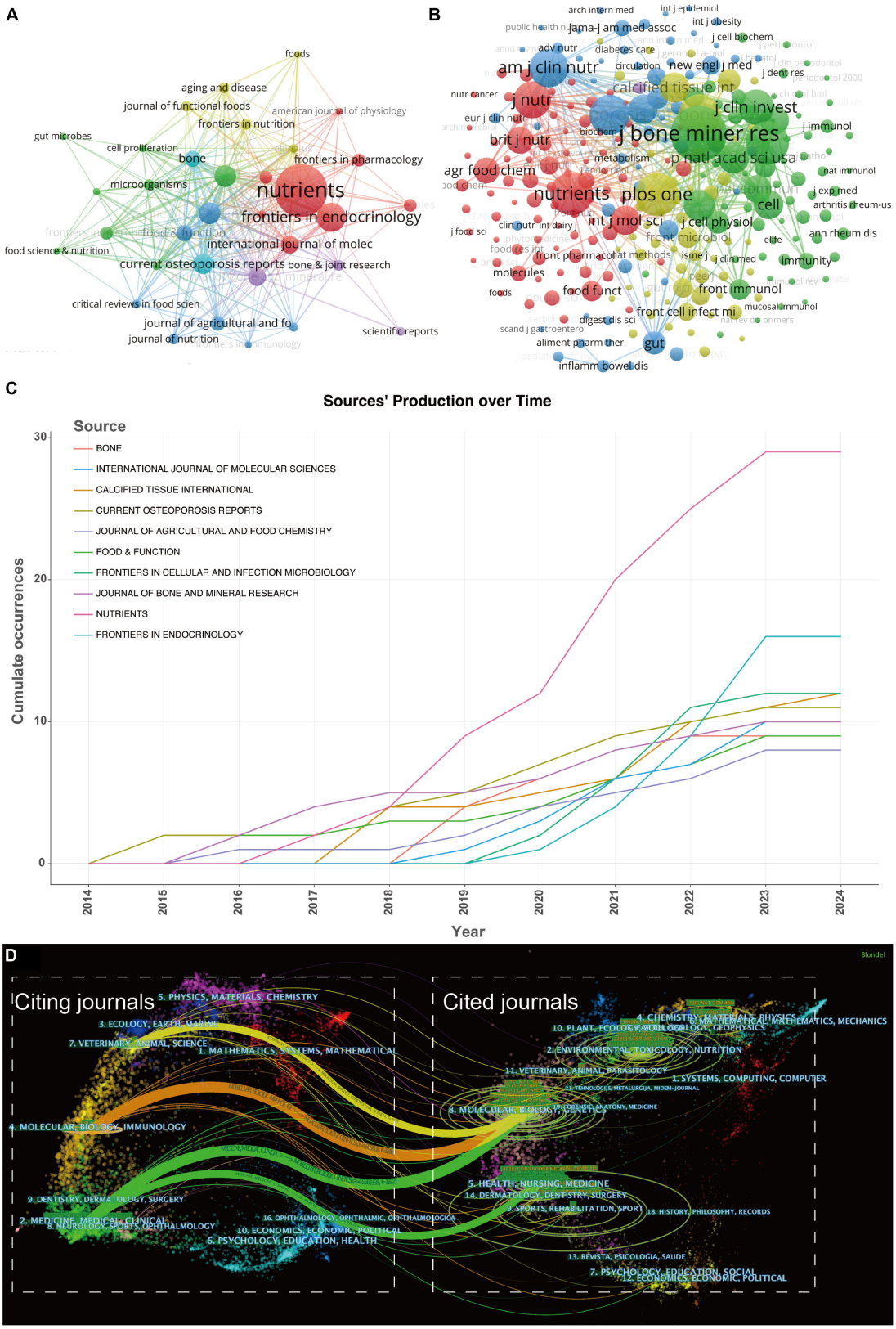

FIGURE 5
(A) The visualization of journals cooperation networks based on CiteSpace and **(B)** network visualization of co-cited journals based on VOSviewer. **(C)** Top 10 journals' production over time. **(D)** The dual-map overlay of journals related to gut microbiota-osteoporosis. The overlay segments into two main areas: journals citing others on the left, and journals being cited on the right, connected by a trajectory curve representing citation paths. Ellipses in the diagram denote the publication volume of each journal, with the ellipse's width indicating the diversity of contributing authors and its height reflecting the total number of articles published by the journal.

TABLE 2 Top 10 journals and co-cited journals for gut microbiota in osteoporosis.

Rank	Journal	Counts	IF	Q	Co-cited journal	Citations	TLS
1	Nutrients	29	6.71	Q1	J Bone Miner Res	1,096	85,399
2	Frontiers in Endocrinology	16	6.05	Q1	PLoS One	727	55,021
3	Frontiers in Cellular and Infection Microbiology	12	6.07	Q1	Nature	725	57,100
4	Calcified Tissue International	12	4.20	Q1	Nutrients	713	58,480
5	Current Osteoporosis Reports	11	5.16	Q2	Bone	694	54,481
6	Journal of Bone and Mineral Research	10	6.39	Q1	Osteoporosis Int	649	50,281
7	International Journal of Molecular Sciences	10	5.61	Q1	Am J Clin Nutr	607	52,133
8	Bone	9	4.62	Q2	J Nutr	565	46,210
9	Food & Function	9	5.98	Q1	J Clin Invest	531	45,583
10	Journal of Agricultural and Food Chemistry	8	6.12	Q1	Sci Rep-UK	427	33,344

TABLE 3 Top 10 authors and co-cited authors on research of gut microbiota in osteoporosis field.

Rank	Author	Counts	H-index	Co-cited Author	Citations	Total link strength
1	Ohlsson C	9	5	Li YJ	205	4,724
2	Hernandez CJ	9	6	Ohlsson C	197	4,266
3	Li YJ	8	6	Yan J	180	4,059
4	Rui YF	8	5	Weaver CM	134	2,890
5	Zhang YW	8	6	Britton RA	125	2,692
6	Sjogren K	8	4	Whisner CM	112	2,573
7	Xiao HH	8	4	Mccabe LR	99	2,253
8	Cao MM	7	4	Sjogren K	97	1,803
9	Fu LG	7	5	Tyagi AM	91	2,243
10	Parameswaran N	7	6	Schepper JD	90	2,295

In the third cited article authored by Yan J, they demonstrate that the resident gut microbiota not only stimulate bone formation but also resorption, with prolonged exposure to microbiota resulting in overall skeletal growth (22). The microbiota triggers the hormone insulin-like growth factor 1 (IGF-1), a key player in bone growth and remodeling. SCFAs, generated when microbiota ferment fiber, also stimulate IGF-1, hinting at a mechanism through which microbiota can impact bone health.

In essence, these significant studies primarily address the function of the gut microbiome in bone health, including discussions on how the composition and metabolites of the gut microbiome affect bone mass and bone mineral density, with special emphasis on the immune system and inflammation. Furthermore, some papers also explored how diet and probiotics (such as *L. reuteri*) affect bone health by acting on the gut microbiota. The research areas covered in these papers likely reflect the evolving hotspots in the field of gut microbiota and osteoporosis studies. Further analysis on this will be conducted in the discussion section of our paper.

3.5.2 Analysis of keywords

Keywords reflect the core or the main points the author wishes to express in an article. Therefore, keyword analysis in bibliometrics allows for exploration of hot topics and trends in the field. The keyword co-occurrence analysis facilitates the prompt identification of research focal points within a given area. Table 6 enumerates the 20 terms exhibiting the highest frequency within this field. The leading four keywords from the co-occurrence analysis include: gut microbiota (285 occurrences), osteoporosis (262 occurrences), inflammation (69 occurrences), and health (69 occurrences), all key terms associated with thematic research. It is noteworthy that "inflammation" and "probiotics" have appeared over 50 times, possibly indicating that investigations into how dysbiosis-induced inflammation in the gut microbiome contributes to osteoporosis, along with the potential of probiotics to manage and treat osteoporosis by altering the gut microbiota, are likely major research priorities in this area.

The keyword burst analysis by CiteSpace (Figure 7B) and the trend topic analysis by the R package "Bibliometrix" (Figure 8A) allow us to understand the hot research areas of particular periods and the most recent trends in this research domain. In the keyword burst analysis, the keyword "estrogen receptor alpha" had the highest burst strength (strength = 4.27), with a burst period from 2015 to 2018. The term "fecal microbiota" exhibited the longest burst duration, spanning 4 years. Additionally, keywords emerging in the past 3 years, including fecal microbiota transplantation

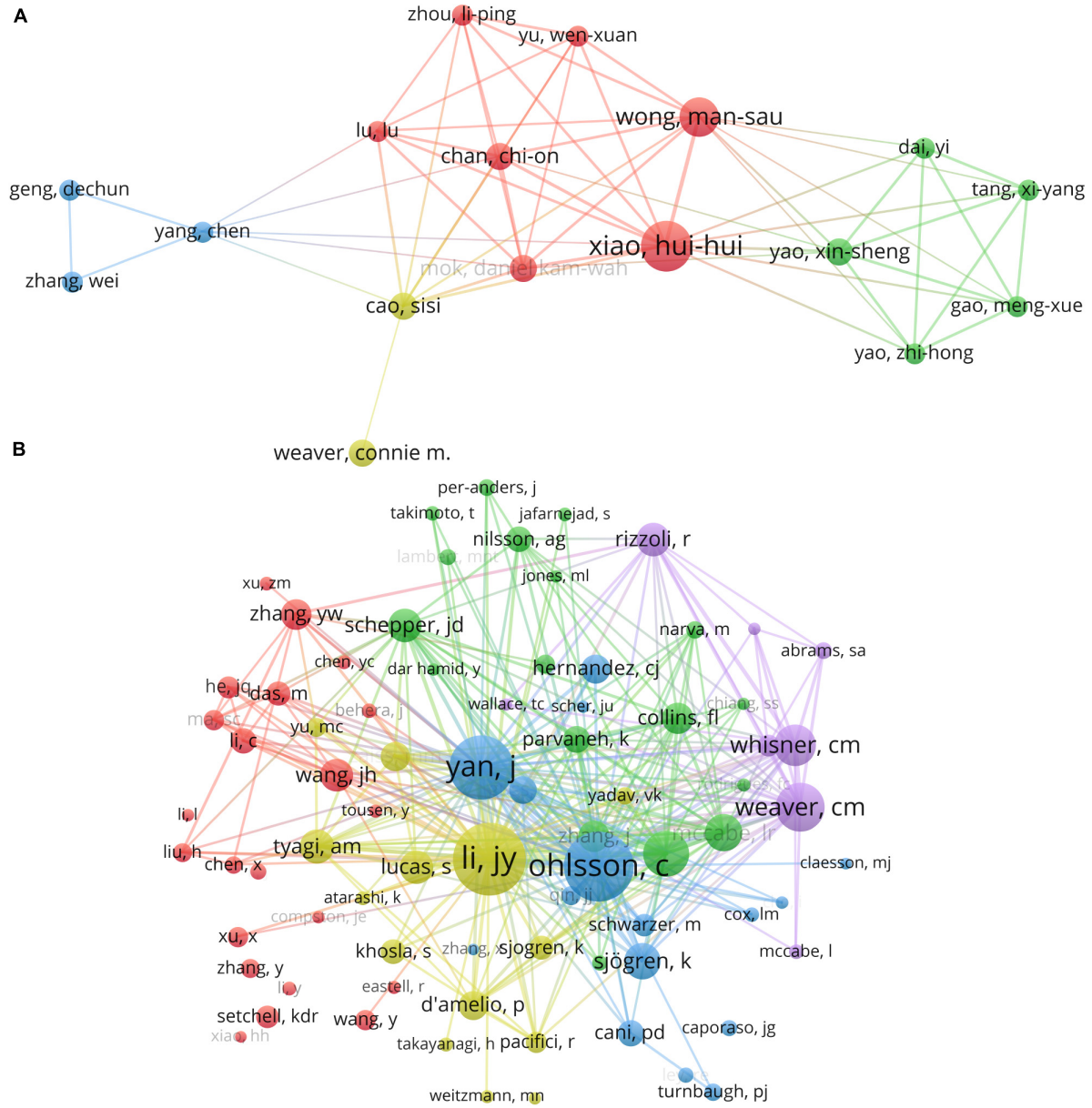

FIGURE 6
(A) The visualization of authors and **(B)** co-cited authors cooperation networks based on VOSviewer.

(strength = 1.65), microbiota (strength = 1.72), and bioavailability (strength = 1.63), represent emerging fields.

Using a minimum co-occurrence threshold of 5, we included 230 keywords in a cluster analysis using VOSviewer. As depicted in Figure 8B, the blue cluster is very clear, with keywords in this cluster primarily including "probiotics," "t-cells," "chain fatty-acids," "mineral density," and "TNF-α." Figure 8C displays high-frequency keywords in an overlay graph, with the colors indicating the average publication year. Combining this with the trend topic map in Figure 8A, it is evident that scholars have been actively investigating the role of the interaction between gut microbiota and the immune system in the progression of osteoporosis, as well as the inflammation initiated by the gut microbiota.

In summary, the keyword analysis section clearly highlights that dysbiosis-induced inflammation is a primary area of focus in the study of gut microbiota-osteoporosis. This focus is evidenced by the prevalent occurrence of terms such as "inflammation" and "probiotics." Furthermore, the term "fecal microbiota transplantation" emerges as a noteworthy area of interest, especially noted for its increasing relevance in recent years. This suggests that fecal microbiota transplantation could be a promising future research direction, potentially effective in modulating gut microbiota to combat osteoporosis.

4 Discussion

Using the WOSCC database, this study searched for literature related to the gut microbiota and osteoporosis from 2014 to 2024. Subsequently, several bibliometric analysis tools were utilized to

TABLE 4 The top 10 documents with the most local citations.

Rank	Title	LC	Journal	IF	PY	Author
1	Sex steroid deficiency–associated bone loss is microbiota dependent and prevented by probiotics	143	J Clin Invest	15.9	2016	Li JY
2	Diversity analysis of gut microbiota in osteoporosis and osteopenia patients	78	PeerJ	2.7	2017	Wang JH
3	*Lactobacillus reuteri* reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial	66	J Intern Med	11.1	2018	Nilsson AG
4	Effects of the gut microbiota on bone mass	65	Trends Endocrinol Metab	10.9	2015	Ohlsson C
5	Gut microbiota alterations associated with reduced bone mineral density in older adults	62	Rheumatology	5.5	2019	Das M
6	Probiotics (*Bifidobacterium longum*) increase bone mass density and upregulate Sparc and Bmp-2 genes in rats with bone loss resulting from ovariectomy	60	Biomed Res Int	0.0	2015	Parvaneh K
7	Gut microbiota composition and bone mineral loss—epidemiologic evidence from individuals in Wuhan, China	60	Osteoporos Int	4.0	2019	Li C
8	Gut microbiota and metabolite alterations associated with reduced bone mineral density or bone metabolic indexes in postmenopausal osteoporosis	57	Aging	5.2	2020	He JQ
9	Diet, gut microbiome, and bone health	56	Curr Osteoporos Rep	4.3	2015	Weaver CM
10	Involvement of the gut microbiota and barrier function in glucocorticoid-induced osteoporosis	48	J Bone Miner Res	6.2	2020	Schepper JD

visually analyze the literature, to further understand the research status of gut microbiota in osteoporosis over the past decade, and to explore the research hotspots and frontiers in this field.

We summarized the top co-cited documents and references with citation bursts, finding that these highly valuable papers primarily focus on how the composition and metabolites of the gut microbiome affect bone mass and bone mineral density, with a particular emphasis on the immune system and inflammation.

Additionally, by analyzing the frequency of keywords, overlay displays, and burst detection results, we discovered that research on inflammation caused by dysbiosis in osteoporosis is a research hotspot and frontier in this field.

4.1 Dysbiosis-induced inflammation: central to the pathophysiology of osteoporosis

The gut microbiota profoundly and subtly impacts host bone health (23), both directly [for example, through microbe-associated molecular patterns (MAMP) or pathogen-associated molecular patterns (PAMP)] and indirectly (for instance, via metabolite production), by modulating innate pro-inflammatory or anti-inflammatory reactions (24).

Eubiosis, colloquially known as a "healthy microbiota," is recognized as a crucial factor in preserving the physiological and metabolic integrity of the organism. Traditionally, eubiosis is conceived as a harmonious balance within the gut microbiome ecosystem, characterized by a predominance of beneficial bacterial species (25). These beneficial microbiota consortia reinforce intestinal epithelial barrier integrity through direct, cooperative mechanisms. Furthermore, they shape the host's immune landscape indirectly by generating a variety of essential metabolites.

Studies indicate that SCFAs are the most significant metabolites known to positively affect Treg cells, particularly butyrate, which enhance Treg cell differentiation and function (26). This is achieved by promoting the expression of relevant genes and by inhibiting the activity of histone deacetylases. Moreover, indole-3-acetic acid (IAA) and indole-3-propionic acid (IPA), metabolites produced by the gut microbiota from tryptophan, can promote the differentiation of Treg cells as well, but through activating the aryl hydrocarbon receptor (AhR) (27). Consequently, an increase in Treg cells triggers the release of anti-inflammatory cytokines and reduces inflammatory signaling pathways, such as NF-κB, thereby enhancing intestinal immune tolerance and maintaining an anti-inflammatory milieu (28).

Contrary to eubiosis, "dysbiosis" denotes alterations in the composition and function of the primary microbial communities, linked to the emergence of various diseases (25).

Dysbiosis in the gut microbiota disrupts the "functional balance" between pro-inflammatory and anti-inflammatory microbes, altering intestinal immunity and biasing the immune system toward a pro-inflammatory response (**Figure 9**). This alteration affects the differentiation of naive CD4+ T cells. In particular, specific bacteria, including segmented filamentous bacteria (SFB), *Bifidobacterium adolescentis*, and *Eggerthella lenta*, are known to augment Th17 cells (29). This leads to an increase in the secretion of pro-inflammatory cytokines, such as IL-17 and TNF-α from Th17 cells, further promoting inflammatory responses and bone resorption (30). Conversely, certain bacterial species, including *Lactobacillus rhamnosus* GG (LGG), *L. reuteri*, and *Bifidobacterium breve*, can positively impact the abundance and function of Treg cells (29). However, the reduction of these

A **Top 25 References with the Strongest Citation Bursts**

References	Year	Strength	Begin	End	2014 - 2024
Britton RA, 2014, J CELL PHYSIOL, V229, P1822, DOI 10.1002/jcp.24636, DOI	2014	18.34	2015	2019	
Ohlsson C, 2014, PLOS ONE, V9, P0, DOI 10.1371/journal.pone.0092368, DOI	2014	16.75	2015	2019	
Sjögren K, 2012, J BONE MINER RES, V27, P1357, DOI 10.1002/jbmr.1588, DOI	2012	7.55	2015	2017	
Cho I, 2012, NATURE, V488, P621, DOI 10.1038/nature11400, DOI	2012	5.03	2015	2017	
Whisner CM, 2013, BRIT J NUTR, V110, P1292, DOI 10.1017/S000711451300055X, DOI	2013	4.65	2015	2018	
Ohlsson C, 2015, TRENDS ENDOCRIN MET, V26, P69, DOI 10.1016/j.tem.2014.11.004, DOI	2015	11.51	2016	2020	
Mccabe LR, 2013, J CELL PHYSIOL, V228, P1793, DOI 10.1002/jcp.24340, DOI	2013	9.51	2016	2018	
Whisner CM, 2014, BRIT J NUTR, V112, P446, DOI 10.1017/S0007114514000981, DOI	2014	5.84	2016	2019	
Cox LM, 2014, CELL, V158, P705, DOI 10.1016/j.cell.2014.05.052, DOI	2014	4.78	2016	2019	
Li JY, 2016, J CLIN INVEST, V126, P2049, DOI 10.1172/JCI86062, DOI	2016	20.07	2017	2021	
Yan J, 2016, P NATL ACAD SCI USA, V113, PE7554, DOI 10.1073/pnas.1607235113, DOI	2016	15.32	2017	2021	
Weaver CM, 2015, CURR OSTEOPOROS REP, V13, P125, DOI 10.1007/s11914-015-0257-0, DOI	2015	13.59	2017	2020	
McCabe L, 2015, CURR OSTEOPOROS REP, V13, P363, DOI 10.1007/s11914-015-0292-x, DOI	2015	9.94	2017	2020	
Parvaneh K, 2015, BIOMED RES INT, V2015, P0, DOI 10.1155/2015/897639, DOI	2015	8.12	2017	2020	
Schwarzer M, 2016, SCIENCE, V351, P854, DOI 10.1126/science.aad8588, DOI	2016	7.95	2017	2021	
Hernandez CJ, 2016, J BONE MINER RES, V31, P1638, DOI 10.1002/jbmr.2887, DOI	2016	6	2017	2021	
Zhang J, 2015, ENDOCRINOLOGY, V156, P3169, DOI 10.1210/EN.2015-1308, DOI	2015	7.68	2018	2020	
Yan J, 2017, CURR OSTEOPOROS REP, V15, P376, DOI 10.1007/s11914-017-0382-z, DOI	2017	5.38	2018	2021	
Whisner CM, 2016, J NUTR, V146, P1298, DOI 10.3945/jn.115.227256, DOI	2016	4.3	2018	2021	
Garcia-Vieyra MI, 2014, J MED FOOD, V17, P1247	2014	4.1	2018	2019	
Wang JH, 2017, PEERJ, V5, P0, DOI 10.7717/peerj.3450, DOI	2017	5.29	2019	2021	
Koh A, 2016, CELL, V165, P1332, DOI 10.1016/j.cell.2016.05.041, DOI	2016	4.16	2020	2021	
Ling CW, 2021, J CLIN ENDOCR METAB, V106, PE3852, DOI 10.1210/clinem/dgab492, DOI	2021	4.97	2022	2024	
Tu Y, 2021, J LEUKOCYTE BIOL, V110, P525, DOI 10.1002/JLB.3MR0321-755R, DOI	2021	4.76	2022	2024	
Rettedal EA, 2021, JBMR PLUS, V5, P0, DOI 10.1002/jbm4.10452, DOI	2021	4.55	2022	2024	

B **Top 25 Keywords with the Strongest Citation Bursts**

Keywords	Year	Strength	Begin	End	2014 - 2024
blood pressure	2014	1.5	2014	2017	
estrogen receptor alpha	2015	4.27	2015	2018	
calcium absorption	2015	2.14	2015	2016	
fecal microbiota	2015	1.99	2015	2019	
expression	2015	1.62	2015	2016	
rats	2016	3.09	2016	2019	
mice	2016	2.39	2016	2018	
metabolism	2016	2.03	2016	2017	
induced obesity	2017	2.14	2017	2019	
adipose tissue	2017	1.95	2017	2018	
osteoclast differentiation	2017	1.92	2017	2018	
intestinal microbiota	2017	2.83	2018	2020	
health	2015	2.71	2018	2019	
model	2018	2.58	2018	2019	
regulatory t cells	2018	1.79	2018	2020	
osteoclast formation	2019	2.5	2019	2020	
fermented milk	2019	2.04	2019	2020	
hip fracture	2019	1.9	2019	2021	
dysbiosis	2019	1.83	2019	2021	
vitamin k	2020	2.38	2020	2021	
nf kappa b	2020	1.88	2020	2021	
protein	2020	1.58	2020	2021	
fecal microbiota transplantation	2021	1.65	2021	2022	
microbiota	2020	1.72	2022	2024	
bioavailability	2022	1.63	2022	2024	

FIGURE 7
(A) Top 25 references with strongest citation bursts of publications regarding gut microbiota in osteoporosis. **(B)** Top 25 keywords with the strongest citation bursts based on CiteSpace.

beneficial bacterial species can inhibit the differentiation of regulatory T cells (Tregs), leading to a decrease in the secretion of anti-inflammatory cytokines derived from Tregs, such as IL-4, IL-10, and TGF-β. This ultimately promotes osteoclastogenesis and inhibits bone formation. The dysbiosis-induced alteration in the Th17/Treg balance reduces the host's immunosuppressive

TABLE 5 The top 10 most co-cited references in the field of gut microbiota in osteoporosis.

Rank	Title	TC	Journal	IF	PY	Author
1	Sex steroid deficiency–associated bone loss is microbiota dependent and prevented by probiotics	118	J Clin Invest	19.4	2016	Li, JY
2	Probiotic *L. reuteri* treatment prevents bone loss in a menopausal ovariectomized mouse model	98	J Cell Physiol	6.5	2014	Britton RA
3	Gut microbiota induce IGF-1 and promote bone formation and growth	89	PANS	12.7	2016	Yan J
4	The gut microbiota regulates bone mass in mice	76	J Bone Miner Res	6.3	2012	Sjögren K
5	Probiotics protect mice from ovariectomy-induced cortical bone loss	70	PLoS One	3.7	2014	Ohlsson C
6	Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss	69	Nat Commun	17.6	2018	Lucas S
7	Diversity analysis of gut microbiota in osteoporosis and osteopenia patients	63	PeerJ	3.0	2017	Wang JH
8	The microbial metabolite butyrate stimulates bone formation via T regulatory cell-mediated regulation of WNT10B expression	54	Immunity	43.4	2018	Tyagi AM
9	*Lactobacillus reuteri* reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial	51	J Intern Med	13.0	2018	Nilsson AG
10	Gut microbiota alterations associated with reduced bone mineral density in older adults	50	Rheumatology	7.04	2019	Das M

TABLE 6 Top 20 keywords on research of gut microbiota in osteoporosis.

Rank	Keywords	Occurrences	Rank	Keywords	Occurrences
1	Gut microbiota	285	11	Bone loss	52
2	Osteoporosis	262	12	Intestinal microbiota	47
3	Inflammation	69	13	Double-blind	47
4	Health	69	14	Microbiota	45
5	Mineral density	64	15	Obesity	44
6	Bone-mineral density	64	16	Chain fatty-acids	42
7	Gut microbiome	61	17	Microbiome	40
8	Probiotics	54	18	Women	38
9	Bone	53	19	Oxidative stress	37
10	Postmenopausal women	52	20	Differentiation	35

capacity, which exacerbates the inflammatory state of the intestinal microenvironment and directly impacts bone metabolism.

As mentioned above, dysbiosis can also be defined as alterations in the composition of the primary microbial communities, typically characterized by a reduction in beneficial bacteria and/or an increase in harmful bacteria. This reduction in beneficial microbes can not only directly compromise the integrity of connections between intestinal epithelial cells, diminishing the thickness and quality of the gut mucosal mucus layer, thus reducing barrier function and enhancing intestinal permeability, a condition commonly referred to as "leaky gut" (31). Furthermore, the proliferation of pathogenic bacteria and their toxins (such as lipopolysaccharide, LPS) can stimulate intestinal mucosal immune cells: dendritic cells (DCs) in the intestine can capture and process antigens, activating T cells, and promoting the differentiation of Th17 cells (32). Macrophages then trigger inflammatory responses through pattern recognition receptors (such as Toll-like receptors), resulting in the production of excessive pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β (33). These inflammatory factors, secreted by intestinal mucosal immune cells in response to pathogens and toxins, not only increase local intestinal inflammation but can also directly damage intestinal epithelial cells, leading to reduced expression of tight junction proteins (such as occludin and claudin), thereby further increasing intestinal permeability. Consequently, bacteria, toxins, and other foreign antigens can more easily enter the bloodstream, leading to a systemic inflammatory response.

The impact of inflammation on bone metabolism is well recognized (34, 35). Chronic systemic inflammation, through the activation of immune cells such as macrophages and T cells, leads to the production of inflammatory cytokines (TNF-α, IL-1, and IL-6) that directly influence bone metabolism. Specifically, TNF-α

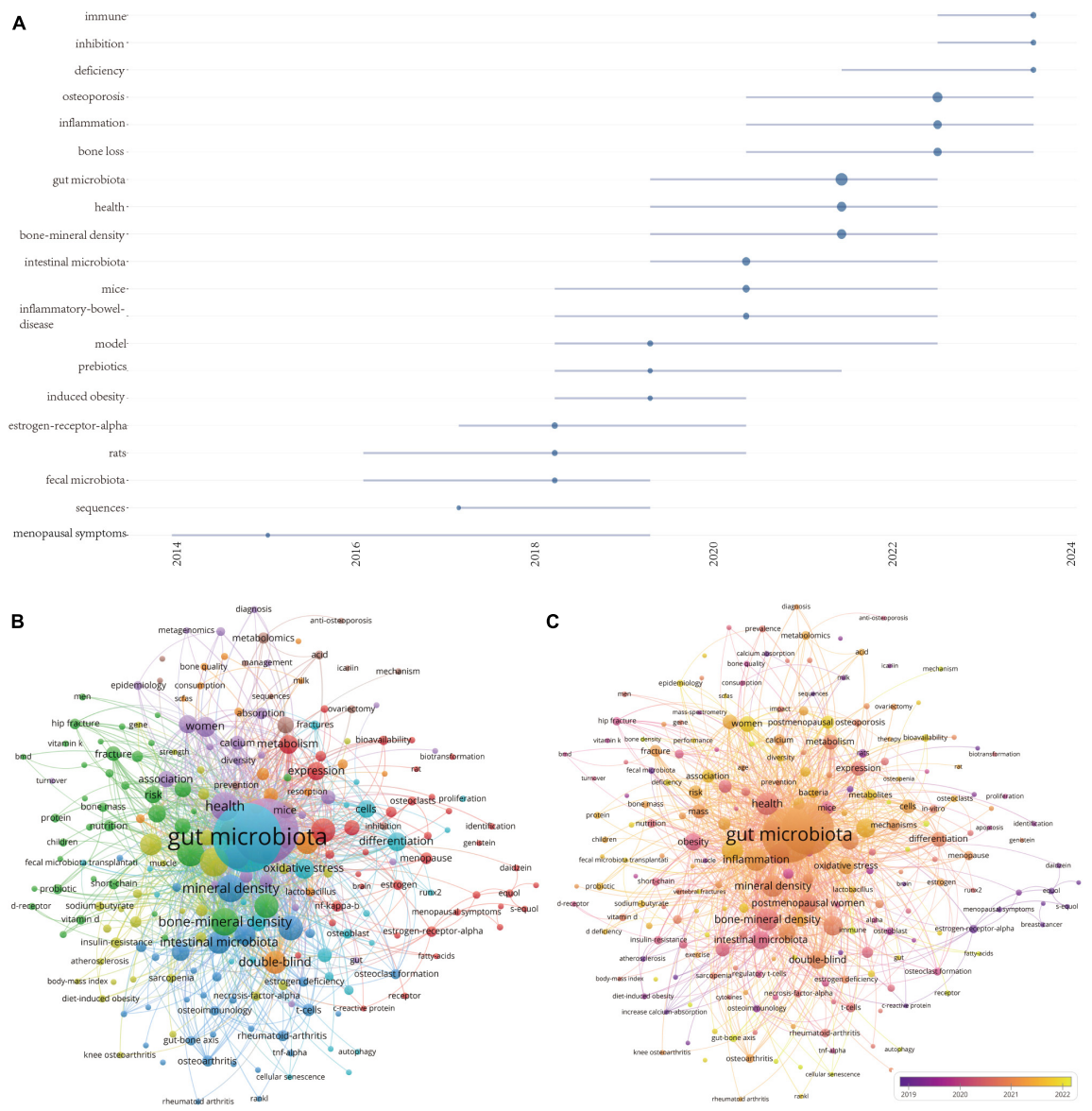

FIGURE 8
(A) Visualization map of trend topics analysis. **(B)** Keywords co-occurrence network and **(C)** overlay visualization of the keywords network based on VOSviewer.

and IL-1 are known to promote the differentiation and maturation of osteoclast precursors, leading to increased bone resorption (36, 37). Additionally, IL-6 facilitates osteoclastogenesis by upregulating RANKL expression and drives bone immune reactions via the JAK/STAT signaling pathway, thus accelerating bone resorption (38). Moreover, IL-17 boosts osteoclast activity by fostering RANKL expression, thereby influencing bone resorption, and it may indirectly affect osteoblast function by inhibiting the Wnt/β-catenin signaling pathway (39). Furthermore, the inflammatory milieu amplifies the interaction between RANKL (a key osteoclast differentiation factor) and its receptor RANK, further enhancing osteoclast formation and activity (40).

Overall, the inflammation initiated by gut microbiota dysbiosis and the resulting immune disorder play a pivotal role in osteoporosis progression. Inflammatory responses triggered by dysbiosis lead immune cells, such as macrophages and dendritic cells, to release an array of inflammatory mediators, directly impacting bone metabolism through key pathways such as the NF-κB signaling pathway and the RANKL/OPG/RANK system, among others. Additionally, the inflammatory state from dysbiosis disturbs the dynamic equilibrium between Th17 and Tregs, not only facilitating osteoclast differentiation but also suppressing bone formation, thus laying the groundwork for osteoporosis development. These mechanisms highlight the critical importance of understanding the interplay between gut microbiota and the host immune system, suggesting that regulating this intricate network might provide novel prevention and treatment options for osteoporosis.

Among current drug treatments for osteoporosis, a range of options such as bisphosphonates, SERMs, PTH and its analogs, RANKL inhibitors, and Sclerostin inhibitors have been confirmed to effectively increase bone density, decelerate bone loss, and

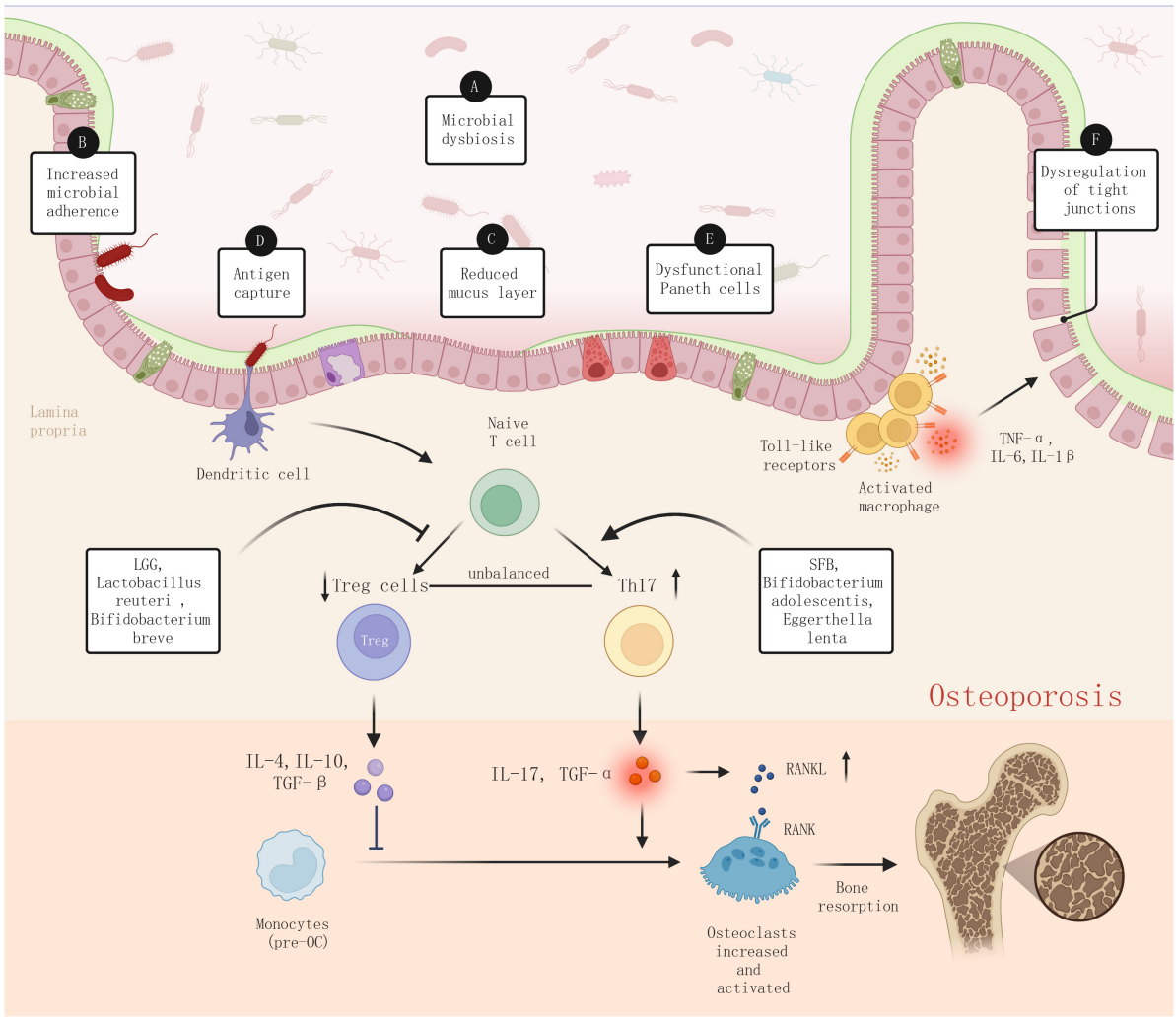

FIGURE 9
Dysbiosis-induced inflammation in pathophysiology of osteoporosis.

lower fracture risks (41, 42). Although these drugs offer viable treatments for osteoporosis, their application is faced with several limitations. These limitations range from potential safety issues with long-term use, such as jaw osteonecrosis and atypical femur fractures associated with bisphosphonates, to significant patient-to-patient variability in treatment outcomes. Additionally, the high costs of developing new drugs and an inadequate understanding of complex disease mechanisms present further challenges. Furthermore, existing treatments mainly target a single aspect of the pathological process and often fail to address the multifactorial etiology of osteoporosis, highlighting the need for more comprehensive treatment strategies.

4.2 Targeting gut microbiota: FMT and diet in osteoporosis treatment

Given the crucial role of gut microbiome dysbiosis and resultant inflammation in the pathogenesis of osteoporosis, focusing research on the gut microbiome as a treatment target for osteoporosis is not only a response to the limitations of current treatments but also a logical extension of a comprehensive understanding of osteoporosis's complex pathophysiological mechanisms. Keywords with the strongest citation bursts, such as "fecal microbiota transplantation," "fermented milk," "protein," "bioavailability," "nf kappa b," highlight that regulating the gut microbiome through methods like fecal microbiota transplantation or specific dietary patterns to control inflammation represents a promising future research direction for enhancing bone health and preventing or treating osteoporosis (Figure 10).

Diet has a significant impact on bone health throughout the entire lifespan and is a major determinant of the types and proportions of microbes in the host organism. Thus, when assessing the impact of changes in the gut microbiome on bone health, diet should be considered an important confounding factor (43).

