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Organ system crosstalk in degenerative musculoskeletal diseases

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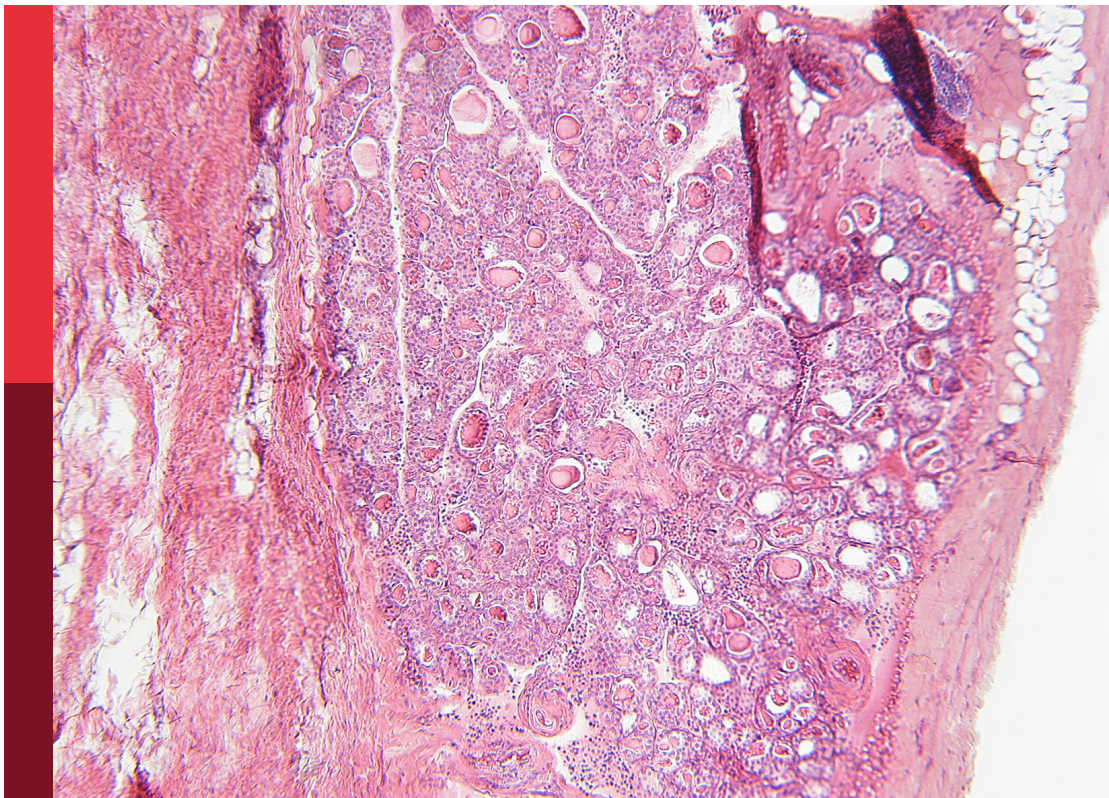

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Organ system crosstalk in degenerative musculoskeletal diseases

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Editorial: Bone-organ axis: an expanding universe

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Editorial on the Research Topic
Bone-organ axis: an expanding universe

The musculoskeletal system, long regarded as a biomechanical scaffold, is increasingly recognized as a dynamic player in systemic physiology, profoundly interconnected with other organ systems (Figure 1). From a physiological perspective, bone functions as an endocrine organ that coordinates bodily functions, thereby enhancing the perception of the surrounding environment and improving athletic performance (1). Pathologically, degenerative musculoskeletal diseases such as osteoporosis, osteoarthritis (OA) and intervertebral disc degeneration (IVDD) are not merely localized bone/cartilage disorders but manifestations of broader systemic imbalances such as metabolic syndrome (2), sarcopenia (3, 4), senescence (5), and systemic inflammation. The crosstalk between bone and other organs—mediated by a complex interplay of biochemical signals, metabolic pathways, and endocrine functions—offers new perspectives on the pathogenesis of these diseases and potential avenues for therapeutic innovation. This Research Topic of *Frontiers in Endocrinology* explores these intricate relationships, with a particular emphasis on the bone-organ axis.

The bone-endocrine interface: a central regulator

Osteokines such as osteocalcin, fibroblast growth factor 23 (FGF23), and sclerostin serve as critical mediators in the crosstalk between bone and other organs. For instance, osteocalcin, particularly its undercarboxylated form, has been shown in animal studies to regulate glucose metabolism and insulin sensitivity in peripheral tissues, thereby establishing a link between bone health and metabolic disorders like diabetes mellitus (DM) (6, 7). Of note, these effects have yet to be conclusively demonstrated in human. Conversely, metabolic derangements seen in DM, such as chronic hyperglycemia and advanced glycation end products (AGEs), impair bone remodeling, increase fracture risk, and exacerbate degenerative joint diseases (8, 9). In this Research Topic, the link between metabolic dysfunction and bone disorders was revealed in several publications. Azami et al. conducted a systematic review that underscored the reciprocal relationship by identifying musculoskeletal complications as underdiagnosed yet significant comorbidities in DM. Additionally, Zhang et al. demonstrated an association between primary frozen shoulder and dyslipidemia, especially in individuals with underlying conditions such as diabetes and

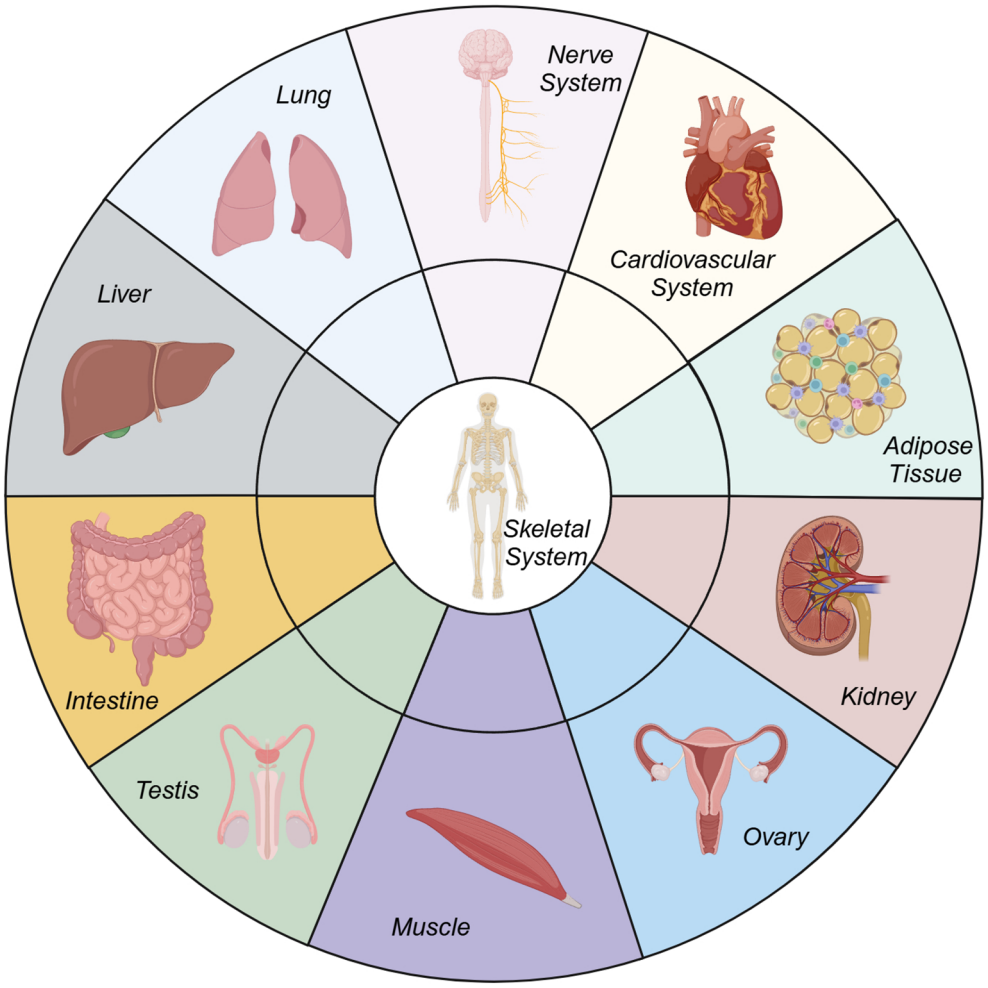

FIGURE 1
Crosstalk between bone and other organs.

thyroid dysfunction, suggesting a potential metabolic contribution to its pathogenesis. Furthermore, Fu et al. reviewed the mechanisms by which gut microbiota metabolites influence bone health. Collectively, these findings advocate for a comprehensive approach to musculoskeletal health that incorporates metabolic regulation as a fundamental component.

Bone-muscle crosstalk: a synergistic partnership

The relationship between bone and muscle extends beyond mere mechanical interaction to encompass biochemical and endocrine communication. Myokines, such as irisin, which are released during muscle contraction, play a significant role in influencing bone density and remodeling by promoting osteoblast activity (10). Conversely, signals derived from bone, including transforming growth factor-beta (TGF-β) and sclerostin, modulate muscle mass and function (11). This bidirectional relationship highlights the interdependence of muscle and bone in the maintenance of musculoskeletal integrity. Disruption of this balance contributes to conditions such as sarcopenia and osteoporosis, which frequently coexist and exacerbate the progression of degenerative diseases. In their review of the current literature, Hurly-Novatny et al. summarized the implications of Duchenne muscular dystrophy (DMD) on bone complications. Li et al. investigated the relationship between degeneration of cervical intervertebral disc and paravertebral muscles. Additionally, Jiang et al. explored the potential link between fat infiltration in paraspinal muscles and spinal degeneration.

The bone-adipose tissue axis: inflammatory mediators in degeneration

Adipose tissue plays a dual role in musculoskeletal health through the secretion of adipokines like leptin, adiponectin, and resistin. While leptin enhances osteoblast activity and bone formation under physiological conditions, its overexpression in obesity contributes to low-grade systemic inflammation, accelerating bone resorption and cartilage degeneration (12–14). In the context of IVDD, He et al. reviewed the role of adipokines in mediating the

inflammatory and catabolic cascades that disrupt intervertebral disc homeostasis. Adiponectin, traditionally regarded as protective, displays context-dependent effects, highlighting the nuanced interplay between adipose tissue and skeletal structures. Targeting this axis through modulation of adipokine signaling holds promise for mitigating the impact of obesity on degenerative musculoskeletal diseases.

Bone and the central nervous system: the neural-endocrine loop

Emerging research also highlights a neural-endocrine connection between bone and the central nervous system (CNS). Sensory neurons in bone regulate bone remodeling via neuromodulators such as calcitonin gene-related peptide (CGRP) and substance P (15–17). Simultaneously, bone-derived osteocalcin crosses the blood-brain barrier, influencing memory and mood through CNS pathways. In degenerative conditions like OA and IVDD, chronic pain originating from bone and joint pathology can alter neural signaling, leading to a feedback loop of neurogenic inflammation that exacerbates tissue damage (18, 19). Understanding this connection opens the door to integrated treatments addressing both nociceptive and systemic contributors to disease progression.

Bone and other organs: the expanding horizon

The influence of bone extends beyond muscle, adipose tissue, and the central nervous system to encompass additional physiological systems. Bone marrow functions as a reservoir for immune cells, while inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α), exert a direct impact on bone resorption (20). Chronic systemic inflammation, commonly observed in autoimmune diseases, accelerates degenerative changes in both bone and cartilage. Notably, a Mendelian randomization study conducted by Li et al. established a link between hepatitis B virus (HBV) infection and the development of osteoporosis. Furthermore, bone-derived fibroblast growth factor 23 (FGF23) plays a critical role in regulating phosphate homeostasis and vitamin D metabolism, thereby creating a feedback loop with renal function (21, 22). Impaired renal function disrupts mineral metabolism, which contributes to bone loss and vascular calcification. Vascular calcification, a characteristic feature of atherosclerosis, is associated with dysregulated pathways of bone mineralization, mediated by common factors such as matrix Gla-protein and osteoprotegerin (23). These interconnected pathways highlight the systemic nature of both cardiovascular and skeletal diseases. Additionally, the role of bone in reproductive health has gained increasing attention. Osteocalcin, beyond its metabolic functions, has been implicated in the regulation of male gonadal function. Notably, Oury et al. demonstrated that osteocalcin acts through a pancreas-bone-testis axis to influence testosterone synthesis and fertility in both mice and humans (24). This emerging axis underscores the endocrine capacity of bone in orchestrating systemic physiological processes beyond traditional musculoskeletal functions.

Implications for precision medicine

The insights presented in this Research Topic emphasize the need for a systemic perspective in understanding and treating degenerative musculoskeletal diseases. Integrating bone health into the broader context of metabolic, endocrine, and inflammatory regulation offers a paradigm shift in degenerative disease management. Thus, the future of research lies in unraveling the molecular mechanisms of bone-organ interactions using advanced technologies like single-cell sequencing and organ-on-a-chip models. Longitudinal studies are needed to establish causal links and identify early biomarkers of systemic dysregulation. Finally, translating these findings into clinical practice requires multidisciplinary collaborations that integrate endocrinology, orthopedics, rheumatology, and metabolic medicine. By recognizing the bone-organ axis as a central player in systemic health, this Research Topic of *Frontiers in Endocrinology* sets the stage for holistic approaches to understanding and managing degenerative musculoskeletal diseases.