Diet directly affects the gut microbiome, modifying its composition or metabolic outputs, potentially contributing to disease progression or maintaining bodily balance (44). A high-fiber diet rich in fruits, vegetables, legumes, and whole grains provides various polysaccharides and oligosaccharides indigestible

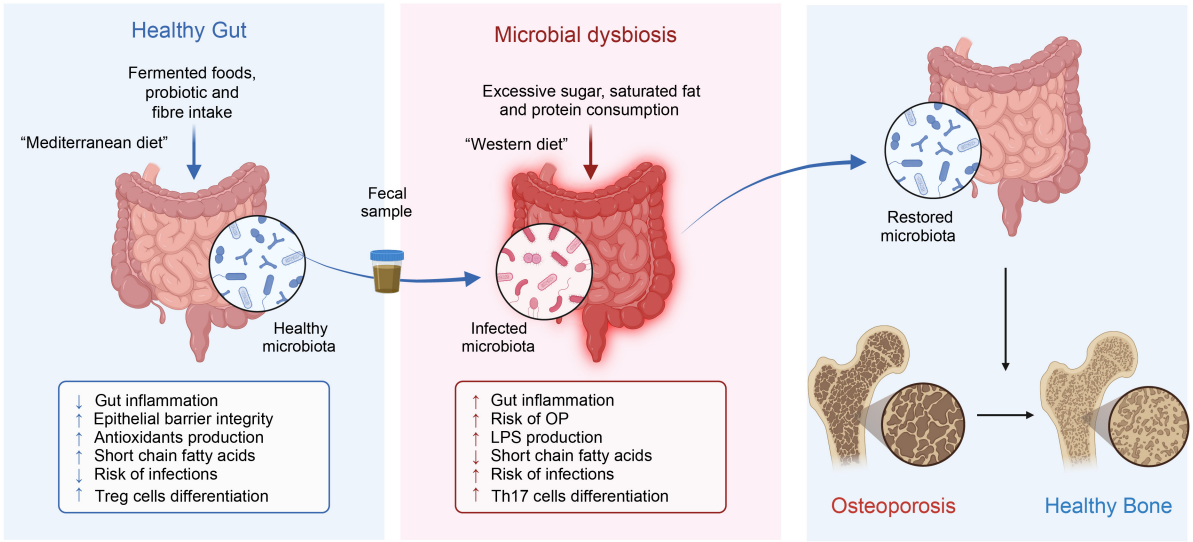

FIGURE 10
Fecal microbiota transplantation and diet in osteoporosis treatment.

by human enzymes. These dietary fibers act as prebiotics, specifically nourishing beneficial gut bacteria and promoting the production of SCFAs (such as butyrate, propionates, and acetates) (45). Fermented foods such as yogurt, kefir, sauerkraut, and kimchi are rich in live microorganisms that contribute to the increased diversity of the gut microbiota. By consuming fermented foods, one can increase the abundance of beneficial bacteria, improve gut barrier function, and have anti-inflammatory effects (46). The Mediterranean diet, enriched with vegetables, fruits, nuts, seeds, legumes, whole grains, fish, and olive oil, is associated with increased microbial diversity and the promotion of beneficial bacterial growth. This diet, rich in monounsaturated fats, polyphenols, and fiber, not only positively affects the gut microbiome but also enhances the production of SCFAs, thereby improving gut barrier function, and reducing inflammation as common benefits (47).

High-protein diets, especially those rich in animal proteins, alter the gut microbiome by increasing the abundance of bacteria capable of fermenting protein. Consequently, this could lead to the production of potentially harmful by-products, including ammonia, amines, and sulfides (48). Western diets, marked by high fat, high sugar, and low fiber, lead to a gut microbiota composition inclined toward increased inflammation levels and decreased diversity (49). Such dietary patterns commonly result in increased intestinal permeability ("leaky gut") and systemic inflammation.

In recent years, fecal microbiota transplantation (FMT) has been widely used to treat a variety of diseases (50), including Crohn's disease, metabolic syndrome (51), diabetes (52), and neurological disorders (53). Remarkably, FMT has also demonstrated considerable potential in treating osteoporosis (54). Unlike individual or mixed bacteria, FMT maximally retains the original diversity and quantity of active microbial communities, thereby enabling a quicker reinstatement of gut microbiota stability in osteoporosis patients (54). In a study by Zhang et al., transplanting the gut microbiota of children into ovariectomized (OVX) mice effectively prevented bone loss caused by ovariectomy and increased the bone strength of the mice. Moreover, 16S rRNA gene sequencing revealed that transplanting the fecal microbiota of children reversed the OVX-induced reduction in *Akkermansia* abundance, while direct supplementation with *Akkermansia* could prevent bone loss in OVX mice (54).

Yet, the choice of FMT donors, the presence of numerous harmful bacteria in the transplants, and the long-term outcomes of the treatment approach continue to cast doubt on its clinical safety (55, 56). Future research should focus on identifying specific microbial strains or consortia that have the most significant impact on bone metabolism, exploring the optimal timing and frequency of FMT for the best bone health outcomes, and understanding individual variations in response to FMT. Clinical trials that monitor the gut microbiota composition, bone density, and bone metabolism markers before and after FMT are essential to ascertain the effectiveness of this method in preventing or treating osteoporosis.

Research on various interventions targeting the gut microbiota is still in its initial stages, and we emphasize the necessity for future studies, particularly the need for more high-quality, large-scale, long-term clinical and mechanistic studies to validate the efficacy and safety of gut microbiota intervention measures.

5 Conclusion

Through a bibliometric analysis of literature on osteoporosis and gut microbiota published between 2014 and 2024, this paper investigates the impact of gut microbiome dysregulation and its associated inflammation on osteoporosis progression, a topic that has garnered international research interest in recent years. Additionally, our study delves into the potential of fecal microbiota transplantation or specific dietary interventions as promising avenues for future research.

Recent human studies have demonstrated a significant role of gut health in bone metabolism, highlighting the potential of the

gut microbiota as a therapeutic strategy in osteoporosis. However, variations in the test environment, the genetic background of the host, and the sources of gut microbiota present major challenges in controlling variables in research. These factors contribute to the heterogeneity and some contradictory conclusions in current studies. As a result, transitioning gut microbiota research from basic studies to clinical trials and practical applications remains a challenge. A critical priority is to continue searching for effective gut microbial strains for the treatment of osteoporosis and to carefully evaluate their quality, safety, dosage, stability, and interactions with other drugs. Moreover, ongoing and future studies must rigorously validate these findings in larger human cohorts to establish a more definitive link between the gut microbiota and osteoporosis. We eagerly anticipate further high-quality research that sheds light on the intricate dynamics between the gut microbiota and osteoporosis, ultimately charting new courses for osteoporosis prevention and therapy.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

This study did not include any patient information. Thus, the requirement for ethics approval was waived.

Author contributions

ZL: Writing – original draft. YH: Conceptualization, Writing – original draft. ZC: Data curation, Writing – original draft. CL: Methodology, Writing – original draft. BZ: Supervision, Writing – review & editing. XG: Investigation, Writing – review & editing. ZS: Data curation, Funding acquisition, Writing – review & editing. TW: Project administration, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The causal role of circulating inflammatory markers in osteoporosis: a bidirectional Mendelian randomized study

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Background: Osteoporosis (OP) associated with aging exerts substantial clinical and fiscal strains on societal structures. An increasing number of research studies have suggested a bidirectional relationship between circulating inflammatory markers (CIMs) and OP. However, observational studies are susceptible to perturbations in confounding variables. In contrast, Mendelian randomization (MR) offers a robust methodological framework to circumvent such confounders, facilitating a more accurate assessment of causality. Our study aimed to evaluate the causal relationships between CIMs and OP, identifying new approaches and strategies for the prevention, diagnosis and treatment of OP.

Methods: We analyzed publicly available GWAS summary statistics to investigate the causal relationships between CIMs and OP. Causal estimates were calculated via a systematic analytical framework, including bidirectional MR analysis and Bayesian colocalization analysis.

Results: Genetically determined levels of CXCL11 (OR = 0.91, 95% CI = 0.85–0.98, P = 0.008, P_{FDR} = 0.119), IL-18 (OR = 0.88, 95% CI = 0.83–0.94, P = 8.66×10−5, P_{FDR} = 0.008), and LIF (OR = 0.86, 95% CI = 0.76–0.96, P = 0.008, P_{FDR} = 0.119) were linked to a reduced risk of OP. Conversely, higher levels of ARTN (OR = 1.11, 95% CI = 1.02–1.20, P = 0.012, P_{FDR} = 0.119) and IFNG (OR = 1.16, 95% CI = 1.03–1.30, P = 0.013, P_{FDR} = 0.119) were associated with an increased risk of OP. Bayesian colocalization analysis revealed no evidence of shared causal variants.

Conclusion: Despite finding no overall association between CIMs and OP, five CIMs demonstrated a potentially significant association with OP. These findings could pave the way for future mechanistic studies aimed at discovering new

treatments for this disease. Additionally, we are the first to suggest a unidirectional causal relationship between ARTN and OP. This novel insight introduces new avenues for research into diagnostic and therapeutic strategies for OP.

KEYWORDS

Mendelian randomization, circulating inflammatory protein, osteoporosis, Bayesian colocalization analysis, artemin (ARTN)

1 Introduction

Osteoporosis (OP) is a severe skeletal disorder characterized by a high incidence and mortality rate. It is characterized by decreased bone strength, increased fragility, and a propensity for fractures, which can lead to cardiovascular diseases and even premature mortality (1–7). Currently, OP affects approximately 18.3% of the global population, and its incidence is increasing due to environmental pollution and an aging population, highlighting the need for vigilance (8). However, regrettably, the current approach to OP treatment primarily focuses on prevention, utilizing pharmacological interventions to slow bone loss while ensuring adequate nutrition. Nevertheless, the clinical diagnosis of OP relies heavily on dual-energy X-ray absorptiometry (DEXA), and a convenient method for widespread use is not available. This may explain the higher prevalence of OP in underdeveloped regions. Therefore, in-depth research on the pathogenesis of OP is urgently needed for the early prevention and accurate diagnosis of OP and to develop effective treatment strategies.

Circulating inflammatory markers (CIMs) are garnering increasing interest in medical research because of their potential to offer critical insights into early disease diagnosis, prognosis evaluation, and therapeutic strategies. These small molecule proteins, which are produced by immune cells, play pivotal roles in regulating and controlling immune and inflammatory responses. Through intercellular signal transduction, CIMs regulate and coordinate the body's responses to infections, injuries, or other stimuli. These CIMs are widely present within the body and can interact with various immune cells, including macrophages, lymphocytes, and granulocytes. As crucial modulators of the inflammatory process, they participate in key biological processes, including cell proliferation, differentiation, migration, and apoptosis. Common CIMs include tumor necrosis factor (TNF), interleukin (IL) family members, interferon (IFN) family members, and chemokines. These CIMs can be synthesized and released by activated immune cells, thereby regulating and promoting the inflammatory process. They play essential roles in the immune system, modulating cell proliferation, mediating inflammation, and clearing pathogens, among other physiological and pathological processes. Nevertheless, excessive or abnormal production of CIMs may lead to chronic inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, and autoimmune diseases (9–13). Consequently, further research on CIMs is paramount for understanding the mechanisms of inflammation and developing treatment methods for related diseases. Various inflammatory mediators, including IL-1, IL-6, IL-8, TNF-α, and IL-12, are known to be involved in the onset and progression of osteoporosis (14). These mediators interact with proteins related to bone resorption, impairing the function of osteoblasts and ultimately leading to osteoporosis.

However, the relationship between the levels of CIMs and OP is not yet clear and may be related to the relatively high concentrations of these proteins in the body. Currently, some scholars believe that there is an association between CIMs and OP (9, 13), but the mechanisms underlying this association remain unclear. In recent years, Mendelian randomization (MR) has emerged as an innovative statistical method and has gained widespread attention in the fields of medicine and biology. This method uses genetic variations as instrumental variables (IVs) to assess the causal relationships between exposures and outcomes (15), thus offering new insights into many diseases that have traditionally been difficult to elucidate.

In this study, we performed bidirectional MR analysis to investigate the causal effects of CIMs on OP and vice versa. This may provide a theoretical basis for further elucidation of the causal relationship between them.

2 Materials and methods

2.1 Study design

The MR analysis in our study was based on three assumptions: (1) the genetic instrumental variables (IVs) are strongly associated with exposure; (2) the selected genetic IVs are not associated with potential confounders; and (3) the genetic IVs can only affect the risk of outcome dependently through exposure (16). This bidirectional MR analysis was performed in two steps. First, CIMs were investigated as exposures, and OPs were investigated as outcomes in the first step. In the second step, this was reversed.

Figure 1 shows an overview of the three assumptions and study design. The confounders are listed in Supplementary Table 1.

2.2 Data sources

The summary-level data used in this study are deidentified public data and are available for download. Each GWAS involved in this study was approved by the ethics committee of the respective institution.

OP-related GWAS summary statistics were extracted from the FinnGen consortium, including data from 365,314 individuals of European ancestry and 7300 individuals with OP. More details for phenotype and modeling, genotype quality control, and related association analyses can be found on the FinnGen website (https://www.finngen.fi/en/) (17). Associations were tested after adjusting for covariates, including heel bone mineral density, heel bone mineral density left, heel bone mineral density right, bone density, lumbar spine bone mineral density, self-reported osteoporosis, and carbamazepine-induced cutaneous adverse drug reactions.

Summary statistics for CIMs were publicly available from the GWAS Catalog (accession numbers from GCST90274758 to GCST90274848) (http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90274001-GCST90275000). A total of 91 phenotypes were included. The original GWAS of immune traits was performed using data from European individuals, and there were no overlapping cohorts. The corresponding information for these 91 CIMs can be found in Supplementary Table 2.

2.3 Genetic instrumental variable (IV) selection

1. The IVs selected for analysis are highly related to the corresponding exposures, and we chose significant single nucleotide polymorphisms (SNPs) based on a loose cutoff of $p < 1 \times 10^{-5}$ to ensure sufficient IVs for screening.
2. The IVs are mutually independent and avoid the offset caused by linkage disequilibrium (LD) between the SNPs (r2 < 0.1, LD distance > 500 kb).
3. We eliminated IVs with an F-statistic <10 to minimize potential weak instrument bias F = R2 (n-k-1)/k (1-R2) (n is the sample size, k is the number of included IVs, and R2 is the exposure variance explained by the selected SNPs).

2.4 MR analysis

The inverse variance weighted (IVW), weighted median, weighted mode, simple mode, and MR–Egger methods were used to evaluate the bidirectional relationships between CIMs and OP as

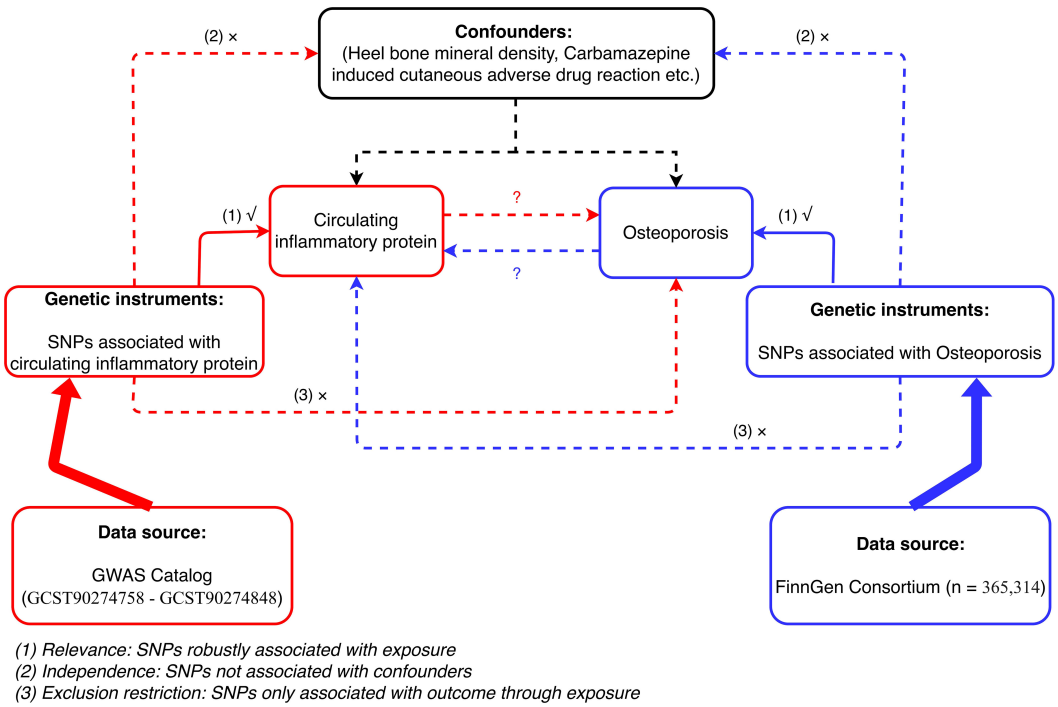

FIGURE 1
Flowchart of bidirectional MR analysis between CIMs and OP.

the main statistical approach (https://mrcieu.github.io/TwoSampleMR/). The IVW method was considered the most accurate method for estimating causal relationships if there was no clear evidence for the presence of directional pleiotropy (p for MR–Egger intercept > 0.05) (16, 18). When there was insufficient evidence of heterogeneity (p for MR heterogeneity > 0.05) in these selected genetic IVs, a random-effects model was used; otherwise, a fixed-effects model was used. A weighted median method was also used, which can generate effective causal estimates when at least 50% of the selected genetic IVs are valid (18). MR-PRESSO was used to test for pleiotropy and detect outliers. Considering multiple testing of OP and CIMs, we applied a moderate approach (false discovery rate, FDR) by adjusting the p values separately to correct for multiple hypothesis testing (19, 20). FDR q-values less than 0.3 were considered to indicate statistical significance.

2.5 Bayesian colocalization analysis

Bayesian colocalization analysis can evaluate the shared local genetic architecture between two traits and is valuable for further identifying MR associations caused by LD confounding (21). In this study, the "COLOC" package was used to perform Bayesian colocalization analysis (22). This package incorporates sophisticated algorithms and models to estimate the probability of colocalization between genes associated with the two traits under investigation. To determine whether colocalization occurred, a threshold was set based on previous studies. Specifically, PPH4 represents the probability that both traits share the same causal variant. If the posterior probability of hypothesis 4 (PPH4) exceeded 80%, it was considered significant evidence supporting the colocalization of genes (23).

2.6 Statistical analysis

To assess the causal relationship between OP and 91 CIMs, the "Mendelian-Randomization" package (version 0.4.3) (24) was primarily used to carry out the IVW (25), weighted median-based (18), and mode-based methods (26). To test for heterogeneity among certain IVs, Cochran's Q statistic and related p values were used. Random effects IVW, as opposed to fixed effects IVW, was employed in the event that the null hypothesis was rejected (25). MR–Egger, a widely used approach that assumes the presence of horizontal multiplicity if its intercept term is large, was employed to eliminate the effect of horizontal pleiotropy (27). Moreover, a potent technique known as the MR pleiotropy residual sum and outlier (MR-PRESSO) method was applied to exclude any potential horizontal pleiotropic outliers that might have a significant impact on the estimation outcomes within the MR-PRESSO package (28). Furthermore, funnel plots and scatter plots were generated. Scatter plots demonstrated that outliers had no effect on the results. Funnel plots showed that there was no heterogeneity, and the correlation was robust. The bidirectional MR effect between each CIM and OP and its corresponding SNPs can be found in the Supplementary Material named dat.csv. The positions of the valid SNPs for OP and each CIM in the context of the annotation can be accessed in the sheet corresponding to the CIM accession ID, with the suffix csv-dat.xlsx.

3 Results

3.1 Exploration of the causal effect of CIMs on OP onset

To explore the causal impact of CIMs on OP, two-sample MR analysis was performed. The threshold for statistical significance was set as an FDR below 0.3, and the quantity of SNPs for each metric was considered to augment the robustness of the genetic IVs. All these genetic IVs met the requirements of linkage disequilibrium (LD)-independent (r2 < 0.1) and achieved a genome-wide significance level ($p < 1 \times 10^{-5}$).

In our examination of CIMs as an exposure variable, we employed a multitude of SNPs as instrumental variables to strengthen our analysis. The IVW approach indicated a statistically significant association between 10 CIMs and the occurrence of OP, and the number of CIMs was reduced to five after eliminating horizontal pleiotropy of variables by MR-PRESSO. Only three CIMs were found to play a protective role in OP pathogenesis, including 11 SNPs for chemokine (C-X-C motif) ligand 11 (CXCL11) (IVW, OR = 0.91, 95% CI = 0.85–0.98, P = 0.008, P_{FDR} = 0.119), 58 SNPs for interleukin 18 (IL-18) (IVW, OR = 0.88, 95% CI = 0.83–0.94, P = 8.66×10^{-5}, P_{FDR} = 0.008), and 24 SNPs for leukemia inhibitory factor (LIF) (IVW, OR = 0.86, 95% CI = 0.76–0.96, P = 0.008, P_{FDR} = 0.119). Two CIMs were found to be risk factors for the pathogenesis of OP, including 34 SNPs in artemisinin (ARTN) (IVW, OR = 1.11, 95% CI = 1.02–1.20, P = 0.012, P_{FDR} = 0.119) and 18 SNPs in interferon gamma (IFNG) (IVW, OR = 1.16, 95% CI = 1.03–1.30, P = 0.013, P_{FDR} = 0.119) (Figure 2). The causal effects of all five CIMs on OP are listed in Supplementary Table 3.

3.2 Exploration of the causal effect of OP onset on CIMs

Conversely, when considering OP as the exposure variable, no CIM achieved statistical prominence in the conventional purview [ARTN (IVW, OR = 1.03, 95% CI = 0.98–1.07, P = 0.205, P_{FDR} = 0.881), CXCL11 (IVW, OR = 1.01, 95% CI = 0.97–1.04, P = 0.791, P_{FDR} = 0.965), IFNG (IVW, OR = 1.02, 95% CI = 0.98–1.07, P = 0.315, P_{FDR} = 0.881), IL-18 (IVW, OR = 0.98, 95% CI = 0.95–1.02, P = 0.404, P_{FDR} = 0.918), LIF (IVW, OR = 1.03, 95% CI = 0.99–1.08, P = 0.157, P_{FDR} = 0.881)] (Figure 3). However, significant linkages might not be precluded under the more accommodating FDR benchmarks of the present study. Notably, the MR–Egger intercept and the global test from MR-PRESSO discounted the possibility of horizontal pleiotropy, thereby strengthening the validity of our results. The consistency of these findings was also supported by scatter plots and funnel plots, which indicated the

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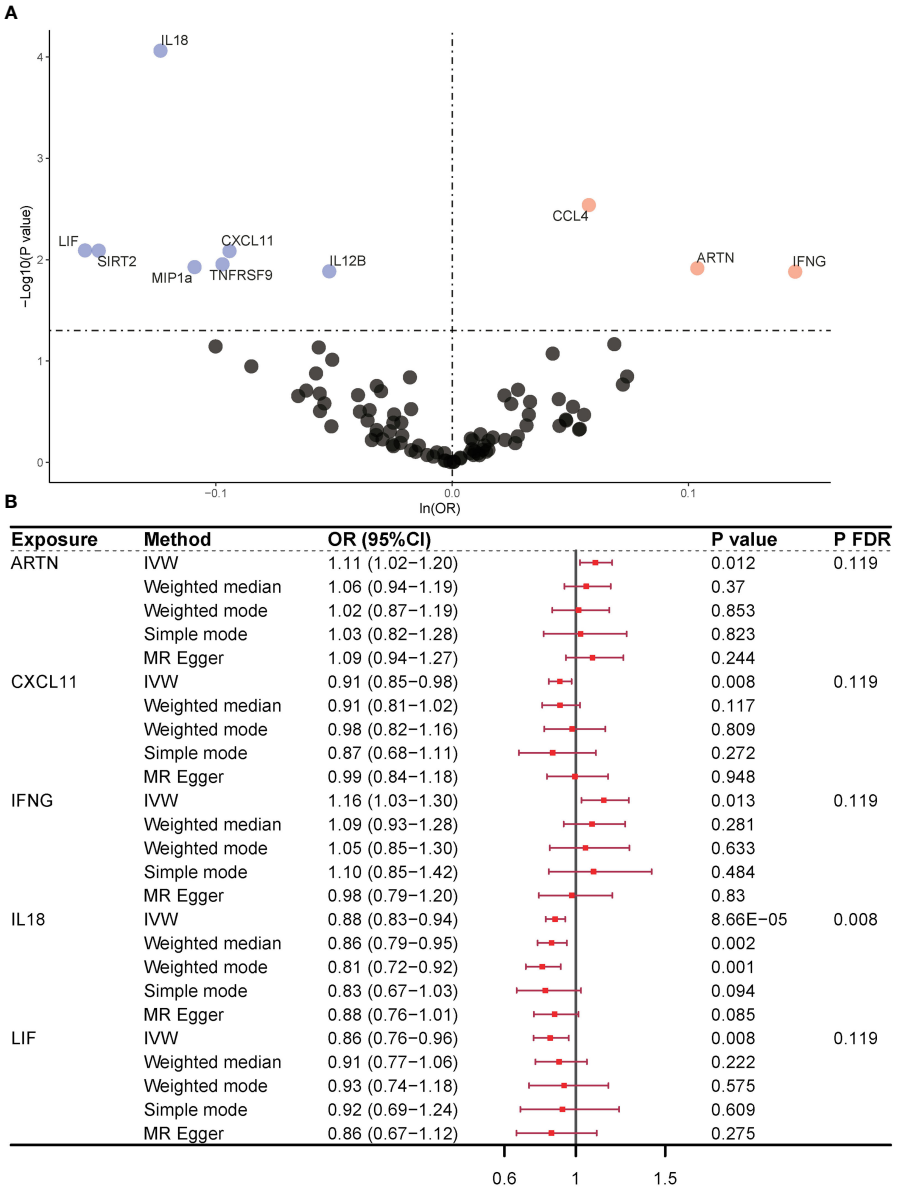

FIGURE 2
Causality outcomes for CIMs and OP. **(A)** Volcano plot showing the association between various CIMs and OP risk. Significant CIMs are labeled. **(B)** Forest plot presenting ORs with 95% CIs for selected CIMs using different MR methods. Five CIMs were identified as being associated with OP after MR analysis. ARTN and IFNG were risk factors for OP. CXCL11, IL-18, and LIF were identified as protective factors against OP. CIM, circulating inflammatory marker; OP, osteoporosis; MR, Mendelian randomization; OR, odds ratio; FDR, false discovery rate; nsnp, number of single nucleotide polymorphisms; ARTN, Artemin; IFNG, interferon gamma; CXCL11, chemokine (C-X-C motif) ligand 11; IL-18, interleukin 18; LIF, leukemia inhibitory factor.

reliability of our conclusions regarding the relationship between CIMs and OP. The results of the reverse causal effect of OP on all CIMs are listed in Supplementary Table 4.

3.3 Bayesian colocalization analysis of CIMs and OP onset

Using Bayesian colocalization analysis, we examined the associations between several CIMs (ARTN, CCL11, IFGN, IL18, and LIF) as exposure factors and OP as an outcome. We found no evidence to support the presence of shared causal variants using COLOC analysis in the training and testing cohorts (ARTN: PPH4 = 0.030; CCXL11: PPH4 = 0.052; IFGN: PPH4 = 8.23e×10−6; IL-18: PPH4 =0.042; LIF: PPH4 =0.005) for the association between CIMs and OP (rs17490485, ARTN; rs4859679, CCXL11; rs438211, IFNG; rs735622, IL-18; rs116967415, LIF). The regional colocalization plots for these associations and the detailed results of the shared genetic IVs and located genes are listed in Supplementary Table 5; Supplementary Figure 1.

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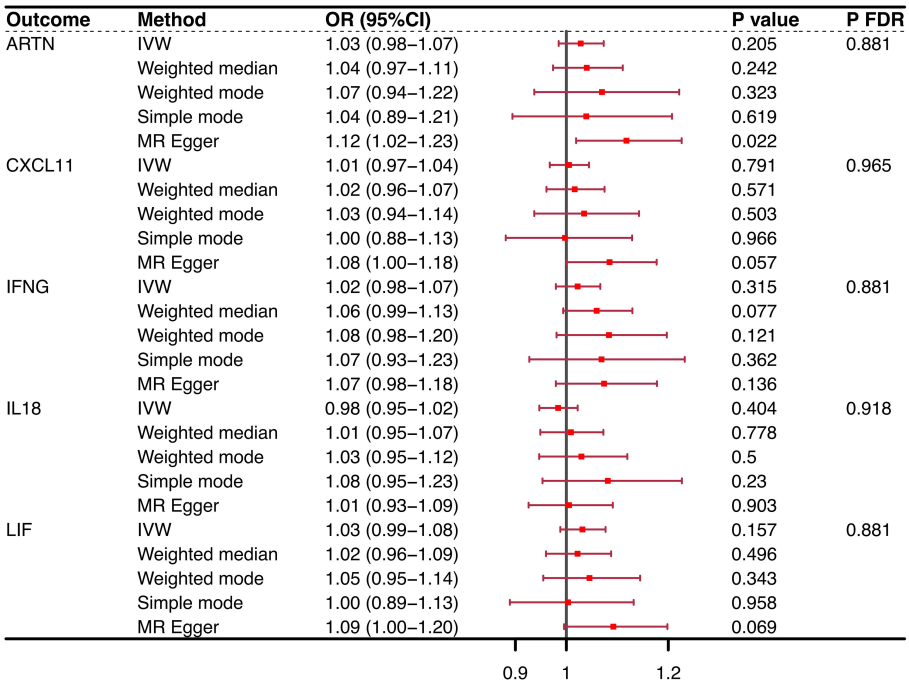

FIGURE 3
Reverse causality outcomes for CIMs and OP. After MR analysis, no CIM achieved statistical prominence when OP was the exposure variable. None of the PFDRs were less than 0.3. CIM, circulating inflammatory marker; OP, osteoporosis; MR, Mendelian randomization; OR, odds ratio; FDR, false discovery rate; nsnp, number of single nucleotide polymorphisms; ARTN, Artemin; IFNG, interferon gamma; CXCL11, chemokine (C-X-C motif) ligand 11; IL-18, interleukin 18; LIF, leukemia inhibitory factor.

4 Discussion

In our study, we performed bidirectional Mendelian randomization to investigate the potential causal associations between OP and 91 CIMs. Based on relatively large publicly available GWAS meta-analyses, we found positive unidirectional causal associations between CIMs and OP. Five CIMs (ARTN, CXCL11, IL-18, LIF, and IFNG) demonstrated a potential association with OP, and OP occurrence was not associated with an alteration in CIMs. These unidirectional associations were consistent with the sensitivity analyses but not supported by the colocalization analyses.

After MR analysis, we identified three protective factors for OP: CXCL11, LIF and IL-18.

CXCL11 is a small cytokine of the CXC chemokine family and has been shown to inhibit osteoclast differentiation of CD14+ monocytes (29). In our study, CXCL11 was shown to play a protective role in OP pathogenesis. The potent inhibition of osteoclastogenesis by IFN-β is partly mediated by the chemokine CXCL11. Similarly, our study revealed that CXCL11 plays a protective role in the pathogenesis of OP.

Several studies have shown a role for LIF in stimulating bone formation *in vivo* (30, 31). The expression level of LIF mRNA is increased upon osteogenic differentiation, resulting in LIF production by osteoblasts (32). Bozec et al. showed a 40% decrease in bone volume in newborn LIF-mutant animals. These animals did not have altered osteoblasts but demonstrated significant increases in osteoclast number and size, relative osteoclast surface area, and bone resorption (33). These findings suggest that LIF prevents OP by controlling osteoclast survival and size and explains why LIF was a protective factor against OP in our study.

IL-18 demonstrated a protective effect against osteoporosis in our study, possibly because IL-18 promotes osteogenic differentiation of hBMSCs through the SLC7A5/c-MYC pathway. SLC7A5 and c-MYC play important roles in the IL-18-induced expression of osteogenic markers in hBMSCs. IL-18 upregulates the expression of SLC7A5 and c-MYC at the early stage of hBMSC osteogenic differentiation, and SLC7A5 and c-MYC inhibition blocks the osteogenic differentiation induced by IL-18 (34). The bone density of elderly patients with osteoporosis increases significantly after anti-osteoporosis treatment. This may be related to the ability of IL-18 to inhibit osteoclast activity, induce the proliferation and differentiation of bone marrow-derived lymphoid progenitor cells, and promote NK cell proliferation and cytotoxicity (35, 36).

We also found two risk factors associated with OP by MR analysis: ARTN and IFNG.

As a member of the glial cell-derived neurotrophic factor (GDNF) ligand family, the major function of ARTN is to drive the molecule to induce sympathetic neuron migration and axon projection (37), and ARTN has been implicated in pain signaling, including that derived from the joint and bone (38–40). Some studies suggest that ARTN/GFRα3 signaling is involved in the pathogenesis of bone pain, and inhibition of this process could be used to treat pain in osteoarthritis (OA) when pathological features are present in the subchondral bone (38, 39). Other studies have suggested that

ARTN is regulated by estrogen and mediates estrogen resistance in breast cancer (41, 42). It has been well documented that estrogen is closely related to osteoporosis, especially postmenopausal osteoporosis in women (43–45). In bone, estrogen inhibits osteoclast formation and bone resorption activity by binding to the estrogen receptor, promoting osteoprotegerin (OPG) expression, and inhibiting the action of nuclear factor-κβ ligand (RANKL). However, to date, research on the relationship between ARTN and OP remains relatively limited, and there is currently no conclusive evidence of a direct association between ARTN and OP. Our study is the first to find that ARTN is positively correlated with the risk of OP from a genetic point of view.

INFG is a classic proinflammatory mediator that is overexpressed in the course of OP (46). Comprehensive studies have confirmed that circulating IFNG levels are significantly elevated in patients with OP and that their upregulation also correlates with a severe OP phenotype (47, 48). This finding is consistent with the results of our study, which revealed that IFNG is a risk factor for OP.

Previous studies have focused on disease-to-disease or symptom-to-disease relationships. To our knowledge, no MR analysis focused on the causal relationship between OP and multiple CIMs has yet been reported. Our study used several variants summarized from large-scale GWASs on OP and CIMs to increase the statistical power to detect causal associations. This approach extends the findings of previous studies. Moreover, this approach can be applied to identify potential novel molecular pathways relevant to the diagnosis and treatment of OP, as the pathophysiological mechanisms of this relationship have not yet been fully elucidated. Therefore, we performed a colocalization analysis to identify five causal variants and localize them to their respective genes. Current evidence that the five CIMs mentioned above (ARTN, CXCL11, IFGN, IL-18, and LIF) are involved in the pathogenesis of CIMs and OP is inconclusive. Thus, further in-depth studies are needed.

Our study has limitations. First, although we obtained positive sensitivity analysis results in MR analysis, colocalization analysis suggested that the association between CIMs and OP remained uncertain. Second, our MR analysis suggests a potential causal link between CIMs and OP risk, but this does not confirm direct causality. MR results rely on the assumption that the genetic variant is randomly associated with both CIMs and OP and is not influenced by confounders. Violations of these assumptions may bias the findings. Due to MR design limitations, we lack specific biomarker-level data. These results should be interpreted alongside other study evidence. Recognizing these limitations, our study provides a hypothesis that can guide future research investigating the specific levels and biological roles of these biomarkers in relation to OP risk. Third, because we used summary-level MR data, we were unable to perform subgroup-specific analysis.

5 Conclusion

In conclusion, we found unidirectional causal relationships between CIMs, including ARTN, CXCL11, IFGN, IL-18, and LIF, and OP in our MR sensitivity analysis, and a potential association between ARTN and OP was proposed for the first time. This association was not supported by colocalization analysis but still has great value for further in-depth study.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

QD: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. JW: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. HZ: Writing – review & editing, Visualization, Writing – original draft, Formal analysis, Data curation. LL: Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Formal analysis, Conceptualization. WW: Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1412298/full#supplementary-material

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The influence of body fat content and distribution on bone mass in healthy Chinese adults

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Background: Previous studies have reported a close relationship between body mass index (BMI) and bone mineral density (BMD). However, the effects of fat on bone mass remain controversial, particularly for fat tissue distribution. The aim of this study was to evaluate the association between regional fat percentage and BMD using a population-based database.

Methods: This study included participants who were referred to the Department of Radio Diagnosis for dual-energy X-ray absorptiometry (DEXA) scan from January 2018 to December 2020. The relationships between BMI and regional fat percentage with BMD were assessed using multiple linear regression and generalized additive models. The risk of low bone mass was determined using logistic regression.

Results: There was a negative relationship between the regional fat percentage and femoral neck BMD (FN BMD) or lumbar spine BMD (LS BMD) in both genders ($p < 0.05$). In females, an inverted U-shaped relationship was observed between regional fat percentage and BMD at both the femoral neck and lumbar spine. The impact of trunk fat percentage on LS BMD was associated with the highest OR of low bone mass in females (OR 3.1, 95% CI 2.6 to 3.7, p for trend <0.001), while the impact of abdomen fat percentage on FN BMD was associated with the highest OR of low bone mass in males (OR 2.2, 95% CI 1.8 to 2.7, p for trend <0.001).

Conclusion: There was an inverted U-shaped relationship between regional fat percentage and BMD. Excessive regional fat percentage may be harmful to bone health in both genders. To promote bone health, males should restrict their abdomen circumference and avoid abdominal adiposity, while females should control their trunk circumference.

KEYWORDS

bone mass, fat percentage, osteoporosis, bone mass index, bone mineral density

Introduction

Obesity and osteoporosis are significant global public health concerns, particularly in aging populations. Body mass index (BMI) is a widely used measure to classify adults into categories such as underweight, normal weight, overweight, and obese (1). However, the relationship between BMI and bone mineral density (BMD) remains contentious in the literature (2).

Existing studies suggest that a high BMI may have a protective effect on bone mass, primarily because increased body weight imposes greater mechanical load on bones, thereby stimulating bone formation (3). On the contrary, other studies indicate that excessive BMI may be detrimental to bone health due to metabolic abnormalities associated with obesity that negatively impact bone metabolism. Specifically, adipose tissue is not merely an energy storage organ; it secretes various hormones and cytokines that influence bone metabolism (4, 5).

In examining the impact of body weight on bone mass, the relative importance of fat mass and lean mass has been extensively discussed. For instance, El Hage et al. (6–8) investigated the relative importance of lean and fat mass on BMD in adolescent boys and girls, finding that lean mass is a strong determinant of L1–L4 BMD in boys and that fat mass is a stronger determinant of whole body BMD in girls.

Despite several studies exploring the overall relationship between fat mass and bone mass, the impact of fat distribution on bone mass remains underexplored. Specifically, the effects of regional fat distribution (e.g., trunk fat and abdominal fat) on BMD at different skeletal sites have not been fully elucidated. Lorenzo et al. (9) found that a significant number of male and female subjects could not be classified as obese based solely on their BMI, suggesting that body fat percentage might be a useful indicator. This study aimed to utilize a large-scale population database to investigate the association between regional fat percentage and BMD at the femoral neck (FN) and lumbar spine (LS), thereby advancing the understanding of the complex relationship between adiposity and skeletal health.

Materials and methods

Study population

The present retrospective study was conducted at a single study center in China from January 2018 to December 2020. Participants who were referred to the Department of Radio Diagnosis for a DEXA scan were selected. Participants were excluded from the study: (1) History of metabolic bone diseases such as hyperparathyroidism, hyperthyroidism, Cushing's syndrome, osteomalacia, renal failure, and diabetes mellitus. (2) Those who were taking medications known to influence bone metabolism such as bisphosphonates, estrogen preparations, antiepileptic drugs, corticosteroids, thyroxine, and anticoagulants. (3) Bilateral trunk replacements or previous spinal fusion. (4) non-Han ethnic individuals.

Clinical measurements

BMI (kg/m2) was calculated as the body weight in kilograms divided by the squared height in meters. BMD was measured at the femoral neck and lumbar spine (L1-L4) as the primary outcome of this study using dual-energy X-ray absorptiometry (DXA, GE-Lunar, Madison, WI, United States). Data on trunk and abdomen fat percentage were extracted from DXA. Based upon the World Health Organization (WHO) classification, a low bone mass (osteopenia or osteoporosis) was defined as a BMD T-score < -1.0 aged above 50 years or a Z-score < -1.0 aged below 50 years. The tests were performed by a trained technician on appropriately calibrated equipment before every session. Densitometers showed stable long-term performances [coefficient of variation (CV) <0.5%] and satisfactory *in vivo* precision (CV 0.8% for lumbar spine; 0.9% for femoral neck).

Statistical analysis

Continuous variables are presented as the mean ± standard deviation (SD). Comparisons between the males and females were made using independent-samples *t*-test. Linear regression analysis was used to assess the relationships between BMI, trunk, and abdomen fat percentage with BMD in each gender. To obtain greater flexibility in representing the relationships between the dependent variable and predictor variables compared to linear regression, generalized additive models (GAMs) were used to generate graphic representations of the dose–response relations of BMI, trunk, and abdomen fat percentage with BMD in each gender. We performed multiple logistic regression analyses to generate odds ratios (ORs) (95% CI) that compared the odds of low BMD (T-score < -1.0 or Z-score < -1.0) for participants in each of the higher three fat percentage quartile to the odds of the participants in the lowest quartile after adjusting for age, weight, height, and BMI. All analyses were performed using IBM SPSS (version 17, IBM, Chicago, Illinois, United States) and R (version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria), and $p < 0.05$ (two-tailed) was considered statistically significant.