Author contributions

PZ: Writing – original draft, Visualization. YW: Writing – original draft, Visualization. YG: Writing – review & editing. XL: Writing – review & editing, Funding acquisition.

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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 role of targeting glucose metabolism in chondrocytes in the pathogenesis and therapeutic mechanisms of osteoarthritis: a narrative review

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Osteoarthritis (OA) is a common degenerative joint disease that can affect almost any joint, mainly resulting in joint dysfunction and pain. Worldwide, OA affects more than 240 million people and is one of the leading causes of activity limitation in adults. However, the pathogenesis of OA remains elusive, resulting in the lack of well-established clinical treatment strategies. Recently, energy metabolism alterations have provided new insights into the pathogenesis of OA. Accumulating evidence indicates that glucose metabolism plays a key role in maintaining cartilage homeostasis. Disorders of glucose metabolism can lead to chondrocyte hypertrophy and extracellular matrix degradation, and promote the occurrence and development of OA. This article systematically summarizes the regulatory effects of different enzymes and factors related to glucose metabolism in OA, as well as the mechanism and potential of various substances in the treatment of OA by affecting glucose metabolism. This provides a theoretical basis for a better understanding of the mechanism of OA progression and the development of optimal prevention and treatment strategies.

KEYWORDS

osteoarthritis, glucose metabolism, cartilage, glycolysis, therapeutic

1 Introduction

Articular cartilage (AC) is a complex connective tissue, which is responsible for bearing, shock absorption and lubrication of joints of the whole body (1). Chondrocyte is the main cell type in normal human cartilage, which is differentiated from mesenchymal cells and accounts for 1-5% of cartilage tissue (2). Chondrocytes are surrounded by the pericellular matrix (PCM), which is considered a buffer of physical forces between chondrocytes and the extracellular matrix (ECM) (3). When chondrocytes are subjected to abnormal

mechanical stimulation, such as overloading or joint injury, their metabolic balance is altered, leading to matrix loss and tissue degeneration, which can lead to osteoarthritis (OA) (4, 5). OA is one of the most common and diverse degenerative diseases with complex pathogenesis that affects all joint tissues, characterized by cartilage degeneration, osteophyte formation, meniscal degeneration, subchondral bone remodeling, joint inflammation and fibrosis of the infrapatellar fat pad (6–12). The main clinical manifestations of OA patients are AC degeneration, subchondral bone sclerosis, synovitis, osteophyte formation, joint pain and disability (13). Globally, OA affects 10% - 12% of the adult population and this number is expected to increase rapidly over the next two decades (6). Although many risk factors are known to contribute to OA, including aging, obesity, genetics, joint biomechanics, and previous joint damage, the pathogenesis of OA remains unclear (14, 15). Due to the difficulty of cartilage recovery after damage, at present, there is no other more effective treatment except the use of analgesic drugs and the selection of surgical joint replacement as the final treatment option for OA (16, 17). Considering the huge population affected by OA, it not only affects the quality of life of patients, but also imposes a huge economic burden on society (7). It is crucial to investigate the pathogenesis of OA and explore new therapeutic approaches.

Energy metabolism is an important regulator of cellular function (18). Under adverse conditions, most mammalian cells undergo an energy metabolic transition (i.e., switching from a static regulatory state to an active metabolic state) to maintain energy homeostasis and promote cell survival (19–21). It has been shown that energy metabolism is critical for cartilage homeostasis and is drastically altered in chondrocytes of OA cartilage (16). In addition, recent studies have shown that chondrocytes can undergo metabolic alterations in response to different stimuli (18, 22). Normally, chondrocytes *in vivo* tend to produce ATP through glycolysis rather than oxidative phosphorylation (OXPHOS) due to the relatively hypoxic environment and maintain a stable ECM by balancing anabolic and catabolic processes (16). However, the TCA cycle and electron transport chain (ETC) efficiently produce ATP in the presence of oxygen through OXPHOS (23). Furthermore, stressed cells, such as immune cells and cancer cells, are capable of generating ATP by aerobic glycolysis when oxygen is available (18). Such cellular metabolic alterations are not only important for the maintenance of energy balance, but probably also critical for alterations in cellular function and signaling (18). Hence, regulation of metabolism may play a vital role in the treatment of OA.

As the main content of energy metabolism, lipid metabolism, amino acid (AA) metabolism and glucose metabolism play a crucial role in the pathogenesis of OA (21). The pathogenesis of OA involves a variety of lipid metabolic changes in cartilage, subchondral bone, and periosteum, which interact with inflammatory mediators to affect the development of lesions (24). Furthermore, some adipokines can directly regulate inflammation and promote the development of OA, thereby affecting joint health (25). Since a variety of AA have been found to be abnormally expressed in chondrocytes, it has also been proposed that the establishment and development of OA are related to the alterations in AA metabolism and profiles, including glutamate- and arginine-family AA as well as their related metabolites (26). Additionally, increasing evidence has shown that glucose metabolism plays a key role in maintaining cartilage homeostasis. Disorders of glucose metabolism can lead to chondrocyte hypertrophy and ECM degradation, contributing to the development of OA (27). It is suggested that targeting enzymes and factors affecting glucose metabolism may be a major breakthrough in the treatment of OA.

At present, many reviews have summarized the role of lipid metabolism and AA metabolism in the pathogenesis of OA in detail. However, no article has specifically targeted a comprehensive summary of the association between glucose metabolism and OA. This narrative review focused on the research literature related to glucose metabolism and OA, aiming to summarize the potential mechanisms of glucose metabolism in the regulation of OA, and provide a reference for further research on the role of glucose metabolism in the treatment of OA and other related diseases. We used “osteoarthritis,” “glucose,” “metabolism,” “mechanism” and “therapeutic” as keywords. Then we searched PubMed, Embase and Web of science for methodological papers on articles from inception to September 2023, especially recently 5 years. Inclusion criteria included: (a) research on OA and its related diseases, (b) the experimental subjects were humans or animals, (c) targeting proteins or factors related to glucose metabolism. Exclusion criteria included: (a) article was not written in English, (b) the full-text was not available or published, (c) conference papers, (d) reviews or meta-analysis. A total of 950 relevant articles were retrieved, and 81 were finally selected for this review.

2 An overview of glucose metabolism

Glucose plays a crucial role in cartilage development, stability, repair, and remodeling (19). Like most cells, chondrocytes use glucose metabolism to produce ATP as their primary energy source, and at the same time, also require glucose as a structural precursor for glycosaminoglycan synthesis (28). There are three major pathways involved in glucose metabolism: glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle (28–30). Among them, glycolysis is tightly regulated as it is required to provide energy for cell survival and specific cellular functions such as inflammation (31). Dysregulation or inhibition of any pathway can lead to lactic acidosis and/or energy deficit, ultimately resulting in abnormal cellular processes that contribute to the formation of several chronic diseases (31). Notably, abnormal glucose metabolism dysregulation has been demonstrated in human cancers as well as abnormal vascular smooth muscle cells, endothelial cells, and human rheumatoid arthritis (RA) chondrocytes, suggesting that glucose metabolism disorders are associated with a variety of diseases (32).

OA is now considered to be a metabolism-related disease, and its establishment and development are closely related to inflammation and metabolism (33). In OA, low-grade inflammation leads to chondrocyte hypoxia, which changes energy metabolism from a resting state to an active state (34). At

this time, as the primary energy source for chondrocytes, glycolysis increases to meet the energy demand of chondrocytes (35). Specifically, unlike many tissues, AC has no blood, nerves, or lymphatic vessels, and the synovial fluid in the joint is its source of nutrients (36). Therefore, oxygen and glucose are more difficult to obtain compared to plasma. Its relatively hypoxic environment results in lower energy production by OXPHOS (27). Since glycolysis is a process of rapid ATP generation, chondrocytes are extremely dependent on glycolysis for energy production. However, excessive enhancement of glycolysis also leads to decreased energy acquisition, which inhibits cell proliferation and differentiation. Disturbance of glycolytic metabolism can lead to chondrocyte hypertrophy and ECM degradation (27, 37). Accumulating evidence indicated that glucose metabolism disorders are the cause of OA. Among them, different enzymes and factors play an important role in the process of glucose metabolism, and may also be involved in the pathogenesis of OA. Targeting glucose metabolism is expected to make a major breakthrough in the pathogenesis and treatment of OA. Therefore, in the following few sections, we summarized the potential targets such as related enzymes and factors involved in glucose metabolism in OA as well as therapeutic substances, respectively.

3 Role of glucose metabolism mediated by different enzymes in OA

3.1 Role of GLUT1-mediated glucose metabolism in OA

Since glucose is a large molecule, its diffusion across a membrane is difficult. Hence, in most cell types, glucose is transported across the plasma membrane through facilitated diffusion along a concentration gradient and does not require energy (38, 39). Glucose metabolism in normal AC has been demonstrated to respond to anabolic and catabolic signals by regulating the expression of multiple facilitative glucose transporters of the Glut family, such as glucose transporter type 1 (GLUT1). GLUT1 is up-regulated under hypoxic and glucose-deprived conditions to enhance glucose uptake and decreased under high-glucose conditions to balance glucose levels in chondrocytes (40). Earlier studies *in vitro* showed that the loss of *GLUT1* gene leads to AC cytopenia and proteoglycan loss in mice, indicating that GLUT1-mediated glucose metabolism is required for chondrocyte growth (41). Recent studies have found in mice that genetic defects in chondrocyte GLUT1 during development cause chondrodysplasia, which may result in structural and mechanical abnormalities of AC and lead to OA in adulthood (41–43). In addition, Li et al. (44) revealed that GLUT1 programming changes play an important role in increased AC matrix degradation and susceptibility to OA in female prenatal caffeine exposure (PCE) offspring rats. At the same time, they also established a PCE-induced intrauterine growth retardation (IUGR) model in rats to elucidate the role of altered glucose transport function mediated by altered GC- insulin-like growth factor-1 (IGF1)-GLUT1 axis programming in the development of fetal-originated OA and its epigenetic mechanism, providing a theoretical basis for the early prevention and treatment of OA. Furthermore, using genetic and metabolic pathways, Wang et al. (28) found in mice that GLUT1-mediated glucose metabolism is required for normal growth plate and showed that GLUT1 loss-of-function (LOF) causes GP cartilage and AC abnormalities. The latest evidence found in mouse suggests that forced expression of GLUT1 in AC is able to prevent OA progression. Notably, *in vitro* studies of human OA chondrocytes, the sustained elevation of GLUT1 expression can increase glucose uptake and produce excess advanced glycation end products (AGEs), which degrade cartilage (45). Moreover, Pfander et al. (46) found in human OA AC that the amount of GLUT1 increased with the severity of OA. A study of human OA chondrocytes reported that although OA chondrocytes were able to adapt to glucose deprivation, high glucose exposure failed to down-regulate GLUT-1 expression. This may be a crucial pathogenic mechanism by which diseases characterized by hyperglycemia promote degenerative changes in chondrocytes, thereby contributing to the progression of OA (47).

Overall, the GLUT1 transport function is essential for glucose uptake in cartilage. Targeting GLUT1 to regulate glucose metabolism may alter chondrocyte maturation processes, including cell proliferation, hypertrophy and cartilage matrix production, suggesting that GLUT1-mediated glucose metabolism plays a key role in cartilage ossification and development. Therefore, GLUT1 may be a potential target for the treatment of OA. However, the exact mechanisms by which glucose metabolism maintains AC health may be multifaceted and warrant further investigation.

3.2 Role of HK2-mediated glucose metabolism in OA

Hexokinases (HKs) catalyze the first step of glucose metabolism, that is, the conversion of glucose to glucose-6-phosphate (G6P) (48). Hexokinase 2 (HK2), an isoform of HK, has a high affinity for glucose compared with other enzymes. Notably, to maintain a concentration gradient, HK2 initiates major glucose utilization pathways such as glycolysis, pentose phosphate pathway, and OXPHOS while promoting glucose entry into the cell (29, 49). Recently, Bustamante et al. (50) explored the role of HK2 in bone and cartilage injury, and found that overexpression of HK2 can lead to increased RNA expression levels of proinflammatory cytokines such as interleukin (IL)-6 and IL-8 in human OA fibroblast-like synoviocytes (FLS, a heterogenous cell population of the synovium that produces certain components of the synovial fluid and AC), suggesting a potential therapeutic effect of HK2 on OA and may be safer than overall glycolysis inhibition (51). Additionally, Wu et al. (31) investigated the energy metabolism of human primary chondrocytes in OA, as well as the role of glycolysis on AC under inflammatory conditions and found that gene expression of *HK2* was increased in severe OA cartilage. Although no significant changes in HK2 were found at the overall protein level, an increase in G6P was found at the metabolomic level of cartilage collected from severely injured region, indicating an elevated level of HK2 activity in OA.