Results

General characteristics of the participants

A total of 18,263 participants (8,969 males and 9,294 females) aged 20 to 100 years old were included in the analysis. The demographic details and key clinical data for all participants are shown in Table 1. The mean age of the participants was 48.3 (13.4) years for males and 52.6 (14.3) years for females ($p < 0.001$). Although males had slightly but significantly higher BMI compared to females (24.7 (3.1) vs. 23.0 (3.3) kg/m^2, $p < 0.001$), they had significantly lower trunk and abdomen fat percentage (18.8 (4.5) vs. 25.6 (5.4) and 26.3 (8.1) vs. 29.2 (8.8), respectively, $p < 0.001$). Both FN BMD and LS BMD were significantly higher in males than in females (0.95 (0.14) vs. 0.86 (0.15) and 1.13 (0.17) vs. 1.06 (0.20), respectively, $p < 0.001$).

Associations of BMI, trunk, and abdomen fat percentage with BMD in each gender

Regression coefficients from the linear regression models with BMI (Model 1), trunk fat percentage (Model 2), or abdomen fat percentage (Model 3) as the predictor variables are presented in Table 2. In both females and males, BMI serves as a positive predictor of FN BMD and LS BMD (β: 0.20 to 0.32 in females; 0.17 to 0.30 in males, all $p < 0.001$), whereas trunk fat percentage and abdomen fat percentage act as negative predictors of FN BMD and LS BMD (β: −0.04 to −0.18 in females; −0.03 to −0.14 in males, all $p < 0.05$).

TABLE 1 Descriptive statistics of the study population.

	Female (n = 9,294)	Male (n = 8,969)	P^a
	Mean ± SD	Mean ± SD	
Age (y)	52.6 ± 14.3	48.3 ± 13.4	<0.001
Anthropometric data			
Height (cm)	158.6 ± 5.4	170.7 ± 5.8	<0.001
Weight (kg)	57.9 ± 8.7	72.0 ± 10.4	<0.001
BMI (kg/m^2)	23.0 ± 3.3	24.7 ± 3.1	<0.001
DXA data			
FN BMD (g/cm^2)	0.86 ± 0.15	0.95 ± 0.14	<0.001
TF (%)	25.6 ± 5.4	18.8 ± 4.5	<0.001
FN T-score (SD)	−0.6 ± 1.3	−0.2 ± 1.1	<0.001
FN BMC (g/cm)	3.9 ± 0.8	5.0 ± 0.9	<0.001
LS BMD (g/cm^2)	1.06 ± 0.20	1.13 ± 0.17	<0.001
AF (%)	29.2 ± 8.8	26.3 ± 8.1	<0.001
LS T-score (SD)	−0.7 ± 1.7	0.2 ± 1.4	<0.001
LS BMC (g/cm)	29.8 ± 7.1	37.0 ± 7.1	<0.001

Values are mean ± SD unless otherwise stated.
TF (%), trunk fat percentage; AF (%), abdomen fat percentage; FN, femoral neck; LS, lumbar spine; BMD, BMI, body mass index; bone mineral density; BMC, bone mineral content. a Student's t-test.

Figure 1 depicts the dose–response relationships of each BMI (Model 1), trunk fat percentage (Model 2), and abdomen fat percentage (Model 3) with BMD in each gender using the generalized additive models. For BMI, there is a positive relationship with BMD at both femoral neck and lumbar spine across all BMI values in males, whereas in females, BMD increases with BMI until BMI reaches approximately 33 kg/m2, after which there is an apparent decline in BMD at the lumbar spine. For regional fat percentage in males, there appears to be no consistent relationship between BMD and regional fat percentage, whereas, in females, an inverted U-shaped relationship between regional fat percentage and BMD was observed at both femoral neck and lumbar spine. The percentage of variation in the BMD measures explained by the GAM was apparently higher in females compared to males (adjusted R^2: 0.366 to 0.459 vs. 0.056 to 0.238, Table 2), indicating that the relationships were better represented in females by the GAM.

TABLE 2 Regression coefficients of models with BMI (kg/m2), TF (%), or AF (%) as the predictor variable for lumbar spine and femoral neck BMD (mg/cm^2).

	Female		Male	
	B	GAM Adjusted R^2	β	GAM Adjusted R^2
Model 1	0.32***	0.456	0.30***	0.232
Model 2	−0.07***	0.459	−0.10***	0.238
Model 3	−0.06***	0.458	−0.11***	0.238
LS BMD				
Model 1	0.20***	0.366	0.17***	0.056
Model 2	−0.18***	0.387	−0.14***	0.070
Model 3	−0.04**	0.366	−0.03*	0.056

Model 1: BMI.
Model 2: TF (%), trunk fat percentage.
Model 3: AF (%), abdomen fat percentage.
β: standard regression coefficient.
*P < 0.05; **p < 0.01; ***P < 0.001 compared to the corresponding linear regression model.
Covariates adjusted in both linear regression and GAM included age, weight (for Models 2 and 3 only), and height.
Linear regression analysis with BMD as the dependent variable and BMI (Model 1), TF (%) (Model 2), or AF (%) (Model 3) as the predictor variable.

Associations of trunk and abdomen fat percentage with the odds of low bone mass in each gender

As Figure 2 shows, multiple logistic regression analyses showed that the risk of low bone mass was significantly higher in the highest quartile of regional fat percentages compared to that in the lowest quartile in females (ORs ranging from 1.5 to 3.1). The impact of trunk fat percentage on LS BMD was associated with the highest OR of low bone mass in females (OR 3.1, 95% CI 2.6 to 3.7, p for trend <0.001). For males, the risk of low bone mass was significantly higher in the highest quartile of regional fat percentage compared to that in the lowest quartile (ORs ranging from 1.2 to 2.2). The impact of abdomen fat percentage on FN BMD was associated with the highest OR of low bone mass in males (OR 2.2, 95% CI 1.8 to 2.7, p for trend <0.001).

Discussion

This study has shown that there was a positive relationship with BMD at both femoral neck and lumbar spine across all BMI values in males, whereas in females, BMD increased with BMI until BMI reached approximately 33 kg/m^2, after which there was an apparent decline in BMD at the lumbar spine. In females, there was an inverted U-shaped relationship between regional fat percentage and BMD at both the femoral neck and lumbar spine. When analyzed by quartiles

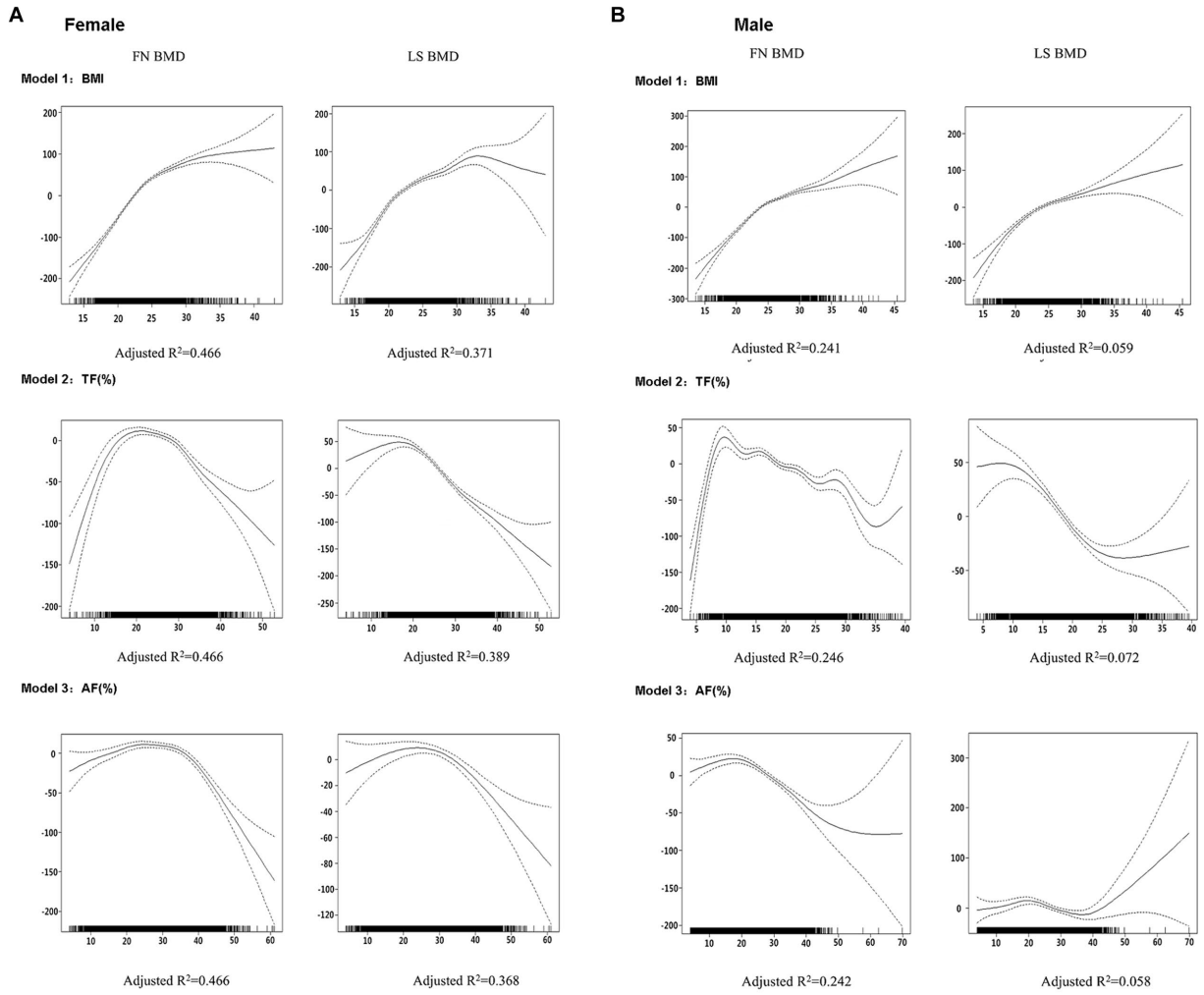

FIGURE 1
Graphic presentation of the dose–response relationship between BMI (Model 1), TF (%) (Model 2), or AF (%) (Model 3) in males **(A)** and females **(B)** obtained by generalized additive regression models. The models were adjusted for age, weight (for Models 2 and 3 only) and height as covariates. The dotted lines represent 95% confidence intervals. The reference value for the BMD is the value associated with the mean BMI, TF (%), or AF (%) for all participants in each gender. The rug plot along the bottom of each graph depicts each observation.

of regional fat percentage, high trunk fat percentage in females is associated with the highest risk of having low LS BMD, while high abdomen fat percentage in males is associated with the highest risk of having low FN BMD.

The role of body composition on bone health has been extensively investigated, but the results regarding the effect of fat mass on BMD have been controversial. In this study, we found that BMI has shortcomings as a predictor of BMD because it does not separate lean mass from fat mass and does not explore the influence of fat distribution on bone mass. In this study, a positive relationship was observed between BMD at both the femoral neck and lumbar spine across all BMI values in males. However, in females, BMD increased with BMI until reaching approximately 33 kg/m^2, after which there was an apparent decline in BMD at the lumbar spine. This finding aligns with the results reported by Li (5), who identified an inverted U-shaped association between BMI and lumbar BMD in females, with the point of inflection at approximately 50 kg/m^2. The variation in the inflection point value may be attributed to racial differences, which could be the result of genetic risk factors, lifestyle, and other factors (10, 11).

In this study, we confirmed that the regional fat percentage was negatively associated with the BMD in males and females using multiple linear regression models. To further investigate the dose–response relationship between the regional fat percentage and the BMD, generalized additive models were performed. In females, the data from our study showed that the relationship between the regional fat percentage and the two-site BMD appeared to be inverted U-shaped, indicating that the effect of the regional fat percentage on the BMD was non-linear. According to these data, we may infer that an increase in regional body fat is weakly protective against bone loss, but this effect becomes detrimental as we move toward morbid obesity. Our results seem consistent with the conclusion from Kim (12) who claimed that overweight may be protective against trunk fractures in Asian adults but not morbid obesity, particularly in women.

Interestingly, through multiple logistic regression models, we found that a high trunk fat percentage in females is associated with the highest risk of having low LS BMD, while a high abdomen fat percentage in males is associated with the highest risk of having low FN BMD. This result was supported by a prospective cohort study

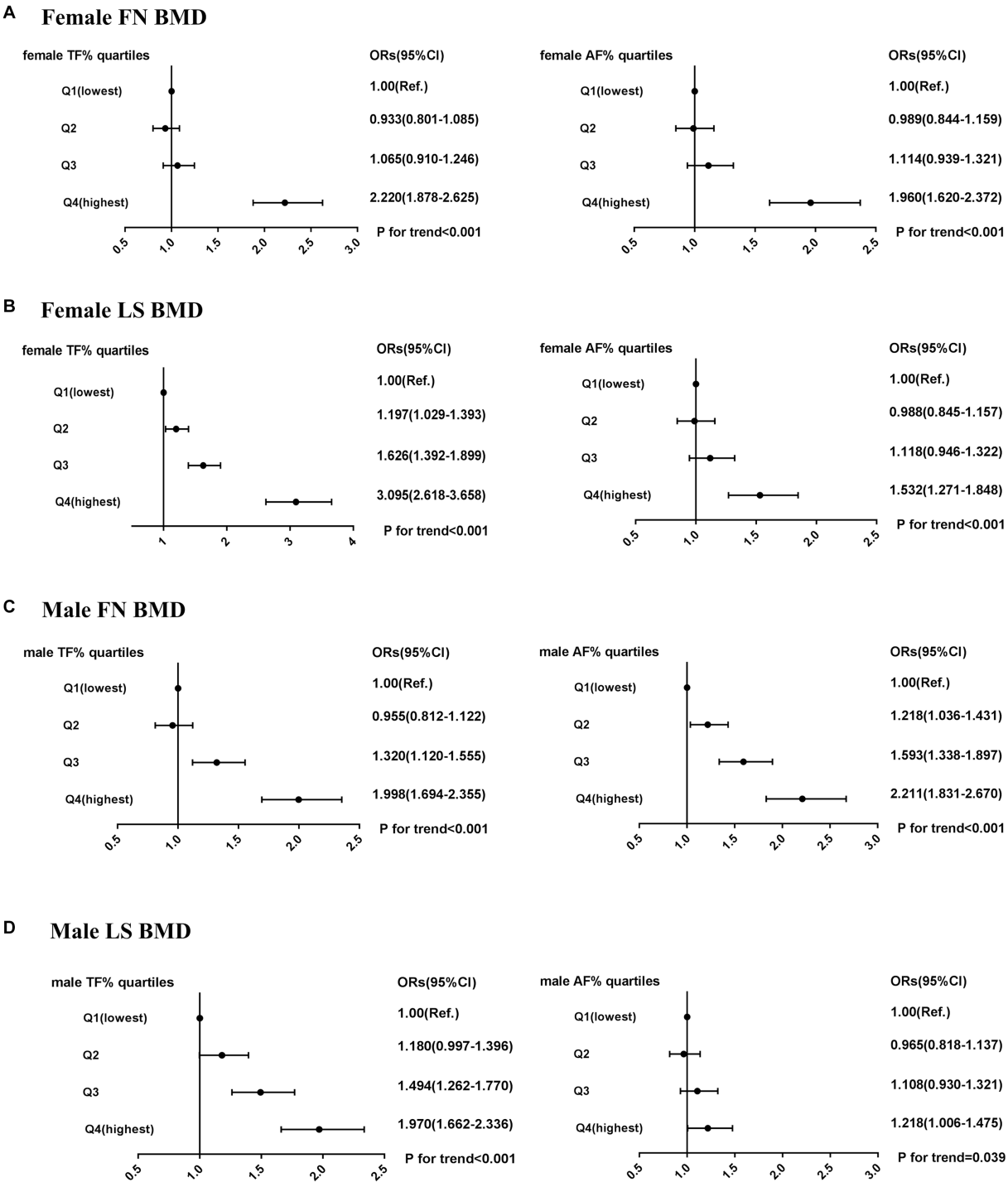

FIGURE 2
Risk of low bone mass (BMD T-score < −1.0) across quartiles of male TF (%), male AF (%), female TF (%), and female AF (%). **(A)** female FN BMD. **(B)** female LS BMD. **(C)** male FN BMD. **(D)** male LS BMD. ORs (95% CI) were calculated using multivariate logistic regression after adjusting for age, height, and BMI; OR, odds ratio; CI, confidence interval.

from Norway of 23,061 men aged 60 to 79 years, wherein males in the highest tertile of abdomen circumference had a 100% higher risk of trunk fractures than males in the lowest tertile (13). In another cross-sectional study of 1,011 participants aged 50–80 years, it was reported that women who had at least one vertebral deformity had a greater percentage of trunk fat than women without vertebral deformities (14), which was also consistent with our findings above. Altogether, our results indicated that males should control their abdomen circumference and avoid abdominal adiposity, while females should focus on their trunk circumference. Actually, not all fat depots are the same: Site-specific effects, rather than simply total body fat, may be crucial in the assessment of the impact of obesity on the BMD (15).

Several underlying mechanisms have been proposed to elucidate the harmful effect of fat tissue on bone health. At the molecular

genetics level, a genome-wide bivariate analysis of Caucasians of European origin identified some suggestive shared genomic regions for both body fat mass and BMD, therefore implying that those two diseases might be influenced by some shared candidate genes or mutual crosstalk between their phenotypes' gene regulatory networks (16). At the cellular level, adipocytes and osteoblasts have common progenitor cells, mesenchymal stem cells (MSCs). A shift of the cell differentiation of MSCs to adipocytes, rather than osteoblasts, will hinder osteogenesis and will consequently result in bone loss (17). Apart from the causations mentioned above, several adipokines, which are secreted by adipocytes, including adiponectin and leptin, have shown a negative effect on bone metabolism. Serum adiponectin is reported to be inversely correlated with BMD in both males and females by inhibiting osteoblast proliferation and promoting apoptosis, altogether decreasing bone formation levels (18–20). Leptin has a detrimental effect on bone formation mainly via the central nervous system, which appears to be mediated by the decreased production of serotonin in hypothalamic neurons (21, 22).

This study has some limitations. First, it was a retrospective study, which limits the exploration of causality regarding the relationship between high regional fat percentage and BMD. Second, some confounding factors such as sex hormones and adipocytokines were not examined, which could affect the results. Third, information on female menopause was not collected, which could have provided additional insights into the relationship between fat distribution and bone mineral density.

In conclusion, we found that in females, BMD increased with BMI until BMI reached approximately 33 kg/m^2. Beyond this point, there was an apparent decline in BMD at the lumbar spine. This may be associated with an inverted U-shaped relationship between regional fat percentage and BMD. To promote bone health, males should restrict their abdomen circumference and avoid abdominal adiposity, while females should control their trunk circumference. Excessive regional fat percentage may be harmful to bone health in both genders.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The requirement of ethical approval was waived by the Ethics Committee of the Second Affiliated Hospital of Soochow University for the studies involving humans because this study did not involve patient demographic information and no potentially identifiable images or data were presented in this study. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this was a retrospective study.

Author contributions

BC: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. GL: Data curation, Investigation, Methodology, Project administration, Software, Validation, Writing – original draft, Writing – review & editing. YW: Supervision, Validation, Writing – original draft, Writing – review & editing. YX: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Role of interleukin-18 in mediating the impacts of celiac disease on osteoporosis: a Mendelian randomization study

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Background: Extensive observational data suggest a link between celiac disease (CeD) and osteoporosis, but the causality and mediating mechanism remain undetermined. Herein, we performed a Mendelian randomization (MR) study to address these concerns.

Methods: We obtained the summary-level statistics for CeD from a large genome-wide association study (GWAS) comprising 4,533 cases and 10,750 controls of European ancestry. The GWAS data for osteoporosis-related traits and inflammatory cytokines were derived from the UK Biobank, FinnGen, IEU OpenGWAS database, or GWAS catalog. Two-sample MR with the inverse variance-weighted methods were employed to evaluate the genetic association between CeD and osteoporosis-related traits. The potential inflammatory mediators from CeD to osteoporosis were explored using two-step mediation analyses.

Results: The primary MR analyses demonstrated causal associations between genetically predicted CeD and osteoporosis (odds ratio [OR]: 1.110, 95% confidence interval [CI]: 1.043–1.182, p=0.001), total body bone mineral density (β: -0.025, p=0.039), and osteoporotic fracture (OR: 1.124, 95% CI: 1.009–1.253, p=0.034). Extensive sensitivity analyses consolidated these findings. Among the candidate inflammatory cytokines, only interleukin-18 was observed to mediate the effects of CeD on osteoporosis, with an indirect OR of 1.020 (95% CI: 1.000–1.040, p=0.048) and a mediation proportion of 18.9%. The mediation effects of interleukin-18 could be validated in other datasets (OR: 1.015, 95% CI: 1.001–1.029, p=0.041). Bayesian colocalization analysis supported the role of interleukin-18 in osteoporosis.

Conclusion: The present MR study reveals that CeD is associated with an increased risk of developing osteoporosis, which may be partly mediated by upregulation of interleukin-18.

KEYWORDS

celiac disease, osteoporosis, inflammation, Mendelian randomization, mediation

Introduction

Celiac disease (CeD) is a chronic immune-mediated enteropathy triggered by intolerance to gluten proteins in genetically predisposed subjects (1). Approximately 0.7–1.4% of the general population worldwide are affected by this illness, and the prevalence appears to be raising over time (2, 3). Within the small bowel of individuals with CeD, gluten digestion products (e.g., omega-5-gliadin) penetrating into the lamina propria can directly, or after deamination by tissue transglutaminases in the submucosa, activate the innate immune system (4). This mechanism induces an abnormal inflammatory cascade followed by damage to the structure and function of intestinal tissue (5). Although CeD primarily attacks the small intestine, it is increasingly recognized as a systemic autoimmune disorder that may present with a diverse of extraintestinal comorbidities, such as type 1 diabetes, autoimmune liver disease, and psoriasis (6).

Osteoporosis is a skeletal disorder featured by low bone mineral density (BMD) and deterioration of bone microarchitecture, with a consequent increase in susceptibility to bone fragility or fractures (7). As a global prevalent disease in the elderly, osteoporosis causes more than 8.9 million pathological fractures each year, casting a heavy economical burden to many regions (8). Nearly 14.4% of patients with CeD are suffering from osteoporosis (9). Accumulating data from population-based studies have also demonstrated that CeD was associated with reduced BMD and might represent an independent risk factor for osteoporotic fracture (10). However, whether these relationships are causal or driven by shared environmental factors remains undetermined, mainly owning to the inherent drawbacks of observational study designs. Traditional observational studies are susceptible to reverse causality as they are always problematic to determine which of two associated variables is the cause; and, confounding bias is more difficult to control for because it is mainly due to social, behavioral, or physiological factors that are difficult to measure and deal with (11). Therefore, previous observational data are insufficient to establish causal insights between CeD and osteoporosis.

Additionally, the etiopathology of osteoporosis in CeD remains largely under-investigated. The osteoporosis may be caused by malabsorption of calcium or vitamin D, but also other factors such as chronic inflammation in CeD can exert an crucial role (12). CeD is characterized by an intestinal Th1 response to dietary gluten, presenting with hypersecretion of proinflammatory proteins in the damaged mucosa or serum, particularly interferon (IFN)-ϒ, tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 (13). These cytokines are implicated in bone metabolism as they regulate the differentiation and activation of osteoblasts or osteoclasts (14). IL-18, also known as an IFN-ϒ inducing factor, are produced linked to gluten intake and associated with Th1 activity in CeD (15). Recent reports have shown that IL-18 maintain a long-standing inflammation status in CeD patients (16) and can up-regulate the expression of key osteoclastogenic regulators (17), pointing towards a mediation role in the CeD-induced osteoporosis.

Mendelian randomization (MR) is an epidemiological strategy widely used to strengthen the causal inference by employing single nucleotide polymorphisms (SNPs) as unbiased instrumental variables (IVs) for exposures (18). Because genetic variants are allocated randomly during gametogenesis and would not be modified by acquired factors, MR procedure furnishes several advantages over observational designs: 1) it ensures the temporality of exposure and outcome, preventing reverse causation; 2) it minimizes the impact of residual or unmeasured confounding factors; 3) it reflects the long-term risk estimates of exposure, as the IVs remain valid throughout a lifetime (19). MR can thus represent an analogue to randomized controlled trials that utilizes genetic variation as the method for randomization ultimately providing causal inferences. In this study, we applied the MR approach to explore the causal association between CeD and osteoporosis-related traits, and simultaneously examined the mediating relationship of inflammatory cytokines between CeD and osteoporosis.

Methods

Study design

The overall design of this work was shown in Figure 1. Briefly, we first employed two-sample MR methods to assess the associations of CeD with osteoporosis-related traits, including osteoporosis, total body BMD, and osteoporotic fracture. Then, two-step MR strategies were leveraged to explore the mediating effects of inflammatory candidates in the associations. MR analyses can provide unbiased causal inference if the selected IVs satisfy the following three assumptions: 1) the IVs must be strongly associated with exposures; 2) the IVs should be free of confounding factors; 3) the IVs affect the outcomes solely through the exposures. This study complies with the Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) statement (seeing the checklist in Supplementary Table S1).

Data source

Our MR study utilized publicly accessible data from genome-wide association studies (GWAS) or databases (Supplementary Table S2). The original articles have provided the ethical clearance and consent to participates, thus there was no need for additional approvals. The summary-level genetic statistics for CeD were derived from a GWAS study involving 4,533 cases and 10,750 controls of European ancestry (IEU OpenGWAS ID: ieu-a-276) (20). The summary-level GWAS data pertaining to osteoporosis and osteoporotic fracture were acquired from the UK Biobank (21) and FinnGen (release 10) (22) consortium, respectively. The former consisted of 6,484 osteoporosis individuals and 401,279 controls, and the latter comprised 1,822 cases and 311,210 controls. The genetic statistics for total body BMD were sourced from a GWAS meta-analysis of 30 cohorts totaling 56,284 European participates (23).

Based on recent literature reviews (13, 24), we examined the mediating effects of nine inflammatory proteins in the CeD-

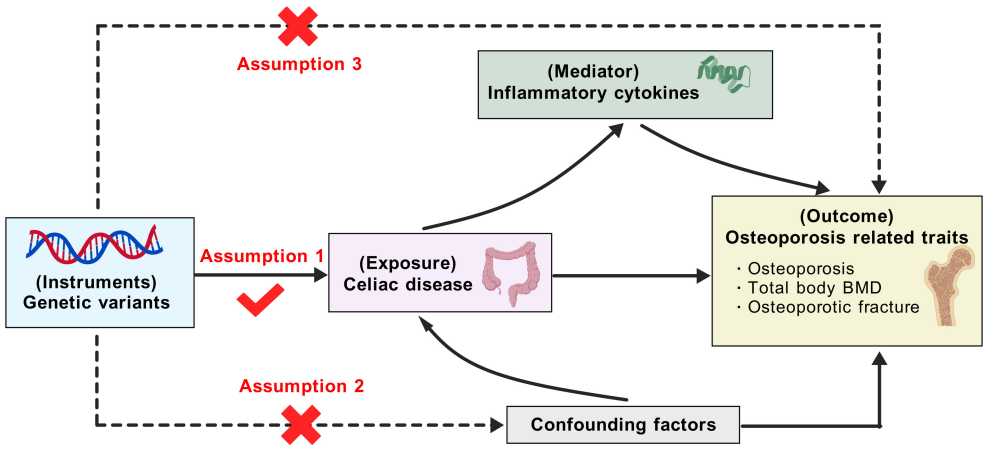

FIGURE 1
Outline of this Mendelian randomization study. BMD, bone mineral density (the figure was created with BioGDP.com). Based on the Mendelian randomization assumptions, the genetic variants are assumed to affect osteoporosis through celiac disease only, not through confounding factors or directly associated with osteoporosis. Then, the mediation effects of potential inflammatory cytokines between celiac disease and osteoporosis were examined.

osteoporosis association, including IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-18, TNF-α, TNF-β, and IFN-γ. The GWAS summary statistics correlated with these cytokines were retrieved from three meta-analyses (25–27) of pQTL, with the number of participates ranging from 3,309 to 21758 as described in Supplementary Table S2. If a cytokine was measured in at least two studies, we used the pQTL data with the largest sample size.

Instrumental variable selection

For CeD, we extracted SNPs that were highly associated with this illness at a genome-wide significance level ($p<5\times10^{-8}$). For each inflammatory protein, cis-pQTLs strongly correlated with the protein level ($p<5\times10^{-8}$) were collected. Cis-pQTLs were defined as the pQTLs locating at ±1 Mb from the encoding gene. To identify independent IVs, the selected genetic variants were pruned through linkage disequilibrium (LD) $R^2<0.001$ within 10-Mb windows, using the 1000 Genomes Project of European ancestry as reference panel (28). Confounding biases were minimized by removing any pleiotropic SNPs using the LDTrait tool (29), and SNPs that directly influenced the outcomes ($p<5\times10^{-8}$) were excluded. During MR harmonization process, SNPs not present in the outcome GWAS data or those being palindromic were further discarded. The remaining genetic variants were screened out as IVs, and their strengths were assessed using the *F* statistics, calculated as dividing the square of β coefficient by the square of standard error.

Statistical analysis

In the primary MR analyses, we used the inverse-variance weighted (IVW) method or the Wald ratio to estimate the causal effects of exposures on outcomes. Heterogeneity was detected using Cochrane's Q-test, with $p>0.05$ indicating no substantial heterogeneity. We applied the MR-Egger intercept test to evaluate potential horizontal pleiotropy, where the difference between the intercept of the MR-Egger regression and zero were tested (30). The IVW approach has the highest statistical power but may be biased when pleiotropy exists; therefore, we further introduced the MR-Egger with bootstrapping (30) and the weighted median (31) methods for reanalysis. The MR-Egger approach can provide genetic estimates corrected for pleiotropy because it allows nearly all of SNPs to be horizontally pleiotropic; and, the weighted median can generate unbiased causal estimates when more than half of the IVs are valid. MR-pleiotropy residual sum and outlier (MR-PRESSO) test was also employed to detect the outliers involved in horizontal pleiotropy and produce the corrected results (32). Leave-one-out analyses were performed to assess the influence of individual SNPs on the MR results.

Two-step MR was conducted to identify the potential inflammatory proteins that mediate the effects of CeD on osteoporosis. In the first step, we performed MR analyses with the IVW method to examine the causal effects of CeD on the nine inflammatory proteins. The proteins that passed the significance threshold ($p<0.05$) were entered into the second step, in which their causal effects on the risk of osteoporosis were investigated. The MR estimates of CeD on osteoporosis, CeD on each protein, and each protein on osteoporosis were recorded as β0, β1, and β2, respectively. For an inflammatory cytokine with both β1 and β2 being significant, we conducted mediation analysis to further elucidate whether it could mediate the effect of CeD on osteoporosis risk. Indirect effect, which refers to the effect of CeD on osteoporosis through the inflammatory cytokine, was computed using the "Product of coefficients" method (33) (β1×β2). We also estimated the corresponding proportion of mediation as the indirect effect divided by the total effect (β1×β2/β0). The 95% confidence intervals (CIs) were obtained from the delta method.

The MR-Steiger test was performed to validate the directionality of causal relationships between CeD, inflammatory mediator, and osteoporosis.

For a cytokine with significant mediating effect, Bayesian colocalization analysis (34) was conducted to reinforce the MR assumption in osteoporosis. This approach calculates the posterior probabilities (PP) for five hypothesis testing: H0 (no causal variants), H1 (causal variant for the cytokine only), H2 (causal variant for osteoporosis only), H3 (separate causal variants for the cytokine and osteoporosis), and H4 (shared causal variant for the cytokine and osteoporosis). We selected all SNPs located within ±100 kb around the lead cis-pQTL of the cytokine encoding gene for colocalization. PP.H4 > 0.5 was considered evidence of colocalization, implying that colocalization is more likely than any other situations combined.

All statistical analyses were implemented using R version 4.1.0 (The R Foundation for Statistical Computing, Vienna, Austria) software. The "TwoSampleMR" (version 0.5.6), "RMediation" (version 1.2.2), and "coloc" (version 5.2.3) R packages were used for the main analyses. A two-sided p value of < 0.05 was deemed as of significance.

Results

Instrument variables for CeD

The correlation analyses and LD clumping generated 12 independent genetic variants that were strongly associated with CeD. Supplementary Table S3 summarized the details of these IVs for CeD. Of them, rs653178 was interfered with other traits such as smoking and diabetes; therefore, we removed this pleiotropic SNP from the subsequent MR analyses of the CeD-osteoporosis association. The F statistics ranged from 29 to 101 for the selected instrumental SNPs, signifying that our MR analyses were robust against weak instrument bias.

CeD and osteoporosis-related traits

The MR associations between CeD and osteoporosis-related phenotypes were exhibited in Figure 2 and Supplementary Table S4. The IVW-MR analyses indicated that genetically instrumented CeD was related to higher risks of developing osteoporosis (odds ratio [OR]: 1.110, 95% CI: 1.043–1.182, p=0.001; Figure 2A) and osteoporotic fracture (OR: 1.124, 95% CI: 1.009–1.253, p=0.034). Meanwhile, we found that genetic liability to CeD could decrease total body BMD (β: -0.025, p=0.039; Figure 2B). The results from the MR-Egger and the weighted median methods, which provide adjustments for potential pleiotropic effects, were directionally consistent with the IVW estimates albeit with noticeably wider CIs (Supplementary Table S4). The Cochran Q-test documented no significant heterogeneity for the above findings (Supplementary Table S5). There were no evidence of horizontal pleiotropy from the MR-Egger regression intercept test and the MR-PRESSO Global test (Supplementary Table S5), implying that the IVW estimates were credible. Leave-one-out sensitivity analyses indicated that our MR results were not driven by a single instrumental SNP (Supplementary Table S6), further reinforcing the robustness of the MR conclusions.

Mediation effects of inflammatory cytokines

Among the nine candidate inflammatory cytokines, we observed that only IL-6 and IL-18 could be affected by genetically

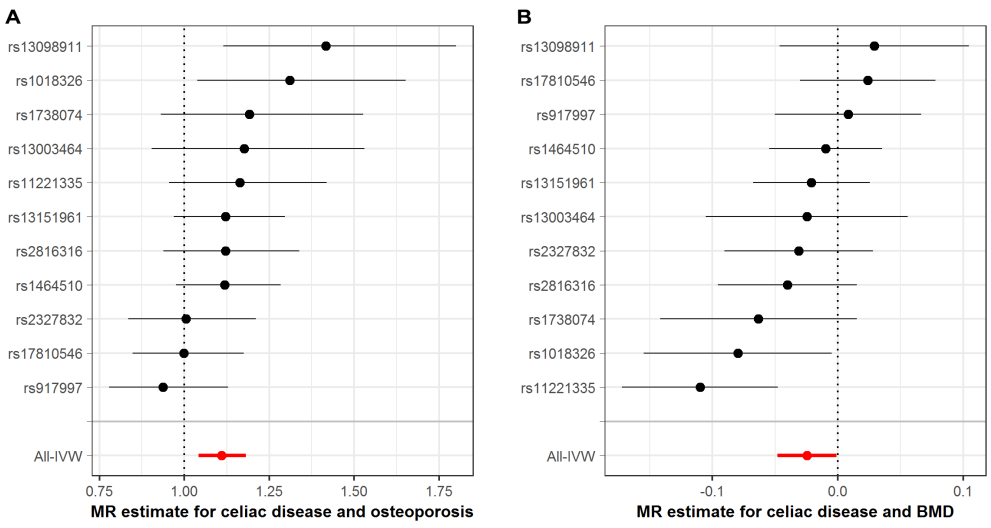

FIGURE 2
Association between genetically proxied celiac disease with osteoporosis-related traits. **(A)**, estimates for celiac disease and osteoporosis risk; **(B)**, estimates for celiac disease and BMD. BMD, bone mineral density; IVW, inverse-variance weighted; MR, Mendelian randomization. The dots represent the odds ratios of estimates, and the bars represent the corresponding 95% confidence intervals.

surrogated CeD. The results showed that CeD was linked to an increased expression of plasma IL-6 (β: 0.038 95% CI: 0.002–0.073, p=0.037) and IL-18 (β: 0.066, 95% CI: 0.018–0.114, p=0.007; Supplementary Table S7). However, there was no significant cis-pQTLs identified for IL-6 in the pQTL datasets; therefore, we only retained IL-18 for the following analyses. By using one cis-pQTL (rs5744249, located in the intron of IL18 gene) as the instrument, we found that plasma level of IL-18 was positively associated with osteoporosis risk (OR: 1.347, 95% CI: 1.115–1.626, p=0.002). These findings indicated that IL-18 might serve as a mediator from CeD to osteoporosis. As expected, the mediation analysis suggested that the indirect effect of IL-18 was 1.020 (95% CI: 1.000–1.040, p=0.048), with a mediation proportion of 18.9% in the CeD-osteoporosis association (Figure 3). The MR-Steiger test confirmed the causal directions from CeD to IL-18 and from IL-18 to osteoporosis (Supplementary Table S8). To validate the mediation effects, we analyzed another two pQTL datasets (25, 26) for IL-18 (seeing Supplementary Table S2). We first combined the MR estimates (β1 or β2) of IL-18 from each pQTL dataset using random-effect meta-analysis and then repeated the aforementioned mediation analyses. The results also demonstrated that IL-18 could mediate the effect of CeD on osteoporosis (indirect effect, OR: 1.015, 95% CI: 1.001–1.029, p=0.041; Supplementary Table S9), with a mediation proportion of 14.0%.

Colocalization between IL-18 and osteoporosis

Considering that the above analysis identified IL-18 as a mediator between CeD and osteoporosis, we further performed colocalization analyses to investigate whether the cis-pQTLs of IL-18 shared the same casual variants with osteoporosis. The results provided evidence for colocalization between the protein expression of IL-18 and osteoporosis (PP.H4 = 0.56, Figure 4 and Supplementary Table S10). This finding reinforces the aforementioned MR results and underlines that IL18 may represent a potential therapeutic target in osteoporosis.

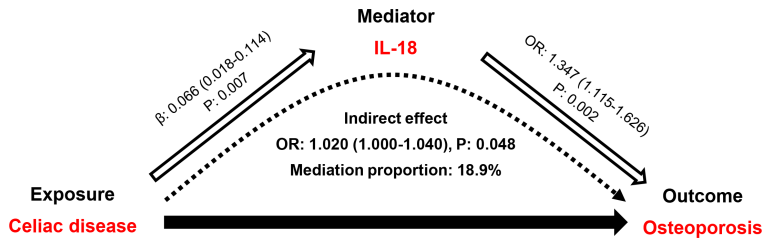

FIGURE 3
Mediation effect of IL-18 in the association between celiac disease and osteoporosis. IL-18, interleukin-18; OR, odds ratio. The indirect effect refers to the effect of celiac disease on osteoporosis through IL-18.

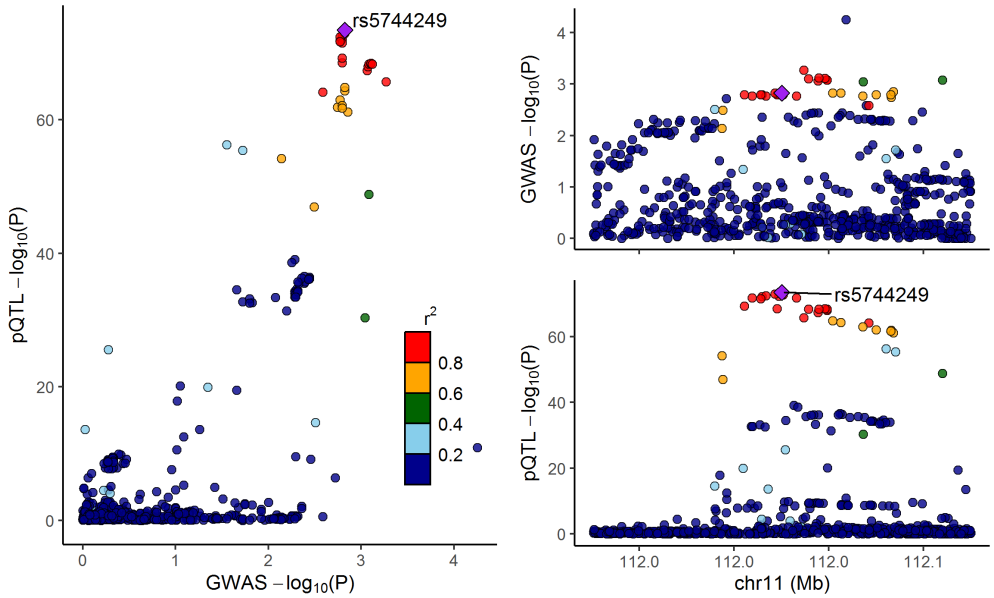

FIGURE 4
LocusCompare plot for the colocalization between interleukin-18 pQTLs and osteoporosis. The dots represent the genetic variants at chromosome 11, with color indicating the magnitude of the p-value. The purple rhombus stands for the shared causal variant, rs5744249.

Discussion

CeD is a chronic immuno-inflammatory disease with a broad spectrum of extraintestinal comorbidities. In the present MR investigation, we found that genetic predisposition to CeD could increase the risks of osteoporosis and osteoporotic fracture and reduce total body BMD. Additionally, plasma IL-18 levels appeared to play an important role in mediating the relationship between CeD and osteoporosis. The colocalization analysis supported the connection between IL-18 and osteoporosis.

CeD has been recognized for decades as a secondary cause of osteoporosis, but most of the relevant data were sourced from observational studies. For example, a nationwide cohort study of 103,361 individuals demonstrated that CeD was an independent risk factor for developing osteoporosis and osteoporotic fractures, both before or after the diagnosis (35). A meta-analysis of prospective cohort studies indicated that CeD at baseline conferred a 30% increase in the risk of any fractures and a 69% increase in the risk of hip fracture (36). As mentioned above, MR is effective in avoiding the bias from observational nature including residual confounding and reverse causation. The present MR analyses revealed causal links of CeD with osteoporosis-related traits, reinforcing the conclusion from conventional epidemiological studies. Our results further highlight the importance of BMD monitoring in the clinical management of patients with CeD, as it proposed in recent guidelines (37, 38).

The mechanisms underlying osteoporosis in CeD remain incompletely understood. One of the theories assumes an impaired intestinal absorption of vitamin D and calcium in CeD, leading to secondary hyperparathyroidism and subsequent osteoclast-mediated bone turnover (13). In symptomatic CeD, the bone loss appears directly associated with intestinal malabsorption of vitamin D, calcium, and other nutrients that are essential to bone health. However, low BMD can be observed even in patients with atypical or asymptomatic CeD at the time of diagnosis (39), raising the possibility of other determinants for the origin of osteoporosis in CeD. In recent years, accelerating evidence have emphasized the role of both local and systemic inflammation in the pathological process of CeD-related bone loss, characterized by a chronic increase in both mucosal and circulating pro-inflammatory cytokines (40). Pro-inflammatory cytokines may imbalance the receptor activator of nuclear kappa-B ligand (RANKL)/osteoprotegerin (OPN) pathway by lowering the OPN to RANKL ratio thus favoring osteoclastogenesis (41). Previous studies have also shown a decreased OPG/RANKL ratio under the condition of CeD, which was positively correlated with BMD at the spine (42). Among the inflammatory cytokines, we observed that IL-18 might mediate the effect of CeD on osteoporosis. IL-18 was reported to be activated at a post-translational level in patients with CeD (15), which in turn sustained a long-standing intestinal inflammation (16). As a powerful inflammatory cytokine, IL-18 can facilitate osteoclast differentiation by boosting inflammatory response via inducing the secretion of critical inflammatory factors (e.g., IFN-γ and TNF -α) as well as acting on T lymphocytes (43). IL-18 also suppress the secretion of osteogenic related proteins or transcription factors, such as Wnt-10b, Runx-2, and BMP-2 (44). Overall, IL-18 seems to play a pivotal role in creating and maintaining a chronic immuno-inflammatory microenvironment that provokes osteoclasis and inhibits osteogenesis in CeD (45). Accordingly, administration of IL-18 antagonists can improve osteoporosis and reduce pro-inflammatory cytokines in ovariectomized mice (46). In this study, we found that the protein expression of IL-18 was colocalized with osteoporosis, further supporting its role in the bone mass deterioration in CeD patients.

Our findings undoubtedly supported the assessment of BMD for CeD at the time of diagnosis, but whether it should be routinely implemented in all patients, particularly in those being atypical or asymptomatic, remains debated in recent guidelines (40). This has risen the need for clinical or biochemical marker to select high-risk patients of developing bone loss. In the present study, we identified IL-18 as mediator between CeD and osteoporosis, reflecting that IL-18 measurement may help the risk classification of osteoporosis in atypical or asymptomatic CeD patients. Currently, a lifelong gluten-free diet (GFD) is still considered to be the mainstay treatment for patients with CeD and bone diseases (47). Nevertheless, some studies suggested that despite strict adherence to GFD, more than half of patients displayed low BMD and continued to experience a higher rate of osteoporosis, denoting that the persistent activation of inflammation should be considered in such residual risk (48, 49). As mentioned above, IL-18 is a dominate cytokine to maintain the long-standing inflammatory nature in CeD. Targeting IL-18 was found to be effective in experimental models of autoimmune diseases including inflammatory bowel disease (50) and rheumatoid arthritis (51), as well as to be safe in clinical patients (52). Alongside with these reports, our results indicated that IL-18 may also serve as a potential target for the prevention and treatment of osteoporosis in patients with CeD.

Several limitations should be acknowledged. First of all, although we have performed extensive analyses to reinforce the MR inferences, the horizontal pleiotropy cannot be completely eliminated. Secondly, due to the lack of relevant data, it is challenging to determine whether our MR findings are affected by potential confounding factors, such as age, gender, reproductive status, smoke, steroid exposure, and endocrine disorders. Thirdly, despite being documented as a potential mediator, the exact biological pathway though which IL-18 promotes osteoporosis in CeD remains unclear. This is a research topic and should be taken into consideration in future works. Fourthly, the original GWAS datasets only include European individuals, limiting the generalization of our results to other ethnics.

In summary, our MR research indicates that patients with CeD are at higher risks of developing osteoporosis, which may be partly mediated by the increased level of IL-18. These findings support the assessment of BMD in CeD management, and put forward that IL-18 may represent a potential target in the management of CeD-induced osteoporosis, which requires further exploration in future researches.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

JX: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. XZ: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing. LL: Conceptualization, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. XY: Conceptualization, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1453657/full#supplementary-material

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Exploring the role of aging in the relationship between obstructive sleep apnea syndrome and osteoarthritis: Insights from NHANES data

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Background: Osteoarthritis (OA) is characterized by high morbidity and disability. While studies have demonstrated that OA is correlated with age-related diseases, few have shown the potential relationship between OA and obstructive sleep apnea syndrome (OSAS). OSAS is characterized by intermittent hypoxia and hypercapnia. We hypothesize that these stressors induce aging and increase the prevalence of OA.

Methods: The study included 10,641 participants drawn from the National Health and Nutrition Examination Survey (NHANES) dataset during 2005–2008 and 2015–2018. The correlation between OSAS and OA was analyzed using multivariable logistic regression, aging-related biomarkers were calculated, and the role of aging was explored through mediation analysis.

Results: OSAS was associated with an elevated risk of OA (for quartile 4 vs. quartile 1, odds ratio (OR) 2.31, 95% confidence interval (CI) 1.34 to 3.99; p-value for the trend = 0.004) after adjusting covariates. In the 20–59 years and > 60 years subgroup, the OSAS patients showed a similar trend (for quartile 4 vs. quartile 1, OR 5.69, 95% CI 2.75 to 11.8; p-value for the trend <0.001; OR 2.42, 95% CI 1.23 to 4.76; p-value for the trend = 0.004, respectively). Further mediation analysis revealed that aging acted as a mediator between OA and OSAS. The mediation proportions for biological age (BA) and phenotypic age (PA) were 13.82 and 52.94%, respectively, both with $p < 0.001$.

Conclusion: These findings suggest that individuals with OSAS may have an increased prevalence of OA, with aging also being involved in the association.

KEYWORDS

aging, NHANES, osteoarthritis, obstructive sleep apnea syndrome, mediation analysis

1 Introduction

Osteoarthritis (OA) is a degenerative disease caused by the erosion of joint cartilage, leading to pain and disability. Estimates suggest that 250 million people have been affected by this condition (1). Previous studies have shown that elderly individuals diagnosed with arthritis are at a higher risk of developing chronic comorbidities, such as cardiovascular diseases (CVDs) and hypertension (2–4).

Obstructive sleep apnea syndrome (OSAS), primarily attributed to obesity and airway collapse, triggers a cascade of pathophysiological processes, including intermittent hypoxia and hypercapnia. These processes subsequently lead to hypertension, metabolic disorders, and inflammation, all of which share many similarities with chronic diseases (5). Existing studies have demonstrated that aging is associated with a decline in sleep quality. It has been frequently observed that sleep-disordered breathing and cognitive changes occur with age (6–8).

The incidence of OA and OSAS increases with age (7, 9). As people age, there is a growing trend toward the coexistence of multiple diseases, making the treatment process more challenging (10, 11). Although many studies have reported that OA is associated with chronic disease, few have shown the association between OA and OSAS in a large number of participants. The role of aging in these two diseases is still unknown.

Therefore, we used the National Health and Nutrition Examination Survey (NHANES, 2005–2008 and 2015–2018) database to explore the risk of OA and OSAS and the role of aging in this process. By managing OSAS and delaying aging, pain can be relieved and quality of life can be improved for elderly patients with OA.

2 Methods

2.1 Data source

The NHANES is a database managed by the National Center for Health Statistics, created through representative sampling and supplemented with questionnaires, physical examinations, laboratory tests, and data collected from mobile examination centers. The Information Collection Review Office approved the research protocol involving human participants. We selected 2005–2008 and 2015–2018 as the study cycles because they included both OA and OSAS questionnaire data. From these cycles, we screened 21,861 individuals older than 20 years for this study (Figure 1), and 10,641 participants were included in the final analysis, with covariables incorporated. All participants provided written informed consent, and all collected information was maintained with strictly confidentiality.

2.2 Assessment of osteoarthritis

Studies have shown that 81% of osteoarthritis data collected through questionnaires are consistent with clinical diagnoses (12). Therefore, we used questionnaires from the NHANES dataset to collect relevant data. Individuals with self-reported osteoarthritis were included in the OA group, whereas those who were healthy, had other types of arthritis, and did not specify the type were included in the non-OA group.

Abbreviations: ACME, Average Causal Mediation Effects; ADE, Average Direct Effects; BA, Biological Age; COPD, Chronic Obstructive Pulmonary Disease; CI, Confidence Interval; NHANES, National Health and Nutrition Examination Survey; OSAS, Obstructive Sleep Apnea Syndrome; OA, Osteoarthritis; OR, Odds Ratio; OSAS.MAP10, OSAS Multivariable Apnea Prediction index value × 10; PA, Phenotypic Age; PIR, Family Income Ratio; ACME + ADE, Total Effect.

2.3 Diagnosis of OSAS and the multivariable apnea prediction index

According to a previous study by Maislin et al., we used the OSAS questionnaire to calculate the multivariable apnea prediction (MAP) index and OSAS multivariable apnea prediction index value × 10 (OSAS.MAP10) based on information provided about snoring, sleep apnea, and fatigue severity (13–15) (see Supplementary material for details).

2.4 Measurement of the biological aging markers

Previous studies have shown that KDM biological age (BA) and phenotypic age (PA) more accurately predict an individual's aging level through algorithmic assessments (16). We obtained data on individual BA biomarkers (C-reactive protein [CRP], serum creatinine, glycosylated hemoglobin, serum albumin, serum total cholesterol, serum urea nitrogen, serum alkaline phosphatase, and systolic blood pressure) and chronological age. We utilized the NHANES III data to compute BA and subsequently incorporated our data from the 2005–2008 and 2015–2018 cycles into the fitting process (17). Similarly, we obtained data on individual PA biomarkers (albumin, creatinine, glucose, C-reactive protein, lymphocyte percentage, mean cell volume, red blood cell distribution width, alkaline phosphatase, and white blood cell count) and used the BioAge R package to calculate phenotypic age (see Supplementary materials for details).

2.5 Potential confounders/other variables

We collected demographic and lifestyle data, including age (18), sex, ethnicity, educational background, family income ratio (PIR), smoking habits, and alcohol consumption, through detailed questionnaires. BMI and blood pressure data (the average of three blood pressure measurements, including those taken while using blood pressure-lowering drugs) were obtained from physical examinations and the medical conditions component of the questionnaires. By synthesizing self-reported health histories, medication records, and chronological age information, we evaluated the presence of cardiovascular diseases. This encompassed conditions such as coronary heart disease, congestive heart failure, myocardial infarctions, cerebrovascular accidents (strokes), and angina pectoris, as well as other chronic diseases including diabetes, asthma, and chronic obstructive pulmonary disease (COPD) (15).

2.6 Statistical analysis

This study used RStudio (version 4.4.0) for the statistical analysis of the weighted data. We grouped the participants according to their osteoarthritis status, and the Wilcoxon rank-sum test and the chi-squared test were used for demographic analysis. Quartiles were utilized to transform the continuous variable OSAS.MAP10 into a categorical variable. Multivariable logistic regression

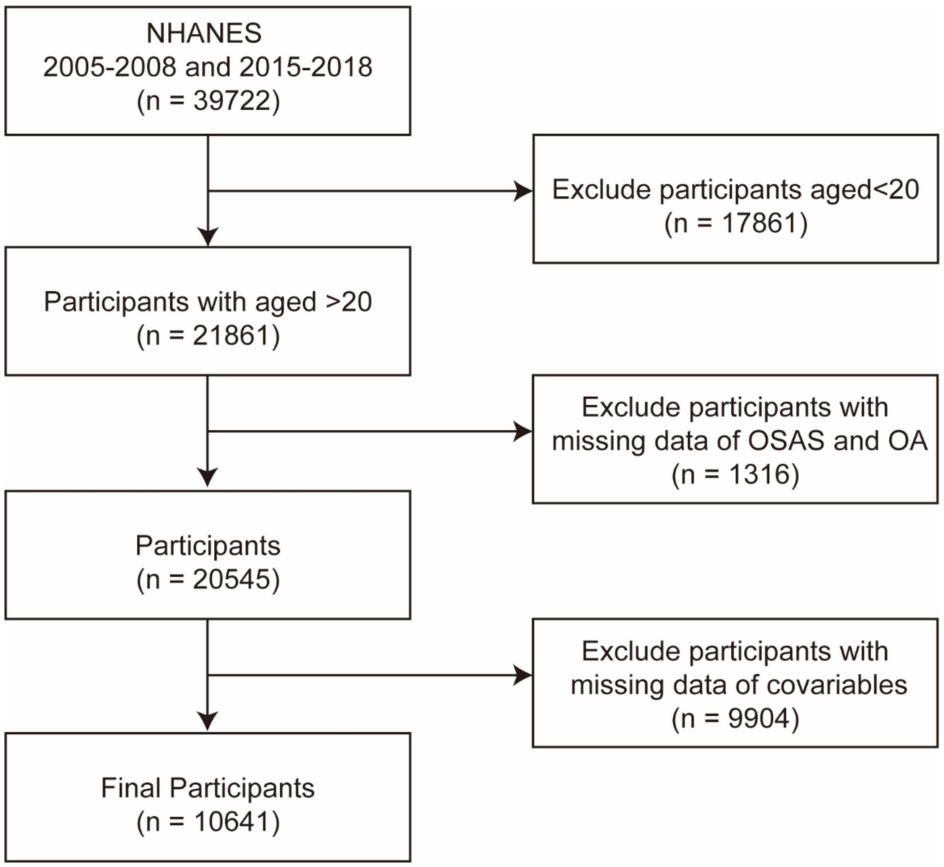

FIGURE 1
Flow chart of the participants in this study. NHANES, National Health and Nutrition Examination Survey; OA, osteoarthritis; and OSA, obstructive sleep apnea syndrome.

analysis was conducted to estimate the odds ratio (OR) and its 95% confidence interval (CI) for the association between OSAS.MAP10 and OA risk, adjusting for age, race, education level, PIR, BMI, smoking status, alcohol consumption, CVDs (coronary heart disease, stroke, heart attack, chronic heart failure, and angina pectoris), hypertension, diabetes, and COPD. Subsequently, the mediating role of aging in the relationship between OSAS.MAP10 and OA was investigated using the mediation package in RStudio. The quasi-Bayesian Monte Carlo method was employed, involving 1,000 simulations based on the normal approximation, to estimate the indirect effect (ACME, average causal mediation effect) and direct effect (ADE, average direct effect). The proportion of mediation was calculated by dividing the ACME by the total effect (ACME + ADE) (18).

3 Results

3.1 Basic clinical characteristics of the study participants

Table 1 shows the demographic characteristics according to the OA status. In this study, we screened 1,027 osteoarthritis patients aged 20 years or older. The OA group was older (60.47 ± 12.65) than the non-OA group (44.17 ± 15.29; $p < 0.001$). The OSAS.MAP10 score was higher in the OA group (4.82 ± 2.54; $p < 0.001$) compared to the non-OA group (OR 3.73 ± 2.53; $p < 0.001$). The participants who were female, had a high income, were obese, smoked, and consumed alcohol were more likely to have osteoarthritis (all p-values for the trend <0.001). The participants with a history of CVDs, hypertension, diabetes, or COPD also had a higher prevalence of osteoarthritis (all p-values for the trend <0.001). However, the participants with asthma did not show a significant difference in the prevalence of osteoarthritis (p-value for the trend = 0.2). Therefore, CVDs, hypertension, diabetes, and COPD were included in model 3.

3.2 Associations between OSAS and OA risk

As shown in Table 2, the association between OSAS and OA was investigated using a multivariable logistic regression model, adjusting for potential covariates. In the unadjusted statistical model (model 1), quartile 4 of the OSAS.MAP10 scores was associated with an increased incidence of OA (OR 3.48, 95% CI 2.60 to 4.65, all p-values for the trend <0.005) compared to quartile 1. The observed trend was consistent with that of model 2 (OR 2.36, 95% CI 1.37 to 4.08, all p-values for the trend = 0.003, quartile

TABLE 1 Baseline characteristics of the participants with and without OA.

Characteristic	Non-OA, *N* = 9,614 (89%)[1]	OA, *N* = 1,027 (11%)[1]	*p*-value[2]
Age, mean (SD), years	44.17 (15.29)	60.47 (12.65)	<0.001
Sex			<0.001
Male	5,206.00 (52.64)	406.00 (37.01)	
Female	4,408.00 (47.36)	621.00 (62.99)	
Race			<0.001
Mexican American	1,676.00 (8.63)	80.00 (2.35)	
Other Hispanic	850.00 (4.99)	56.00 (1.92)	
Non-Hispanic White	4,262.00 (70.25)	663.00 (84.78)	
Non-Hispanic Black	1,897.00 (9.49)	147.00 (5.35)	
Other/multiracial	929.00 (6.64)	81.00 (5.59)	
Education			0.045
Less than 9th grade	696.00 (3.27)	44.00 (1.93)	
9–11th grade	1,186.00 (8.48)	97.00 (6.85)	
High school graduate/GED	2,210.00 (22.98)	226.00 (22.06)	
Some college or AA degree	3,066.00 (32.83)	341.00 (32.43)	
College graduate or above	2,456.00 (32.43)	319.00 (36.74)	
PIR			<0.001
Low income	2,357.00 (15.98)	215.00 (13.22)	
Medium income	3,796.00 (35.02)	362.00 (29.77)	
High income	3,461.00 (49.00)	450.00 (57.01)	
Smoke			<0.001
Never	4,851.00 (51.92)	451.00 (45.56)	
Current	2,389.00 (23.29)	173.00 (15.34)	
Former	2,369.00 (24.80)	402.00 (39.09)	
Drinking	1,498.00 (14.29)	70.00 (5.68)	<0.001
BMI			<0.001
Normal	2,654.00 (29.65)	212.00 (19.90)	
Underweight	139.00 (1.36)	5.00 (0.52)	
Overweight	3,243.00 (32.44)	328.00 (32.04)	
Obese	3,578.00 (36.55)	482.00 (47.55)	
CVDs	743.00 (5.45)	192.00 (16.05)	<0.001
Hypertension	2,992.00 (27.32)	540.00 (47.64)	<0.001
Diabetes	906.00 (6.98)	157.00 (12.69)	<0.001
COPD	353.00 (3.21)	103.00 (9.04)	<0.001
Asthma	1,354.00 (14.49)	190.00 (16.53)	0.2
Arthritis	1,652.00 (15.49)	1,027.00 (100.00)	<0.001
OSA	4,907.00 (49.33)	613.00 (58.48)	<0.001
OSAS.MAP10	3.73 (2.53)	4.82 (2.54)	<0.001

[1]median (IQR) for continuous variables; *n* (%) for categorical variables.
[2]The Wilcoxon rank-sum test for complex survey samples.
The chi-squared test with Rao & Scott's second-order correction. The continuous variables are presented as median. The categorical variables are presented as *n* (%). OA, osteoarthritis; OSA, obstructive sleep apnea syndrome; PIR, the ratio of family income to poverty; BMI, body mass index; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; OSAS. MAP10, OSAS multivariable apnea prediction index value × 10; SE standard error, *n*, number of participants; and %, weighted percentage. Median (SD) or median (IQR) for the continuous variables; *n* (%) for the categorical variables.

4 vs. quartile 1), in which we adjusted for age, sex, race, education level, PIR, BMI, smoking status, and alcohol consumption. Model 3 (in which the variables in model 2 and CVDs, hypertension, diabetes, and COPD were adjusted for) showed a similar trend (OR 2.31, 95% CI 1.34 to 3.99, all *p*-values for the trend = 0.004, quartile 4 vs. quartile 1).

TABLE 2 The association between the OSAS.MAP10 quartiles and OA.

	Model 1		Model 2		Model 3	
Characteristic	OR[1] (95% CI[1])	*p*-value	OR[1] (95% CI[1])	*p*-value	OR[1] (95% CI[1])	*p*-value
OSAS.MAP10 (continuous)	1.18 (1.14, 1.22)	<0.001	1.17 (1.09, 1.26)	<0.001	1.17 (1.09, 1.26)	<0.001
OSAS.MAP10 quartile						
Q1	Reference		Reference		Reference	
Q2	1.94 (1.46, 2.59)	<0.001	1.31 (0.92, 1.86)	0.129	1.30 (0.91, 1.85)	0.142
Q3	2.16 (1.60, 2.93)	<0.001	1.46 (0.98, 2.18)	0.063	1.47 (0.99, 2.18)	0.057
Q4	3.48 (2.60, 4.65)	<0.001	2.36 (1.37, 4.08)	0.003	2.31 (1.34, 3.99)	0.004
p-value for the trend	<0.001		0.003		0.004	

[1]OR, Odds Ratio; CI, 95% CI.
Model 1: no covariates were adjusted. Model 2: adjusted for age, sex, race, education level, PIR, BMI, smoking status, and alcohol consumption. Model 3: adjusted for the covariates in model 2 and CVDs (coronary heart disease, stroke, heart attack, chronic heart failure, and angina pectoris), hypertension, diabetes, and COPD. OSAS, obstructive sleep apnea syndrome and OSAS. MAP10, OSAS multivariable apnea prediction index value × 10.

3.3 The associations between OSAS and OA risk in age and sex subgroups

Table 3 shows the associations between OSAS and OA risk in different age and sex subgroups. We found that the highest quartile of OSAS.MAP10 (OR 3.08, 95% CI 2.04 to 4.64, $p < 0.001$) in the 20–59 years subgroup was associated with an increased risk of OA after adjustment in model 1 compared to quartile 1. Model 2 (OR 8.18, 95% CI 4.24 to 15.8, $p < 0.001$) and model 3 (OR 5.69, 95% CI 2.75 to 11.8, $p < 0.001$) showed similar trends. However, OSAS.MAP10 in the >60 years subgroup did not show the trend (OR 1.01, 95% CI 0.58 to 1.77, $p = 0.664$) in model 1. After adjusting for the covariates, OSAS.MAP10 restored the trend in model 2 (OR 2.29, 95% CI 1.19 to 4.38, $p = 0.006$) and model 3 (OR 2.42, 95% CI 1.23 to 4.76, $p = 0.004$).

Table 3 shows that the highest quartile of OSAS.MAP10 in the male subgroup was associated with an increased risk of OA in all three models (all *p*-values for the trend <0.005). However, quartile 4 of OSAS.MAP10 in the female subgroup was associated with an increased risk of OA in model 1 (OR 6.24, 95% CI 4.26 to 9.14, $p < 0.001$) and model 2 (OR 2.32, 95% CI 1.25 to 4.31, $p = 0.043$), but there was no significant difference in model 3 (OR 2.16, 95% CI 1.14 to 4.07, $p = 0.096$).

3.4 Aging-mediated effects on the association between OSAS and OA risk

Furthermore, the mediating role of aging in the relationship between OSAS.MAP10 and OA is shown in Figure 2. In the phenotypic age group, the ACME was 0.00559 (95% CI: 0.00464, 0.01), the ADE was 0.00497 (95% CI: 0.00390, 0.01), and the proportion of mediation was 52.9%. In the biological age group, the ACME was 0.00145 (95% CI: 0.00104, 0.00), the ADE was 0.00905 (95% CI: 0.00824, 0.01), and the proportion of mediation was 13.8% (all $p < 0.001$).

4 Discussion

In this study, we identified two novel findings. First, OSAS was positively associated with OA in this cross-sectional investigation, which included 10,641 participants. Second, after adjusting for the covariates, aging was found to play a mediating role between OSAS and OA.

OSAS is a sleep disorder characterized by intermittent hypoxia and fragmented sleep, leading to oxidative stress and systemic inflammation. This condition is not only associated with aging-related diseases but may also accelerate the aging process (19, 20) and contribute to the hallmarks of aging, such as telomere attrition, mitochondrial dysfunction, and intercellular communication (21–24). Studies have found that OA shares the above risk factors (25–27). Silva et al. found that elderly patients with OA in the knees with OSAS are more prone to pain, stiffness, and physical dysfunction compared with those without OSAS (28). Carroll et al.'s investigation uncovered a connection between OSAS and shortened leukocyte telomere length, suggesting an association with accelerated biological aging (29). Vicente etal. found a reduction in certain biomarkers of inflammation in pharyngeal lavage samples collected from moderate-to-severe OSAS patients, who were treated with continuous positive airway pressure (30). In our study, compared to the control group, the participants diagnosed with OSAS exhibited higher levels of aging-related clinical biomarkers, including CRP and immunity-related cells.

Aging-induced alterations in articular cartilage particularly contribute to the progression of OA. Loeser et al. reported several mechanisms associated with aging that contribute to OA. Among these are inflammaging, cellular senescence, mitochondria dysfunction, and heightened oxidative stress (31). A recent study discovered that senolytic therapies, which specifically remove senescent cells, effectively decreased oxidation-modified proteins in aged arthritic knee joints, reduced pain, and promoted cartilage development (32, 33). It suggested that aging plays a critical role in OSAS and OA by promoting inflammation and oxidative stress. Our study is consistent with the findings of the studies mentioned above.

We used the NHANES database to explore the mediating effect of aging on OSAS and OA. Based on these findings, efficiently managing OSAS might serve as a strategy for delaying aging in clinical practice and alleviating patients' pain. This approach offers new perspectives on minimizing the incidence of OA and enhancing the overall well-being of elderly patients.

TABLE 3 The association between OSAS.MAP10 and OA in the age and sex subgroup.

		Model 1		Model 2		Model 3	
Group	Characteristic	OR[1] (95% CI[1])	*p*-value	OR[1] (95% CI[1])	*p*-value	OR[1] (95% CI[1])	*p*-value
20–59 years	OSAS.MAP10 quartile		<0.001		<0.001		<0.001
	Q1	Reference		Reference		Reference	
	Q2	1.52 (0.99, 2.34)		2.22 (1.37, 3.59)		2.01 (1.24, 3.25)	
	Q3	1.81 (1.20, 2.72)		3.56 (2.19, 5.80)		2.92 (1.78, 4.79)	
	Q4	3.08 (2.04, 4.64)		8.18 (4.24, 15.8)		5.69 (2.75, 11.8)	
	p-value for the trend	<0.001		<0.001		<0.001	
60+ years	OSAS.MAP10 quartile		0.664		0.006		0.004
	Q1	Reference		Reference		Reference	
	Q2	1.18 (0.66, 2.11)		1.24 (0.70, 2.19)		1.27 (0.71, 2.27)	
	Q3	0.97 (0.58, 1.61)		1.36 (0.78, 2.38)		1.44 (0.81, 2.55)	
	Q4	1.01 (0.58, 1.77)		2.29 (1.19, 4.38)		2.42 (1.23, 4.76)	
	p-value for the trend	0.664		0.006		0.004	
Male	OSAS.MAP10 quartile			<0.001			0.022
	Q1	Reference		Reference		Reference	
	Q2	2.25 (0.70, 7.22)		1.11 (0.34, 3.59)		1.09 (0.34, 3.52)	
	Q3	4.47 (1.71, 11.7)		1.24 (0.46, 3.36)		1.20 (0.44, 3.26)	
	Q4	13.7 (5.33, 35.1)		2.37 (0.82, 6.83)		2.27 (0.79, 6.55)	
	p-value for the trend	<0.001		0.022		0.028	
Female	OSAS.MAP10 quartile			<0.001			0.043
	Q1	Reference		Reference		Reference	
	Q2	2.70 (1.98, 3.69)		1.29 (0.85, 1.97)		1.29 (0.84, 1.97)	
	Q3	3.94 (2.76, 5.63)		1.60 (0.90, 2.85)		1.60 (0.91, 2.83)	
	Q4	6.24 (4.26, 9.14)		2.32 (1.25, 4.31)		2.16 (1.14, 4.07)	
	p-value for the trend	<0.001		0.043		0.096	

[1]OR, Odds Ratio; CI, 95% CI.

Model 1: no covariates were adjusted. Model 2: adjusted for age, sex, race, education level, PIR, BMI, smoking status, and alcohol consumption. Model 3: adjusted for the covariates in model 2 and alcohol consumption, CVDs (coronary heart disease, stroke, heart attack, chronic heart failure, and angina pectoris), hypertension, diabetes, and COPD. OSAS, obstructive sleep apnea syndrome and OSAS.MAP10, OSAS multivariable apnea prediction index value × 10.

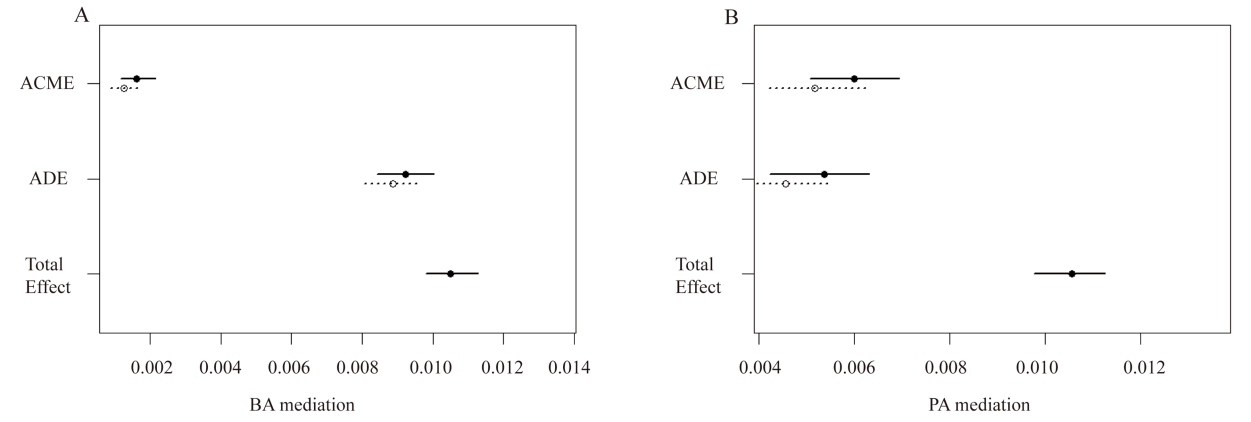

FIGURE 2
The aging-mediated plot illustrates the effects of aging on the association between OSAS with OA risk. (A) BA mediated the association between OSAS and OA. (B) PA mediated the association between OSAS and OA. BA, biological age; PA, phenotypic age; ACME, average causal mediation effect; and ADE, average direct effect; Total effect, ACME + ADE.

Our study has certain strengths. We explored the association between OSAS and OA using a large population database. Furthermore, we evaluated the mediating effect of biological aging on the relationship between OSAS and OA, providing a more nuanced understanding of the relationship. This study includes some limitations. First, while we found a positive correlation between OSAS and OA, the observational study design precluded establishing causality. Future research should include longitudinal data collection and prospective studies to further explore this connection. Second, the data on OA and OSAS were self-reported, which introduced recall bias and decreased the credibility of the results. Third, although we adjusted for the survey cycles, the data on self-reported OA and OSAS were only available in four specific cycles. Therefore, future studies are needed to verify, evaluate, and reinforce our findings.

5 Conclusion

Our results suggest that OSAS may increase the prevalence of OA. In addition, we explored the mediating effects of aging on these two diseases. Our study could provide new insights into the management of various health concerns in the elderly population.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by the Information Collection Review Office. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XL: Methodology, Investigation, Formal analysis, Writing – original draft, Conceptualization. MC: Writing – original draft, Software, Methodology, Investigation, Conceptualization. JX: Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1486807/full#supplementary-material

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Prevalence and risk factors of osteopenia in adults with short bowel syndrome: a retrospective longitudinal cohort study

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Background: Metabolic Bone Disease (MBD) is common in patients with short bowel syndrome (SBS). This study was to investigate the incidence and risk factors of osteopenia in adult SBS patients.

Methods: Hospital records from January 2010 to December 2019 were used to identify all eligible patients. Logistic regression and a nomogram were used to analyze the data.

Results: A total of 120 patients with SBS were included in this study, and 76 patients (63.3%) developed osteopenia during the 10-year observation period, The multivariate analysis using the logistic regression model demonstrated that age (OR = 1.070; 95%CI: 1.016–1.126, p = 0.010), female (OR = 5.098; 95%CI: 1.211–21.456, p = 0.026), tumor history (OR = 4.481; 95%CI: 1.125–17.854, p = 0.033), duration of SBS (OR = 1.0862; 95%CI: 1.022–1.103, p = 0.002) and remnant ileum (OR = 4.260; 95%CI: 1.210–15.002, p = 0.024) were independent risk factors for osteopenia in adults with SBS. The area under the curve (AUC) for the joint model (age, female, tumor history, duration of SBS, remnant ileum) was 0.848 and the corresponding sensitivity and specificity were 0.855 and 0.705, respectively. The C-index was 0.849 (95% CI: 0.778–0.917); thus, the predictions made by the model were close to the actual outcomes. In the decision curve analysis, the nomogram performed well and was feasible to make beneficial clinical decisions.

Conclusion: This study reveals the high prevalence of osteopenia in SBS patients and highlights the importance of early identification and treatment of osteopenia. A nomogram may provide personalized prediction and guidance for medical intervention.

KEYWORDS

short bowel syndrome, osteopenia, risk factor, prevalence, parenteral nutrition

Introduction

Short bowel syndrome (SBS) is a clinicopathological condition characterized by malabsorption and malnutrition following the surgical removal of a section of the small intestine. This condition leads to an inadequate capacity for the gut to absorb nutrients, often necessitating the reliance on parenteral nutrition (PN) for many SBS patients to maintain adequate nutrition supplement (1, 2). PN involves the continuous infusion of nutritional

preparations through veins to meet the patient's energy demands, making it a life-sustaining treatment for SBS patients (3). However, long-term use of PN or an inappropriate oral diet can lead to complications such as liver injury, kidney stones, and osteoporosis in SBS patients (4, 5).

The pathogenesis of metabolic bone disease (MBD) is likely associated with underlying conditions such as disease, malabsorption, inflammation, and certain medications like corticosteroids. PN treatment can also impact bone health (6), with factors like aluminum toxicity, vitamin D effects, and hypercalciuria. Micronutrient deficiencies or toxicities may contribute, but currently, there is no conclusive evidence linking abnormal micronutrient levels to MBD in patients on HPN (4). In a study conducted by ESPEN in 2002 with 165 patients, the prevalence of MBD was assessed using dual-energy X-ray absorptiometry (DXA). This study found that 138 patients (84%) met the criteria for osteopenia, while osteoporosis was present in 41% of the patients (7). The pathogenesis of osteopenia involves various complex factors, including those related to PN treatment, underlying diseases, and general lifestyle factors (8). Patients with SBS who have PN have an increased susceptibility to MBD, which is due to altered calcium and phosphate metabolism, vitamin D deficiency, and other nutritional deficiencies that impair bone formation and resorption. Osteopenia significantly increases the risk of fractures, impacting the quality of life for patients with SBS (9). Therefore, early identification and management of bone health issues are important for SBS patients.

This study aims to provide long-term, comprehensive data on the occurrence of osteopenia in a cohort of adult SBS patients and to identify risk factors for osteopenia early enough to prevent its development. Patients at risk of bone loss should be encouraged to take appropriate calcium and vitamin D supplements, increase their sunlight exposure, and consider milk supplementation as early as possible.

Materials and methods

Study design

The study utilized a database detailed in previous published studies (10) and was approved by the ethics committee of the Jinling Hospital, which followed the ethical guidelines outlined in the 1964 Helsinki Declaration and its subsequent revisions. From January 2010 to December 2019, the hospital records database in this study was used to retrospectively identify all patients with SBS who were admitted to the Intestinal Failure Clinical Nutrition Center. A remaining small intestine length of 200 cm or less was generally agreed upon as fitting the criteria for SBS (11). The study included patients with or without a history of PN use and waived informed consent since it was a retrospective study.

The patient inclusion criteria for this study were adult patients diagnosed with SBS who had regular bone mineral density (BMD) monitoring once a year. Exclusion criteria were: (1) patients aged over 80 years (Physical decline and coexistence of multiple underlying diseases), (2) prior history of bone disorders or bone loss before the diagnosis of SBS, (3) patients with active cancer or acquired immunodeficiency syndrome, (4) primary hyperparathyroidism, and (5) a history of long-term hormone use.

Demographic and clinical variables

The study collected the following patient information based on the date of BMD assessment: sex, age, body weight, height, body mass index (BMI), drinking/smoking history, presence of diabetes mellitus, presence of hypertension, patient-generated subjective global assessment (PG-SGA) grade, nutritional risk screening 2002 (NRS-2002) score, and some laboratory blood examinations. Furthermore, the duration of SBS, causes of SBS, remaining length of the small intestine, and PN-dependence were also recorded.

Diagnosis of osteopenia

BMD was measured at the double femur and lumbar spine using dual-energy X-ray absorptiometry (GE Medical Systems Lunar Prodigy). Before measurement, ensure DEXA is calibrated and its ray output stability checked. Explain the process to the patient to relieve anxiety and have them remove metal items to prevent measurement interference. Position the patient based on the measurement site. For the lumbar spine, the patient lies supine with knees bent. For the hip joint, the patient lies supine with legs straight and slightly internally rotated. Set parameters and scanning range according to the patient's situation. After scanning, check data, calculate T- and Z-values against reference values, and generate a report with basic info, measurement values, interpretations, and clinical suggestions to assist doctors in decision-making. The WHO criterion was used to classify patients' bone mass. For teenagers, men <50 years, and premenopausal women, a Z-score > −2.0 indicated that the patient's BMD range was normal as compared to peers, while a Z-score ≤ −2.0 indicated that the patient had lower BMD range than his peers. For postmenopausal women and men older than 50, the T-score was recommended. A T-score > −1.0 indicated normal bone mass, while a T-score from −1.0 to −2.5 indicated reduced bone mass (osteopenia). A T-score ≤ −2.5 indicated severely reduced bone mass (osteoporosis) and severe osteoporosis could be diagnosed when there is low BMD with a clear evidence of a fragility fracture (8, 12). For convenience in statistical analysis, the study divided patients into the non-osteopenia group (Z-score > −2.0 or T-score > −1.0) and the osteopenia group (Z-score ≤ −2.0 or T-score ≤ −1.0).