In general, HK2 plays an important role in cartilage glycolysis by regulating cellular metabolism, which greatly expands our understanding of glucose metabolism in the pathogenesis of OA. New treatments for OA or related alternative outcomes may be developed in the future, especially small-molecule drugs that target HK2 downstream proteins or kinases.

3.3 Role of LDHA-mediated glucose metabolism in OA

Lactate dehydrogenase A (LDHA), the reduced form of nicotinamide-adenine dinucleotide (NADH), is one of the key enzymes in aerobic glycolysis, which is mainly used to catalyze the last step of glycolysis, i.e. the conversion of pyruvate and finally into lactate via the alternative oxidation of the cofactor, from reduced form of NADH to oxidized form (NAD+) (32, 52). In the normoxic environment, glucose is catalyzed to produce pyruvate, which then enters the mitochondria and undergoes OXPHOS through the TCA cycle. Conversely, under a hypoxic state, high-throughput glycolysis occurs primarily in the cytoplasm via LDHA (32). Previous studies have reported that LDHA is highly expressed in aerobic glycolytic cancer lineages, suggesting that the up-regulation of LDHA can be induced in a hypoxic state (53). In addition, the aberrant expression of LDHA may be an important initiator of RA joints (54, 55). A recent study explored the effect of glycolytic inhibition on hyaluronic acid synthetase 2 (HAS2) synthesis and inflammation development in synovial fibroblasts (SFs) from TMJOA patients and found that the use of a specific inhibitor of LDHA inhibited lactate secretion and promoted HAS2 and hyaluronic acid (HA) synthesis in temporomandibular joint OA (TMJOA) SFs (32). HA is an important matrix component in cartilage. High molecular weight HA can participate in the anti-inflammatory process by forming a protective coating of chondrocytes (56). Interestingly, recent *in vivo* results in a rat OA model showed that the combination of HA and lactose-modified chitosan (Chitlac; CTL) inhibited cartilage degradation more than HA alone (57). This provides a new therapeutic approach for OA. Furthermore, Arra et al. (18) have shown in studies of mouse and human OA chondrocytes that a metabolic shift in cellular metabolism toward glycolysis and LDHA reprogramming may occur in chondrocytes under inflammatory conditions. Moreover, they also found a pro-reactive oxygen species (ROS) formation function of LDHA in chondrocytes under inflammatory states (18). ROS are considered to be one of the potential factors leading to OA disease due to their ability to modify proteins and lipids, damage DNA and other cellular adverse reactions (58–61). Although ROS play an important signaling function under normal physiological conditions, elevated ROS as a mediator of disease progression in OA is considered to carry significant pathological risks (62). Besides, it has been specifically reported that upregulation of pyruvate kinase M2 (PKM2) and LDHA expression was found in OA chondrocytes compared to healthy controls, indicating an increased activity of glycolysis (18, 63). This suggests a shift from anti-inflammatory dependent OXPHOS and TCA cycle to pro-inflammatory dependent glycolytic energy in OA chondrocytes, identifying LDHA as a potential target for OA therapy.

In summary, LDHA is a key glycolytic enzyme that promotes the conversion of pyruvate to lactate and ROS production in chondrocytes under inflammatory conditions. Since there are many pathways for ROS formation, further studies are needed to prove its source. Previous studies have shown that LDHA plays an important role in the development of RA as well as TMJOA, suggesting the potential of LDHA in the treatment of OA.

3.4 Role of PKM2-mediated glucose metabolism in OA

PKM2 is one of the major rate-limiting enzymes in glycolysis and plays an important role in the regulation of glycolysis by catalyzing phosphoenolpyruvate (PEP) to pyruvate and ATP (63). Yang et al. (63) investigated the involvement of PKM2 in the pathogenesis of OA by exploring its role in glycolysis and collagen matrix production in OA chondrocytes. PKM2 expression has been reported to be upregulated in isolated OA chondrocytes compared to healthy control chondrocytes. In addition, glucose consumption and lactate production were significantly inhibited in the PKM2 knockout group, while GLUT1, LDHA and hypoxia-inducible factor 1α (HIF-1α) were decreased, suggesting that PKM2 knockout inhibited glycolysis in OA chondrocytes. Furthermore, the hydrolysis of cartilage ECM proteins is the main pathogenesis of OA, and *PKM2* gene knockout reduces the production of extracellular collagen matrix, which in turn leads to the degeneration of AC in OA. Interestingly, when PKM2 expression is upregulated in OA chondrocytes, lactate accumulates under the catalysis of LDHA, forming an acidic environment that inhibits chondrocyte matrix synthesis and possibly promotes OA cartilage degeneration (64, 65).

In conclusion, PKM2 expression and activity were increased in OA chondrocytes, while PKM2 knockdown inhibited chondrocyte glycolysis and OA cartilage proliferation, suggesting that PKM2 can regulate glycolysis and ECM production, which provides new insights into PKM2 as a potential target for the treatment of OA.

3.5 Role of PFKFB3-mediated glucose metabolism in OA

Fructose 2, 6-diphosphate (F2,6BP) is one of the important regulators of glycolysis and an allosteric activator of phosphofructokinase-1 (PFK-1). F2,6BP is mainly synthesized and degraded by 6-phosphofructose-2-kinase/fructose 2, 6-bisphosphatase (PFKFB1/2/3/4). Among them, PFKFB3 has a high kinase/phosphatase activity, therefore, it is the most widely expressed in the human tissues, and is also the main studied isomerase (66). At present, it has been confirmed that PFKFB3 can affect the physiological activities of various tissues. Interestingly, PFKFB3 expression can regulate glycolysis in tumor cells and cancer cells (67, 68). In addition, inhibition of PFKFB3 was found to be associated with decreased insulin-stimulated glucose uptake, GLUT4 translocation, AKT signaling, and glycolytic flux in cancer cells and adipocytes (69). Based on this, PFKFB3 may play a role in

OA cartilage and its dysfunction may participate in the pathogenesis of OA. Data from Qu et al. showed that PFKFB3 expression was decreased in OA cartilage tissue as well as tumor necrosis factor (TNF)-α- or IL-1β-regulated chondrocytes, accompanied by decreased glucose utilization, ATP production, and lactate production, suggesting that PFKFB3 is impaired in OA and contributes to glycolysis disorders (70). Similarly, inhibition of PFKFB3 in FLS cells from synovium of patients with inflammatory arthritis correlated with decreased glucose uptake and lactate production (54, 71). More importantly, PFKFB3 was reported to enhance the cell viability of chondrocytes through the PI3K/AKT/C/EBP homologous protein (CHOP) signaling pathway (70). It is also capable of reducing caspase 3 activation and promoting aggrecan and type II collagen expression in OA cartilage explants and chondrocytes, possessing potential to be a target for OA prevention and treatment.

3.6 Role of AMPK-mediated glucose metabolism in OA

AMP-activated protein kinase (AMPK) is a major regulator of energy metabolism and balance, possessing the potential to be a therapeutic target for metabolic disorders and aging-related diseases, including OA (7, 72, 73). A reduction in AMPK activity was found in both human and mouse knee OA cartilage as assessed by phosphorylation of specific threonines in AMPKα1, the AMPK-activated α subunit, suggesting that AMPK activity in chondrocytes plays an important role in maintaining joint function stability and the development of OA (74). Indeed, Zhou et al. (75) found in a mouse model that using cartilage-specific, tamoxifen-inducible AMPKα knockout mice exhibited accelerated severity of surgically induced OA compared to wild-type mice, and at 12 months, male mice developed severe spontaneous aging-related OA lesions. These results demonstrate that chondrocyte-specific AMPKα depletion in adulthood leads to accelerated OA progression (29, 74). Notably, the activation of AMPK is partially achieved by maintaining glycolysis. Specifically, OA-associated degradation features in AC are a downstream consequence of altered metabolism of resident chondrocytes. These OA features include enhanced production of extracellular proteases such as MMPs. Ohashi et al. (76) found that chondrocytes were more dependent on glycolysis as a source of cellular ATP after il-1β treatment, while only a small fraction was derived from OXPHOS. Blocking IL-1β-enhanced MMP13 levels using galactose substitution (a known activator of OXPHOS and inhibitor of glycolysis) in OA chondrocytes led to a reverse increase in phospho-AMPK-related biomarkers, confirming AMPK as a downstream regulator during OA glycolysis.

Current studies have confirmed the important role of AMPK and aerobic glycolysis in the pathogenesis of OA and provided clues for further exploration.

All in all, the role of glucose metabolism mediated by different enzymes in OA is shown in Table 1 and Figure 1.

4 Role of glucose metabolism mediated by different factors in OA

4.1 Role of HIF-1α-mediated glucose metabolism in OA

HIF-1 is a highly conserved transcription factor and a major regulator of cellular adaptation to hypoxic stimulation (77). The activity of HIF-1 is primarily dependent on its alpha subunit (HIF-1α) (78). Because cartilage is relatively thick, chondrocytes at the surface of the joint are exposed to only about 6% to 10% of oxygen, while the deepest chondrocytes have access to even less oxygen in the cartilage (79). In hypoxic cells, HIF-1α can induce a switch from oxidative to glycolytic metabolism. Moreover, it is of critical importance in maintaining chondrocytes activity and integrity, cartilage energy production, and regulating chondrogenesis, energy metabolism, and matrix synthesis (80, 81). Studies have shown that the treatment of OA can significantly down-regulate the expression of *HIF-1α* gene, suggesting that HIF-1α plays an important role in the pathological mechanism of OA (82). Yudoh et al. (83) revealed that OA chondrocytes lacking HIF-1α fail to produce energy and maintain ECM growth under both normoxic and hypoxic conditions, suggesting that chondrocytes may regulate ATP levels and ECM production during OA through adaptive functions of the *HIF-1α* gene. Metabolically, activated OA chondrocytes rely on HIF-1 to increase glucose uptake and utilization to promote anaerobic ATP production, thereby compensating for the accelerated energy expenditure during OA (84). HIF-1α also increases the expression of glycolytic enzymes, such as HIF-1α augments the expression of phosphoglycerate kinase, which converts 1, 3-diphosphoglycerate to 3-phosphoglycerate during glycolysis (85). Besides, under the influence of the complex of HIF-1α, HIF-1β and HREs, the expression of GLUT1, G6P dehydro-genase (G6PD), phosphoglycerate kinase 1 (PGK1) and pyruvate dehydrogenase kinase 1 (PDK1) is increased, which leads to promoting glucose transfer and anaerobic glycolysis (79, 86). Furthermore, vascular endothelial growth factor (VEGF) and erythropoietin (EPO) have been reported to increase oxygen and nutrient delivery, as well as metabolic adaptations, thereby preventing chondrocyte death in the growth plate. Under hypoxia, HIF-1α promotes glycolysis by inducing the expression of both (87). Therefore, further studies on the role of HIF-1α in OA and its potential mechanism may provide a new therapeutic target for the treatment of OA.

4.2 Role of TGF-β1-mediated glucose metabolism in OA

Transforming growth factor (TGF)-β1 is a multifunctional cell regulatory factor, mainly involved in the process of growth and development, proliferation and differentiation, ECM synthesis and immune response (88, 89). Recent studies have indicated that TGF-β1 can inhibit glycolysis by inducing natural regulatory T cells and down-regulating the expression of critical enzymes of glycolysis

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TABLE 1 The role of targeted regulation of glucose metabolism in OA.

Regulator	Role in glucose metabolism	Mechanisms of action in OA (Cell source)	References
Role of glucose metabolism mediated by different enzymes in OA			
GLUT1	Glucose uptake	1. Enhance glucose uptake, balance glucose levels in cells, and produce excess AGEs (human OA chondrocytes); 2. The loss of *GLUT1* gene leads to GP cartilage, articular cartilage abnormalities, cytopenia and proteoglycan loss in articular cartilage (mice chondrocytes)	(16, 22–28)
HK2	Initiate major glucose utilization glycolysis, pentose phosphate pathway, and OXPHOS; convert glucose to G6P	1. Promote glucose entry into the cell; 2. Lead to increased RNA expression levels of proinflammatory cytokines (human OA FLS); 3. Gene expression of *HK2* was increased in severe OA cartilage (human primary chondrocytes)	(17, 19, 29–31)
LDHA	Key enzyme in aerobic glycolysis, catalyze the conversion of pyruvate into lactate and ROS production	1. Inhibit lactate secretion and promoted HAS2 and HA synthesis (SFs from TMJOA patients); 2. mediate high-throughput glycolysis	(9, 20, 32)
PKM2	Rate-limiting enzyme in glycolysis; catalyze PEP to pyruvate and ATP	1. PKM2 knockout inhibits glycolysis and reduces the production of extracellular collagen matrix; increase lactate accumulation; inhibit chondrocyte matrix synthesis and promotes OA cartilage degeneration (human OA chondrocytes)	(38–40)
PFKFB3	Regulators of glycolysis	1. Contribute to glycolysis disorders	(41–45)

(Continued)

TABLE 1 Continued

Regulator	Role in glucose metabolism	Mechanisms of action in OA (Cell source)	References
Role of glucose metabolism mediated by different enzymes in OA			
		(human OA cartilage); 2. decrease glucose uptake and lactate production (FLS cells from synovium of patients with arthritis)	
AMPK	Major regulator of energy metabolism and balance	1. A downstream regulator during OA glycolysis (human OA chondrocytes)	(19, 49, 50)
Role of glucose metabolism mediated by different factors in OA			
HIF-1α	Induce a switch from oxidative to glycolytic metabolism	1. Increase glucose uptake and utilization to promote anaerobic ATP production (human OA chondrocytes); 2. increase the expression of glycolytic enzymes; increase the expression of GLUT1, G6PD, PFK1, and PDK1 (mice primary articular chondrocytes)	(52–59)
TGF-β1	Inhibit glycolysis; induce OXPHOS conversion to aerobic glycolysis; maintain chondrocyte homeostasis and articular cartilage integrity	1. Increase glucose consumption and lactate production, as well as a decrease in ATP levels; elevate the expression of key glycolytic enzymes such as GLUT1 and HK2 to increase glycolysis and lactate production (human OA chondrocytes)	(7, 17, 62, 63, 65)
NF-κB	Increase the production of downstream cytokines	1. Increase the expression of cartilage degradation genes and promote chondrocytes dysfunction; inflammation can shift cellular metabolism toward NF-κB-mediated aerobic glycolysis (mice chondrocytes)	(9, 66–69)

(Continued)

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TABLE 1 Continued

Regulator	Role in glucose metabolism	Mechanisms of action in OA (Cell source)	References
Potential therapeutic substances for OA that affect glucose metabolism			
Icariin	An inducer of HIF-1α and an activator of glucose metabolism in chondrocytes	1. Promote the up-regulation of HIF-1α and PDK1 expression in chondrocytes, leading to the increased expression of key targets (G6PD and PGK1) and the inhibition of mitochondrial OXPHOS, ultimately inducing glucose metabolism in a glycolytic manner; promote the expression of GLUT1 and other glycolytic enzymes; promote anaerobic glycolysis and increased cell viability in OA chondrocytes (mice chondrocyte)	(58, 70–72)
2-deoxy-D-glucose	An inhibitor of glycolysis with the potential to affect glucose metabolism	1. Altered glucose use and reduced lactate production in an inflammatory environment (human OA chondrocytes)	(1, 19, 73, 74)
Glucocorticoids	Shift carbohydrate metabolism from mitochondrial respiration to glycolysis	1. Anti-inflammatory; regulate glucose and lipid metabolism; up-regulate PDK4 (human OA chondrocytes); 2. affect IGF1 and GLUT1 in cartilage tissue through GC-IGF1-GLUT1 axis (mice chondrocyte)	(28, 75–78)
Galactose	Galactose substitution has been proposed as an OXPHOS activator with the effect of inhibiting glycolysis to produce ATP	1. Enhance chondrocyte dependence on the glycolytic pathway for ATP production (bovine chondrocytes)	(50, 63, 79)
Glucose	Provide adequate glucose to maintain the major anaerobic metabolism of	1. Affect the balance of matrix synthesis and degradation; affect glucose uptake as well as matrix	(80, 81)

(Continued)

TABLE 1 Continued

Regulator	Role in glucose metabolism	Mechanisms of action in OA (Cell source)	References
Potential therapeutic substances for OA that affect glucose metabolism			
	articular chondrocytes	production and maintenance, and promote the anti-inflammatory activity of differentiated chondrocytes (equine chondrocytes)	

OA, osteoarthritis; GLUT, glucose transporter; AGEs, advanced glycation end products; HK2, hexokinase 2; OXPHOS, oxidative phosphorylation; G6P, glucose-6-phosphate; FLS, fibroblast-like synoviocytes; LDHA, lactate dehydrogenase A; ROS, reactive oxygen species; HAS2, hyaluronic acid synthetase 2; HA, hyaluronic acid; TMJOA, temporomandibular joint osteoarthritis; SFs, synovial fibroblasts; PKM2, pyruvate kinase M2; PEP, phosphoenolpyruvate; PFKFB3, 6-phosphofructose-2-kinase/fructose 2, 6-bisphosphatase 3; AMPK, AMP-activated protein kinase; HIF-1α, hypoxia-inducible factor 1α; G6PD, glucose-6-phosphate dehydrogenase; PFK1, phosphoglycerate kinase 1; PDK1, pyruvate dehydrogenase kinase 1; TGF-β1, transforming growth factor-β1; NF-κB, nuclear factor kappa B; PGK1, phosphoglycerate kinase 1; PDK4, pyruvate dehydrogenase kinase 4; IGF1, insulin-like growth factor-1.

such as GLUT1 and HK2 (29, 90). TGF-β1 stimulation can promote the glycolytic process in dermal fibroblasts, while inhibition of glycolysis is able to impede TGF-β1-induced profibrosis, suggesting that TGF-β1 plays a dominant role in inducing OXPHOS conversion to aerobic glycolysis (91). Additionally, accumulating evidence supports that the maintenance of AC morphology and the intracellular environment of chondrocytes relies on the regulatory function of TGF-β1 (16, 21). Wang et al. (16) found an increase in glucose consumption and lactate production, as well as a decrease in ATP levels, in OA chondrocytes from humans treated with TGF-β1. Moreover, TGF-β1 stimulation significantly elevated the expression of key glycolytic enzymes such as GLUT 1 and HK2 to increase glycolysis and lactate production. Interestingly, HK1 was also found to be upregulated under TGF-β1 treatment (16). Likewise, Ni et al. detected that TGF-β1 increased HK1 and HK2 expression and simultaneously upregulated HK activity in c-Kit cell lines, suggesting that TGF-β1 has the potential to directly regulate HK and providing important clues for TGF-β1-mediated OA pathogenesis (92). However, the current therapeutic potential of TGF-β1 in OA is still controversial, as it plays both protective and detrimental roles in OA. Therefore, the molecular mechanisms of glucose metabolism in OA targeting TGF-β1 deserve further attention to provide insights into the regulation of energy metabolism and lead to exploring new therapeutic approaches for effective management of OA by modulating cellular metabolism.

4.3 Role of NF-κB -mediated glucose metabolism in OA

Nuclear factor kappa B (NF-κB) is a transcription factor that widely presents in a variety of cell lines and participates in various

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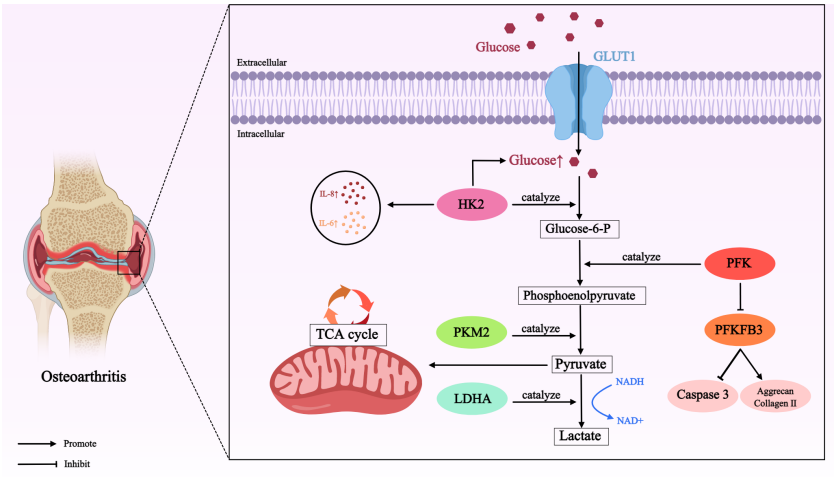

FIGURE 1
Role of glucose metabolism mediated by different enzymes in OA. In OA cartilage, glucose is transported by GLUT1 (a passive transporter that relies on the concentration gradient as the driving force for the movement of glucose molecules across the membrane) into chondrocytes for metabolism. Many enzymes involved in glucose metabolism, such as HK2, PKM2, LDHA, PFK, PFKFB3, initiate the major pathways of glucose utilization (including glycolysis) in this process, thereby directly or indirectly affecting the development of OA. Glucose transporter type 1 (GLUT1); hexokinase 2 (HK2); interleukin-8 (IL-8); interleukin-6 (IL-6); phosphofructokinase (PFK); 6-phosphofructose-2-kinase/fructose 2, 6-bisphosphatase 3 (PFKFB3); pyruvate kinase M2 (PKM2); lactate dehydrogenase A (LDHA); nicotinamide-adenine dinucleotide (NADH).

cellular inflammatory responses (93). Mechanistically, in chondrocytes, NF-κB activation may play an important role in the progression of OA by increasing the expression of cartilage degradation genes and promoting chondrocytes dysfunction (18). An earlier study found that NF-κB activation is involved in regulating cartilage differentiation (94). In addition, Sang et al. (95) found that glycolysis and microenvironment remodeling could be promoted by activating the Ca^{2+}/NF-κB axis to increase the production of downstream cytokines, suggesting that the role played by NF-κB signaling is critical in the regulation of glycolytic activity. In addition, Wang et al. (96) reported a positive correlation between HK2 expression and inflammatory cell infiltration and cartilage destruction in arthritic rats treated with glycolysis inhibitors, implying that HK2 glycolysis inhibitors are closely related to NF-κB activation and inhibition. Furthermore, Arra et al. (18) showed that inflammation can shift cellular metabolism toward aerobic glycolysis mediated by NF-κB, further confirming the strong links between inflammatory and metabolic responses in OA chondrocytes. The above evidence clearly establishes that NF-κB activation can mediate glycolytic process to drive OA pathological changes, but further studies are needed to reveal the precise association and underlying mechanism.

In conclusion, the role of glucose metabolism mediated by different factors in OA is presented in Table 1 and Figure 2.

5 Potential therapeutic substances for OA that affect glucose metabolism

Targeting glucose metabolism switches, especially glucose transporters and regulatory factors involved in mediating glucose metabolism, is challenging but has potential therapeutic implications in OA. The following substances (summarized in Table 1) play an important role in inhibiting OA development by affecting glucose metabolism.

5.1 Icariin

Icariin (ICA), a flavonoid compound extracted from Herba Epimedii (HEP), is known to be an inducer of HIF-1α and an activator of glucose metabolism in chondrocytes (86, 97). As reported by Wang et al. (86) in chondrocyte experiments in mice, ICA treatment upregulated the expression of SOX9, a key ECM regulator that contributes to the chondrocyte phenotype, suggesting that ICA can promote chondrocyte viability and formation. Specifically, as a glucose carrier and a downstream target of HIF-1α, GLUT1 is activated after ICA treatment. Similarly, ICA treatment also enhanced the expression of PGK1, which is both a critical enzyme in anaerobic glycolysis and a downstream target of HIF-1α (86, 98). PDK1 is capable of limiting oxygen consumption by preventing TCA cycle, thereby promoting anaerobic glycolysis. Additionally, ICA has been shown to up-regulate the expression of G6PD, which is a rate-limiting enzyme in the pentose phosphate pathway and an important regulator of energy expenditure and glucose metabolism. Mechanistically, ICA treatment promoted the up-regulation of HIF-1α and PDK1 expression in chondrocytes, leading to the increased expression of key targets (G6PD and PGK1) and the inhibition of mitochondrial OXPHOS, ultimately inducing glucose metabolism in a glycolytic manner (86). To sum up, ICA treatment promoted the expression of GLUT1 and other glycolytic enzymes, which contributed to anaerobic glycolysis and increased cell viability in OA chondrocytes (99). Therefore, ICA is expected to represent a novel treatment for OA.