Statistical analysis

Continuous variables were presented as mean ± standard deviation, while categorical variables were expressed as absolute counts and percentages. To ensure normality, the Kolmogorov–Smirnov test was used. Proportions were compared using Chi-squared or Fisher's exact tests, while the student t-test or Mann–Whitney U-test was used for continuous variables when appropriate. Logistic regression was used to assess potential risk factors associated with osteopenia in adults with SBS, and the results were presented in terms of odds ratio (OR) with 95% confidence intervals (CI). The R package 'rms' was used to generate nomograms of multivariable models, and the accuracy of the nomogram was evaluated using the receiver-operating characteristic (ROC) curve (13). In addition, the predictive performance of the model was validated by repeating bootstrap resampling 1,000 times. Model fitting, nomogram display, model

validation, and prediction effectiveness evaluation were carried out using R 4.1.1 programming software.

All tests were two-sided, and statistical significance was set at $p < 0.05$. SPSS (version 23.0; IBM Corp, Armonk, NY, United States) was used for statistical analysis.

Results

Demographic and clinical characteristics of the study population

A total of 385 patients with SBS were assessed, of which 265 were excluded due to various reasons, including 225 without BMD assessments, 21 with known bone disorders, 13 aged under 18 years, and six aged over 80 years. Ultimately, 120 SBS patients were enrolled, with 76 patients in the osteopenia group and 44 in the non-osteopenia group (Figure 1).

The main demographic characters and the results of some laboratory tests for the included patients are shown in Table 1. The mean age of the patients was 53.6 ± 13.8 years with 68.3% of them being male. The differences in height and weight were as expected, and unexpectedly, we found no significant difference in BMI. Hypertension and diabetes were present in 23.5 and 5% of patients, respectively. Most patients (93.3%) suffered moderate to severe malnutrition (mean NRS-2002 score 4.1, 112 patients had PG-SGA grades B and C). A history of malignant tumors was reported by one-third of patients (30.8%), and just under half required HPN (40.8%). The median duration of SBS was 47 months. Nutritional indicators and relevant hematological results can be found in Supplementary Tables 1, 2, whereas underlying diseases in adult patients with SBS are shown in Supplementary Table 3.

In the study of intestinal anatomy among SBS patients, the results showed that 44 patients (36.7%) had a jejunostomy (type I), 31 (25.8%) had a jejunocolic anastomosis (type II), and 45 (37.5%) had a jejunoileal anastomosis (type III). Of the patients in the osteopenia group, 34.2% had type III, while 31.8% of non-osteopenia group patients had type I. The mean length of the remaining small intestine was 93.2 ± 53.8 cm. In total, of 69 patients (57.5%) had a predominantly jejunum residual small intestine, 65% had an intact ileocecal valve, and 73.3% had colon integrity (Table 2).

The prevalence of osteopenia in patients with SBS

Kaplan–Meier curve shows the cumulative incidence of osteopenia (Supplementary Figure 1). The incidence of osteopenia in SBS patients was 63.3% (76/120) over an observation period of 10 years. With increased duration of SBS, the incidence rate of bone disease goes up progressively. Patients were divided into the osteopenia group ($n = 76$) and the non-osteopenia group ($n = 44$) according to the BMD assessment. Significant differences were found in age, gender, tumor history, and duration of SBS between groups ($p < 0.05$; Table 1).The osteopenia group had a mean age of 57.2 ± 13.2 years with 29 (38.2%) of them being male and 30 (39.5%) with a tumor history. The average duration of SBS was 50.6 months according to our data. The two groups also differed significantly in nutritional and mineral indicators, such as albumin, HDL, GH, and zinc ($p < 0.05$; Supplementary Table 1). The remaining small intestine type, as a feature of intestinal anatomy, was significantly different between the groups; the proportion of patients with remnant ileum in the osteopenia group was higher than that in the non-osteopenia group [41 (53.9%) vs. 10 (22.7%), $p = 0.001$] (Table 2).

The risk factors for osteopenia in SBS patients

We modeled the probability of developing osteopenia using univariable exact logistic regression and multivariable logistic regression. Univariable analysis showed a significant relationship between the risk of developing osteopenia and age, sex, tumor history, duration of SBS, remaining small intestine type, Zn, and Alb. These factors were further assessed in the multivariable model, where age

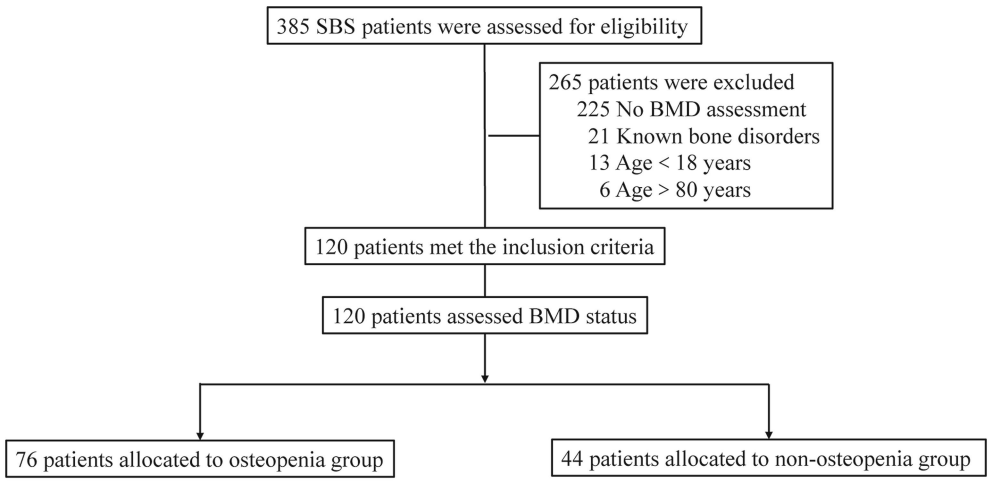

FIGURE 1
Flow diagram of patients with short bowel syndrome included in the study.

TABLE 1 Demographic and laboratory tests for the study population.

Characteristic	ALL	Osteopenia	Non-osteopenia	*p* value
Number of patients (%)	120	76(63.3)	44(36.7)	
Age (years)	53.6 ± 13.8	57.2 ± 13.2	47.4 ± 12.7	**<0.001**
Sex (%)				**0.045**
Female	38(31.7)	29(38.2)	9(20.5)	
Male	82(68.3)	47(61.8)	35(79.5)	
Height (cm)	166.3 ± 7.9	164.7 ± 7.4	169.4 ± 8.1	**0.003**
Body weight (kg)	50.9 ± 11.4	48.8 ± 10.3	55.2 ± 12.5	**0.005**
BMI (kg/m^2)	18.3 ± 3.3	17.9 ± 3.2	19.1 ± 3.3	0.091
Diabetes mellitus (%)	5(4.2)	3(4.1)	2(4.5)	1.000
Hypertension (%)	28(23.5)	17(22.7)	11(25)	0.772
Smoking (%)	17(17.3)	11(16.9)	6(18.2)	0.877
Alcohol (%)	20(20.4)	12(18.5)	8(24.2)	0.502
NRS2002 (%)	4.1 ± 1.1	4.0 ± 1.1	4.1 ± 1.0	0.875
PG-SGA (%)				0.388
A	7(5.9)	5(6.6)	2(4.7)	
B	60(50.4)	35(46.1)	25(58.1)	
C	52(43.7)	36(47.4)	16(37.2)	
Tumor (%)				**0.007**
Yes	37(30.8)	30(39.5)	7(15.9)	
No	83(69.2)	46(60.5)	37(84.1)	
PN dependence (%)				0.126
Yes	49(40.8)	35(46.1)	14(31.8)	
No	71(59.2)	41(53.9)	30(68.2)	
Duration of SBS (months)	47.0(39.0–70.0)	50.5(39.0–80.5)	42.5(38–51.8)	**0.008**

Values were presented as n (%), or mean ± SD, or median (first-to-third interquartile range). $p < 0.05$ is indicated by black bold. BMI, body mass index; PG-SGA, Patient-Generated Subjective Global Assessment; NRS-2002, nutritional risk screening 2002; PN, parenteral nutrition.

(OR = 1.070; 95%CI: 1.016–1.126, $p = 0.010$), being female (OR = 5.098; 95%CI: 1.211–21.456, $p = 0.026$), tumor history (OR = 4.481; 95%CI: 1.125–17.854, $p = 0.033$), duration of SBS (OR = 1.0862; 95%CI: 1.022–1.103, $p = 0.002$), and remnant ileum (OR = 4.260; 95%CI: 1.210–15.002, $p = 0.024$) were identified as independent risk factors for developing osteopenia (Table 3). Supplementary Tables 4, 5 provide information about BMD*T- and Z-scores in adult patients with SBS with and without osteopenia, sorted by bone mass, sex, and age. Regardless of the age - and - gender - based grouping, significant differences in bone mineral density (BMD) are observed.

Model development for the prediction of osteopenia in SBS patients

The logistic regression analysis results allowed us to create a joint model using all identified independent risk factors (age, being female, tumor history, duration of SBS, and remnant ileum) to predict osteopenia in patients with SBS. A nomogram illustrating the predictive variables and corresponding point scales is displayed in Figure 2 (14). Meanwhile, The ROC curve, calibration curve, decision curve analysis, and clinical impact curve for the nomogram are shown in Figure 3. The calibration of the model was assessed with calibration curves, which measure the relationship between the outcomes predicted by the model and the observed outcomes in the cohort. The calibration curve would lie on the diagonal 45-degree line in an ideal nomogram. The dashed line indicates the performance of an ideal nomogram, and the solid line indicates the performance of the present nomogram. The joint model's area under the curve was 0.848, with corresponding sensitivity and specificity values of 0.855 and 0.705, respectively. We conducted a multiple bootstrap procedure ($n = 1{,}000$ bootstraps) to estimate the significance of these analyses, and the C-index was 0.848 (95% CI: 0.778–0.917). The decision curve analysis indicated that the nomogram was useful for making beneficial clinical decisions.

Discussion

The present study is the first to investigate osteopenia in adult patients with short bowel syndrome (SBS). Through analysis of clinical data, several variables, including age, duration of SBS, and sex, were identified as impacting the loss of bone mass in individuals with intestinal failure (IF). Using these risk factors, a nomogram was developed to identify osteopenia as early as possible. This tool has the

TABLE 2 The intestinal anatomy of adult patients with SBS with and without osteopenia.

Characteristic	ALL	Osteopenia	Non-osteopenia	*p* value
Number of patients (%)	120	76(63.3)	44(36.7)	NA
Anatomy type (%)				0.608
Jejunostomy (type I)	44(36.7)	26(34.2)	18(40.9)	
Jejunocolic anastomosis (type II)	31(25.8)	19(25.0)	12(27.3)	
Jejunoileal anastomosis (type III)	45(37.5)	31(40.8)	14(31.8)	
Remaining small intestine length (cm)	93.2 ± 53.8	97.4 ± 59.5	85.9 ± 41.8	0.221
Intestine length type (%)				0.081
≤100	78(65)	45(59.2)	33(75)	
>100	42(35)	31(40.8)	11(25)	
Remaining small intestine type (%)				**0.001**
Jejunum predominantly	69(57.5)	35(46.1)	34(77.3)	
Ileum predominantly	51(42.5)	41(53.9)	10(22.7)	
Ileocecal valve intact (%)				0.874
Yes	78(65)	49(64.5)	29(65.9)	
No	42(35)	25(35.5)	15(34.1)	
Colon integrity (%)				0.458
Yes	88(73.3)	54(71.1)	34(77.3)	
No	32(26.7)	22(28.9)	10(22.7)	

Values were presented as n (%), or mean ± SD, or median (first-to-third interquartile range). $p < 0.05$ is indicated by black bold. SBS, short bowel syndrome.

TABLE 3 Univariate and multivariate analysis of the independent variables associated with osteopenia in the entire population of patients with SBS study.

Independent variable	Univariable analysis		Multivariable analysis	
	OR(95% CI)	*p* value	OR(95% CI)	*p* value
Age (years)	1.058(1.026–1.092)	**<0.001**	1.070(1.016–1.126)	**0.010**
Sex Female/Male	2.400(1.009–5.707)	**0.048**	5.098(1.211–21.456)	**0.026**
Tumor Yes/No	3.447(1.361–8.734)	**0.009**	4.481(1.125–17.854)	**0.033**
Duration of SBS	1.028(1.009–1.047)	**0.003**	1.062 (1.022–1.103)	**0.002**
Ileocecal valve intact Yes/No	1.065(0.488–2.325)	0.874		
Colon in continuity Yes/No	1.385(0.585–3.280)	0.459		
Remaining small intestine type ileum/jejunum predominantly	3.983(1.725–9.198)	**0.001**	4.260(1.210–15.002)	**0.024**
PN dependence Yes/No	1.829(0.840–3.984)	0.128		
Intestine length type >100/≤100	0.484(0.213–1.100)	0.083		
GH	1.289(0.977–1.700)	0.072		
Zn	0.87(0.767–0.987)	**0.030**	0.974(0.818–1.160)	0.770
Alb	0.933(0.872–1.000)	**0.048**	0.974(0.865–1.097)	0.664
HDL	4.102(0.972–17.306)	0.055		

p value <0.05 is indicated by black bold. SBS, short bowel syndrome; OR, odds ratio; CI, confidence interval. Zn, zinc; GH, growth hormone; Alb, albumin; HDL, high density lipoprotein.

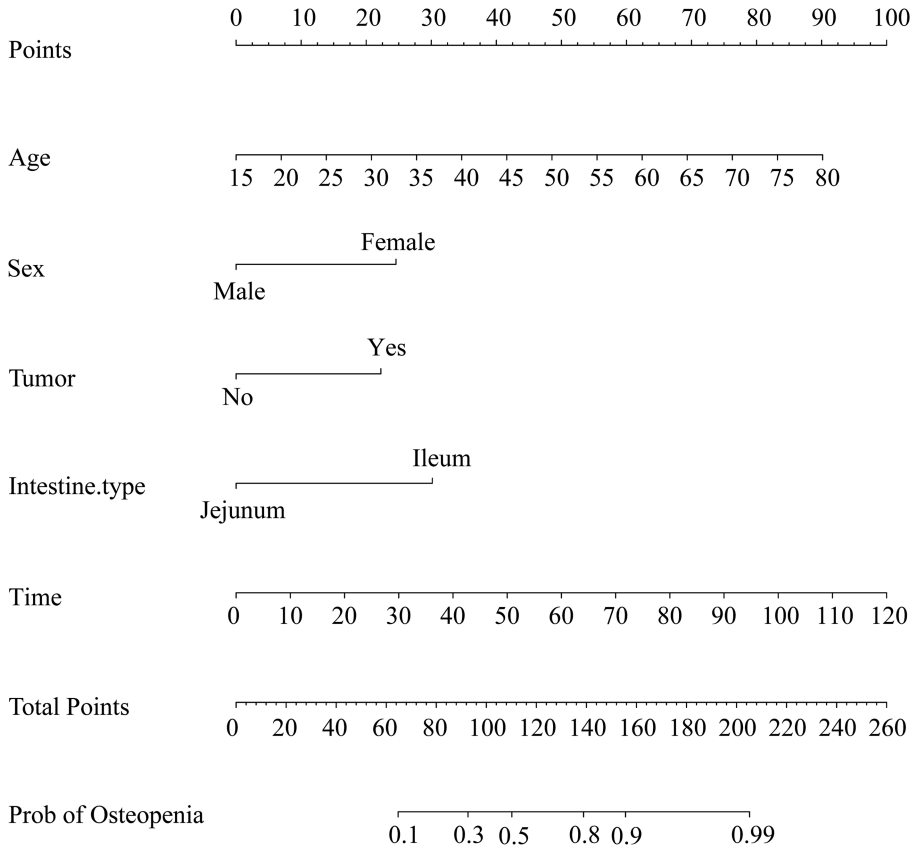

FIGURE 2
Nomogram for the prediction of osteopenia. As an example, an adult patient with SBS, 55 years old, scored 55 points; a female patient scored 25 points; a tumor history scored 22.5 points; the rest of the small intestine was mainly ileum, scored 30 points; 100 months history of SBS, scored 82.5 points; the total score was 215 points; therefore, the possibility of bone loss was greater than 99%.

potential to improve the management and treatment of osteopenia in patients with SBS, particularly by enabling earlier intervention to prevent the development of more severe bone deficiencies.

According to a previous study, the frequency of bone loss in patients with an ostomy due to inflammatory bowel diseases was 29.4% and was attributed to an average age of 45 years old (15). However, our study showed a higher frequency of bone loss at 63.3%, with an average age of 53.6 years. In a multicenter study, 84% of patients receiving home parenteral nutrition (HPN) had bone disease according to the bone mineral density (BMD) T-score, and 62% according to the BMD Z-score, with half of these patients classified as having severe osteopenia (7). Patients with SBS at a rate of 40% also exhibited osteopenia. In these patients, osteopenia was associated with a tendency toward prolonged PN treatment compared to normal patients (11.58 vs. 2.39, $p = 0.094$) (16). Weaning rates from PN among individuals with IF range from 20 to 50% after 2 years (17). Our study found that 40.8% (49/120) of patients were PN-dependent, and among them, 46.1% (35/76) had osteopenia. A follow-up study of patients with IF showed no link between HPN and a decrease in BMD; instead, BMD was negatively correlated with being female and older age (18). Similarly, the results of our study showed that PN-dependency was not the primary influencing factor ($p = 0.128$). However, subsequent investigations involving comparatively large patient cohorts suggest that prolonged HPN is not necessarily linked to a reduction in BMD, and in certain instances, bone density may actually rise during HPN treatment (18).

A randomized controlled trial of growth hormone (GH) showed a significant increase in blood osteocalcin levels after 12 weeks of treatment (+62%; $p < 0.05$) (19) However, our study did not find a statistically significant difference. While GH may promote calcium absorption, bone development, and anabolic activity, it may not be the main factor influencing bone mass in patients with SBS. Instead, an imbalanced distribution of nutrients, particularly mineral insufficiency resulting from reduced dietary intake and impaired absorption of metallic elements, may contribute to increased bone loss and deterioration of bone microstructure in elderly patients. This could be the primary factor in the pathophysiological mechanism of osteopenia (20). Micronutrients such as iron, zinc, and copper play a significant role in the development and transformation of new bones, and their levels in the body can affect the synthesis of bone matrix and bone minerals (21). Our study showed no significant differences in minerals between the two groups, except for Zn ($p = 0.03$), which is an essential micronutrient for bone formation and maintenance of homeostasis. Imbalances in iron metabolism can increase osteoblast development and death, while inhibiting osteoblast proliferation and differentiation, leading to osteoporosis (22). However, our research revealed no such differences, which may be attributed to the poor nutritional status of our patients. The integrity of the colon is essential

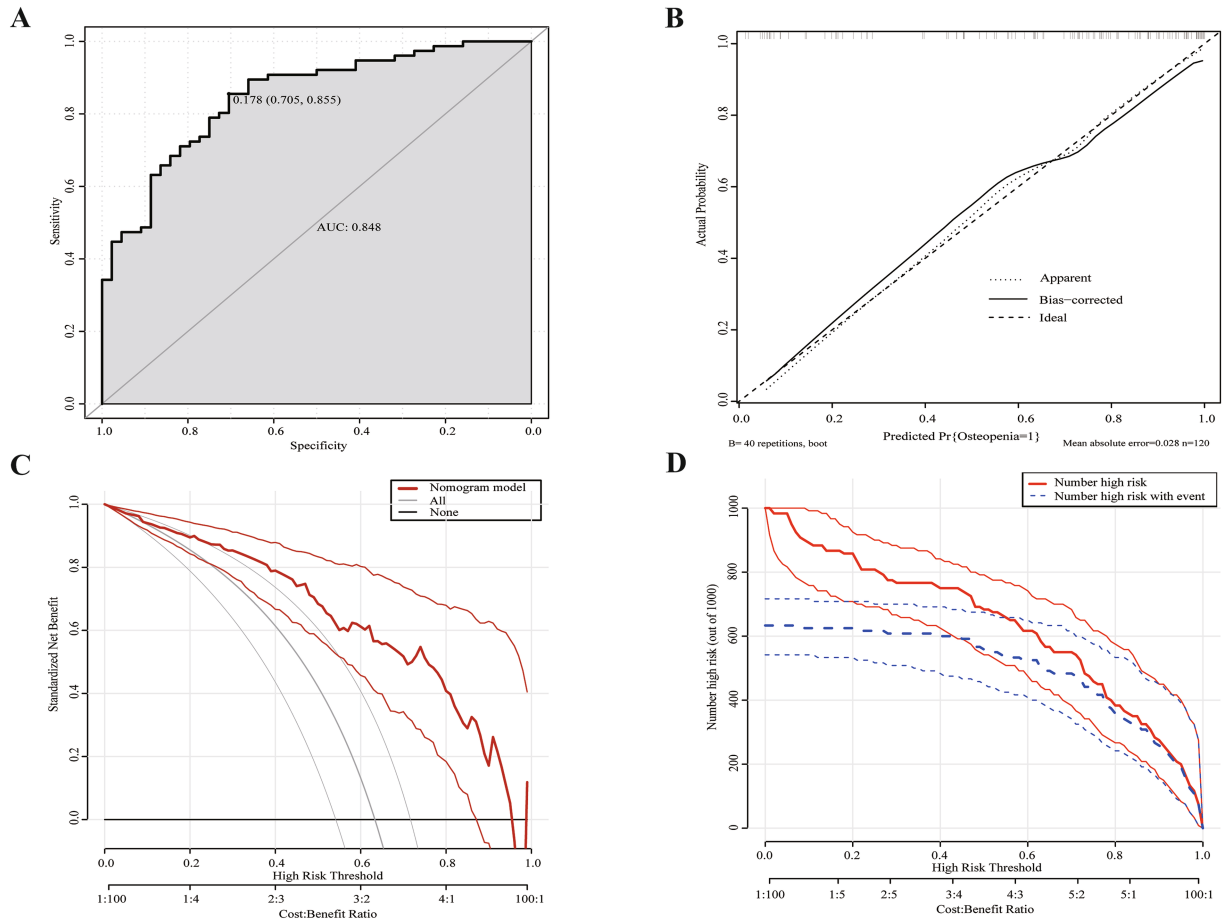

FIGURE 3
Evaluation of nomogram model. **(A)** ROC curve for the nomogram evaluation. The cut-off value was 0.178 with an area under the curve of 0.848. **(B)** Calibration curves of the nomogram. **(C)** DCA for the nomogram. Using multivariate combined nomographs to predict osteopenia has a higher net benefit than treating either no or all patients. **(D)** CIC for the nomogram. The red curve (the number of individuals at high risk) indicates the number of persons who are classified as positive (high risk) by the prediction model at each threshold probability; the blue curve (the number of individuals at high risk with osteopenia) is the number of true positives at each threshold probability. Two light colored lines represent a 95% confidence interval.

for compensatory absorption of triglycerides and fatty acids released from bacterial breakdown in patients with SBS, providing energy production (16). Our results showed no significant differences in BMD between SBS patients with or without colonic integrity, suggesting that while the colon may play a role in calorie clearance, it does not have a significant impact on BMD. Certain researchers hypothesize that ileectomy may contribute to bone loss, as this area is vital for the absorption of vitamin D and the synthesis of glucagon-like peptide 2 (GLP-2), which has been demonstrated to enhance bone mineral density (BMD) in certain patients (23). Moreover, long-term use of HPN may also lead to complications such as cholestasis, affecting the absorption of fat-soluble vitamins (such as vitamins A, D, E, and K). The application of antidiarrheal drugs may also affect the metabolism of nutrients such as calcium and other essential minerals in the body, thereby having a negative impact on bone density (5).

Risk factors for osteopenia in SBS patients can be identified by obtaining comprehensive bone health history, monitoring laboratory values, and conducting nutrition-focused physical examinations. Based on the results, it is advisable for all SBS patients to undergo DXA follow-ups every 1–3 years (24). When oral medication is needed for the prevention or treatment of osteoporosis, special consideration should be given to the high incidence of impaired gastrointestinal absorption. Direct injection is recommended for patients with impaired gastrointestinal absorption (25).

Despite the identification of independent risk factors for osteopenia in SBS patients and the provision of helpful aids for clinical decision-making, our study still has some limitations. Firstly, our sample population consists of older individuals, which may introduce bias as age itself is closely related to BMD. Additionally, sex also plays a role in osteopenia, particularly in post-menopausal women. Subgroup analyses by gender and age were not conducted, but the stability of our model was ensured through bootstrap sampling. The objective of this study was to identify risk factors for osteopenia in patients with SBS and provide data from China on this condition. Secondly, data on vitamin D (102/120) and parathyroid hormone (34/120) only partially available, there may be bias in the results, and we will continue to pay attention to the effect of PTH on BMD in the follow-up. Studies indicate that deficiencies in vitamin D impact calcium and phosphorus metabolism, bone mineralization, and the attainment of peak bone mass. Even slight deficiencies in vitamin D are linked to skeletal homeostasis and/or the inability to achieve peak bone mass (26, 27). But, when vitamin D less than

<20 ng/mL was defined as deficiency, the percentage of vitamin D deficiency was 87% (89/102) in our study. By this standard, vitamin D insufficiency or deficiency is globally widespread, accounting for about 50 to 80 percent of the total population (28, 29). Surveys in Chinese cities at different latitudes have shown that vitamin D insufficiency or deficiency is common in the population (30, 31). Vitamin D is synthesized in the skin under ultraviolet radiation B (UVB) from sunlight or comes from food (natural and fortified). SBS patients are at high risk of vitamin D insufficiency/deficiency due to poor intake, absorption, and lack of UVB. They need more vitamin D supplements or sunlight (32). We tried to analyze that in the non-osteopenia group 13.79 ± 5.60 ng/mL, 13.24 ± 4.51 in Osteopenia group with a p value of 0.58, there was no significant difference between the two groups. Therefore, most patients have vitamin D deficiency, and vitamin D may not be a key factor in the reduction of bone mass in patients with short bowel syndrome, which may be more related to age, parenteral nutrition-related factors, and the condition of the intestinal tract itself. A prospective study by Shengxian fan (6) found vitamin D deficiency in 95% (57/60) of patients with short bowel syndrome. L. Ellegard et al. investigated 78 patients with intestinal failure vitamin D and bone mineral density, there was no significance association between vitamin D deficiency and low BMD (6, 33). We speculate that due to individual differences, some populations may have low detection values, but their vitamin D receptors on bone cells function well and can efficiently utilize limited vitamin D to regulate processes such as bone remodeling are still functional and can effectively utilize scarce vitamin D to regulate processes like bone remodeling (34). Genetic factors can promote traits that help keep bone density normal, even with low vitamin D levels. This can lessen the harmful effects of vitamin D deficiency on bone density (35). Furthermore, Mendelian randomization analysis also revealed no genetic causality between vitamin D and BMD (36). Finally, future studies involving larger sample sizes are required. Hence, timely detection of osteopenia in patients is crucial, and appropriate supplementation of trace elements based on the patient's condition should be considered to prevent further deterioration. Intramuscular injection of vitamin D and zoledronic acid may also be considered as preventive measures. Furthermore, our aim is to gather more relevant data to validate the accuracy of our model and provide further valuable insights for SBS research.

Conclusion

SBS in adult patients is associated with a significantly high risk of developing osteopenia. Independent predictors for osteopenia include the type of residual small intestine, age, sex, duration of SBS, and a history of tumors. In order to minimize the adverse effects of osteopenia, close monitoring and preventive interventions are recommended.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the ethics committee of the Jinling Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

GS: Formal analysis, Writing – original draft, Writing – review & editing. YuX: Data curation, Writing – review & editing. HW: Data curation, Writing – review & editing. YaX: Investigation, Writing – review & editing. LZ: Conceptualization, Writing – review & editing. YZ: Methodology, Writing – review & editing. XG: Conceptualization, Project administration, Writing – review & editing. XW: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1422596/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Kaplan–Meier plot showing adult patients with short bowel syndrome without osteopenia during the 10-year period.

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Risk factors for new vertebral fractures after percutaneous vertebroplasty or percutaneous kyphoplasty in the treatment of osteoporotic vertebral compression fractures

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Object: This study aims to conduct a prospective analysis of patients with osteoporotic vertebral compression fractures (OVCF) who underwent percutaneous vertebroplasty (PVP) or percutaneous kyphoplasty (PKP), and further analyze the risk factors for new vertebral fracture following treatment.

Methods: A prospective study was conducted from November 2020 to March 2022 at the First Hospital of Longyan City to select patients with OVCF who underwent treatment in the Department of Spinal Surgery. Data collection during the follow-up period focused on various factors that could potentially be associated with new vertebral fractures after PVP/PKP procedures. Patients were divided into two groups based on whether they experienced new vertebral fractures within two years after discharge: the new fracture group (n = 186) and the non-fracture group (n = 64), and statistical analysis was conducted accordingly.

Results: All cases were followed up for 12 to 24 months, with an average of 14.7 months. Differential analysis revealed that age, diabetes, hemoglobin (HB), total protein (TP), serum albumin (ALB), b-C-terminal telopeptide of type I collage (β-CTX), 25-hydroxyvitamin D (25-OH-D3), number of fractured vertebrae, bone mineral density (BMD), regular exercise after discharge, anti-osteoporosis treatment after discharge, cross-sectional area (CSA), and fatty degeneration ratio (FDR) were associated with new vertebral fractures (all $P < 0.05$). Multivariate analysis showed that age (OR = 1.519, P = 0.032), diabetes (OR = 3.273, P = 0.048), and FDR (OR = 1.571, P = 0.027) were positively associated with the occurrence of new vertebral fractures, while bone mineral density (OR = 0.108, P = 0.044), 25-OH-D3 (OR = 0.871, P = 0.032), CSA (OR = 0.564, P = 0.009), regular postoperative exercise (OR = 0.259, P = 0.025), and osteoporosis treatment (OR = 0.291, P = 0.045) were negatively associated with the occurrence of new vertebral fractures.

Conclusion: Patients with osteoporosis fractures who are older, have poor glycemic control, lower bone mineral density, lower levels of 25-OH-D3, weaker paraspinal muscles, and higher fat infiltration are at increased risk of new vertebral fractures after undergoing PKP/PVP. On the other hand, maintaining regular physical activity and adhering to osteoporosis treatment can help prevent new vertebral fractures.

KEYWORDS

osteoporotic vertebral compression fractures, senior citizen, new vertebral fractures, percutaneous vertebroplasty, percutaneous kyphoplasty, risk factors

Introduction

Osteoporosis is a metabolic disorder distinguished by diminished bone strength and alterations in bone microstructure, resulting in heightened bone fragility (1). The condition is prevalent among the elderly. According to a Canadian multicentre osteoporosis study, 21.5% of men and 23.5% of women aged over 50 years exhibit at least one vertebral compression deformity (2). Osteoporotic vertebral compression fractures (OVCF) are fractures of the vertebral bodies caused by external forces acting on an osteoporotic bone structure. In elderly patients, the decline of various physiological functions often necessitates bed rest following a fracture, resulting in reduced mobility and further bone loss. Moreover, the fracture and the associated treatment can exacerbate bone loss, thereby increasing the risk of subsequent fractures (3, 4). Symptomatic OVCF can induce considerable pain and diminish a patient's mobility, significantly affecting their quality of life (5).

Over the past few decades, percutaneous vertebroplasty (PVP) and percutaneous kyphoplasty (PKP) have been widely used to treat OVCF due to their rapid pain relief and functional improvement (6). However, new vertebral fractures after PVP/PKP remain a common issue. Previous studies have reported varying frequencies and timing of new vertebral fracture after treatment. Kim et al. (7) found that refracture occurred, on average, 3.4 months after PKP, with an incidence rate of 12.5%. Similarly, Chen et al. (8) conducted a retrospective analysis, reporting that 9.7% of 134 OVCF patients treated with PKP experienced re-collapse of the cemented vertebrae, resulting in severe back pain and dysfunction.

A study encompassing 178 patients with OVCF demonstrated that 68 patients (38.2%) encountered new vertebral fractures (9). The risk of experiencing a subsequent fracture within one year for osteoporotic vertebral fracture patients is approximately 2.7 times higher than for non-fracture patients (10). Previous studies have confirmed that patient characteristics such as gender, age, body mass index (BMI), and chronic diseases, as well as factors related to cement injection, may influence the occurrence of new vertebral fractures after PVP/PKP (11). Since osteoporosis is closely associated with lifestyle factors such as smoking, alcohol consumption, and physical activity, it is believed that these same factors may affect the incidence of postoperative new vertebral fractures.

Bone turnover markers are parameters that reflect the dynamic state of osteoporosis. By measuring serum bone metabolism markers, the bone turnover status in OVCF patients can be indirectly assessed. Several studies have explored the relationship between bone turnover markers and osteoporosis, including parathyroid hormone (PTH), C-terminal telopeptide of type I collagen (β-CTX), N-terminal propeptide of type I procollagen (PINP), osteocalcin (OC), serum 25-hydroxyvitamin D3 (25-OH-D3), and alkaline phosphatase (ALP) (12). In addition, some researchers have suggested that paraspinal muscle and fat infiltration are associated with OVCF (13, 14). However, no studies have comprehensively analyzed all of these potential risk factors. Therefore, the risk factors for new vertebral fractures after PVP/PKP in OVCF patients remain inadequately defined. Based on a large dataset from our hospital, this study aims to provide insights into the risk factors for new vertebral fractures after PVP/PKP in OVCF patients through differential analysis and multivariate regression analysis.

Materials and methods

Data sources

This study utilized a prospective design and selected 250 patients with OVCF who received surgical treatment in the Spinal Surgery Department of Longyan First Hospital from November 2020 to March 2022 as the research subjects. The patients were divided into a new fracture group and a non-fracture group based on whether they experienced another fracture within two years after discharge. There were 111 male and 139 female patients, with an age range of 60 to 80 years and an average age of 72.89 $\pm$ 7.93 years. Inclusion criteria were as follows: (1) $80 \geq$ age ≥ 60 years; (2) diagnosed with OVCF based on diagnostic criteria and confirmed through X-ray/CT scan/MRI imaging (15); (3) initial admission for PKP or PVP treatment; (4) consent obtained from patients and their families for participation in the study; (5) availability of complete medical records, the willingness of patients to answer questions during follow-up, and provision of the necessary information for the study. The exclusion criteria were as follows: (1) neoplasms of the vertebral column; (2) history of vertebral fracture, spinal surgery, and low back soft tissue injury or surgery; (3) any other comorbidity or chronic diseases that could

significantly affect bone or soft tissue metabolism (for example liver and kidney disease, chondromalacia, thyroid disorders, ankylosing spondylitis, diffuse idiopathic skeletal hyperostosis, or connective tissue disease); (4) history of certain drug use (hormonal drugs, anti-osteoporosis drugs, or diet pills); (5) severe cardiopulmonary diseases or coagulation dysfunction; and (6) incomplete clinical data. This study has been approved by the Medical Ethics Committee of Longyan First Hospital (L2022005). All participants in the study received written and oral information prior to giving written consent, and the study was performed following the Declaration of HELSINKI.

Surgical procedure

Surgical procedure PVP/PKP was performed within three days of hospitalization for all patients. Every operation was conducted by the specified spinal surgeons with sufficient clinical experience, and the skills designed by Garfin et al. (16). The patients first placed in a prone position on the operating table were administered under local anesthesia (2% lidocaine). With the guidance of two single-plane mobile C-arms, the anterior–posterior and lateral views of the fractured vertebra were confirmed. After incision of the skin, two 11-gauge needles were placed parallel to the superior and inferior edges of the pedicle, percutaneously into the anterior part of the vertebral body with a transpedicular or perpendicular approach. For vertebrae with excessive anterior compression or significant kyphotic deformity, PKP surgery was performed. Under the guidance of a C-arm X-ray machine, a balloon was inserted, and the pressure was gradually increased to 200 PSI. Once the balloon was fully expanded and satisfactory vertebral height restoration was achieved, the balloon was removed. The injection of polymethylmethacrylate (PMMA) cement (Stryker, Kalamazoo, USA) into the fractured vertebral body was ceased until the cement reached the posterior one-fourth of the body or if PMMA extravasated outside the bone. The volume of bone cement inserted during the operation for each vertebra was recorded. Thoracic-lumbosacral orthosis was supplied to all patients for one month, and osteoporotic medications were used postoperatively.

Study methods

Based on the electronic medical records system of elderly patients with OVCF admitted to the hospital, relevant patient information was obtained. We employed differential analysis and logistic regression analysis to identify the influencing factors for new vertebral fractures after discharge.

The following information was recorded for each patient: basic demographic data (gender, age, height, and weight), adverse lifestyle habits (smoking, alcohol consumption), presence of chronic diseases (hypertension, diabetes, coronary atherosclerosis), preoperative bone mineral density (BMD), bone metabolism-related markers, blood biochemical indicators, number of vertebral fractures, amount of bone cement injected per vertebra, the condition of cement leakage, postoperative exercise, and adherence to anti-osteoporosis treatment. During each postoperative follow-up, patients were asked whether they followed the doctor's advice for exercise and whether they received osteoporosis treatment. Exercise was defined as aerobic activities, including brisk walking, jogging, dancing, swimming, and hiking, aimed at increasing heart rate for at least 30 min, at least four times per week. Osteoporosis treatment medications included calcitonin and bisphosphonates. According to the treatment plan prescribed at discharge, patients adhered to the medication regimen (including calcitonin and bisphosphonate used in our hospital) for at least 12 months during the follow-up period.

Image analyses

Magnetic resonance imaging (MRI) examinations were completed using the 3.0T (Siemens Healthineers, Erlangen, Germany) scanner. T2-weighted images, parallel to the inferior endplate of the L3 vertebral body, were selected for analysis. The cross-sectional area (CSA) of the bilateral multifidus and erector spinae, and vertebral body size (VB) were separately outlined with the graphic cursor and measured on images using the hospital PACS digital imaging system (Figure 1). The fatty degeneration ratio (FDR) of the paraspinal muscle was analyzed and calculated using the ImageJ software for Windows (ImageJ version 1.53k, National Institutes of Health, Bethesda, MD, USA). Using yellow lines in ImageJ, the CSA was outlined and labeled. The pseudocolor tool in ImageJ was employed to identify adipose tissue regions, which were highlighted in red. The intersection between the CSA and the red adipose regions represented the paraspinal fatty area (FA). The FA obtained was then divided by the previously measured CSA to calculate the FDR.

Statistical analysis

Statistical Methods Continuous data are expressed as means ± standard deviations, and the independent samples *t*-test was used for comparison between groups. Categorical data were analyzed by a chi-square test. A univariate analysis was used to identify potential influencing factors for new vertebral fracture after PVP/PKP. Multivariable logistic regression analysis was performed using the variables that showed statistical significance in the differential analysis. All statistical analyses were performed using SPSS Statistics for Windows, version 23.0 (IBM Corp, Armonk, NY, USA). A P-value of < 0.05 was considered statistically significant.