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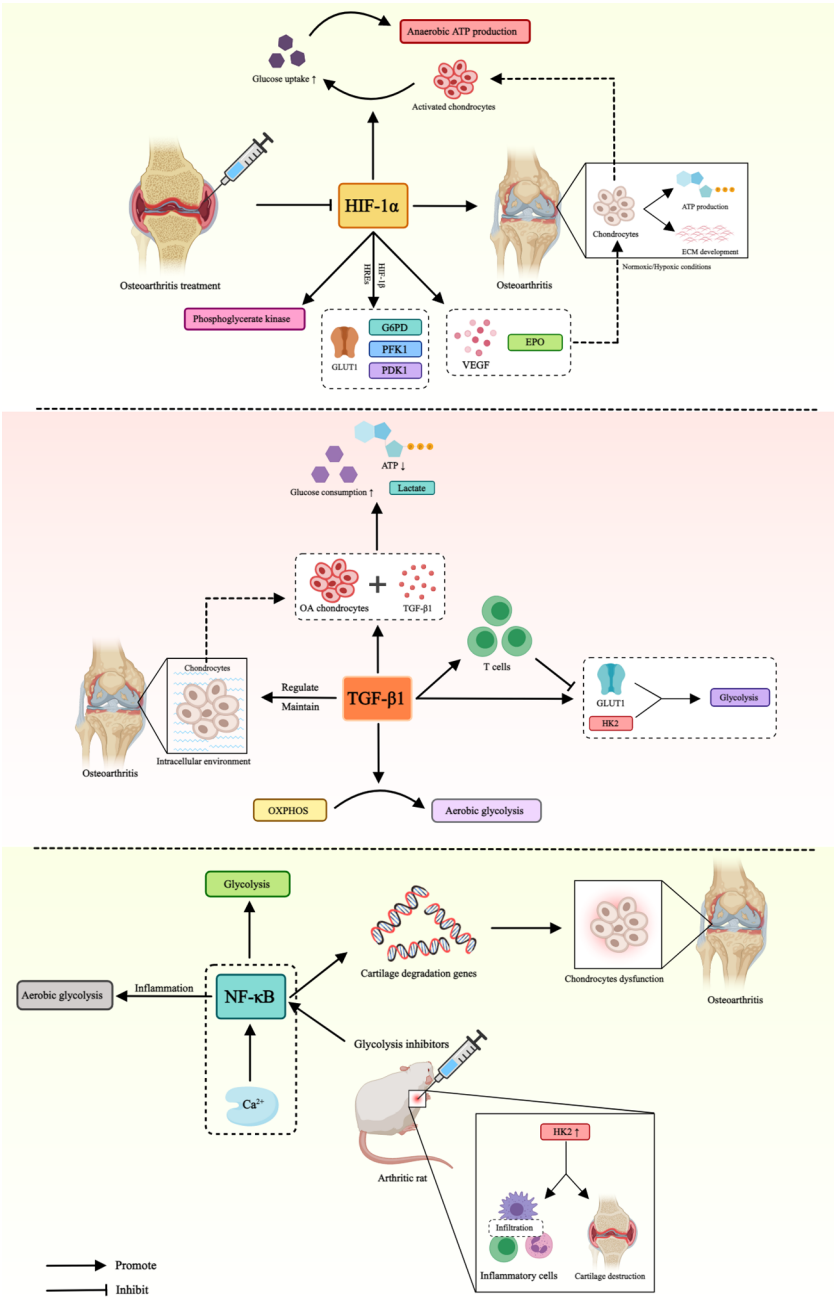

FIGURE 2
Role of glucose metabolism mediated by different factors in OA. This figure illustrates that HIF-1α, TGF-β and NF-κB affect the pathogenesis of OA by mediating different pathways of glucose metabolism. Hypoxia-inducible factor-1α (HIF-1α), glucose transporter type 1 (GLUT1), hypoxia-inducible factor-β1 (HIF-β1), hypoxia-responsive elements (HREs), glucose-6-phosphate dehydro-genase (G6PD), phosphoglycerate kinase 1 (PFK1), pyruvate dehydrogenase kinase 1 (PDK1), vascular endothelial-derived growth factor (VEGF), erythropoietin (EPO), transforming growth factor-β1 (TGF-β), hexokinase 2 (HK2), oxidative phosphorylation (OXPHOS), nuclear factor kappa B (NF-κB).

5.2 2-deoxy-D-glucose

2-deoxy-D-glucose (2-DG) is widely known as an inhibitor of glycolysis with the potential to affect glucose metabolism (100). Ying et al. (6) demonstrated that glucose metabolism is an inflammatory target for OA progression *in vivo* by intraperitoneal injection of 2-DG. The results suggest that 2-DG attenuates the development of OA in mice, and the underlying mechanism may be related to the inhibition of subchondral bone sclerosis and cartilage degeneration. Meanwhile, they also found that 2-DG had a protective effect on destabilization of the medial meniscus surgery (DMM)-induced OA mice by inhibiting glycolysis. Previous studies have recommended an effective dose of 2-DG to sufficiently inhibit glycolytic activity in animals, but it remains important to optimize this dose to prevent cartilage damage in OA (101). In addition, Wu et al. (31) found in primary chondrocytes collected from patients

undergoing total knee arthroplasty surgery that altered glucose use and reduced lactate production in an inflammatory environment have been proposed as potential mechanisms by which 2-DG inhibits the expression of cartilage catabolic genes by suppressing glycolysis. Terabe et al. (102) also demonstrated that 2DG is a potent blocker of the glycolytic pathway in chondrocytes in IL1β-induced human OA chondrocytes and bovine chondrocytes. Overall, the glycolytic inhibitor 2-DG provides an experimental therapeutic option for OA, but further studies are still needed to observe the potential effects of 2-DG on chondrogenesis and overall anabolic activity.

5.3 Glucocorticoids

Glucocorticoids (GCs) are steroid hormones produced endogenously by the adrenal cortex. In target cells, they bind to glucocorticoid receptors (GR) to form complexes and then migrate into the nucleus (103, 104). Clinically, intra-articular injection of GCs is widely used to relieve various types of inflammation and pain (103, 105). There are two potential mechanisms underlying the anti-inflammatory effects: 1) enhancing the expression of anti-inflammatory genes through the binding of GR-steroid complexes to glucocorticoid response elements (GREs); 2) inhibiting the activity of inflammatory transcription factors such as NF-κB and activator protein 1 (AP-1) (103). More importantly, GCs are also recommended for the treatment of knee OA. In OA chondrocytes, in addition to their anti-inflammatory effects, GCs also partially mediates their effects on OA cartilage by regulating glucose and lipid metabolism. Interestingly, *pyruvate dehydrogenase kinase 4 (PDK4)* was one of the most significantly up-regulated genes by dexamethasone (a glucocorticoid). Since this gene is able to inhibit pyruvate dehydrogenase activity and prevent the progression of pyruvate produced by glycolysis to OXPHOS, the role of GCs may be to shift carbohydrate metabolism from mitochondrial respiration to glycolysis (106). Furthermore, Li et al. (44) reported that GCs could affect IGF1 and GLUT1 in cartilage tissue through the GC-IGF1-GLUT1 axis. In detail, IGF1 can promote the up-regulation of GLUT1 expression, leading to the accumulation of AGEs in chondrocytes, thereby promoting the inflammation and matrix degradation of AC, and ultimately inducing OA. Therefore, future studies to further elucidate the role of GCs in glucose metabolism associated with OA pathophysiology may be an interesting avenue to investigate.

5.4 Galactose

Galactose substitution has been proposed as an OXPHOS activator with the effect of inhibiting glycolysis to produce ATP (91). In addition, galactose substitution is a common method to study the downstream effects of metabolic function in cancer or normally proliferating cells. Typically, G6P converted from galactose is involved in the glycolytic pathway, and the conversion rate of galactose to G6P is slower than that of glucose (107). Thus, pyruvate produced through the glycolytic metabolism of galactose produces less ATP, forcing the cell to increase its dependence on ATP produced by OXPHOS. Ohashi et al. (76) observed in bovine and human primary articular chondrocytes a strong dependence of chondrocytes on ATP produced by the glycolytic pathway in glucose-rich medium, which became more prominent upon stimulation with IL-1β and, in turn, echoed the absence of ATP produced by OXPHOS. In contrast, chondrocytes in galactose-substituted medium showed a significant reduction in glycolysis and an increase in ATP produced by mitochondrial activity due to the enhanced overall mitochondrial function, suggesting that repairing mitochondrial dysfunction may be one of the effective strategies for the treatment of OA by targeting catabolism. Similarly, Lane et al. (108) found significant reductions in lactate production and maximal lactate dehydrogenase activity in healthy bovine primary chondrocytes with increasing time of galactose culture, suggesting inhibition of chondrocyte glycolysis and decreased dependence of cellular ATP production on the glycolytic pathway, confirming the ability of galactose culture to alter chondrocyte metabolism.

5.5 Glucose

Glucose plays an essential role in the maintenance of chondrocyte metabolism, and adequate glucose supply is required to maintain the major anaerobic metabolism of articular chondrocytes. In addition, glucose is also a key precursor for the synthesis of ECM macromolecules by these cells (109). Therefore, inflammatory diseases such as OA may have a higher glucose requirement, but the demand for chondrocytes is limited due to the avascular nature of cartilage tissue. It should be emphasized that limited glucose supply carries the risk of impairing cellular function and may lead to an imbalance in matrix synthesis and degradation, resulting in OA (56). Of interest, Sopasakis et al. (56) investigated the pivotal role of glucose as a fuel source for matrix generation and cartilage repair in conditions of OA using differentiated equine chondrocytes pellets in a 3D model *in vitro*. The results showed that in the presence of inflammatory conditions associated with OA, heightened glucose levels not only facilitate glucose uptake, HA synthesis and enhance matrix production and maintenance, but also promote the anti-inflammatory activity of differentiated chondrocytes. This indicates that sufficient glucose supply to chondrocytes promotes cartilage repair by interrupting the harmful inflammatory cycle and inducing the synthesis of HA, suggesting that intra-articular injection of glucose is beneficial to the early treatment of OA, and its combination with other therapies is expected to improve the therapeutic effect by regulating chondrocytes metabolism and inducing anti-inflammatory processes. Notably, a large number of studies have shown that both diabetes mellitus and type 2 diabetes mellitus are significantly associated with OA, and diabetic patients have a higher risk of OA, suggesting that hyperglycemia may induce or aggravate OA (110, 111). Li et al. (112) demonstrated in high glucose-stimulated rat FLS in the *in vitro* cocultivation system that hyperglycemia leads to the accumulation of AGEs in FLS through the HIF-1α-GLUT1 pathway, thereby increasing the release of FLS

inflammatory factors, which in turn induces chondrocyte degradation and promotes OA development. Of note, after being activated by AGEs, the receptor of AGEs (RAGE) can trigger downstream inflammatory signals to participate in a variety of degenerative diseases. Therefore, RAGE inhibitors are expected to block the proinflammatory effects of high glucose.

Prolotherapy is a safe nonsurgical regenerative injection technique that stimulates the production of growth factors and cytokines, and promotes growth of normal cell and tissue by injecting small amounts of a stimulating solution (including hypertonic dextrose, morrhuate sodium, dextrose/phenol/glycerin solution, or platelet-rich plasma) into painful and degenerative joints, ligaments, and adjacent joint spaces, leading to the regeneration of previously damaged ligaments, tendons, and other intra-articular structures (113). A previous systematic review suggests that dextrose prolotherapy may benefit patients with knee OA by promoting tissue repair and improving range of motion and joint stability. OA patients treated with dextrose showed significant improvement compared to those receiving saline injection and normal treatment (114). In addition, another systematic review including 10 articles indicated that dextrose prolotherapy has been shown to have better therapeutic effects on pain in patients with OA compared to exercise, and that it has similar effects to platelet-rich plasma and steroid injections (115). However, although the latest systematic review showed that dextrose prolotherapy is beneficial in reducing pain and improving function in patients with knee OA, there is a high risk of bias and heterogeneity in the results (116, 117). While individuals may experience short-term relief, questions remain about the long-term durability of the treatment. This uncertainty can make it challenging to assess the suitability of treatment for sustained management of knee OA. Additionally, the dextrose concentration/volume, interval and duration of treatment, as well as injection site and technique may differ. The absence of standardized treatment protocols for dextrose prolotherapy contributes to variability in how the procedure is performed. Therefore, despite the promising results, there is still a need for larger clinical trials with a standardized treatment regimen and long-term findings to show improvement in the quality of life consistently.

6 Conclusions

In conclusion, improving the glucose metabolism is conducive to treating OA, mainly through regulating the glycolysis. Due to the hypoxic environment, chondrocytes are highly dependent on glycolysis, which is the main source of energy for chondrocytes. Thus, in OA chondrocytes, the regulation of glucose transport and glycolytic pathways is thought to influence the pathogenesis of the disease, and the process involves the responses of multiple enzymes. Among them, GLUT1, HK2, LDHA, PKM2, PFKFB3, and AMPK are closely related to cell growth, proliferation, metabolism, and apoptosis, and play a vital role in the development of OA mainly by affecting the glycolytic process. In addition, many studies have reported key factors in the process of glucose metabolism, suggesting their involvement in the pathogenesis of OA. This review emphasizes the potential mechanisms of HIF-1α, TGF-β1, NF-κB and their regulation pathway in energy metabolism and progression of OA. They are also important targets for the regulation of glycolysis. More importantly, ICA, 2-DG, GC, galactose and glucose have been shown to inhibit OA by regulating glucose metabolism, which provides new insights into the treatment of OA. However, different administration methods, concentrations and dosages still need to be further explored to ensure the best results.

7 Future perspectives

Energy metabolism is central to the maintenance of cartilage function. Accumulating studies are interested in the role of glucose metabolism in the pathogenesis and therapeutic mechanisms of OA. In an adverse microenvironment, energy conversion to glycolysis-dominated glucose metabolism is critical for immune response and inflammatory pathway activation during OA progression. From a translation perspective, the pathogenesis of OA is considered to be related to differences in glucose utilization between normal and diseased chondrocytes (16, 31). Targeting glucose transporters and rate-limiting enzymes during glucose metabolism may be an important strategy for the treatment of OA. However, future studies are needed to further confirm the comprehensive details of the specific regulatory mechanisms and signaling pathways. In addition, studies on the key factors affecting glucose metabolism such as HIF-1α, TGF-β1, NF-κB, and their mediated signaling pathways are expected to further reveal the potential mechanisms regulating energy metabolism in OA, which will contribute to developing small molecule drugs targeting the downstream proteins or kinases. Furthermore, metabolomics techniques have suggested some potential metabolic pathway alterations in OA. Future research of OA should focus on specific phenotypes and related molecular pathways. The disease-related alterations of specific components and regulatory molecules in glucose metabolism pathways such as glycolysis, TCA cycle, and their interactions in OA progression warrant further investigation. From a therapeutic perspective, it will also be critical to explore the potential of glucose metabolism shifts in current effective OA therapies, such as physical exercise and weight loss, and the response to experimental drugs that affect cellular glucose metabolism. Similarly, immunometabolism should also be used as a key means of understanding OA pathophysiology in the future.

Author contributions

PP: Writing – original draft, Writing – review & editing. LZ: Writing – original draft, Writing – review & editing. ZZ: Writing – review & editing. KZ: Writing – review & editing. BH: Writing – review & editing. XB: Writing – review & editing. YW: Conceptualization, 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

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Roles of organokines in intervertebral disc homeostasis and degeneration

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The intervertebral disc is not isolated from other tissues. Recently, abundant research has linked intervertebral disc homeostasis and degeneration to various systemic diseases, including obesity, metabolic syndrome, and diabetes. Organokines are a group of diverse factors named for the tissue of origin, including adipokines, osteokines, myokines, cardiokines, gastrointestinal hormones, and hepatokines. Through endocrine, paracrine, and autocrine mechanisms, organokines modulate energy homeostasis, oxidative stress, and metabolic balance in various tissues to mediate cross-organ communication. These molecules are involved in the regulation of cellular behavior, inflammation, and matrix metabolism under physiological and pathological conditions. In this review, we aimed to summarize the impact of organokines on disc homeostasis and degeneration and the underlying signaling mechanism. We focused on the regulatory mechanisms of organokines to provide a basis for the development of early diagnostic and therapeutic strategies for disc degeneration.

KEYWORDS

intervertebral disc homeostasis, intervertebral disc degeneration, organokines, organ crosstalk, signaling pathway

1 Introduction

Intervertebral disc degeneration (IVDD) is the main contributor to the development of low back pain, leading to a remarkable loss of disability-adjusted life years as well as a substantial economic burden on society (1, 2). Healthy discs are cartilaginous structures that contribute one-third of the spine height and act as "elastic cushions" providing essential support, absorbing mechanical stress through compression, and providing flexibility. At the core of discs lies the gel-like nucleus pulposus (NP), which is surrounded by a concentric layer-arranged annulus fibrosus (AF) and two semi-rigid thin cartilage endplates (CEPs) that lie beneath the adjacent vertebrae. Various biological processes, including inflammation modulation, prevention of neovascularization, cell homeostasis, and matrix metabolism balance, are essential for preserving the disc

homeostasis (3). IVDD, which is characterized mainly by persistent inflammation and matrix metabolism imbalance, refers to the progressive deterioration of the disc structure, leading to disc herniation, disc height loss, and nerve compression (3). The therapeutic options for IVDD are limited due to a poor understanding of the underlying mechanisms.

The healthy disc is not isolated from other tissues, despite having long been known as a unique organ without blood vessels, nerves, or immune cell infiltration (4). An increasing number of studies have shown that organokines, the bioactive factors secreted by diverse tissues, may have a vital impact on disc homeostasis. The expression of organokines can be induced by several factors, including physical activity, diet, aging, and metabolic alterations like obesity and diabetes (5, 6). Through autocrine, paracrine, or endocrine mechanisms, organokines have been linked to several inflammatory diseases, such as rheumatoid arthritis (7, 8). However, the role of organokines in IVDD is not completely understood.

In this review, we aimed to summarize the molecular and biochemical characteristics of organokines from specific tissues and their association with disc homeostasis and degeneration. The organokines treated in this review include adipokines, osteokines, myokines, cardiokines, gastrointestinal hormones, and hepatokines. Common hormones, growth factors, cytokines, and chemokines are excluded (Figure 1). Organokines play regulatory roles in cellular behavior, inflammation, and matrix metabolism in intervertebral disc homeostasis by binding to their receptors and activating downstream signaling pathways. We hope that this review will deepen the understanding of IVDD in the view of organ crosstalk and pave the way for the development of novel therapeutic interventions.

2 Adipokines

Obesity, characterized by excessive adipose tissue, has been recognized as a significant risk factor for disc degeneration (9). In recent decades, adipose tissue has been considered as an endocrine organ that secretes various bioactive factors named adipokines (10) (Figure 2, Table 1). The cell-signaling proteins, such as leptin, adiponectin, and progranulin (PGRN) are secreted from adipose tissues and act like cytokines in the obesity-related impact on non-adipose tissues (33, 34). Research suggests that adipokine signaling is involved in the regulation of intervertebral disc homeostasis by several conditions, including disc tissue disruption by vertebral

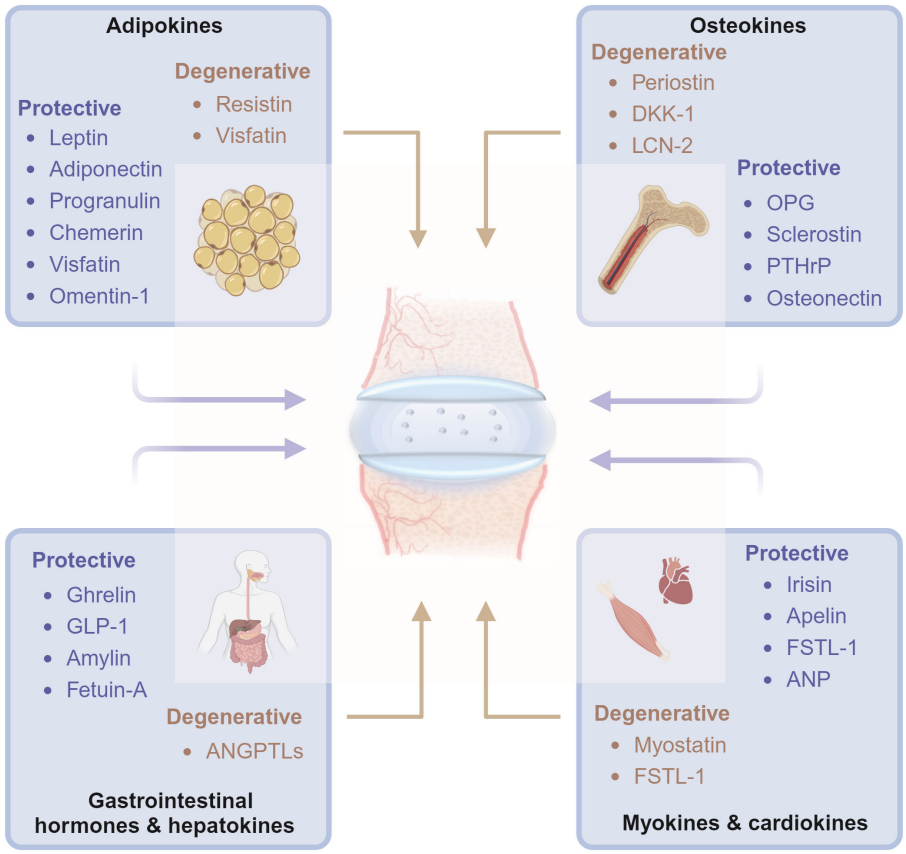

FIGURE 1
Diagrams illustrate that organokines, including adipokines, osteokines, myokines, cardiokines, gastrointestinal hormones and hepatokines, mediates the cross-organ regulation of disc homeostasis under physiological and pathological conditions from the major tissues of endocrinory ability. OPG, osteoprotegerin; DKK-1, dickkopf-1; PTHrP, Parathyroid Hormone-Related Protein; SPARC, Secreted protein acidic and rich in cysteine; BMPs, Bone morphogenetic proteins; ANP, atrial natriuretic peptide; GLP-1, Glucagon-like peptide-1; ANGPTLs, angiopoietin-like proteins; FSTL-1, Follistatin-like-1; LCN-2, lipocalin-2. Graphic elements were created using biorender.com.

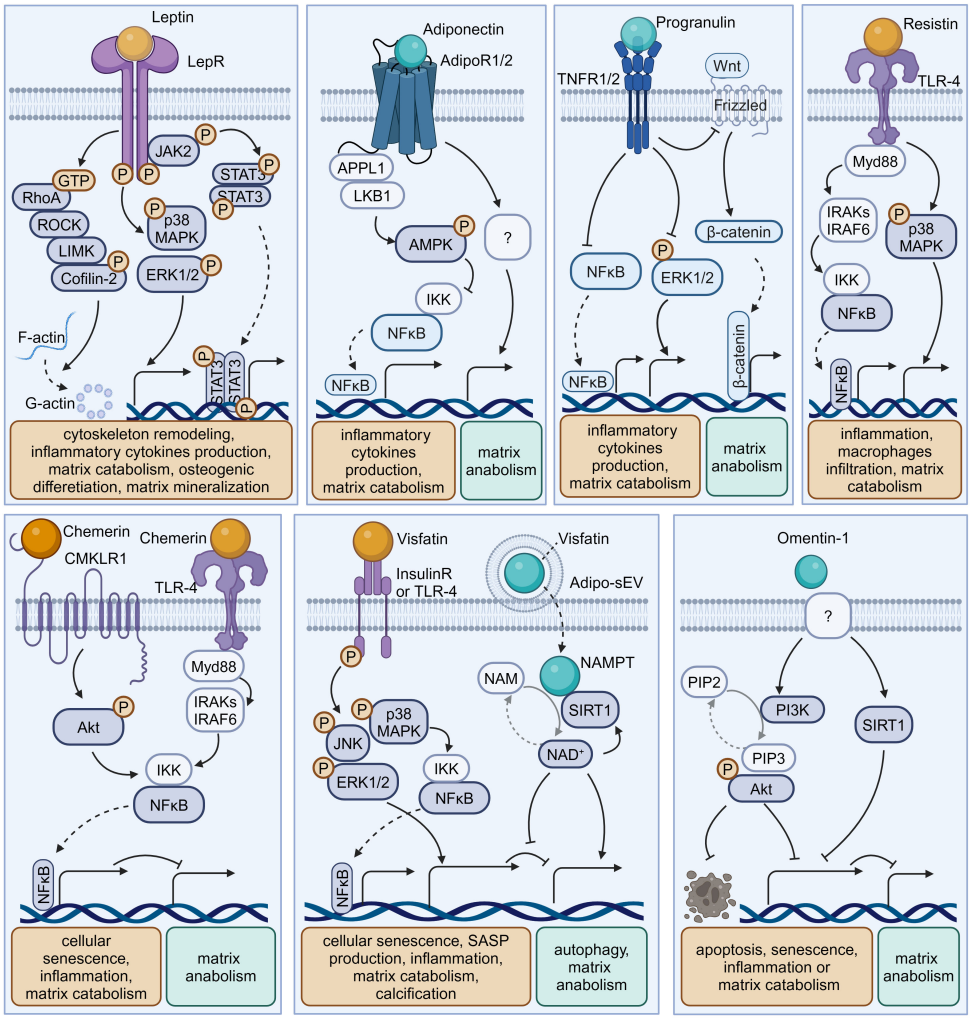

FIGURE 2
Schematic plots illustrate the signaling mechanism of various adipokines in intervertebral disc cells. Leptin, binding to LepR, activates JAK-2/STAT-3, MAPK, ERK1/2, RhoA/ROCK/LIMK/Cofilin-2 pathways, promoting disc degeneration. Adiponectin, binding to AdipoR1/2, activates AMPK, inhibiting the NF-κB pathway to exert a protective effect. Progranulin activates TNFR1/2, inhibiting NF-κB, ERK1/2, and Wnt/β-catenin pathways, providing a protective effect. Resistin, binding to TLR-4, inhibits NF-κB or MAPK pathways, exhibiting a protective effect. Chemerin, binding to CMKLR1 or TLR-4, activates the NF-κB pathway, promoting disc degeneration. Visfatin, binding to Insulin receptors (InsulinR) or TLR-4, activates JNK, MAPK, ERK1/2, and NF-κB pathways, promoting disc degeneration. However, the intracellular NAMPT activity of Visfatin delivered by Adipo-sEV could promote NAD^+ biogenesis and SIRT activity, exerting a protective effect. Omentin-1 activates PI3K/Akt and SIRT1 pathways, providing a protective effect, though its receptor remains incompletely understood. Graphic elements were created using biorender.com.

osteomyelitis, disc inflammation by ectopic adipose tissue infiltration, and osteonectin deletion-induced disc degeneration in mice (35–38).