Results

All cases were followed up for 12 to 24 months, with an average of 14.7 months. The study comprised 250 patients, among whom 64 experienced new vertebral fractures two years after follow-up, while 186 remained fracture-free (25.60%, 64/250) (Representative case shown in Figure 2). There were no statistically significant differences observed between the new fracture group and the non-fracture group concerning gender, BMI, habit (smoking/alcohol consumption), hypertension, coronary heart disease, red blood cell (RBC), white blood cell (WBC), platelet, uric acid (UA), blood glucose (Glu), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), serum calcium, serum phosphorus, ALP, OC, PINP, fracture site, PKP, the average volume of bone cement,

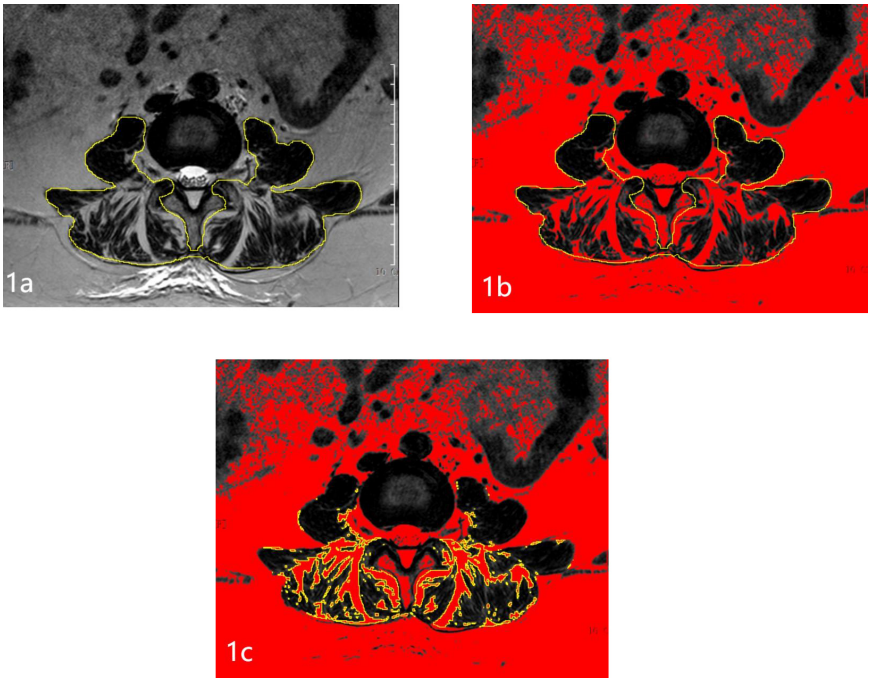

FIGURE 1
Schematic diagram of measuring paraspinal muscles and fat on MRI. **(A)** Measurement of the paraspinal muscle area. **(B)** Red represents all adipose tissue. **(C)** The intersection of the CSA and adipose tissue indicates the paraspinal FA.

cement leakage ($P > 0.05$). However, statistically significant variances ($P < 0.05$) were noted between the new fracture group and the non-fracture group in terms of age, diabetes, hemoglobin (HB), total protein (TP), serum albumin (ALB), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), β-CTX, PTH, 25-OH-D3, number of fractured vertebrae, BMD, exercise regularly after discharge, anti-osteoporosis after discharge. Please refer to Table 1 for detailed information.

Magnetic resonance imaging measurements

The VB of the two groups were (15.40 ± 2.36, 16.16 ± 2.44) cm^2, with no statistically significant difference. The CSA of the two groups were (30.36 ± 3.57, 33.84 ± 4.18) cm^2, and the difference was statistically significant ($P = 0.003$). The FDR of the two groups were (32.32 ± 4.58, 29.48 ± 4.54)%, and the difference was statistically significant ($P = 0.032$). Please refer to Table 2 for detailed information.

Multivariate logistic regression analysis

Based on the results of the differential analysis, variables with a significant probability of $P < 0.05$ were included in the subsequent multivariable logistic regression analysis. Therefore, the sixteen variables, including Age, Diabetes, HB, TP, ALB, HDL-C, β-CTX, PTH, CSA, VB, FDR, 25-OH-D3, Number of fractured vertebrae, BMD, Exercise regularly after discharge, Anti-osteoporosis after discharge, were included in the multivariate Logistic regression model. Multivariate analysis showed that age (OR = 1.519, $P = 0.032$), diabetes (OR = 3.273, $P = 0.048$), and fat infiltration (OR = 1.571, $P = 0.027$) were positively associated with the occurrence of new vertebral fractures ($B > 0$), while bone mineral density (OR = 0.108, $P = 0.044$), 25-OH-D3 (OR = 0.871, $P = 0.032$), paraspinal muscle area (OR = 0.564, $P = 0.009$), regular postoperative exercise (OR = 0.259, $P = 0.025$), and osteoporosis treatment (OR = 0.291, $P = 0.045$) were negatively associated with the occurrence of new vertebral fractures ($B < 0$). The specific analysis results are shown in Table 3.

ROC curve and results

The receiver operating characteristic (ROC) curves for the variables of age, diabetes, CSA, FDR, 25-OH-D3, BMD, regular exercise after discharge, and anti-osteoporosis treatment after discharge are shown in Figure 3. The figure presents the cutoff values, specificity, sensitivity, positive and negative predictive values, and diagnostic efficiency for each factor. Among these variables, CSA demonstrated the highest AUC for predicting new vertebral fractures (0.84, 95% CI: 0.7902–0.8944). Both CSA and 25-OH-D3 had AUC values exceeding 0.8, while age, FDR, and BMD had AUC values greater than 0.7.

Discussion

PVP and PKP are widely used for the treatment of osteoporotic thoracolumbar fractures due to their minimally invasive nature and rapid pain relief (17, 18). However, new vertebral fractures are a

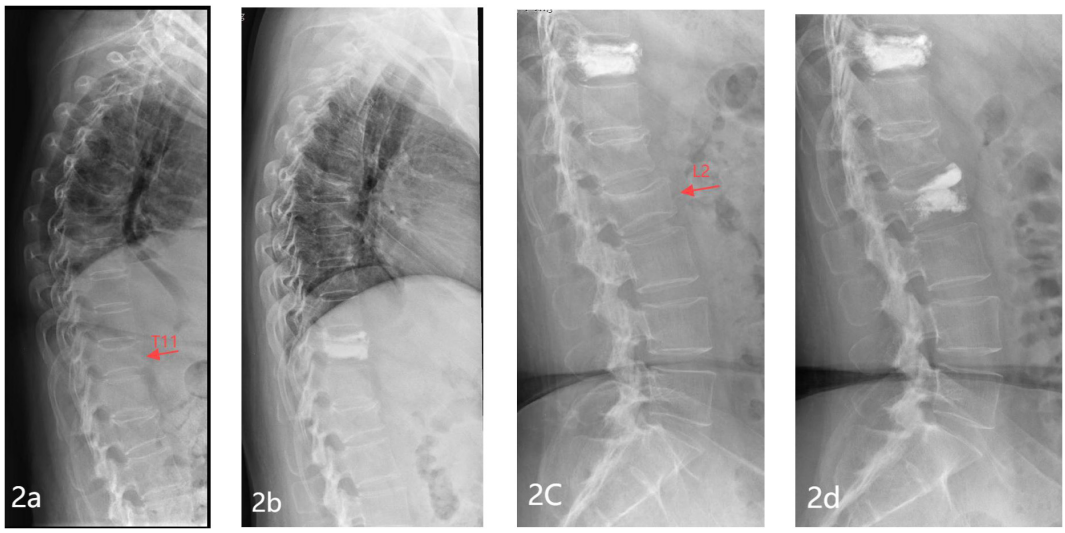

FIGURE 2
Representative images of a typical case of new vertebral fracture in a patient with OVCF after PVP/PKP. **(A)** Preoperative lateral X-ray of the thoracic spine showing a T11 vertebral compression fracture; **(B)** lateral X-ray of the thoracic spine 1 day post-operation showing T11 filled with bone cement; **(C)** lateral X-ray of the lumbar spine 1 year post-operation showing a new vertebral compression fracture at L2; **(D)** Lateral X-ray of the lumbar spine 1 day post-operation showing L2 filled with bone cement.

common long-term complication following these procedures (19, 20), and there is still considerable debate regarding the associated risk factors. Through differential analysis and multivariable logistic regression analysis, this study identified advanced age, diabetes, and fat infiltration as independent risk factors for new vertebral fractures after PVP/PKP in osteoporotic thoracolumbar fractures, while CSA, 25-OH-D3, regular exercise after discharge, and anti-osteoporosis treatment after discharge were found to be protective factors. In this study, the incidence of new vertebral fractures after PVP/PKP was 25%, slightly higher than in previous studies (21–23), which may be due to the older age of the study population and the longer follow-up period.

The incidence of subsequent fractures in elderly patients with OVCF post-discharge significantly exacerbates the decline in patients' quality of life (24). Subsequent fractures not only subject patients to heightened levels of pain and inconvenience in daily life but may also exacerbate the initial spinal deformity, thereby amplifying the risk of paralysis and mortality (25). Hence, acquiring a comprehensive understanding of and effectively intervening in factors associated with subsequent fractures post-discharge is of paramount importance for enhancing patients' quality of life and averting future fracture occurrences (26).

This study found that advanced age is an independent risk factor for new vertebral fractures after PVP/PKP in osteoporotic thoracolumbar fractures, consistent with the findings of Zhang et al. (23). The possible reasons are as follows: First, with aging, bone mass gradually decreases. Warden et al. (27) suggested that in older adults, reduced calcium intake and increased excretion lead to secondary hyperparathyroidism and a decrease in calcium ions within the bones. Additionally, Russell and Kahn (28) proposed that reduced sex hormone production in the elderly, along with increased oxidative stress, inhibits osteoblast activity, further exacerbating osteoporosis. Second, reduced physical activity in older adults further worsens osteoporosis. Finally, the elderly often have poorer self-care ability, making them more prone to falls and other accidents.

There is substantial and growing evidence that diabetes is associated with osteoporosis, despite bone mineral density usually remaining normal. This study found that diabetes is an independent risk factor for new vertebral fractures after PVP/PKP in osteoporotic thoracolumbar fractures. Given that diabetic patients tend to have worse fracture outcomes (including higher mortality rates), careful consideration should be given to fracture prevention management for this highly vulnerable group (29). Osteoporosis is emerging as a complication of diabetes. Compared to the general population, individuals with diabetes have a significantly higher risk of fractures. Osteoporosis in diabetes results from complex, poorly understood mechanisms at the cellular level, involving vascular, inflammatory, and mechanical disruptions (30). Both intrinsic bone factors, such as accumulation of advanced glycation end products, low bone turnover, and changes in bone microstructure, and extrinsic factors, such as hypoglycemia induced by treatment, diabetic peripheral neuropathy, muscle weakness, visual impairment, and certain glucose-lowering medications that affect bone metabolism, may contribute to impaired bone strength and an increased risk of fragility fractures (31). Bone mineral density serves as a crucial indicator of bone strength, intricately linked to the pace and quality of fracture healing (32). Low bone mineral density commonly signifies diminished internal bone tissue strength, compromised structural integrity, decreased load-bearing capacity, and stability, rendering bones more susceptible to injury or collapse even under minor external forces (33).

Previous research by Lee et al. (11) found that patients with lower BMD had a higher risk of new vertebral fractures. Similarly, Wang et al. (34) identified BMD as an independent risk factor for new vertebral fractures following PKP. Li et al. (35) demonstrated

TABLE 1 Baseline characteristics of new fracture group and non-fracture group.

Variables	New fracture group (n = 64)	Non-fracture group (n = 186)	P-value
Gender (male/female)	24/40	87/99	0.198
Age (year)	75.64 ± 8.01	70.28 ± 7.94	0.021*
BMI (kg/m^2)	22.04 ± 2.15	21.88 ± 2.86	0.824
Smoking (Yes/No)	22/42	42/142	0.069
Alcohol consumption (Yes/No)	16/48	36/150	0.337
Hypertension (Yes/No)	23/41	56/130	0.387
Diabetes (Yes/No)	20/44	44/142	0.032*
Coronary heart disease (Yes/No)	18/46	38/148	0.203
RBC ($\times 10^{12}$/L)	4.34 ± 0.38	4.51 ± 0.43	0.151
HB (g/L)	114.32 ± 11.36	122.23 ± 13.96	0.031*
WBC ($\times 10^9$/L)	7.75 ± 1.48	7.25 ± 1.26	0.207
Platelet ($\times 10^9$/L)	237.24 ± 51.94	223.89 ± 53.51	0.577
TP (g/L)	61.69 ± 4.31	65.64 ± 4.43	0.002*
ALB (g/L)	37.36 ± 3.66	40.88 ± 4.69	0.005*
UA (umol/L)	334.40 ± 31.72	345.17 ± 41.33	0.291
Glu (mmol/L)	5.65 ± 0.73	5.85 ± 0.84	0.398
TC (mmol/L)	5.34 ± 0.33	5.22 ± 0.38	0.229
TG (mmol/L)	1.47 ± 0.28	1.57 ± 0.26	0.163
HDL-C (mmol/L)	1.31 ± 0.25	1.19 ± 0.24	0.107
LDL-C (mmol/L)	3.32 ± 0.47	3.41 ± 0.44	0.506
Serum calcium (mmol/L)	2.27 ± 0.19	2.33 ± 0.17	0.248
Serum phosphorus (mmol/L)	1.14 ± 0.19	1.11 ± 0.20	0.582
ALP (U/L)	80.64 ± 10.27	82.21 ± 10.82	0.604
β-CTX (ng/ml)	0.80 ± 0.15	0.67 ± 0.14	0.003*
OC (ng/ml)	23.38 ± 8.41	21.06 ± 7.73	0.315
PINP (ng/ml)	58.52 ± 9.51	56.88 ± 14.17	0.633
PTH (ng/ml)	50.36 ± 9.41	56.72 ± 10.48	0.029*
25-hydroxyvitamin D (nmol/L)	33.16 ± 10.01	41.81 ± 12.13	0.008*
Site (Thoracic /Lumba)	24/40	64/122	0.655
Number of fractured vertebrae			0.039*
1	38	136	
> 1	26	50	
Percutaneous kyphoplasty			0.539
Yes	44	120	
No	20	66	
The average volume of bone cement	4.48 ± 0.92	4.62 ± 1.02	0.635
Cement leakage			0.771
Yes	10	32	
No	54	154	
BMD (T-score)	−3.74 ± 0.74	−3.26 ± 0.63	0.015*
Exercise regularly			0.002*
Yes	19	96	
No	45	90	
Anti-osteoporosis			0.001*
Yes	14	108	
No	50	78	

Asterisk symbol (*) indicates p-value < 0.05, meaning a statistically meaningful. BMI, body mass index; RBC, red blood cell; HB, hemoglobin; WBC, white blood cell; TP, total protein; ALB, serum albumin; UA, uric acid; Glu, blood glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; β-CTX, b-C-terminal telopeptide of type I collagen; OC, osteocalcin; PINP N-terminal propeptides of type I procollagen; PTH, parathyroid hormone, BMD, bone mineral density, PKP, percutaneous kyphoplasty.

TABLE 2 Comparison of MRI imaging measurements between the two groups.

Variables	New fracture group (n = 44)	Non-fracture group (n = 106)	P-value
CSA (cm^2)	30.36 ± 3.57	33.84 ± 4.18	0.003*
VB (cm^2)	15.40 ± 2.36	16.16 ± 2.44	0.269
FA (cm^2)	10.66 ± 1.86	9.68 ± 2.13	0.089
FDR (%)	32.32 ± 4.58	29.48 ± 4.54	0.032*

CSA, cross-sectional area; VB, vertebral body size, FA, fatty area; FDR, fatty degeneration ratio. *Indicates p-value < 0.05, meaning a statistically meaningful.

that low BMD increases the risk of new vertebral fractures after PVP or PKP. In a four-year follow-up study of patients with OVCF who underwent PVP or PKP, He et al. (36) found that BMD was the only risk factor for new vertebral fractures. Consistent with these previous studies, this study also confirms that BMD is an independent predictor of new vertebral fractures in OVCF patients post-surgery.

25-OH-D assumes a pivotal role in bone remodeling by fostering enhanced absorption of calcium and phosphorus in the intestines, augmenting bone metabolism, activating osteoblasts for new bone matrix formation, bolstering muscle contraction function, and expediting bone repair and healing processes (37). Postoperative patients with insufficient vitamin D3 levels may experience poor bone healing and further decline in bone mineral density, increasing the risk of new vertebral fractures. Vitamin D3 deficiency can lead to impaired bone mineralization, making bone tissue fragile. Several studies have shown that vitamin D3 supplementation significantly reduces the rate of new vertebral fractures in patients with osteoporosis (12). For instance, a randomized controlled trial found that patients who regularly supplemented with vitamin D3 had a significantly lower incidence of new vertebral fractures within one year post-surgery compared to those who did not supplement. As a crucial factor in maintaining bone health, vitamin D3 has a significant impact on reducing the risk of new vertebral fractures in postoperative osteoporosis patients (38). Future research could further explore its mechanisms of action and optimize supplementation strategies to improve clinical outcomes.

This study confirms that the decline in lumbar muscle mass and muscle fat degeneration are important risk and predictive factors for lumbar vertebral fractures. Previous studies have referred to this muscle condition as sarcopenia, characterized by the progressive loss of muscle mass and the associated reduction in strength and function with aging (39, 40). While the concept of sarcopenia is widely accepted, there is no consensus on how to measure and quantify it. To accurately analyze the qualitative and quantitative differences in lumbar muscle between groups, MRI imaging was used for measurement. Additionally, in this study, MRI-based measurements were identified as independent and sensitive predictors of secondary fracture risk. As shown in the study results, the fracture group exhibited significantly higher FDR and CSA. Similar findings were reported by Tokeshi et al. (14), who confirmed the phenomenon in their study, which included all OVCF participants regardless of injury mechanism. Jeon et al. (13) also reported that paraspinal muscle fat degeneration is a risk factor for progressive vertebral compression in OVCF patients. Wang et al. (34) further identified sarcopenia as an independent predictor of osteoporotic vertebral compression fractures. Even after vertebral augmentation procedures for OVCF, sarcopenic patients face a higher risk of postoperative mortality (41). The paraspinal muscles are crucial for maintaining normal spinal alignment, and the loss of muscle mass and increase in fat degeneration severely impact spinal balance and muscle strength, leading to frailty and an increased risk of fragility fractures. This study indicates that FDR and CSA can predict the risk of fragility fractures in elderly osteoporotic patients, with their predictive power being independent of, and even equal to or greater than, BMD (T-score).

Patients who fail to adhere to regular exercise post-discharge and neglect consistent intake of anti-osteoporosis medications are at an elevated risk of subsequent fractures. Inactivity can result in reduced bone mineral density and diminished muscle strength, exacerbating osteoporosis and the susceptibility to falls. Consistent physical activity not only aids in preserving or augmenting bone mineral density but also fortifies muscle strength, coordination, balance, and flexibility, thereby diminishing the risk of instability and falls (42). Moreover, regular exercise contributes to sustaining the range of motion of joints and muscles, thereby mitigating the risk of fractures stemming from limited mobility. The results of this study indicate that regular exercise is a protective factor against new vertebral fractures following PKP/PVP. Physical activity has been shown to improve BMD in elderly osteoporotic patients after PKP. With the increasing trend of an aging population in China, the number of elderly patients with osteoporosis is on the rise. If elderly patients with OVCF do not receive anti-osteoporosis medication post-surgery, their bone fragility may worsen. Early rehabilitation guidance after discharge can promote functional recovery. Appropriate exercise can improve BMD, reduce bone loss, and help prevent osteoporosis. The mechanism of treatment involves hormonal regulation; aerobic exercise can lower calcium levels in the blood, inhibit thyroid hormone secretion, suppress bone resorption, and promote bone synthesis, significantly increasing BMD. Exercise also enhances neuromuscular function, improving muscle strength and body mass, which helps prevent bone loss, increases BMD and bone strength, and ultimately improves bone and functional outcomes in elderly patients with osteoporosis (43, 44). Some studies have also shown that exercise regulates neuroendocrine function, promotes calcium absorption and utilization, and reduces calcium loss from bones, effectively preventing osteoporosis (45). However, long-term lack of weight-bearing and muscle activity can result in significant calcium loss from bones, exacerbating osteoporosis.

Likewise, irregular adherence to anti-osteoporosis medications disrupts their therapeutic efficacy, diminishes bone protection, weakens bone structure, and heightens the susceptibility to fractures (46). Anti-osteoporosis medications are designed to manage the advancement of osteoporosis and fortify bone integrity. Non-compliance with the medication regimen impedes this process, hastens osteoporosis progression, and complicates the assessment of treatment efficacy and subsequent adjustment of treatment protocols. Recent research has revealed that patients who inconsistently adhere to anti-osteoporosis treatment face a 3.40-fold higher risk of subsequent fractures compared to those who consistently follow the prescribed medication regimen (47).

TABLE 3 Risk factors related to the new vertebral fracture after PVP/PKP in OVCF patients (Multivariate logistic regression analysis).

Clinical parameters	B value	SE value	Wald value	*p*-value	OR value	Odds ratio 95% CI
Age (year)	0.413	0.462	5.327	0.032*	1.519	1.046∼1.852
Diabetes	1.186	0.601	3.897	0.048*	3.273	1.008∼10.621
HB (g/L)	−0.028	0.035	0.629	0.428	0.973	0.909∼1.041
TP (g/L)	−0.152	0.119	1.635	0.201	0.859	0.680∼1.085
ALB (g/L)	−0.065	0.096	0.453	0.501	0.937	0.777∼1.131
HDL-C (mmol/L)	0.015	1.592	0.001	0.993	1.015	0.045∼23.009
β-CTX (ng/ml)	1.665	2.213	0.567	0.452	5.288	0.069∼404.335
PTH (ng/ml)	−0.077	0.046	2.810	0.094	0.926	0.846∼1.013
CSA (cm2)	−0.572	0.219	6.803	0.009*	0.564	0.367∼0.867
VB (cm^2)	−0.309	0.186	2.779	0.096	0.734	0.501∼1.056
FDR (%)	0.451	0.205	4.871	0.027*	1.571	1.052∼2.345
25-OH-D3 (nmol/L)	−0.138	0.064	4.581	0.032*	0.871	0.768 0.988
Number of fractured vertebrae	0.495	0.578	0.735	0.391	1.641	0.529∼5.093
BMD (T-score)	−2.230	1.107	4.057	0.044*	0.108	0.012 0.942
Exercise regularly	−1.350	0.604	4.992	0.025*	0.259	0.079 0.847
Anti-osteoporosis	−1.233	0.616	4.004	0.045*	0.291	0.087 0.975

HB, hemoglobin; WBC, white blood cell; TP, total protein; ALB, serum albumin; UA, uric acid; Glu, blood glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; β-CTX, b-C-terminal telopeptide of type I collagen; OC, osteocalcin; P1NP, N-terminal propeptides of type I procollagen; PTH, parathyroid hormone, BMD, bone mineral density; CSA, cross-sectional area; FDR, fatty degeneration ratio; VB, vertebral body size. *Indicates *p*-value < 0.05, meaning a statistically meaningful.

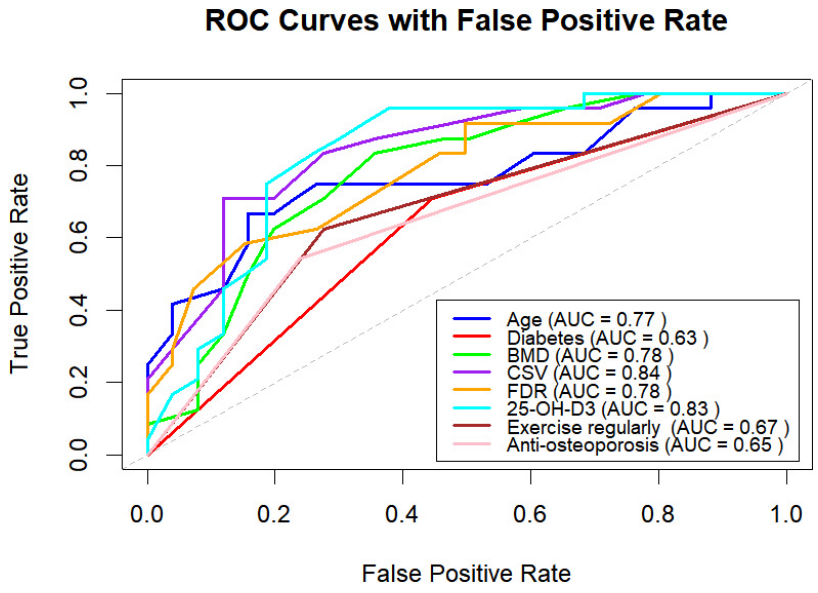

FIGURE 3
ROC curves of the selected variables after screening.

Therefore, to effectively mitigate the risk of subsequent fractures, individuals with osteoporosis should participate in suitable and consistent exercise regimens and adhere to their anti-osteoporosis medication schedules post-discharge. This proactive approach is vital for preserving the health of bones, muscles, and joints (48, 49).

Nevertheless, several limitations persist within this study. Primarily, the study's sample size is modest, inevitably introducing biases. Another limitation of this study is the insufficient sample size, which prevented the integration of various predictive factors to develop a new clinical prediction model for vertebral refracture. Furthermore, the clinical data were solely collected from the orthopedic department of our hospital, thus lacking comparative analysis with other centers. Hence, further large-scale, multicenter prospective studies are imperative to corroborate these findings.

Conclusion

In conclusion, our findings highlight several critical risk factors associated with subsequent fractures in elderly patients with OVCF, including advanced age, low bone mineral density, low 25-OH-D levels, irregular post-discharge exercise, and inconsistent use of anti-osteoporosis medications. Notably, we emphasize that regular physical activity and strict adherence to osteoporosis treatment significantly reduce the risk of new vertebral fractures. These results underscore the importance of personalized interventions targeting modifiable factors to improve long-term outcomes in this vulnerable population.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Medical Ethics Committee of Longyan First Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

WY: Investigation, Methodology, Supervision, Writing – original draft. KZ: Formal analysis, Methodology, Writing – original draft. XL: Conceptualization, Data curation, Formal analysis, Writing – original draft. YY: Methodology, Software, Writing – review and editing. TC: Conceptualization, Data curation, Investigation, Writing – review and editing. XWu: Conceptualization, Data curation, Writing – review and editing. XWa: Investigation, Writing – review and editing. QL: Investigation, Methodology, Project administration, Validation, Writing – review and editing. CH: Data curation, Investigation, Writing – original draft, Methodology, Supervision. WS: Conceptualization, Methodology, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI Statement

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Utility of osteoporosis screening based on estimation of bone mineral density using bidirectional chest radiographs with deep learning models

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Introduction: Osteoporosis increases the risk of fragility fractures, especially of the lumbar spine and femur. As fractures affect life expectancy, it is crucial to detect the early stages of osteoporosis. Dual X-ray absorptiometry (DXA) is the gold standard for bone mineral density (BMD) measurement and the diagnosis of osteoporosis; however, its low screening usage is problematic. The accurate estimation of BMD using chest radiographs (CXR) could expand screening opportunities. This study aimed to indicate the clinical utility of osteoporosis screening using deep-learning-based estimation of BMD using bidirectional CXRs.

Methods: This study included 1,624 patients aged ≥ 20 years who underwent DXA and bidirectional (frontal and lateral) chest radiography at a medical facility. A dataset was created using BMD and bidirectional CXR images. Inception-ResNet-V2-based models were trained using three CXR input types (frontal, lateral, and bidirectional). We compared and evaluated the BMD estimation performances of the models with different input information.

Results: In the comparison of models, the model with bidirectional CXR showed the highest accuracy. The correlation coefficients between the model estimates and DXA measurements were 0.766 and 0.683 for the lumbar spine and femoral BMD, respectively. Osteoporosis detection based on bidirectional CXR showed higher sensitivity and specificity than the models with single-view CXR input, especially for osteoporosis based on T-score ≤ −2.5, with 92.8% sensitivity at 50.0% specificity.

Discussion: These results suggest that bidirectional CXR contributes to improved accuracy of BMD estimation and osteoporosis screening compared with single-view CXR. This study proposes a new approach for early detection of osteoporosis using a deep learning model with frontal and lateral CXR inputs. BMD estimation using bidirectional CXR showed improved detection

performance for low bone mass and osteoporosis, and has the potential to be used as a clinical decision criterion. The proposed method shows potential for more appropriate screening decisions, suggesting its usefulness in clinical practice.

KEYWORDS

bone mineral density, osteoporosis, screening, chest radiograph, artificial intelligence

1 Introduction

Osteoporosis is a systemic bone disease that causes bone fragility due to loss of bone mass (1). Osteoporosis is the most significant risk factor for fragility fractures and has a substantial impact on life prognosis (1–3). Among these, lumbar spine and femur fractures significantly worsen quality of life (4). In addition, osteoporosis typically does not cause symptoms until a fracture occurs (5, 6). Delayed diagnosis of osteoporosis leads to fragility fractures, with an estimated 37 million fragility fractures occurring annually in people aged 55 years and older worldwide between 1990 and 2019 (7). Therefore, early detection of osteoporosis before its progression is essential to prevent fragility fractures (8, 9).

Cost-effective screening is required to reduce the incidence of fragility fractures caused by osteoporosis (10). Osteoporosis is examined by measuring bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is the most accurate method for measuring BMD and remains the gold standard for screening osteoporosis (11, 12). Individual BMD measurements are assessed based on differences and ratios against the young adult mean (YAM) in skeletally healthy young adults. The T-score is an assessment index widely used as an international standard, and is the difference in an individual's BMD measurement against the YAM divided by the standard deviation of young adults' BMD. Corresponding to the World Health Organization (WHO) diagnostic categories, $-2.5 \geq$ T-score indicates osteoporosis, and $-1 >$ T-score > -2.5 indicates low bone mass (13). In some countries, the relative ratio of an individual's BMD to YAM is also used; for example, the diagnostic criteria for osteoporosis and osteopenia in Japan define osteoporosis as a BMD less than 70% of the YAM (14). Thus, osteoporosis can be objectively diagnosed based on BMD measurements. However, the high cost of DXA equipment limits the availability of medical facilities that can perform DXA, and the low uptake of DXA measurements poses a challenge for the early detection of osteoporosis (13).

Effective screening opportunities should be expanded for the early detection of osteoporosis. It would be useful if BMD could be obtained from popular medical data other than bone mineral density testing, the risk of osteoporosis could be assessed, or medical examinations could be encouraged based on objective data. Previous studies have shown that the values obtained by analyzing anatomical features, such as the cortical thickness of the clavicle, spine, and ribs on radiographs, correlate with BMD and bone mass (15–17). Furthermore, many recent studies have used deep learning and other artificial intelligence (AI) methods to analyze medical images and obtain information for purposes other than the original use. Previous studies have used deep learning models to estimate BMD using radiographic images of the lumbar spine and hip joint as inputs (18, 19) and to identify the presence of osteoporosis (20, 21), indicating the possibility of identifying osteoporosis with high accuracy. However, because lumbar and hip radiography are performed mainly as adjuncts to orthopedic consultations, the target population for the method of osteoporosis screening using lumbar and hip radiographs is limited to orthopedic patients. It has been reported that $\geq$ 75% of all individuals for whom DXA testing is recommended do not undergo it (22–27). Thus, it remains important to promote screening more broadly to target individuals without orthopedic consultation or those who have not undergone DXA testing. A method that utilizes medical data obtained at a high frequency regardless of orthopedic symptoms could effectively extend osteoporosis screening opportunities.

Chest radiography is performed consistently in primary care and during health checkups and is the most widely and frequently performed basic diagnostic imaging technique worldwide. It would be promising to expand screening without requiring additional imaging if chest radiographs (CXRs) could be used to accurately

TABLE 1 Summary of data characteristics.

Demographics		
Sex, *n* (%)		
Male	2,682	(41.6%)
Female	3,764	(58.4%)
Age, *mean* [*IQR*] (years)	60.0	[47, 75]
BMI, *mean* [*IQR*] (kg/m^2)	20.3	[17.7, 22.2]
Bone mineral density		
Lumbar BMD, *mean* [*IQR*] (g/cm^2)	0.893	[0.770, 1.012]
Femoral BMD, *mean* [*IQR*] (g/cm^2)	0.616	[0.516, 0.711]
T-score, *n* (%)		
T-score ≥ -1.0	1,373	(21.3%)
$-2.5 <$ T-score < -1.0	2,411	(37.4%)
T-score ≤ -2.5	2,662	(41.3%)
BMD/YAM, *n* (%)		
BMD/YAM $\geq$ 80%	2,650	(41.1%)
70% $<$ BMD/YAM $<$ 80%	1,373	(21.3%)
BMD/YAM $\leq$ 70%	2,423	(37.6%)

Age, BMI, lumbar BMD, and femoral BMD are shown as mean [25, 75 percentiles]. *n* is the number of applicable data-pairs and not the number of participants. BMI, body mass index; BMD, bone mineral density; IQR, Interquartile range; YAM, young adult mean.

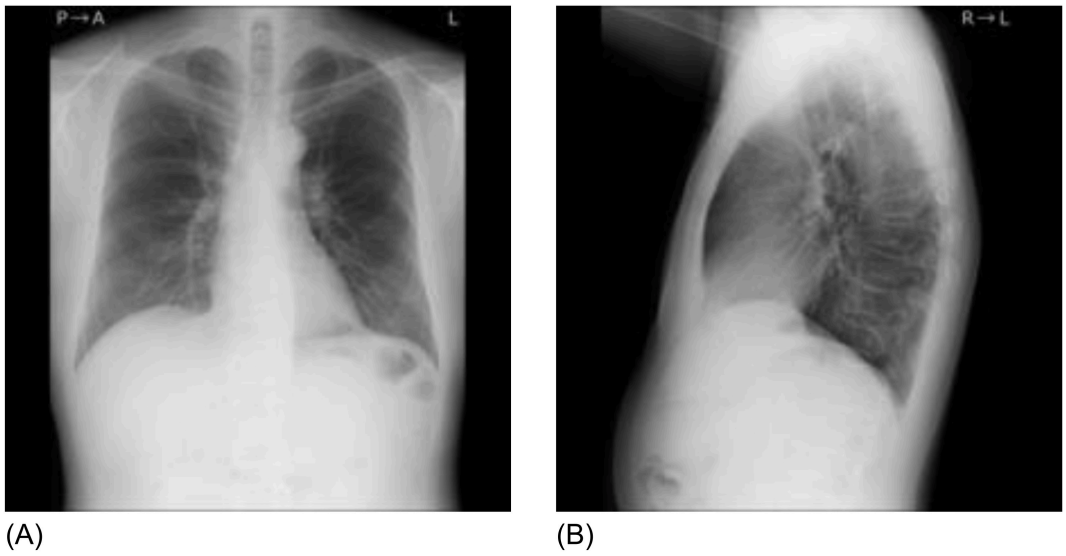

FIGURE 1
Sample chest radiographs of one patient used in this study. **(A)** Frontal chest radiograph, **(B)** Lateral chest radiograph. The original images were down-sampled and zero-padded to 1,024 × 1,024 matrixes with the aspect ratio preserved and resized to 299 × 299 as the input size for the pre-trained model.

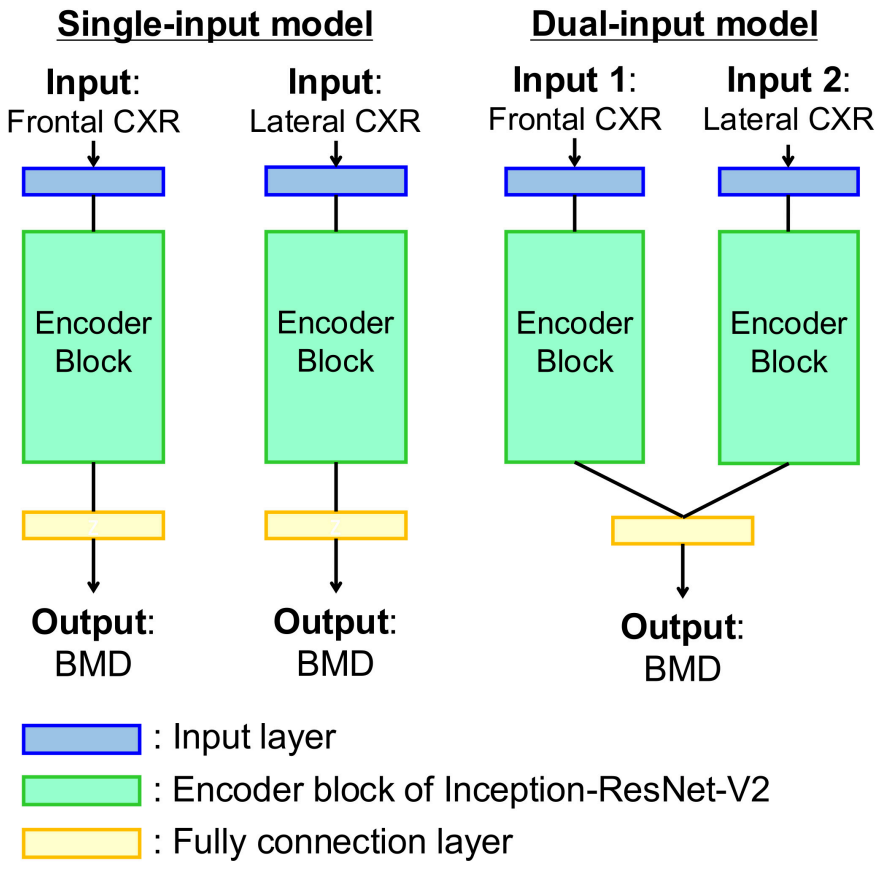

FIGURE 2
Diagrams of the model for estimating bone density from chest radiographs. **(Left)** Single-input model with input of frontal or lateral chest radiograph. **(Right)** Dual-input model with inputs of bidirectional chest radiographs. Each encoder block is composed of a pre-trained Inception-ResNet-V2 network as feature extractor.

assess the risk (28). Studies investigating approaches to detect osteoporosis from CXR have been reported, including studies using deep learning models that directly classified the prevalence of osteoporosis from CXR (29) and estimated BMD from CXR with patient information (30, 31). This BMD estimation approach would be more valuable for flexible use in clinical practice because BMD estimates could be used to identify osteoporosis and/or to consider the necessity for DXA testing. Sato et al. reported the detection of osteopenia with a cutoff T-score of −1.0, based on estimated BMD, and obtained a sensitivity of 90.14% (30). Meanwhile, the detection sensitivity for osteoporosis with a cutoff T-score of −2.5 was relatively low at 77.27%. A study evaluating the recommendation criteria for osteoporosis screening described a sensitivity of > 90% as necessary (32); for example, the National Osteoporosis Foundation guideline criteria in the United States were evaluated and found to yield a sensitivity of 96.2% and specificity of 17.8% for selecting cases with T-score ≤ −2.5 in postmenopausal women aged ≥ 45 years (32). Improving the sensitivity of osteoporosis detection is essential to promote adequate osteoporosis screening opportunities and to provide more persuasive recommendations for medical examinations in recipients undergoing chest radiography.

In primary care and health checkups in many countries, chest radiography with frontal and lateral bidirectional imaging is often performed depending on the patient's symptoms (33). A lateral CXR can visualize the thoracic region up to the upper lumbar spine, and the shape of the vertebrae can be observed. Imaging findings of fragility fractures such as vertebral compression fractures are also diagnostic criteria for osteoporosis (13), and lateral CXRs are effective for detecting vertebral fractures (34). Previous studies using lateral CXR have been reported on osteoporosis screening using lateral CXR with automatic detection using AI (35). Previous studies using AI to estimate BMD from medical images tended to show higher estimation performance when using radiographs in which the region to be measured by DXA was imaged than when it was not (36). Therefore, we hypothesized that AI with input of lateral CXR can estimate BMD effectively, and that combining frontal and lateral CXR images will improve the accuracy of AI estimation compared with using only the frontal image as input.

This study aimed to develop deep learning models to estimate BMD using only bidirectional CXR as input and to clarify their performance improvement over models using only frontal CXR as input, as well as their utility in osteoporosis screening.

2 Materials and methods

2.1 Materials

2.1.1 Data

This study was approved by the institutional review boards of the participating institutions (approval number: 18973-230111). The Institutional Review Board determined that formal informed consent was not required because this study used de-identified clinical data.

TABLE 2 Prediction results for bone mineral density of the lumbar spine and femoral neck by deep learning models with three different input type.