2.1 Leptin

Leptin is a peptide hormone that is mainly synthesized in white adipose tissue and plays a regulatory role in energy metabolism and body weight. Beyond enhancing energy consumption in target cells, leptin can promote the production of pro-inflammatory cytokines, underlying the inflammatory and painful impacts of obesity. Both the leptin protein and the leptin receptor (LepR) have been detected in discs and are positively correlated with age and degeneration severity (11). While leptin can induce osteogenic differentiation in CEPs (17), the levels of leptin and its receptors increase with matrix metalloproteinase (MMP) and cytokine levels in the AF and NP of degenerative discs (12, 16, 39). Mechanistically, leptin can drive matrix catabolism via the Janus kinase-2 (JAK-2)/signal transducer and activator of transcription-3 (STAT-3) and mitogen-activated protein kinase (MAPK) pathways (13, 40). Additionally, leptin activates the ras homolog gene family member A (RhoA)/rho-associated coiled-coil containing protein kinase (ROCK) pathway and cytoskeletal remodeling in response to mechanical signals (15, 41). Although these findings suggest leptin has detrimental effects, whole-body leptin receptor knockout mice display delayed cellular proliferation and differentiation, elevated MMP-3 levels, and higher apoptosis rates, leading to IVDD (14, 18). Moreover, LepR has been identified as a lineage marker and fate modulator of notochord-derived cells at perinatal stages (42). Therefore, the potential

TABLE 1 Main characteristics of adipokines modulating IVD homeostasis and degeneration.

Organokines (Receptors)	Target	Model	Signaling pathway	Cellular behavior or phenotype induced by organokines	Citation
Leptin (LepR)	NPC AFC	Exposure[a]	MAPK↑; PI3K/Akt↑; JAK-2/STAT-3↑	Inflammation (IL-6, TNF-α)↑; NO↑; Lactate↑; Catabolism (MMP-1, 9, 13; ADAMTS-4, 5)↑	(11–14)
	NPC	Exposure[a]	RhoA/ROCK/LIMK/ Confilin-2↑	Cytoskeleton remodeling↑	(15)
	AFC	Exposure[a]	MAPK/ERK1/2↑	Differentiation (Col IX, MMP-13)↑	(16)
	CEP	Exposure[b]	ERK1/2↑; STAT-3↑	Matrix mineralization (RUNX-2)↑; Cartilage and chondrocyte↓; Disc height↓	(17)
	IVD	LepR KO[b]	NA	Proliferation↓; Differentiation↓; Disc height↓; Torsional strength↓	(18)
Resistin (TLR-4)	NPC AFC	Exposure[a]	MAPK↑; NF-κB↑	Infiltration of macrophages (CCL4)↑; Inflammation (NLRP3, caspase-1, IL-1β, IL-6, IL-8)↑; Catabolism (MMP-1, 3, 13; ADAMTS-5)↑	(19, 20)
Adiponectin (AdipoRs)	NPC	Exposure[a]	AMPK/NF-κB↑; TNF-α↓	Inflammation (TNF-α, IL-6)↓; Anabolism (Acan, Col II)↑; Catabolism (MMP-13, ADAMTS4)↓	(21)
	NP, AF	Exposure[b]	AMPK/NF-κB↑	AdipoR1↓, AdipoR2↓; Histological scores↓; DHI↑;	(21, 22)
Visfatin/ NAMPT (Insulin receptor, TLR-4)	NPC CEPC	Adipo-sEV delivery[a,b]	SIRT1/NAD^+↑	Senescence (p16)↓;,SASPs:(TNF-α, IL-6, IL-8)↓; Matrix mineralization (OCN, RUNX2)↓; Anabolism (Acan, Col II)↑; Catabolism (MMP-3, ADAMTS4)↓; Pfirrmann grade↓	(23)
	NPC	KD[a];OE[a]	MAPK/NF-κB↑	Autophagy (Beclin-1, LC3B)↓; Inflammation (TNF-α, NLRP3)↑; Anabolism (Acan, Col II)↓; Catabolism (MMP-3,13; ADAMTS-4, 5)↑;	(24, 25)
	NP	Exposure[b]	MAPK↑; JNK/ ERK1/2↑	Inflammation (IL-6)↑; Anabolism (Acan, Col II)↓; Catabolism (MMP-3)↑; Pfirrmann grade↑	(26)
Progranulin (TNFR1/2)	NPC	Exposure[a,b]	NA	Inflammation (MMP-13, COX-2, iNOS, IL-17) ↓; Chondro-staining density↑; Histological scores↓	(27)
	NPC NP	Analogue (Atsttrin)[a]; TNFR1/ 2 KO[b]	NA	Apoptosis↓; Catabolism (MMP-13)↓; Anabolism (Acan)↑; Histological scores↓; Pfirrmann grade↓	(28)
	NP, CEP, AF	PGRN KO[b]	NF-κB↓ Wnt/β-catenin↓	Inflammation (IL-17↓, IL-10↑)↓; Matrix mineralization (ALP, OCN, Osterix, BSP, Col I, AXIN2, RUNX2)↓; Anabolism (proteoglycan)↑; Catabolism (MMP-13, ADAMTS-5, 7, 12)↓; Pfirrmann grade↓	(29, 30)
Chemerin (CMKLR1, TLR-4)	NPC AFC	KD[a,b]; Exposure[a]	Akt↑; NF-kB↑	Inflammation (COX-2, IL-1β, IL-6, TNF-α)↑; Senescence (SA-β-gal, p53, p16)↑; Anabolism (Acan, Col II, SOX-9)↓; Catabolism (MMP-3, 9; ADAMTS-5)↑; Histological scores↑; DHI↓;	(31)
Omentin-1 (NA)	NPC	Exposure[b]	SIRT1↑	Senescence (SA-β-Gal, p16, p53)↓ Anabolism(Acan, Col II)↑; Catabolism (MMP-13, ADAMT-5)↓;	(32)

↑, increase; ↓, decrease; NA, not available; Exposure[a], exposure in vitro; Exposure[b], exposure in vivo; KD[a], knock down in vitro; KD[b], knock down in vivo; KO[b], knock out in vivo; OE[a], overexpression in vitro; Receptor activation[a], receptor activation in vitro; Receptor activation[b], receptor activation in vivo; LepR, leptin receptor; LRPs, low density lipoprotein-related proteins; TLR-4, toll-like receptor-4; IL, interleukin; Acan, aggrecan; Col II, type II collagen; Col lX, type IX collagen; OCN, osteocalcin; RUNX2, RUNX family transcription factor 2; STAT-3, signal transducers and activators of transcription 3; CCL4, C-C motif chemokine ligand-4; AdipoR, adiponectin receptor; DHI, disc height index; NF-κB, transcription factor-Kb; NLRP3, NLR family pyrin domain containing3; iNOS, inducible nitric oxide synthase; ALP, alkaline phosphatase; BSP, bone sialoprotein; AXIN2, axis inhibition protein 2; LC3, microtubule-associated protein 1A/ 1B-light chain 3; NAMPT, nicotinamide phosphoribosyl transferase; CMKLR1, chemokine-like receptor 1; SOX-9, SRY-box transcription factor -9; SIRT1, NAD-dependent deacetylase sirtuin-1.

fundamental role of leptin–LepR interactions in IVDD requires further exploration.

2.2 Adiponectin

Adiponectin, a glycoprotein that is uniquely expressed by adipocytes, could maintain energy balance and suppress inflammation or apoptosis in various tissues by binding to adiponectin receptors (AdipoR1/2). However, the role of adiponectin and AdipoRs in IVDD is unclear. Previous studies showed that adiponectin expression in degenerative discs was decreased or absent while AdipoR1 and AdipoR2 expression increased or decreased with the Pfirrmann grade of degenerative discs (22, 43). However, plasma adiponectin levels were found to be increased in IVDD patients (44). Recently, administration of the AdipoR agonist AdipoRon was found to effectively reduce the levels of the pro-inflammatory factor tumor necrosis factor α (TNF-α)

and mitigate disc degeneration (21). In future research, the exact role of adiponectin–AdipoR interactions in IVDD needs to be clarified.

2.3 Progranulin

PGRN, a secreted glycoprotein that can be cleaved into granulins by enzymes like elastase, exerts anti-inflammatory effects and plays protective roles by enhancing cell proliferation and through interacting with TNF receptors (TNFRs) or other receptors. Although higher PGRN levels are associated with higher degeneration severity in IVDD patients, current evidence suggests a protective role for PGRN in disc degeneration and aging (3, 45). Knockdown of PGRN in aged mice accelerates disc degeneration by promoting matrix catabolism and cellular dysfunction in AF and CEPs (29). Mechanistically, PGRN competitively binds to TNFR-1, thereby inhibiting the expression of the pro-inflammatory factor interleukin-17 (IL-17) and inflammatory and catabolic pathways (30, 45). Moreover, PGRN promotes anabolism and the production of the anti-inflammatory factor IL-10 via binding to TNFR-2 (28, 30). Additionally, PGRN and its derivatives, like atsttrin, inhibit epoxide synthase-2, IL-6, IL-17, and MMP-13 production, thereby inhibiting IVDD progression (29, 30).

2.4 Resistin

Resistin is a cysteine-rich polypeptide that is secreted by white adipocytes and is involved in insulin resistance. In agreement with the devastating effect of diabetes on IVDs, recent research indicates resistin's involvement in IVDD for its pro-inflammatory properties (46). By targeting Toll-like receptor-4 (TLR-4), resistin activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling to increase the expression of the macrophage inflammatory protein chemokine C-C motif ligand 4 (CCL4), thereby fostering macrophage infiltration into discs (19). In addition, resistin triggers inflammatory cascades through the activation of the MAPK and NF-κB pathways, which increases the NLR family pyrin domain containing 3 protein (NLRP3) inflammasomes and the expression levels of IL-1β, IL-6, IL-8, and MMPs in discs (20, 47).

2.5 Chemerin

Chemerin is an obesity-associated adipokine and is involved in various processes including inflammation by interacting with chemokine-like receptor 1 (CMKLR1). The expression levels of chemerin and CMKLR1 are increased in degenerative NP tissues, especially those of obese individuals (31). Furthermore, the administration of chemerin results in inflammation and tissue degeneration, while CMKLR1 knockdown could slow the progression of needle-induced disc degeneration in rats (31). Furthermore, chemerin exerts pro-senescent and pro-inflammatory effects on NP cells through binding to TLR-4, a well-known receptor activating the NF-κB signaling cascade (31, 48).

2.6 Visfatin

Visfatin, identified as the extracellular form of nicotinamide-phosphate ribosyl transferase (NAMPT), has been known to mediate insulin resistance and inflammation via binding to the insulin receptor or the innate immune receptor TLR-4. Visfatin could induce IL-6 expression and disc degeneration by activating the MAPK pathway, which participates in the inflammatory response (26). In addition, pharmacological inhibition or knockdown of visfatin resulted in the maintenance of metabolism balance by enhancing autophagy in the presence of IL-1β (24). Interestingly, a recent study showed that NAMPT was delivered in small extracellular vesicles derived from adipocytes (Adipo-sMV) and mediated the protective impact of Adipo-sMV through increased nicotinamide adenine dinucleotide (NAD) and NAD-dependent deacetylase sirtuin-1 (SIRT1) activity in senescent NP and CEP cells (23). Considering the lack of a secretion signal sequence, visfatin/NAMPT may play a multifaceted role dependent on its location: serving as the rate-limiting enzyme for NAD^+ biosynthesis in the cytosol or binding receptors on the cellular surface after leakage into the extracellular space.

2.7 Omentin-1

Omentin-1, an anti-inflammatory adipokine, exhibits anti-inflammatory and antioxidant properties. Its expression level is inversely correlated with the progression of various diseases, including diabetes, obesity, and osteoarthritis (49, 50). Recent studies showed that omentin-1 could protect NP cells from ongoing senescence, inflammation, apoptosis, or matrix metabolism imbalance in the presence of IL-1β through activating SIRT1 or the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB, also known as Akt) signaling pathway (32, 49). Therefore, it is valuable to further investigate its *in vivo* therapeutic potential in IVDD treatment.

3 Osteokines

Osteokines are a category of proteins predominantly secreted in bone and can have a significant influence on the homeostasis of bone and extraosseous organs (51–53). The interplay between bone homeostasis regulation and disc degeneration is becoming increasingly recognized. Indeed, the osteogenic potential of discs increases with the progression of degeneration, evidenced by elevated osteogenic differentiation of AF and CEP cells (54, 55). Then, intradiscal ectopic ossifications or calcifications can result in increased tissue stiffness, thereby provoking inflammation, disc degeneration, and low back pain (56–59). Additionally, structural alterations of vertebral bone, such as Modic changes (also known as magnetic resonance imaging [MRI] signal intensity changes in vertebral bone marrow) and vertebral osteoporosis, have been identified as associated with the development of IVDD (60, 61). Therefore, it is imperative to investigate the precise role of these osteokines (Figure 3, Table 2) in the pathophysiology of disc degeneration.