	R [95% CI]		MAPE [95% CI]/%	
Lumbar BMD				
Frontal	0.709	[0.682–0.707]	11.5	[11.3–11.8]
Lateral	0.732	[0.720–0.743]	11.0	[10.8–11.3]
Bidirectional	**0.766**	[0.756–0.776]	**10.6**	[10.4–10.9]
Femoral BMD				
Frontal	0.581	[0.565–0.597]	14.7	[14.3–15.1]
Lateral	0.624	[0.609–0.638]	14.7	[14.3–15.0]
Bidirectional	**0.683**	[0.670–0.696]	**13.8**	[13.5–14.1]

BMD, bone mineral density; CI, confidence interval; *R*, Pearson's correlation coefficient; MAPE, mean absolute percentage error. Bolded values of R and MAPE represent the best value for each BMD.

This study included patients aged ≥ 20 years who underwent bidirectional CXR and BMD measurement using DXA within 1 year at a single medical facility in Japan from April 2010 to July 2022. This study included DXA examinations performed at the facility during the study period and corresponding chest radiographs (frontal and lateral) performed at the facility within 1 year before and after the DXA examination. All the paired data were incorporated within the inclusion period. Data were excluded if the CXR lacked parts of the lungs or clavicles and if the CXR was performed using portable devices. A total of 1,624 cases (520 males, 1,104 females) with 6,446 data pairs (2,682 males, 3,764 females) of multiple BMD measurements and bidirectional CXR images met these criteria. The data attributes are listed in Table 1. A Horizon (Hologic Inc., Marlborough, MA, United States) was used for BMD measurements. Lumbar BMD measured in the lumbar vertebral region and femoral BMD measured in the femoral neck region were targeted. T-scores and BMD/YAM were calculated based on the mean BMD values and standard deviations for each sex of young adults, corresponding to the DXA device used for actual BMD measurements. According to the WHO (Geneva) criteria, the participants were classified based on their T-scores into normal (T-score ≥ −1.0), osteopenia (−1.0 < T-score < −2.5, and osteoporosis (T-score ≤ −2.5) groups (37). All bidirectional CXRs used in this study were obtained using frontal and lateral chest radiography. Figure 1 shows an example of a bidirectional CXR. Each original CXR image of different sizes was resized while preserving the aspect ratio and was zero-padded to a 1,024 × 1,024 matrix.

2.1.2 Dataset splitting

A 10-fold cross-validation method was used to create the datasets; each of the 10 datasets was created by randomly splitting the participants into training, validation, and evaluation ratios of 80%, 10%, and 10%, respectively. In each dataset used for cross validation, there was no contamination by the same participants between the training, validation, and evaluation data. The AI outputs for the ten evaluation datasets were combined, and all data were used for evaluation.

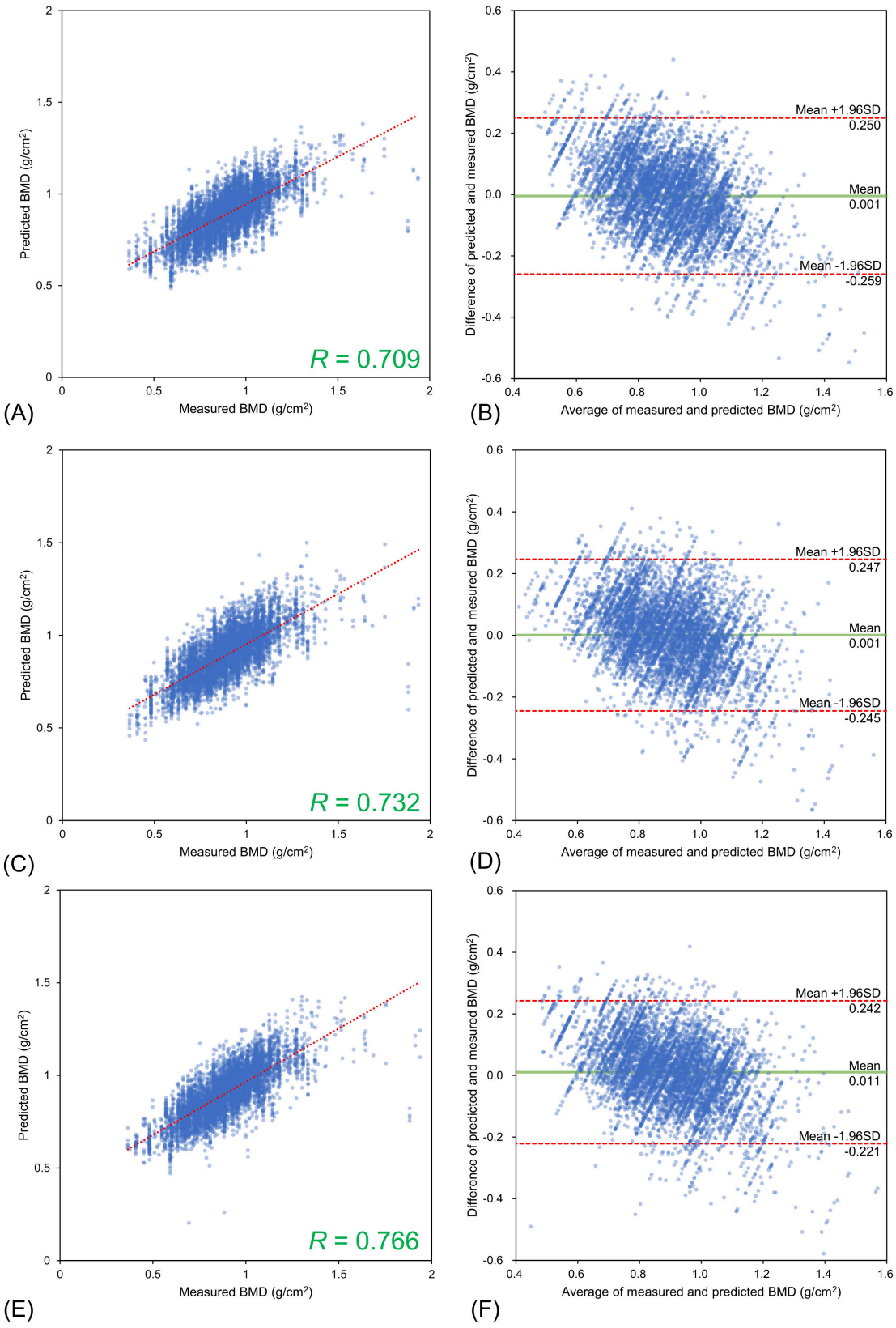

FIGURE 3
Estimation results for bone mineral density of the lumbar spine using three different input types. Upper **(A,B)** Frontal chest radiographs (CXR) input, Middle **(C,D)** Lateral CXR input, Bottom **(E,F)** Bidirectional CXR input. Left **(A,C,E)** Relationships of measured and estimated values, Right **(B,D,F)** Bland–Altman plots. All the models confirmed the agreement between model estimates and dual-energy absorptiometry measurements.

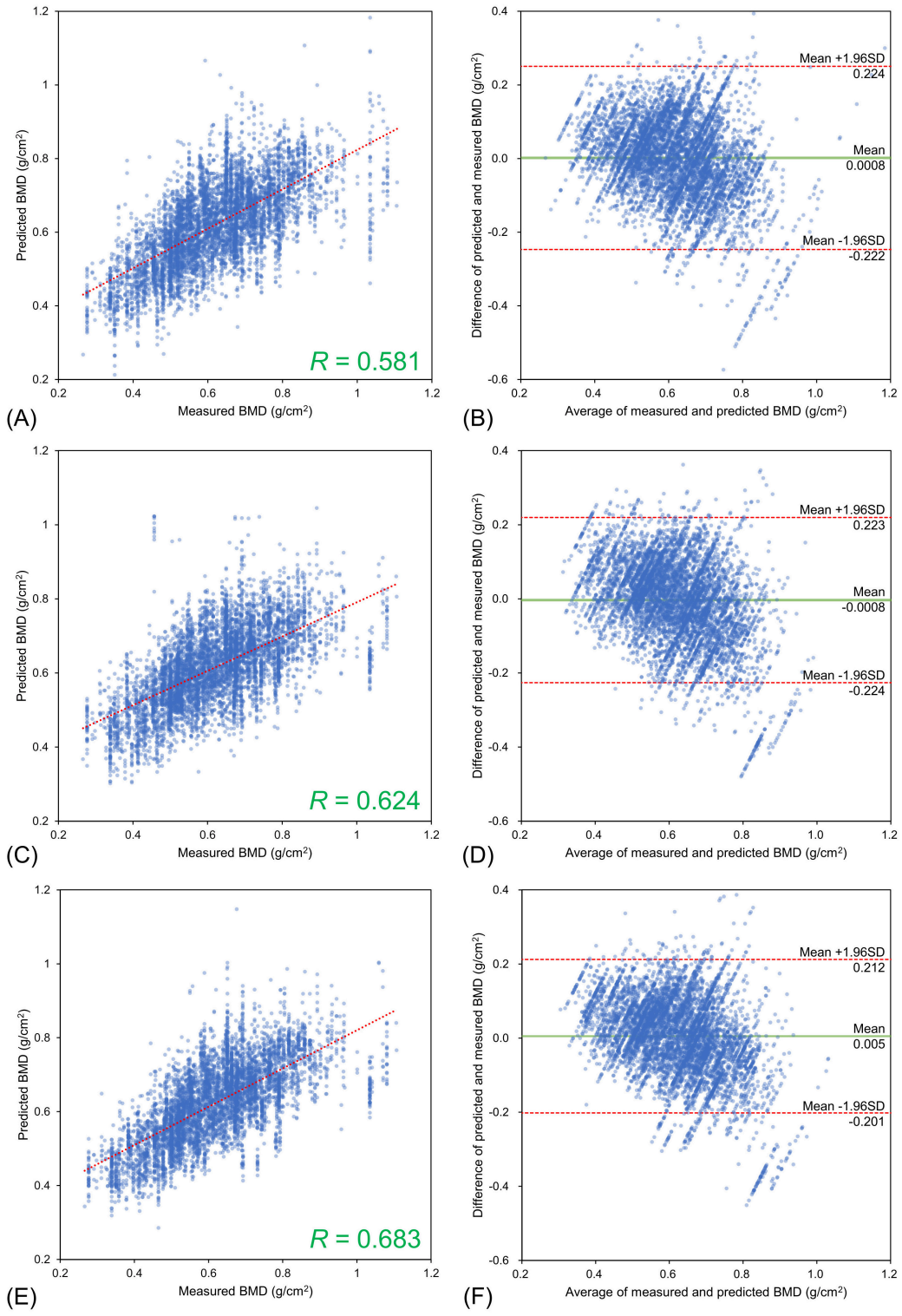

FIGURE 4
Estimation results for bone mineral density of the femoral neck using three different input types. Upper **(A,B)** Frontal chest radiographs (CXR) input, Middle **(C,D)** Lateral CXR input, Bottom **(E,F)** Bidirectional CXR input. Left **(A,C,E)** Relationships of measured and estimated values, Right **(B,D,F)** Bland–Altman plots. All the models confirmed the agreement between model estimates and dual-energy absorptiometry measurements.

2.1.3 Experimental environment

The specifications of the experimental computer were an Intel Core i5-12400 CPU, 16 GB × 2 of RAM, and an NVIDIA GeForce RTX 3090 GPU with a VRAM of 24 GB. The experiment was implemented using MATLAB 2023b (MathWorks, Inc.) on Windows 11 (Microsoft, Inc.). All image processing and deep learning network computations were performed using MATLAB.

TABLE 3 Summary of statistics in Bland–Altman analysis.

	Mean difference [95% CI] (g/cm^2)		Limits of agreement	
			Range (g/cm^2)	Agreement/%
Lumbar BMD				
Frontal	−0.0011	[−0.0079 to −0.0016]	−0.262 to 0.250	96.1
Lateral	0.0009	[−0.0021 to 0.0040]	−0.245 to 0.247	95.6
Bidirectional	0.0106	[0.0077 to 0.0135]	−0.221 to 0.242	95.5
Femoral BMD				
Frontal	0.0017	[−0.0014 to 0.0048]	−0.247 to 0.250	97.2
Lateral	−0.0033	[−0.0061 to −0.0005]	−0.226 to 0.220	95.6
Bidirectional	0.0052	[0.0026 to 0.0078]	−0.202 to 0.212	95.7

CI, confidence interval; BMD, bone mineral density.

TABLE 4 Detection results with predicted values by different models for osteoporosis and osteopenia based on T-score of measured bone mineral density.

	Accuracy	Sensitivity	Specificity	PPV	NPV	AUROC/%
BMD T-score < −1.0						
Frontal	79.5	89.1	43.8	85.4	52.1	79.4%
Lateral	78.9	**90.3**	36.9	84.1	50.7	79.0%
Bidirectional	**80.6**	90.0	**46.0**	**86.0**	**55.4**	**81.5**%
BMD T-score ≤ −2.5						
Frontal	74.5	**64.7**	81.4	71.0	76.6	82.3%
Lateral	72.6	62.1	79.9	68.5	75.0	80.7%
Bidirectional	**76.5**	63.4	**85.6**	**75.6**	**76.9**	**84.2**%

Accuracy, Sensitivity, Specificity, PPV, NPV, and AUROC are shown as percentage values. BMD, bone mineral density; PPV, positive predictive value; NPV, negative predictive value; AUROC, area under the receiver operating characteristic curve. Bolded values represent the best in each BMD T-score category.

2.2 Methods

2.2.1 Experiments

The Inception-ResNet-V2 model (38) was used as the feature extractor. The model data were acquired from the public MATLAB add-on library (39). The model data were used as a pretrained network obtained from a classification task using ImageNet (40). The classifier in the base model was replaced by a single fully connected layer, with a single output class as the regression layer. The models used in the experiments were a single-input model, which used one direction of the CXR as the input, and a dual-input model, which used a bidirectional CXR as the input. Figure 2 shows a structural diagram of the models. Using the single-input model, training was conducted to estimate the BMD using the CXR frontal or lateral views as the input. The dual-input model was implemented using two single-input models trained on frontal and lateral CXR. The trained feature extractors of the single-input models were connected in parallel to the fully connected layer. The dual-input model was trained to estimate the BMD using paired frontal and lateral CXRs inputs. The training conditions were as follows: optimization method Adam, loss function root mean square error, learning rate 1×10^{-3}, 3×10^{-4}, 1×10^{-4}, 3×10^{-5}, 1×10^{-5} (variable), batch size 32, maximum number of epochs 100, and image data augmentation with $\pm$ 5° rotation, horizontal flipping, and $\pm$ 5% scaling. The learning rate was selected to obtain the lowest loss-function value for each model. The input images were resized to the input size of the pretrained model (299 × 299 pixels) during image data augmentation and then used as the model input. The training dataset was used to update the network weights and the validation dataset was used to display the model performance at each epoch. Training was stopped early when the loss value for the validation dataset at the end of each epoch did not update the minimum for 10 consecutive epochs. The weights at the epoch with the smallest loss function output on the validation dataset were saved, thereby completing the training. Model training was conducted separately for each target to be estimated as the lumbar and femoral BMD.

2.2.2 Evaluation

The trained model was used to input chest X-ray images from the evaluation dataset and output BMD estimates. The Pearson's correlation coefficient (R) and mean absolute percentage error (MAPE) between the reference measured values and model estimates were used as the evaluation metrics. A 95% confidence interval (CI) of the correlation coefficient was obtained using Fisher's z-transform. A Bland–Altman analysis was performed using the mean values of the reference measured values and model estimates and the differences between the estimates and measured values. If the following conditions were met, the estimated values and measured values were considered equivalent: (1) The 95% CI of the mean difference between the estimated and measured values included $\pm$ 0.01 g/cm^2, and (2) more than 95% of the evaluation

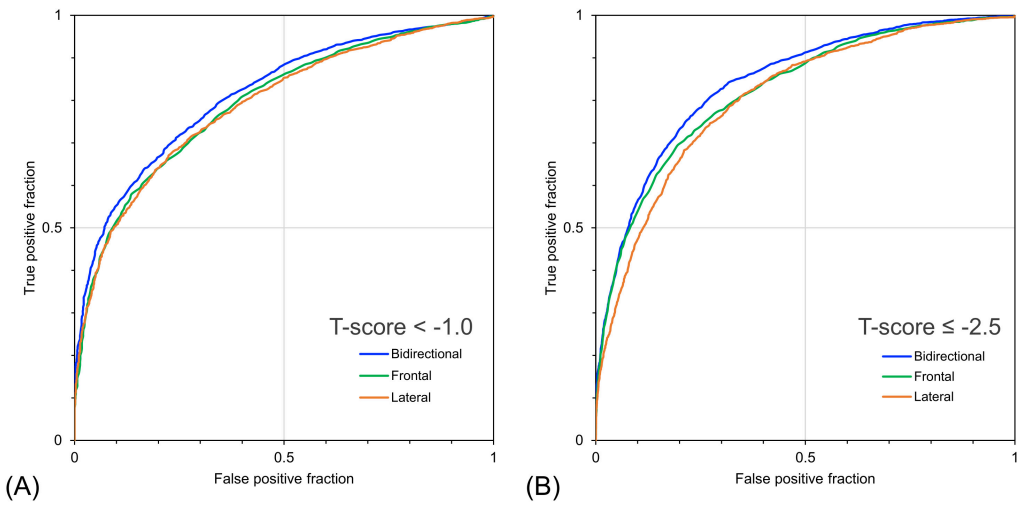

FIGURE 5
Receiver operating characteristic curves for detecting bone loss and osteoporosis based on bone mineral density (BMD) T-score using predicted bone mineral density. **(A)** Detection performance for cases with measured T-score < −1.0 and **(B)** ≤ −2.5. The bidirectional chest radiographs (CXR) input model performed better than the single-view models, particularly for detecting cases with a T-score ≤ −2.5.

TABLE 5 Detection results with predicted values by different models for osteoporosis and osteopenia based on the ratio of measured bone mineral density to the young adult mean.

	Accuracy	Sensitivity	Specificity	PPV	NPV	AUROC/%
BMD/YAM < 80%						%
Frontal	69.4	75.3	61.0	73.5	63.3	77.2%
Lateral	71.0	**79.1**	59.5	73.6	66.5	78.0%
Bidirectional	**71.6**	75.2	**66.3**	**76.2**	**65.1**	**79.7**%
BMD/YAM ≤ 70%						
Frontal	76.2	**61.5**	85.0	71.2	78.6	83.4%
Lateral	74.9	58.7	84.6	69.6	77.3	82.0%
Bidirectional	**77.8**	60.8	**88.0**	**75.4**	**78.9**	**85.6**%

Accuracy, Sensitivity, Specificity, PPV, NPV, and AUROC are shown as percentage values. PPV, positive predictive value; NPV, negative predictive value; AUROC, area under the receiver operating characteristic curve; BMD, bone mineral density; YAM, the young adult mean. Bolded values represent the best in each BMD/YAM category.

data were included within the limits of agreement (LOA) of mean ± 1.96 SD (41).

The predicted T-score and BMD/YAM ratio were calculated using the predicted BMD and actual YAM according to sex. Based on the WHO guidelines, osteoporosis (measured T-score ≤ −2.5) and osteopenia (measured T-score < −1.0) were detected from the calculated predicted T-scores. Additionally, the detection performance for osteoporosis and osteopenia was evaluated. Furthermore, based on Japanese guidelines (13), measured BMD/YAM ≤ 70% (osteoporosis) and < 80% (low bone mass) were detected from the predicted BMD/YAM. The evaluation metrics included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the receiver operating characteristic curve (AUROC).

3 Results

The results of the BMD estimation for each model with different inputs are presented in Table 2. The model with bidirectional CXR inputs showed superior correlation coefficients to models with only frontal or lateral image inputs: $R = 0.766$ (95% CI 0.756–0.776) and 0.683 (95% CI 0.670–0.696) for lumbar and femoral BMD estimation, respectively.

The lumbar BMD estimation results for each input image type relative to the DXA measurements are shown in Figure 3 and the femoral BMD estimation results are shown in Figure 4. The statistics for the Bland–Altman analysis are shown in Table 3. In the Bland–Altman analysis based on lumbar BMD estimates by the three models and actual measurements by DXA, the 95% CIs for the mean of the differences between the estimates and measurements overlapped with the reference range, indicating no fixed errors. The percentage of data within the LOA was greater than 95% for all three models. These results confirm the agreement between the lumbar BMD estimates and measured values in the three models with different input types. Bland–Altman analysis for femoral BMD estimation showed that the 95% CIs of the mean differences between the model estimates and the DXA measurements overlapped with the reference range, indicating no fixed errors. The percentage of data within the LOA was greater

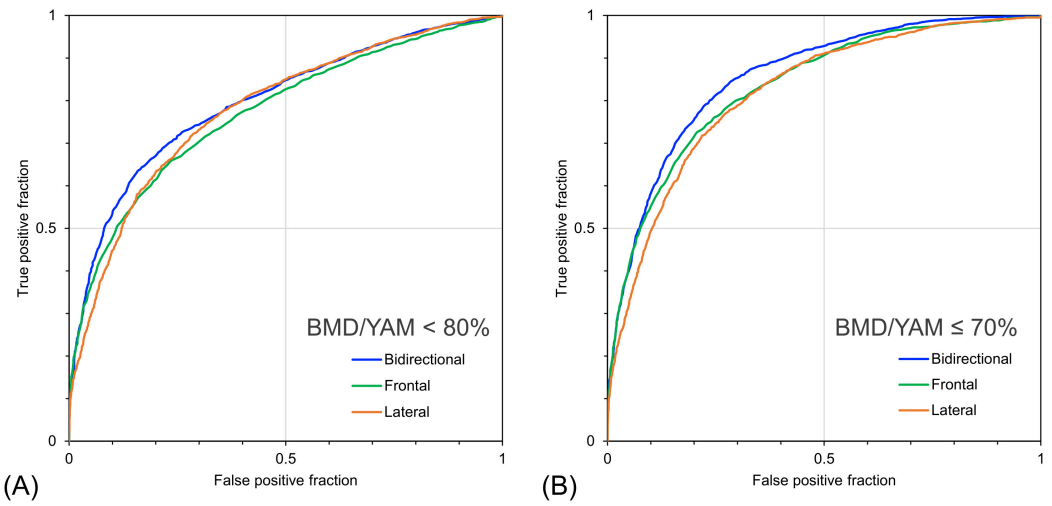

FIGURE 6
Receiver operating characteristic curves for detecting bone loss and osteoporosis based on the ratio of individual bone mineral density to the young adult mean (BMD/YAM) using predicted bone mineral density. **(A)** Detection performance for cases with measured BMD/YAM < 80% and **(B)** ≤ 70%. The bidirectional chest radiographs (CXR) input model performed better than the single-view models, particularly for detecting cases with BMD/YAM ≤ 70%.

TABLE 6 Sensitivities of the models with different inputs in detecting risk groups for osteoporosis based on measured BMD, with variable cutoffs based on model estimates and tuned specificity.

	Sensitivity/%	
	With 40% specificity	With 50% specificity
BMD T-score ≤ −2.5		
Frontal	**93.5**	88.8
Lateral	**92.4**	89.1
Bidirectional	**94.5**	**91.3**
BMD T-score < −1.0		
Frontal	**90.1**	86.1
Lateral	89.7	85.3
Bidirectional	**92.0**	88.4
BMD/YAM ≤ 70%		
Frontal	**94.9**	**90.7**
Lateral	**93.7**	**91.0**
Bidirectional	**95.8**	**92.8**

Bolded values represent sensitivity above 90%, Underlined values are the highest values for the three input types. BMD, bone mineral density; YAM, the young adult mean.

than 95% for all three models. These results confirm the agreement between the femoral BMD estimates and actual measurements for the three models with different input types.

Table 4 shows the detection performance for low bone mass and osteoporosis using the estimated T-score obtained by the models with different inputs. The model with the bidirectional CXR input yielded the highest values for all other indicators, except for sensitivity. In the model with bidirectional CXR inputs, the detection performance for T-score < −1.0 and T-score ≤ −2.5 were 90.0% and 63.4% for sensitivity, 46.0% and 85.6% for specificity, and 81.5% and 84.2% for AUROC, respectively. The model with bidirectional CXR inputs improved specificity and AUROC by approximately 2.2% and 2.1% for T-score < −1.0 and 4.2% and 1.9% for T-score ≤ −2.5, respectively, compared with the model with frontal CXR input. Figure 5 shows the ROC curves for detecting low bone mass (measured T-score < −1.0) and osteoporosis (measured T-score ≤ −2.5) using predicted T-scores. For osteoporosis detection, the model with bidirectional CXRs showed better performance than those with only frontal or lateral CXRs.

Table 5 shows the detection performance for low bone mass and osteoporosis based on the estimated BMD/YAM derived from the models with different inputs. The model with bidirectional CXR yielded the highest values for almost all indices except for sensitivity. The model with bidirectional CXR improved the specificity and AUROC for detecting osteopenia with a BMD/YAM cutoff of 80% by 5.3% and 2.5%, respectively, and for detecting osteoporosis with a BMD/YAM cutoff of 70% by 3.0% and 2.2%, respectively, compared with the model with only frontal CXR input. Figure 6 shows the ROC curves for the detection of low bone mass and osteoporosis using the predicted BMD/YAM ratios. The model with bidirectional CXR showed higher performance for osteoporosis detection than those with only frontal or lateral images.

4 Discussion

The results of this study suggest that ensemble learning models with frontal and lateral CXR inputs yield a higher BMD estimation accuracy than models with single-view CXR inputs. To the best of our knowledge, this is the first study to develop a deep learning model to estimate BMD using only lateral and frontal CXR images. The results of the ensemble model with bidirectional CXR inputs indicated higher accuracy of osteoporosis screening in clinical practice. Furthermore, our findings add value to the previous

studies on BMD estimation using chest radiographs as an input. The lateral CXR images contained information related to BMD, and the effectiveness of the lateral CXR images for estimating BMD using AI was demonstrated.

The results of lumbar spine BMD estimation using bidirectional CXR (frontal and lateral) showed a strong positive correlation of $R = 0.766$ with the actual DXA measurements, indicating the feasibility of obtaining a stronger correlation than that obtained in previous studies. Among previous studies on BMD estimation using chest radiographs, Sato et al. reported the highest estimation performance (30). Using CXR frontal images and patient information (age and sex) as model inputs, they obtained $R = 0.68$ for lumbar BMD estimation and $R = 0.75$ for femoral BMD estimation. Meanwhile, the estimation of femoral BMD using bidirectional CXRs in this study showed a moderate correlation of $R = 0.683$, which was relatively weak. The average of 5,157 training data points in the 10-fold cross-validation in this study was about 58.8% less than the 12,529 training data points used by Sato et al. Generally, the performance of deep learning models increases with the number of training data points. Our model with bidirectional CXR inputs has the potential to achieve even higher estimation performance with greater amounts of training data.

The developed dual-input model with bidirectional CXR inputs is more effective than the single-input model in terms of application to osteoporosis screening. The estimates from the model with bidirectional CXR inputs in this study identified the presence of a measured T-score of < -1.0, with 90% sensitivity and 46% specificity, and a cutoff predicted T-score of –1.0. Regarding triage screening for osteoporosis, a sensitivity of $> 90\%$ and a specificity of approximately 40%–60% or higher are considered acceptable clinical decision criteria (32), and our results with a fixed cutoff fulfilled these criteria for the identification of a T-score < -1.0. Furthermore, suppose that the cut-off of the predicted T-score based on the model output is tuned according to this criterion. In such cases, it can provide even higher sensitivity for the detection of osteoporosis. For the detection task for cases with T-score ≤ -2.5, the cutoff of predicted BMD was modified to 40% and 50% specificity. Table 6 lists the sensitivities of the models with different input information when the specificity was tuned. The model with bidirectional CXR inputs showed a sensitivity of 94.5% at a specificity of 40% and a sensitivity of 91.3% at a specificity of 50% for detecting a measured T-score ≤ -2.5. Thus, the developed model may be helpful for screening for osteoporosis and low bone mass. The sensitivity of the model with bidirectional CXR as input at 50% specificity was 91.3% for T-score ≤ -2.5, which was + 2.5% higher than that of the model with frontal CXR as input. The model with only frontal CXR showed a sensitivity of 88.8%, which was less than acceptable for the clinical decision criteria. These results indicate the usefulness of deep learning methods using bidirectional CXR for osteoporosis screening.

The results of the estimates from the bidirectional CXR model showed the feasibility of effective screening for osteoporosis and osteopenia based on the T-score and BMD/YAM criteria. A T-score ≤ -2.5 is a current global diagnostic criterion for osteoporosis. However, other criteria have been used to diagnose osteoporosis in other regions. For example, the Japanese criteria are based on BMD/YAM (37). Future diagnostic criteria may change based on the medical conditions. In addition, the management of osteoporosis risk groups is essential. Therefore, it is important to provide a robust detection performance for diagnoses based on various cutoff values. As shown in Table 6, the results of this study with bidirectional CXRs met the sensitivity $> 90\%$ and specificity $> 40\%$ levels for multiple cutoffs of T-score ≤ -2.5, T-score < -1.0, and BMD/YAM $\leq 70\%$. Thus, BMD prediction using bidirectional CXRs can provide robust screening performance for osteoporosis and its risk groups based on multiple cut-off values.

This study used only CXR images as model input to estimate BMD and did not use patient information such as age, sex, or clinical covariates. Previous studies estimating BMD from CXR used a model with frontal CXR and patient information (30, 31), although it was not clarified to what extent the AI model performance was affected by the input of patient information. The reason patient information was not input in this study was to eliminate the influence of the input of patient information on the estimation results since the purpose was to clarify the differences in performance depending on the input of CXR imaging direction and number of inputs. Another study reported that the use of patient information such as age, sex, and body mass index improved model performance (42). In future studies, our model may improve the accuracy of osteoporosis screening in primary care and health check-ups by inputting patient information and clinical covariates. This study aimed to propose an expansion of opportunity for osteoporosis screening by estimating BMD from chest radiographs, which are frequently obtained in primary care. Regarding opportunistic screening using chest radiographs, studies have reported on the estimation of pulmonary function (43–45) and the prediction of cardiovascular events (46) using deep learning models. These studies have proposed the secondary use of chest radiographs acquired for other purposes to incidentally detect the risk of lifestyle-related diseases. This study could be applied concurrently with these approaches to provide an opportunity to intervene before disease progression.

This study had two limitations. Firstly, this study used data obtained from a single facility to train and evaluate the model, which may have been affected by the regional characteristics. To validate the generalizability of this study's findings, it is important to evaluate their performance using data collected from different facilities and/or racial groups. Secondly, the diagnostic results of chest radiography were not considered. The results of this study could potentially have been influenced by imaging findings; however, their influence is unclear.

5 Conclusion

This study developed a deep learning model to estimate BMD using frontal and lateral CXRs and demonstrated the utility of the model for osteoporosis screening based on the model estimates. The model with bidirectional CXR inputs showed higher BMD estimation performance than the model with a single CXR input. This suggests the usefulness of a BMD estimation model using bidirectional CXR inputs for screening for osteoporosis and low bone mass.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Niigata University of Health and Welfare. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because this study constitutes an observational study without individually identifiable information.

Author contributions

AY: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and editing. YS: Conceptualization, Data curation, Resources, Supervision, Writing – review and editing. CK: Resources, Validation, Writing – review and editing. YH: Validation, Writing – review and editing. IS: Validation, Writing – review and editing. SK: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review and editing.

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Conflict of interest

YS was previously the president of iSurgery Co., Ltd. YH was employed by TOITU Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Deciphering the role of lncRNA-mediated ceRNA network in disuse osteoporosis: insights from bone marrow mesenchymal stem cells under simulated microgravity

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Background: Disuse osteoporosis (DOP) poses a significant health risk during extended space missions. Although the importance of long non-coding RNA (lncRNA) in bone marrow mesenchymal stem cells (BMSCs) and orthopedic diseases is recognized, the precise mechanism by which lncRNAs contribute to DOP remains elusive. This research aims to elucidate the potential regulatory role of lncRNAs in DOP.

Methods: Sequencing data were obtained from Gene Expression Omnibus (GEO) datasets, including coding and non-coding RNAs. Positive co-expression pairs of lncRNA-mRNA were identified using weighted gene co-expression network analysis, while miRNA-mRNA expression pairs were derived from the prediction database. A mRNA-miRNA-lncRNA network was established according to the shared mRNA. Functional enrichment analysis was conducted for the shared mRNAs using genome ontology and KEGG pathways. Hub genes were identified through protein-protein interaction analysis, and connectivity map analysis was employed to identify potential therapeutic agents for DOP.

Results: Integration of 74 lncRNAs, 19 miRNAs, and 200 mRNAs yielded a comprehensive mRNA-miRNA-lncRNA network. Enrichment analysis highlighted endoplasmic reticulum stress and extracellular matrix (ECM) pathways as significant in the ceRNA network. PPI analysis revealed three hub genes (COL4A1, LAMC1, and LAMA4) and identified five lncRNA-miRNA-hub gene regulatory axes. Furthermore, three potential therapeutic compounds (SB-216763, oxymetholone, and flubendazole) for DOP were identified.

Conclusion: This study sheds light on the involvement of lncRNAs in the pathogenesis and treatment of DOP through the construction of a ceRNA network, linking protein-coding mRNA functions with non-coding RNAs.

KEYWORDS

disuse osteoporosis, lncRNA, WGCNA, competing endogenous RNAs, BMSCs, CMAP

1 Introduction

While space exploration captivates our imagination, it also presents a spectrum of short- and long-term health challenges for astronauts (1). One of the most critical concerns is disuse osteoporosis (DOP), a condition characterized by accelerated bone loss due to mechanical unloading in microgravity environments (2, 3). Astronauts can experience a bone mass reduction of up to 2% per month during spaceflight, equivalent to more than a year's worth of bone loss in postmenopausal women (4). Disuse osteoporosis (DOP), arising from skeletal mechanical unloading, underscores the importance of investigating bone health under microgravity conditions (5).

Bone homeostasis depends on the balance between bone resorption by osteoclasts and bone formation by osteoblasts, processes critically regulated by bone marrow mesenchymal stem cells (BMSCs) (6, 7), which can differentiate into various cell types, including osteocytes, osteoblasts, chondrocytes, adipocytes, and smooth muscle cells (8). Strict regulation of BMSC differentiation is pivotal for maintaining bone homeostasis, as even minor disruptions can lead to orthopedic diseases such as osteoporosis (9). Although previous studies have explored the involvement of protein-coding genes in BMSC differentiation, increasing evidence suggests that non-coding RNAs, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), also play pivotal regulatory roles in this process.

Long non-coding RNA (lncRNA), a subgroup of non-coding RNAs exceeding 200 nucleotides in length, have emerged as key players in diverse cellular processes such as cell differentiation, apoptosis, gene regulation, and cancer progression (10). Recent research has implicated lncRNAs in the regulation of osteogenic differentiation in BMSCs, with aberrant expression potentially disrupting bone homeostasis (11). For instance, decreased expression of lncRNA-H19, induced by mechanical unloading, has been linked to DOP development through Wnt signaling inhibition via increased Dkk4 expression. MiRNAs are known to modulate bone metabolism under mechanical stress and are associated with disuse-induced osteopenia or osteoporosis (12). lncRNAs regulate gene expression through various mechanisms, including acting as ceRNAs that sequester miRNAs from their target mRNAs. This competitive mechanism forms lncRNA-miRNA-mRNA networks, which are involved in many physiological and pathological processes (13). While their importance is recognized, few studies have explored the roles of competing endogenous RNAs (ceRNA) networks in DOP, particularly under microgravity conditions. Understanding these networks could provide valuable insights into the molecular mechanisms of DOP and reveal novel therapeutic targets.

This study addresses a critical gap in the understanding of DOP by focusing on the underexplored role of non-coding RNAs (ncRNAs), particularly lncRNAs, in regulating bone homeostasis under microgravity conditions. While previous research has primarily focused on protein-coding genes, our work highlights the complex interplay within lncRNA-miRNA-mRNA networks and their involvement in key processes, such as endoplasmic reticulum (ER) stress and extracellular matrix (ECM) remodeling. By constructing a comprehensive competitive endogenous RNA (ceRNA) network through multi-omics integration, this study not only advances the molecular understanding of DOP but also identifies novel therapeutic targets.

Furthermore, the use of weighted gene co-expression network analysis (WGCNA) allowed us to systematically identify key regulatory axes, while Connectivity Map (CMap) analysis revealed bioactive compounds with strong translational potential, including SB-216763, oxymetholone, and flubendazole. These findings provide a foundation for developing targeted therapeutic strategies for DOP, bridging the gap between bioinformatics insights and practical applications. By filling these research gaps, our study paves the way for more effective prevention and treatment of DOP, offering significant benefits for astronauts and patients with osteoporosis caused by prolonged bed rest.

2 Materials and methods

A summary of the analysis procedure employed in this study is presented in Figure 1, while fundamental details regarding the two microarray datasets are outlined in Table 1.

2.1 GEO datasets

The relevant RNA sequencing data and microarray data were extracted from the Gene Expression Omnibus (GEO) database[1]. RNA expression profiles were derived from GSE100930 (Microarray on mRNA and lncRNA, comprising seven flight samples and six ground samples) and GSE100932 (miRNA-seq, including six flight samples, and three ground samples). These datasets were generated using the platforms GPL6244 [HuGene-1_0-st] and GPL11154 [Illumina HiSeq 2000], as initially described by Bradamante and co-workers.

2.2 Data preprocessing

The Bioconductor software package 3.8[2] and the R v.3.5.2[3] were utilized to preprocess the gene expression datasets. Specifically, the R software was employed via the Agilent platform to conduct preprocessing and normalization of the Series Matrix Files (.TXT files). Principal component analysis (PCA), a mathematical algorithm commonly integrated into genome-wide expression studies, was employed. PCA condenses data while preserving the bulk of its variability, facilitating visual assessment of sample similarities and differences and enabling grouping determination (14). In this study, PCA was conducted using the PRCOMP function in R software based on singular value decomposition (SVD) of the data matrix.

1 http://www.ncbi.nlm.nih.gov/geo/
2 https://www.bioconductor.org/
3 https://www.r-project.org

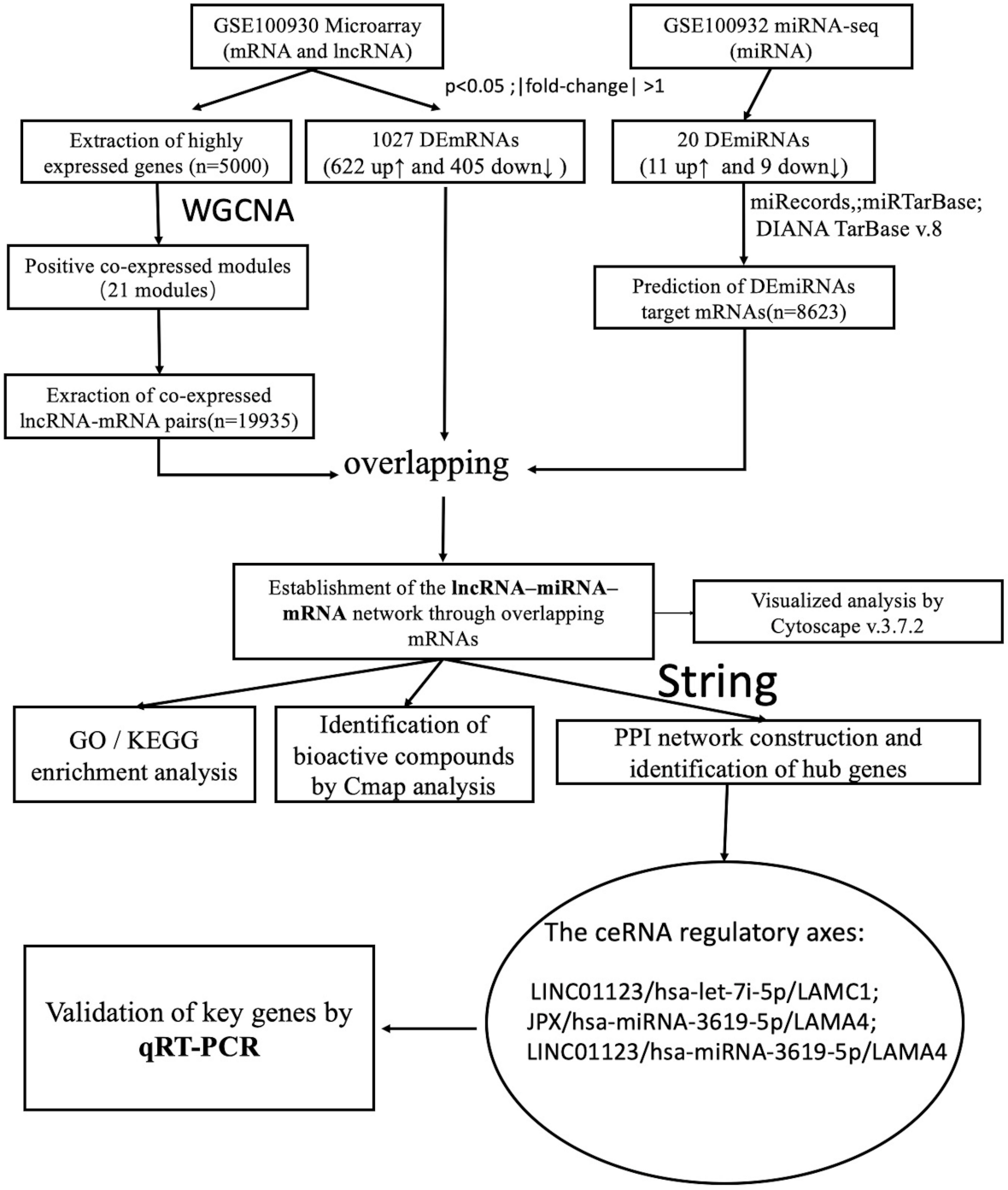

FIGURE 1
Flow chart of the analysis in this study.

TABLE 1 Basic information on the two datasets from Gene Expression Omnibus (GEO).

GEO datasets	Year	Country	Platform	Sample	N	Detected RNA type
GSE100930	2019	Italy	GPL6244	Flight Ground	7 6	mRNA and lncRNA
GSE100932	2019	Italy	GPL11154	Flight Ground	6 3	miRNA

2.3 Identification of DEmRNAs and DEmiRNAs

The limma package in R was employed to perform differential expression analysis of microarray data between Flight and Ground conditions, focusing on mRNA expression changes. Meanwhile, for miRNAs, the edgeR package in R was utilized with RNA sequencing data. A significance threshold of $p < 0.05$ and a log2 (fold-change) > 1 were utilized as cut-off thresholds.

2.4 Prediction of target mRNAs for DEmiRNAs

The multiMiR package in R, comprising 14 databases, was used to predict miRNA-mRNA interactions. The predictions mainly employed data from three databases: miRecords, miRTarBase[4] and DIANA TarBase v.8.

2.5 Construction of co-expressed gene module for lncRNA-mRNA pairs

Weighted gene co-expression network analysis is a state-of-the-art technique for deciphering co-expression patterns among genes (15). Firstly, from the GSE100930 dataset, 5,000 genes exhibiting the highest average expression were identified. Subsequently, the soft-thresholding power was measured, guided by the criterion of approximate scale-free topology fitting indices R^2, whereby the β value was selected once R^2 achieved 0.85. Employing the appropriate soft threshold (β = 12), the weighted network underwent transformation into a topological overlap matrix (TOM), utilized thereafter to compute a gene's network connectivity. Following this, average linkage hierarchical clustering was performed using the TOM-based dissimilarity measure, enabling the classification of genes with analogous expression profiles into distinct gene modules. Ultimately, to acquire positive lncRNA-mRNA co-expression pairs from the gene modules, the TOMType parameter in the WGCNA package was designated as "signed." The detailed code is in the Supplementary Data Sheets 1, 2.

2.6 Establishment of the mRNA-miRNA-lncRNA network

The positive lncRNA-mRNA co-expression pairs and miRNA-mRNA expression pairs were integrated based on shared mRNAs, with those containing DEmRNAs being filtered out. All mRNA-miRNA-lncRNA regulatory axes constituted the ceRNA network, which was visualized using Cytoscape v.3.7.2.

2.7 Enrichment analysis

Functional annotation of the DEmRNAs within the ceRNA network was conducted using the clusterProfiler package in R. GO terms were employed for functional enrichment analysis, while KEGG pathways were utilized for pathway enrichment analysis. A threshold of $p < 0.05$ was utilized as the cutoff for reporting significant GO terms and KEGG pathways.

2.8 Establishment of PPI network and identification of hub genes

The genes identified through the aforementioned analyses were cross-referenced with the STRING database[5] to construct a PPI network. Interactions were restricted to Homo sapiens to ensure the relevance of the results to human biology. A confidence score threshold of ≥ 0.4 (medium confidence) was set to ensure that only interactions with appropriate evidence were included in the analysis. Additionally,the constructed PPI network was imported into Cytoscape software (version 3.7.2) for visualization and analysis. Finally, the CytoHubba plugin in Cytoscape software was utilized to identify the top 10 hub genes using the Maximal Clique Centrality (MCC) algorithm.

2.9 CMap query

The CMap dataset of cellular signatures captures the transcriptional responses of human cells to both genetic and chemical perturbations. This comprehensive dataset encompasses profiles of 27,927 perturbagens, resulting in a total of 476,251 expression signatures (16). Subsequently, the upregulated and downregulated genes were compiled into respective upregulated and downregulated signatures, accessible via https://clue.io. Using pattern-matching algorithms, connectivity scores were computed to discern functional associations among drugs, genes, and diseases based on shared gene expression changes. These scores represent the degree of similarity with the expression profile, enabling the identification of bioactive compounds with the lowest connectivity scores, which are deemed potential therapeutic drugs for treating diseases.

2.10 Cell model

Sprague-Dawley (SD) rat BMSCs were generously supplied by the Stem Cell Bank, Chinese Academy of Sciences. These BMSCs were cultivated in growth medium consisting of α-MEM containing 10% FBS and antibiotics (HyClone, United States). Cultures were maintained at 37°C in a 5% CO_2 atmosphere. BMSCs assigned to the flight group were cultured in the Rotary Cell Culture System (RCCS, Synthecon Company, United States) for 72 h. During this period, the rotation speed was gradually increased from 10 to 20 (Supplementary Figures 1, 2).

4 http://mirtarbase.mbc.nctu.edu.tw/php/index.php

5 https://string-db.org/

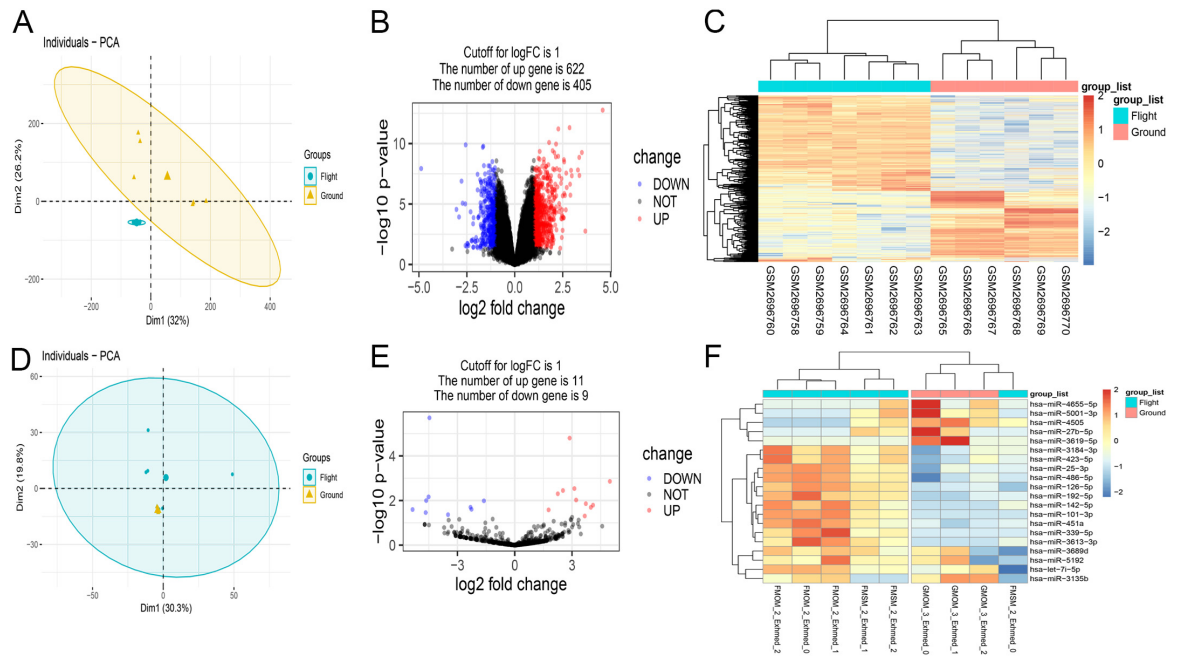

FIGURE 2
Overview of aberrantly expressed mRNAs **(A–C)** and miRNAs **(D–F)** in flight samples. **(A,D)** In principal component analysis (PCA), mRNA samples showed similarities within the group, while ground samples of miRNA did not show good similarities due to the small sample size. **(B,E)** The Volcano plot illustrating the results of mRNA and miRNA differential expression analysis. Each data point corresponds to a single mRNA or miRNA, with red indicating upregulation and blue indicating downregulation. **(C,F)** Hierarchical clustering revealed distinct mRNA or miRNA expression patterns between the flight and ground groups, demonstrating group homogeneity within each set. Each row represents a gene, and each column represents a sample. Genes with upregulation are presented as red, while those with downregulation are presented as blue, reflecting the degree of expression alteration.

2.11 Validation of key genes and ceRNA network by qRT-PCR

Total RNA was isolated using Eastep®-Super-Total-RNA-Extraction-Kit (Promega Biological Products, China). The yield of total RNA was examined using NanoDrop equipment (Thermo-Fisher-Scientific, United States). cDNA synthesis was performed using PrimeScript RT reagent Kit (Takara, Japan). Then, SYBR Green (TianGen, China) was employed to conduct qRT-PCR. All specific primer pairs (5′ to 3′) were shown as follows: (1) GAPDH: F: GGGTGTGAACCACGAGAAAT, R: ACTGTGGTCATGAGCCCTTC; (2) JPX: F: GCGAAGGTCTTG GTCACATCTGTC, R: AGAGGAGGGAAGGAAGGAAGGAAAC; (3) LINC01123: F: AGGAAGGAGGTGCTTGGCTCTC, R: TGAC AACGATGACGAGGAAACTGAC; (4) LAMC1: F: TGGACTT ACTTGCTGACTCATTGACTG, R: ATTGATGGATGGATGGAT GGATGGATG; (5) LAMA4: F: AACTGACCGAGGCTGTCAAG, R: TGAGGTTTCTCACTGCGTCC. The relative expression of genes was determined using the $2^{-\Delta\Delta Ct}$ approach, with GAPDH serving as the internal control.

2.12 Statistical analysis

The findings obtained from qRT-PCR were analyzed by Student's *t*-test using GraphPad Prism 9.0 (GraphPad Software, Inc., United States). Statistical significance was deemed at a threshold of $P < 0.05$.

3 Results

3.1 Comparative analysis of miRNA and mRNA expression profiles in BMSCs from ground and flight samples

In this study, 1,027 significant DEmRNAs (622 with upregulation and 405 with downregulation) were identified using a significance threshold of $p < 0.05$ and a log2-fold change of > 1 (Figures 2A–C). Additionally, 20 DEmiRNAs were discerned in BMSCs using the same criteria, comprising nine with downregulation and 11 with upregulation (Figures 2D–F). In total, 8,623 target mRNAs of the DEmiRNAs were estimated through miRecords, miRTarBase, and DIANA TarBase v.8.

3.2 Construction of co-expressed gene module for lncRNA-mRNA pairs

According to the expression's variance, the top 5,000 genes were first identified from flight samples in the GSE100930 dataset. Subsequently, through optimization, a soft threshold of $\beta = 12$ was determined, achieving an R^2 of 0.85 (Figures 3A, B). When the TOMType parameter in the WGCNA package was set to "signed," 21 modules were delineated via average linkage hierarchical clustering (Figures 3C, D). Ultimately, the analysis yielded 19,935

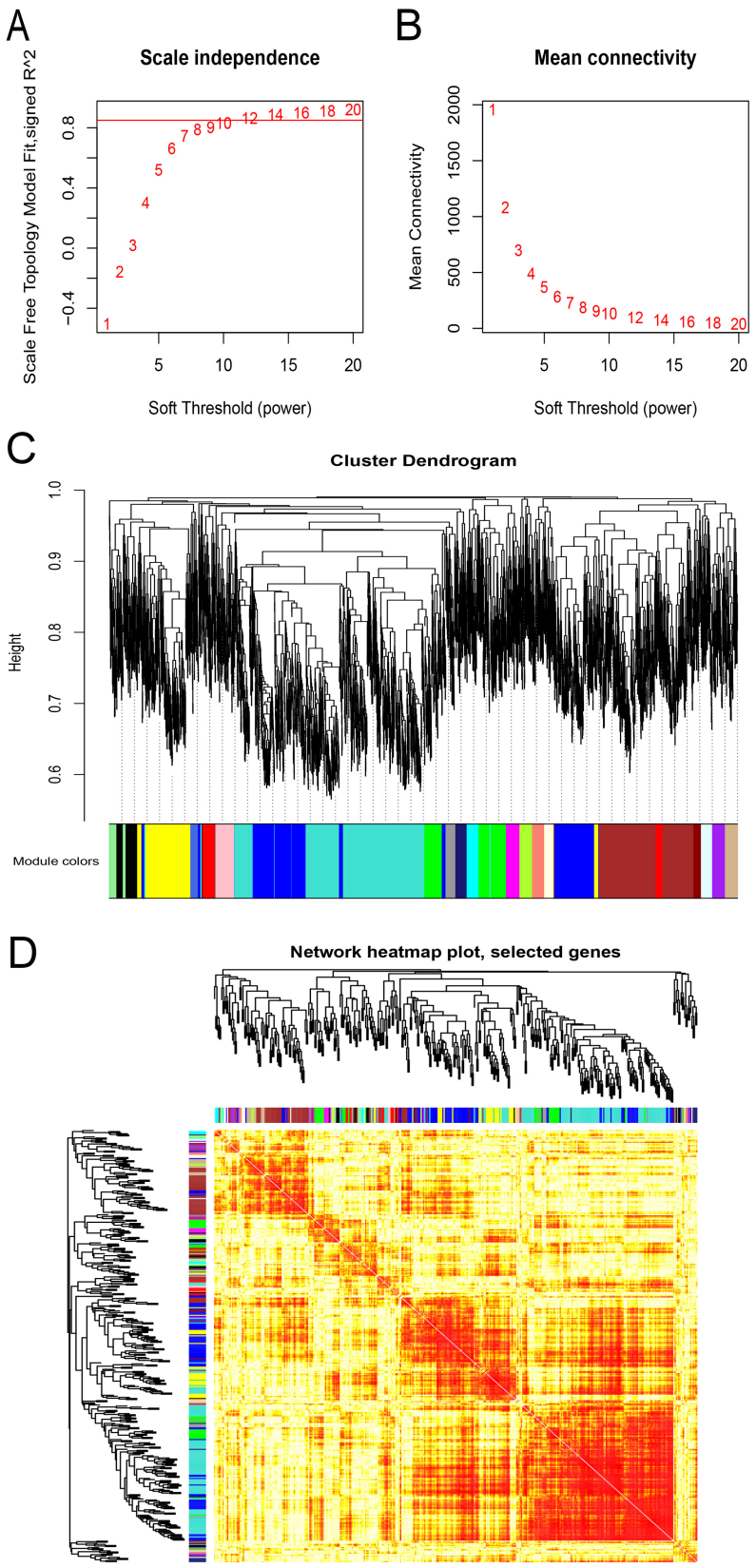

FIGURE 3
Weighted gene co-expression network analysis (WGCNA) of flight samples (GSE100930). **(A)** Assessment of scale-free fit indices across soft-thresholding powers (β). **(B)** Evaluation of mean connectivity across soft-thresholding powers. **(C)** The hierarchical clustering dendrogram was constructed according to the gene dissTOM. Each color-coded row beneath the dendrogram represents a distinct module, with module width indicating the number of co-expressed genes. **(D)** Visualization of inter-gene interactions within co-expression modules. Various colors on the vertical and horizontal axes denote diverse modules, while yellow hues denote inter-module correlations. Significant variations in correlation patterns among modules were observed.

FIGURE 4
Venn diagram showing the number of shared mRNAs. The purple represents target mRNAs predicted by DEmiRNAs. The gray represents mRNAs co-expressed with long non-coding RNA (lncRNAs). The green represents differentially expressed mRNAs.

positive lncRNA-mRNA co-expression pairs across these 21 signed modules, involving 95 lncRNAs and 4,023 mRNAs.

3.3 Establishment of the lncRNA–miRNA–mRNA network

A total of 200 shared mRNAs were obtained from positive lncRNA-mRNA co-expression pairs, predictive miRNA-mRNA expression pairs, and DEmRNAs (Figure 4). A total of 74 lncRNAs, 19 miRNAs and 200 mRNAs were then integrated into a lncRNA–miRNA–mRNA network by shared mRNAs (Figure 5), which consisted of 293 nodes and 2,075 edges.

3.4 Enrichment analysis

The 200 differentially expressed mRNAs (DEmRNAs) implicated in the competing endogenous RNA (ceRNA) network underwent enrichment analysis. Utilizing GO terms for Molecular Functions (MF), Cellular Compartments (CC), and Biological Processes (BP), the primary functions of these DEmRNAs were examined. Notably, significant enrichment was observed for GO terms associated with the collagen-containing extracellular matrix, extracellular matrix structural constituent, and extracellular matrix organization (Figure 6A). Conversely, enrichment analysis of downregulated genes highlighted terms associated with the endoplasmic reticulum chaperone complex and response to endoplasmic reticulum stress as the most significantly enriched (Figure 6B). These processes are likely involved in BMSC differentiation.

Moreover, KEGG pathways were employed for systematic gene characteristic analysis by integrating higher-level pathway information with gene data. Focal adhesion emerged as the most highly enriched pathway among DEmRNAs with upregulation, whereas protein processing in the endoplasmic reticulum ranked as the most enriched pathway among DEmRNAs with downregulation (Figure 6C).

3.5 Establishment of PPI network and identification of hub genes

The selection of 10 hub genes was guided by the Cytohubba plug-in in Cytoscape. The genes, including nine upregulated genes and one downregulated gene, were then ranked according to their Maximal clique centrality (MCC) values (Figure 7). The key genes Laminin-Subunit-Gamma-1 (LAMC1) and Laminin-Subunit-Alpha-4 (LAMA4) were identified. Furthermore, from the constructed ceRNA network, three distinct mRNA-miRNA-lncRNA regulatory axes were delineated: LINC01123/hsa-let-7i-5p/LAMC1, JPX/hsa-miRNA-3619-5p/LAMA4, and LINC01123/hsa-miRNA-3619-5p/LAMA4. Detailed information regarding the two key lncRNAs, LINC01123 and JPX, is provided in Table 2.

3.6 CMap analysis

After submitting 200 DEmRNAs into the CMap Query, including 117 upregulated signatures and 83 downregulated signatures, three bioactive compounds (SB-216763, oxymetholone, flubendazole) were identified as candidate therapeutics for treating DOP since they had the lowest connectivity scores (Table 3).

3.7 Validation of key genes and ceRNA network in DOP

To validate the findings from the microarray analysis, qRT-PCR was employed to detect the expression levels of key genes and the ceRNA network (Figure 8). The microarray analysis indicated upregulation of LAMC1 and LAMA4 in the DOP condition. According to the competitive mechanism, their corresponding lncRNAs were expected to exhibit upregulation in DOP as well. The qRT-PCR results demonstrated high expression levels of JPX, LINC01123, LAMC1, and LAMA4 in the flight group, aligning with our anticipated outcomes.

4 Discussion

Disuse osteoporosis is a prevalent condition experienced by both astronauts during spaceflight and individuals with spinal cord injuries, contributing significantly to deleterious effects on human health (5, 17). Is widely recognized that a majority of human RNA transcripts do not encode proteins but rather play crucial roles in regulating cellular physiology and influencing cellular function. Recent advances in network biology have highlighted the importance of certain properties of biological networks (18), such as scale-free distribution and hierarchical modularization, in

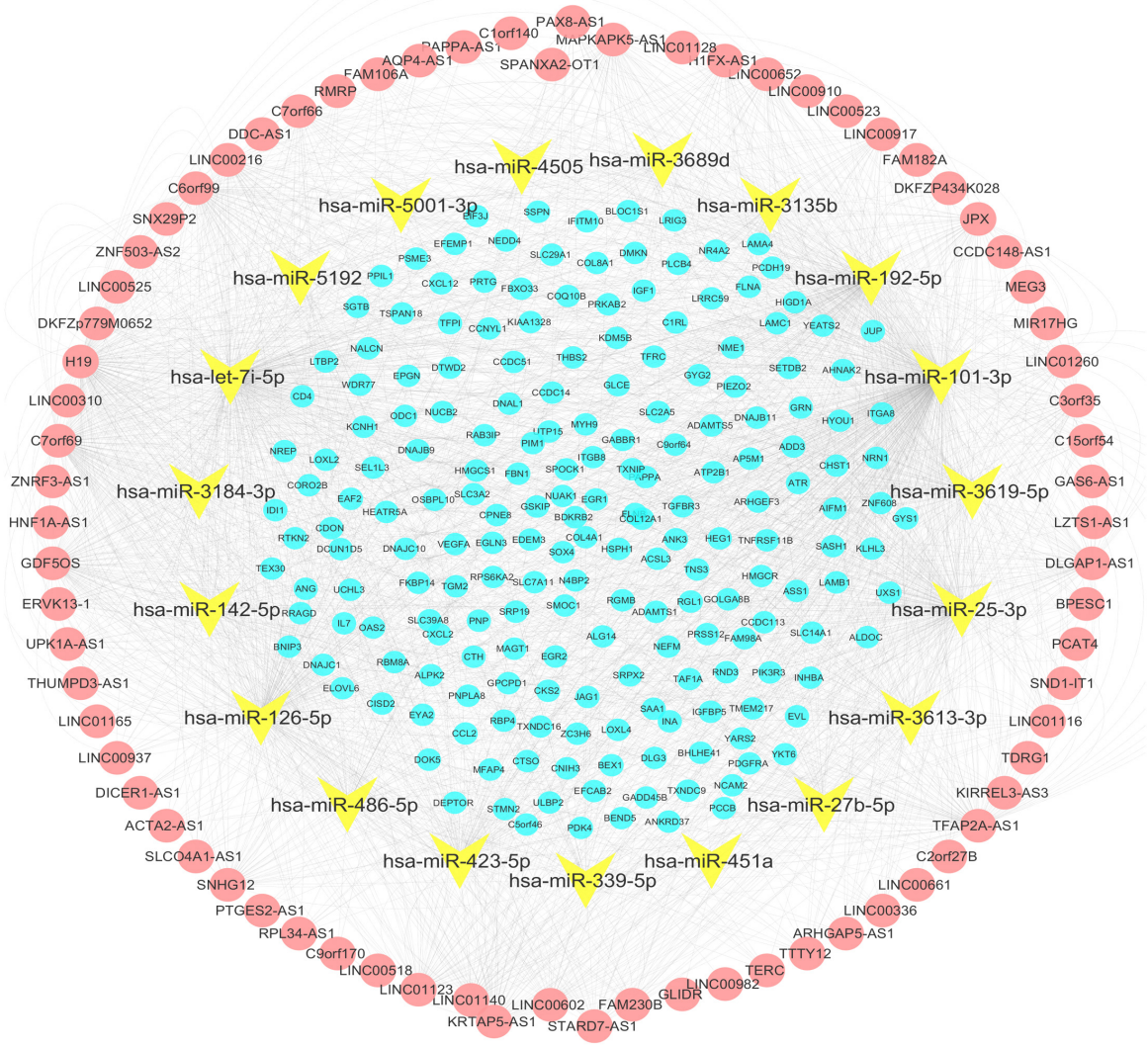

FIGURE 5
The lncRNA–miRNA–mRNA network. Nodes in yellow are miRNAs. The red nodes represent the long non-coding RNA (lncRNAs) positively correlated with the shared mRNAs. The blue nodes represent shared mRNAs. The lines represent relationships between genes.

maintaining the stability of cellular processes. This underscores the potential of regulatory network analysis to offer novel insights into the mechanisms underlying gene regulation and dysfunction in DOP. In this study, we present, for the first time, the construction of a ceRNA network for BMSCs in DOP, linking the functions of protein-coding mRNAs with non-coding RNAs, including lncRNAs and miRNAs.

Through functional enrichment analysis, we propose a hypothesis that following the cessation of mechanical stress, dysregulated lncRNAs may disrupt signaling pathways involved in endoplasmic reticulum (ER) stress and extracellular matrix (ECM) collagen production. This disruption, in turn, could impact bone mineral deposition, turnover, and osteocyte activity (19). ER stress, associated with cellular damage to the protein-folding mechanism, has been reported to inhibit osteoblast formation, increase osteoclast numbers, and enhance their migration and adhesion, consistent with our enrichment analysis findings (20). Notably, focal adhesion emerged as a significant term in both GO and KEGG analyses. Focal adhesion plays a crucial role in mediating cellular interactions with the ECM and transducing mechanical forces and regulatory signals (21). Furthermore, in vivo studies have demonstrated the assembly of focal adhesion complexes in response to mechanical overload, suggesting its potential involvement in DOP development (22, 23). However, further investigation is warranted to validate this observation. This underscores the importance of understanding the effects of microgravity on osteoblast cytoskeletal adhesion and organization as well as osteoclast bone resorption in regulating DOP progression.

The PPI network analysis revealed two hub genes, LAMC1 and LAMA4. LAMC1, a pivotal member of the laminin family, interacts with integrins, promoting cell migration and adhesion (24). Additionally, LAMC1 plays a significant role in aging BMSCs (25). LAMA4, a key component of the bone marrow ECM, influences the proliferation and survival of stem and progenitor cells (26). Both hub genes are associated with the ECM term (GO: 0031012) in GO analysis. The mineralization process of bone tissue exhibits unique biomechanical properties, and the investigation into vertebrate extracellular matrix mineralization remains a focal

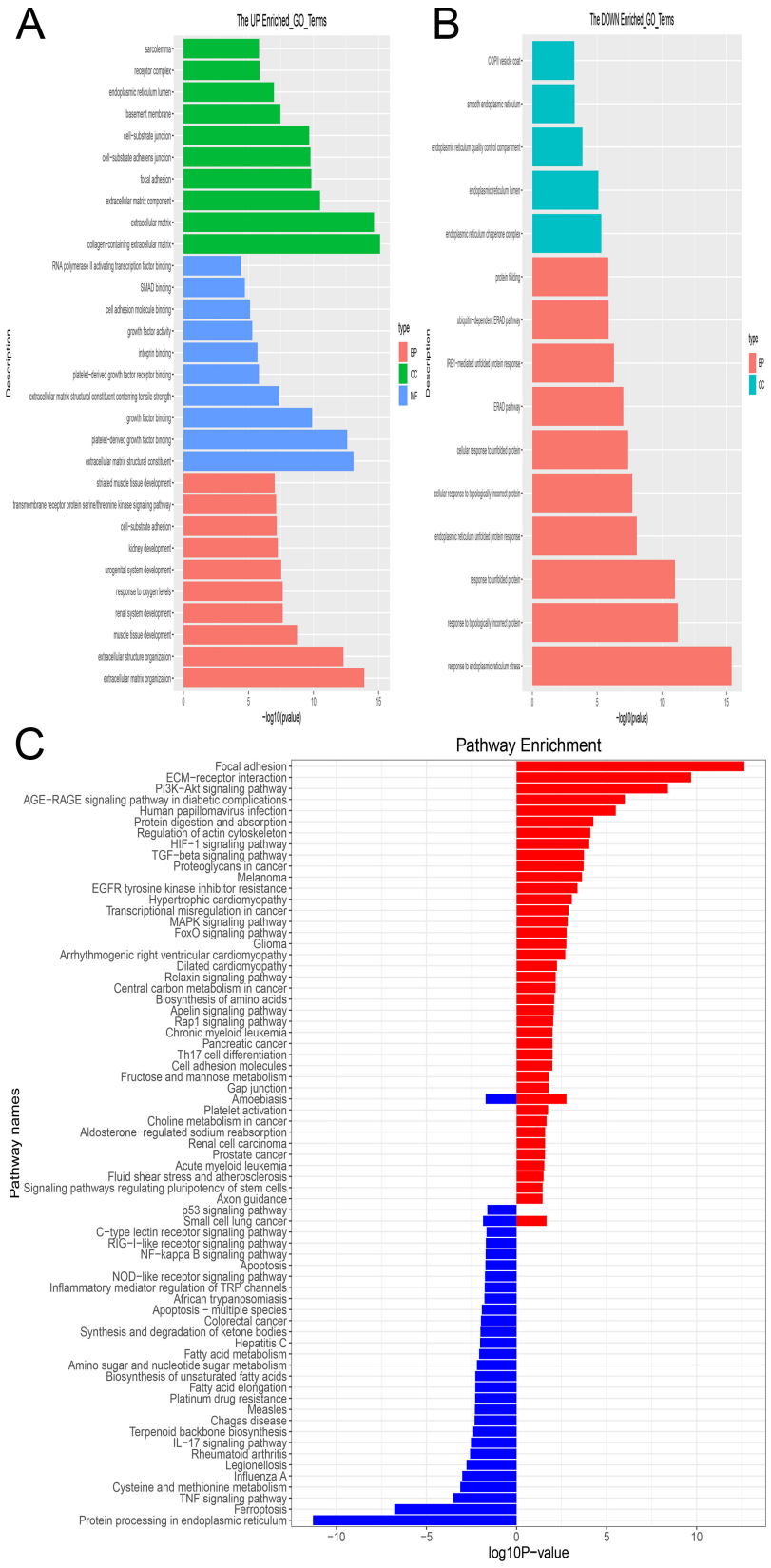

FIGURE 6
The enrichment analysis of shared mRNA. **(A,B)** GO enrichment analysis was performed across three functional categories: Molecular Functions (MF), Cellular Compartments (CC) and Biological Processes (BP). Panel **(A)** indicates enrichment by upregulated genes, while Panel **(B)** indicates enrichment by downregulated genes. The y-axis denotes the enriched GO term, while the x-axis denotes the corresponding *p*-value. **(C)** Enrichment analysis of shared mRNAs using the KEGG pathways. The enriched pathway is depicted in red, comprising upregulated genes, while the enriched pathway is shown in blue, comprising downregulated genes.

TABLE 2 Basic information on the two long non-coding RNAs (lncRNAs).

LncRNA	Entrez_ID	Locus	Genomic length	Strand
JPX	554203	chrX:73,944,182-74,070,832	126651	+
LINC01123	440894	chr2:109,986,583-109,996,414	9832	+

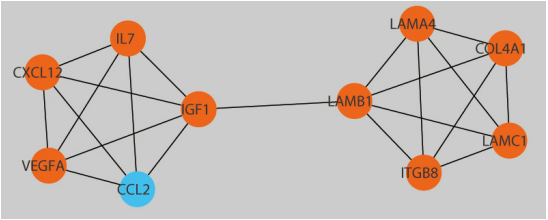

FIGURE 7
Detection of central genes within the PPI network utilizing the Cytohubba plugin in Cytoscape. Hub genes with upregulation are presented as red, while those with downregulation are presented as blue.

point in research (27). Bone comprises hundreds of ECM proteins, and the ECM of various bone tissue compartments directs bone remodeling through the coordinated actions of osteoblasts and osteoclasts (28). Exploring key genes within the ECM could elucidate its role in DOP.

In our study, two key regulatory axes, JPX/hsa-miR-3619-5p/LAMA4 and LINC01123/hsa-let-7i-5p/LAMC1, were identified as critical modulators of ECM remodeling and bone homeostasis. Both JPX and LINC01123 function as competitive endogenous RNAs (ceRNAs), sequestering miRNAs to prevent the suppression of LAMA4 and LAMC1, two essential ECM components. This enhances ECM structural stability, promotes osteoblast attachment and proliferation, and modulates osteoclast activity, thereby influencing bone metabolism.3.

The lncRNA JPX, known as an activator of Xist, can activate Xist both in trans and cis (29). XIST has been implicated in promoting osteoporosis by inhibiting the differentiation of BMSCs (30, 31). Moreover, the Wnt/β-catenin pathway, crucial for DOP development, may be activated by hsa-miR-3619-5p through enhancing the stability of β-catenin (32). Our findings suggest that JPX, acting as a ceRNA, modulates hsa-miR-3619-5p levels, influencing the expression of LAMA4 and the remodeling of ECM. Dysregulation of this axis could destabilize bone matrix integrity and disrupt osteoblast and osteoclast activity. Activation of Wnt signaling via hsa-miR-3619-5p may partially mitigate bone loss associated with DOP, presenting a potential therapeutic strategy. The JPX/hsa-miR-3619-5p/LAMA4 axis thus emerges as a critical regulatory mechanism in ECM remodeling and DOP progresion.

Previous studies have demonstrated the ceRNA crosstalk network involving LINC01123, which acts as a decoy to sequester miRNA-199a-5p from binding c-Myc mRNA, thereby attenuating its suppressive effect on c-Myc expression (33). Although hsa-let-7i-5p has not been extensively studied in bone-related research, it has shown potential as a diagnostic and predictive biomarker in other disease models (34, 35). Further investigation is warranted to elucidate the potential functions of these lncRNAs and miRNAs. In our study, LINC01123 was identified as a competitive endogenous RNA (ceRNA) that sequesters hsa-let-7i-5p, thereby upregulating LAMC1 expression and supporting ECM integrity. Dysregulation of this axis, particularly the overexpression of LINC01123, could impair osteoblast differentiation and enhance osteoclast activity, disrupting bone matrix mineralization and turnover. This dysregulation may exacerbate the pathological progression of disuse osteoporosis (DOP), highlighting the critical role of the LINC01123/hsa-let-7i-5p/LAMC1 axis in maintaining bone homeostasis under mechanical unloading conditions.

Based on CMap analysis, three chemicals, namely, SB-216763, Oxymetholone, and Flubendazole, emerged as potential therapeutic agent for treating DOP. SB-216763, an inhibitor of glycogen synthase kinase 3 (GSK-3), regulates osteoclast differentiation by phosphorylating NFATc1. Moreover, GSK-3, activated by dexamethasone, inhibits the osteoblast differentiation-related cell cycle (36–38). Notably, GSK-3 also plays a crucial role in the Wnt/β-catenin axis, a potential target for osteoporosis treatment (39, 40). Therefore, SB-216763 holds promise as a significant drug candidate for DOP treatment.

Oxymetholone is an androgen and anabolic steroid (AAS) which is used for treating osteoporosis (41). Its anabolic effects are mediated through androgen receptor activation, which stimulates protein synthesis and mineral deposition in bone tissue. Oxymetholone has a well-established clinical history in treating osteoporosis and other conditions associated with bone fragility. Its ability to enhance bone mass and strength makes it a valuable option for managing severe DOP, particularly in patients with significant bone loss due to prolonged immobilization. Our data were consistent with previous results; and to avoid repetition of previously published discussion, the data will not be examined in depth here.

Flubendazole, a tubulin inhibitor, has been extensively evaluated in humans and animals for the treatment of intestinal parasites and systemic worm infections. Tubulin is a major component of microtubules, which are cytoskeletal components needed for cell division, cell transport, and cell integrity (42). The microtubule-disrupting mechanism underpinning flubendazole's antiproliferative effects has garnered significant interest in oncology research. Preclinical studies have demonstrated its broad-spectrum anti-tumor efficacy across diverse malignancies (43, 44). In addition,previous studies showed that another tubulin inhibitor vinpocetine had a similar binding domain like that for flubendazole, and was shown to inhibit RANKL-induced osteoclastogenesis and thereby attenuate bone loss (45). However, the direct effects of flubendazole on bone metabolism, particularly concerning osteoblasts and osteoclasts, remain inadequately explored. As a benzimidazole anthelmintic agent, flubendazole demonstrates a well-established safety profile at standard therapeutic doses, with minimal incidence of severe adverse reactions in clinical practice (46, 47). However, its pharmacological safety requires rigorous reassessment when considering potential applications in bone disorders, particularly in the context of bone-related pathologies where drug-bone cell interactions remain undefined. That flubendazole may be a

TABLE 3 Three compounds with high negative connectivity scores in Connectivity Map (CMap) analysis.

Name	ID	Target	Score	MOA
SB-216763	BRD-K59184148	GSK3B, CCNA2, CDK2, GSK3A	−97.86	Glycogen synthase kinase inhibitor
Oxymetholone	BRD-A23637604	AR	−97.55	Androgen receptor agonist
Flubendazole	BRD-K86003836	TUBB	−96.54	Tubulin inhibitor

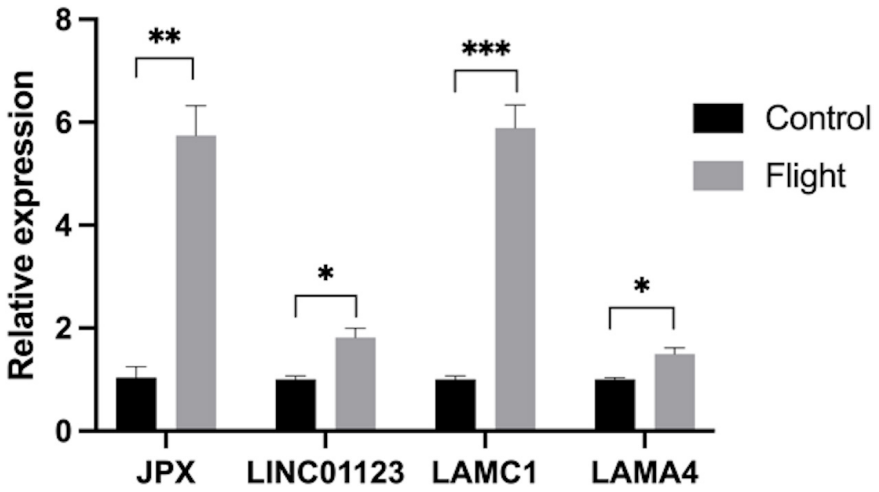

FIGURE 8
Expression of JPX, LINC01123, LAMC1 and LAMA4 in samples from rats in the flight group (in gray) and the control group (in black) (*$P < 0.05$, **$P < 0.01$,***$P < 0.001$).

potential drug for reducing bone loss after DOP is also worthy of further study.

However, we acknowledge several limitations in this research. Firstly, the sample size of BMSCs in the dataset was small, although it met the statistical significance requirements for biological duplication. The relatively small sample size may limit the robustness of miRNA differential expression analysis and we will integrate larger datasets in future research to enhance the reliability of the results. Secondly, although we constructed the ceRNA network, further investigation is needed to elucidate the precise mechanisms of action of these lncRNAs, miRNAs, and mRNAs through targeted studies. Integrating more data in the future will enhance the accuracy and completeness of the ceRNA network. As our future direction, we aim to explore the direct molecular biological mechanisms of DOP-specific ceRNAs and how dysregulated ECM remodeling contributes to DOP. Additionally, further studies on bioactive compounds will investigate their potential in translational medicine.

5 Conclusion

In conclusion, through bioinformatics analysis of BMSC gene expression data under microgravity conditions, we constructed the first mRNA-miRNA-lncRNA network. This network sheds light on the potential role of ceRNA in regulating BMSCs under microgravity and provides novel insights into the regulatory mechanisms and therapeutic targets for DOP.

Data availability statement

The original contributions presented in this study are included in this article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

WW: Validation, Writing – original draft, Writing – review and editing. ZZ: Funding acquisition, Writing – original draft. BL: Data curation, Writing – original draft, Writing – review and editing. ZF: Validation, Visualization, Writing – original draft. JL: Funding acquisition, Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2025.1444165/full#supplementary-material

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