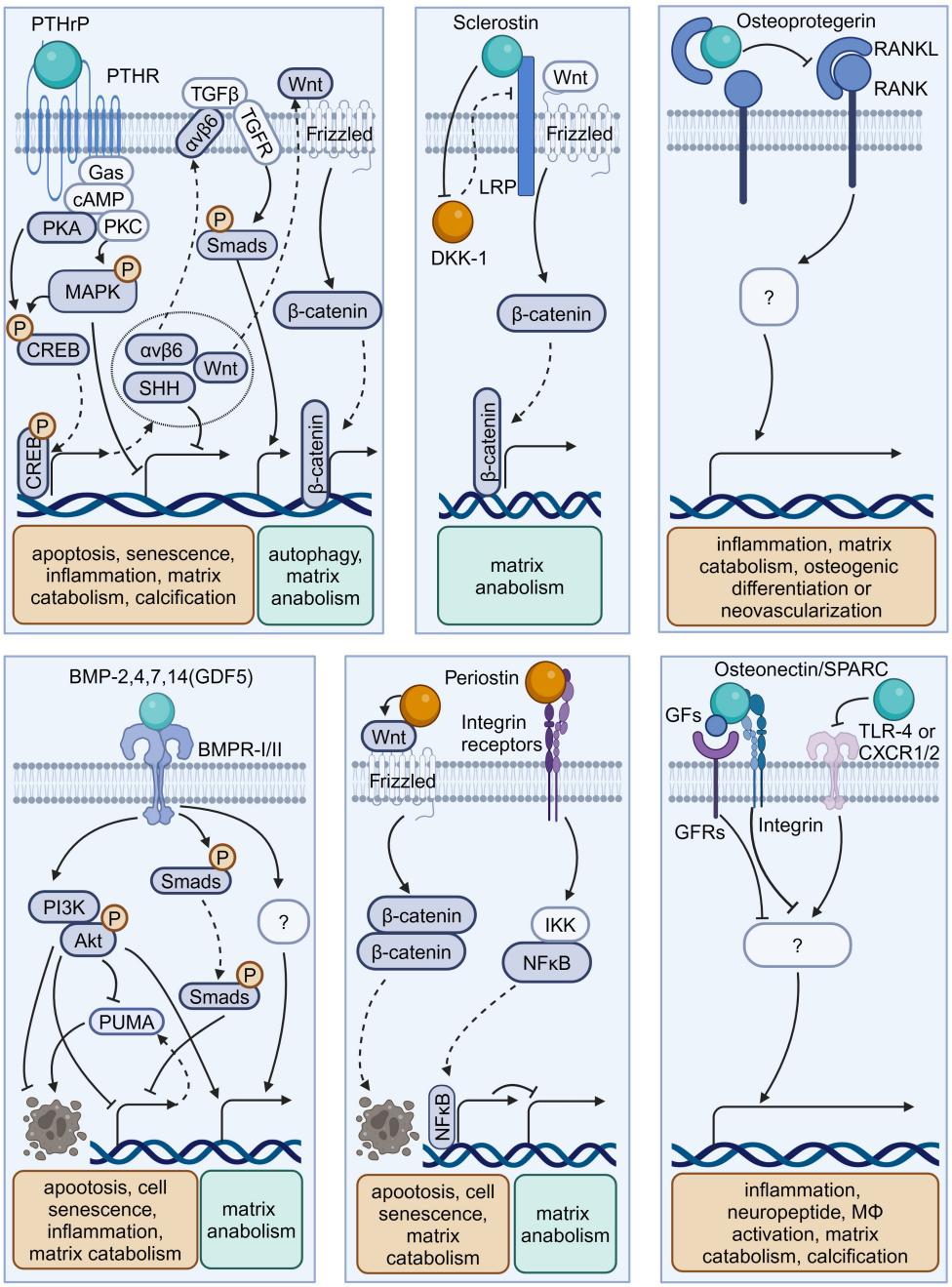

FIGURE 3
Schematic plots illustrate the signaling mechanism of various osteokines in intervertebral disc cells. PTHrP, binding to PTHR, could activate MAPK or PKA/CREB/Hedgehog pathways to protect disc from degeneration. Sclerostin binds to LRPs to activate Wnt/β-catenin pathway and matrix anabolism, while inhibits the expression of DKK-1 that inhibits Wnt/β-catenin pathway. Osteoprotegerin inhibits the RANK-RANKL interactions to protect disc from degeneration, while the intracellular signaling pathway is unknown. BMP-2,4,7,14(GDF5) binds BMPR-I/II to activate PI3K/Akt, Smads pathway and inhibit PUMA expression to protect disc from degeneration. Periostin could interact with Integrin receptors, NF-κB pathway and Wnt/β-catenin pathway to promote disc degeneration. Osteonectin/SPARC could modulate TLR-4, CXCR1/2, or GFs-GFRs interactions to protect disc from degeneration. Graphic elements were created using biorender.com.

3.1 Osteoprotegerin

OPG is a typical osteokine recognized as the regulator of bone mass and the receptor activator of NF-κB (RANK)/RANK ligand (RANKL) pathway, while it exists in various extraosseous tissues including discs. Various studies have shown a significant correlation between the levels of OPG in serum or disc samples and degeneration severity (62, 63, 81, 82). Further, OPG gene polymorphisms and increased OPG expression levels may contribute to IVDD development (81). OPG and RANK/RANKL expression could be upregulated with increased catabolism in AF, NP, or CEP cells exposed to acidic microenvironments or the

TABLE 2 Main characteristics of osteokines modulating IVD homeostasis and degeneration.

Organokines (Receptors)	Target	Model	Signaling pathway	Cellular behavior or phenotype induced by organokines	Citation
OPG (RANK/RANKL)	Disc cell	Exposure[a]	RANKL↓	Inflammation (IL-1β)↑; Catabolism (MMP-3, 13)↑	(62–64)
	CEP	OPG KO[b]	NA	Inflammation (IL-1β, IL-6, TNF-α)↑; Tissue remodeling (TRAP, Rank, MMP-9, Cathepsin K)↑; Chondrogenesis (cartilage area, growth plate thickness, aggrecan)↑; Neovascularization (VEGF-A, CD31, VE-cadherin, CD34)↑;	(65–67)
Sclerostin (LRPs)	NP	Sost KO[b]; Exposure[b]	Wnt/β-catenin↑	Matrix maturation (Col II, FOXA2, Osterix)↑; DDK-1↓; Matrix stiffness (proteoglycan↓; hydration↓)↑; DHI↓	(68)
PTHrP (PTH-1R)	NPC AFC	Analogue (PTH)[a]	mTOR↑; MAPK↑; PKA↑	Autophagy (Beclin-1, p62, LC3B)↑; Senescence (SA-β-gal)↓; Matrix mineralization (Acan↑, Col I↑, COLX↓, calcium release↓)↓	(69, 70)
	NPC CEP	Analogue (PTH)[a,b]	Wnt/β-catenin↑	Anabolism (Acan, Col II)↑; Catabolism (MMP3, 9)↓; Tissue remodeling (endplates calcification↓; micro-vessel density↓, porosity↑, thickness↑)↑; Histological score↓; DHI↑	(71, 72)
	NPC NP	Analogue (PTH)[a,b]	CREB/ Sonic Hedgehog↑	Oxidative stress (SOD-1, 2)↓; Apoptosis (Caspase-3, 8, 9)↓; Inflammation (IL-1β, IL-6, TNF-α)↓	(73)
	IVD	PTH1R KO[b]; Analogue (PTH)[b]	Integrin αvβ6/ TGF-β/CCN2↑	IVD volume↑; IVD height↑; MRI signal intensity↑	(74)
BMP-2,7 (BMPR-I/II)	NPC AFC NP	Exposure[ab]; KD[a]	PI3K/Akt↑; Puma↓	Apoptosis (Apaf-1, cleaved-caspase-3,9)↓; Senescence (SA-β-Gal, G0/G1 arrest, p16, p53)↓; Inflammation (IL-6 and TNF-α)↓; Anabolism (Acan, Col II, SOX-9)↑; Catabolism(MMP-13)↓; DHI↑;	(75–77)
Osteonectin/ SPARC (CXCR1/2, TLR-4)	NP	SPARC KO[b]	NA	Inflammation (CXCL-1, 5)↓; Macrophage activation (ITGAM↓)↑; Endplate calcification↑; DHI↓	(78, 79)
	NP	SPARC KO[b]; Receptor inhibition[a]	NA	Inflammation (C3aR1, COX-2, CCL-7,19)↓; Catabolism (MMP-3, 13↓, TIMP1, 2↑)↓; Neutral zone stiffness↓;	(80)

↑, increase; ↓, decrease; NA, not available; Exposure[a], exposure in vitro; Exposure[b], exposure in vivo; KD[a], konock down in vitro; KD[b], knock down in vivo; KO[b], knock out in vivo; Receptor activation[a], receptor activation in vitro; Receptor activation[b], receptor activation in vivo; RANK, receptor activator of NF-κB; RANKL, receptor activator of NF-κB ligand; PTH, parathyroid hormone; PTH1R, parathyroid hormone type 1 receptor; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; SPARC, secreted protein acidic and rich in cysteine; TRAP, tartrate-resistant acid phosphatase type 5; VEGF-A, vascular endothelial growth factor-A; VE-cadherin, vascular endothelial-cadherin; FOXA2, forkhead box protein a2; SOD, superoxide dismutase; CCN2, communication network factor-2; Apaf-1, apoptotic protease activating factor-1; CXCL, C-X-C motif ligand; ITGAM, integrin subunit alpha M; C3aR1, complement 3a receptor 1; TIMP,tissue inhibitor of matrix metalloproteinase.

inflammatory factor IL-1β (63, 64). However, OPG knockout results in osteoclast-mediated cartilage erosion, leading to disorganized alignment of CEPs, enhanced bone formation or neovascularization, and elevated inflammatory factors in mice (65–67). Thus, the multifaceted role of OPG in disc homeostasis highlights that further research is needed to elucidate its mechanism in IVD biology.

3.2 Sclerostin and Dickkopf-1

Sclerostin and DKK-1 are a pair of physical activity-related osteokines that competitively bind to the Wnt coreceptors lipoprotein receptor-related proteins (LRPs) and mediate the crosstalk between bone and other organs. Recently, sclerostin and DKK-1 have been shown to be involved in spinal pathological conditions, including spinal ligament ossification, spondylarthritis, and disc calcification (66, 67, 83). A recent study illustrated the compensatory increase in DKK-1 levels and the suppression of the Wnt/β-catenin pathway in sclerostin-depleted murine discs, and the administration of antibodies against sclerostin or DKK-1 exhibited beneficial effects on proteoglycan content, disc hydration, and height (84). Considering the complex role of Wnt signaling in disc development and degeneration, it is needed to clarify the exact roles and determinants of these Wnt inhibitors in IVDD (85).

3.3 Parathyroid hormone-related protein

PTHrP, first discovered in malignancy-associated hypercalcemia, has been recognized as an osteokine acting in a paracrine manner on bone and other tissues through binding to the PTH-1 receptor (PTH-1R). PTHrP is involved in intervertebral disc maturation and calcification, delays cellular mineralization and hypertrophy in Col IX knockout mice, and inhibits progressive kyphoscoliosis in fibroblast growth factor receptor-3 (FGFR-3) knockout mice (83–85). By enhancing Hedgehog, transforming growth factor beta (TGF-β), Wnt/β-catenin, mammalian target of rapamycin (mTOR), and MAPK/protein kinase A (PKA) signaling, PTH-1R activation by

PTH administration plays a protective role in NP cell activity and disc homeostasis (69–72, 74). Considering the elevated PTH-1R expression in NP cells, the role of PTHrP–PTH-1R interactions in IVDD ought to be elucidated in future research (73, 86).

3.4 Bone morphogenetic proteins

BMPs are osteokines participating in the formation and maintenance of bone and various non-bone tissues, including cartilage (51, 87). Various studies confirmed the presence of BMPs, including BMP-2, 4, 7, and 14 (also known as growth differentiation factor -5 [GDF-5]), with their receptors (BMPR-I/II) in the IVD (88–92). Mechanistically, BMP-2 and BMP-7 activate various signaling pathways, including the Smad/Puma and PI3K/Akt signaling pathways, to inhibit NP cell apoptosis or senescence (75, 76). Additionally, GDF-5 deficiency in mice results in notable matrix abnormalities and disc degeneration, which could be substantially restored by treatment with recombinant human GDF5 (93, 94). Due to their anti-inflammatory and pro-regenerative effects, recombinant human BMPs are used for bone grafting in vertebral fusion surgery as well as disc tissue engineering (95, 96).

3.5 Osteonectin

Osteonectin, also known as secreted protein acidic and rich in cysteine (SPARC), is one of the most abundantly expressed non-collagenous proteins in mineralized tissues as well as non-mineralized tissues and orchestrates inflammation and tissue remodeling through binding to TLR-4, BMPRs, integrin receptors, and various growth factors. SPARC expression in human disc cells decreases with age and disc degeneration (97). Moreover, SPARC-deficient mice exhibit spontaneous disc degeneration and lower back pain, evidenced by an age-dependent increase in neuron markers like calcitonin gene-related peptide and Neuropeptide-Y within the discs and peripheral nerves (78, 98, 99). Additionally, these mice demonstrate a diminished lumbar neutral zone, increased spinal stiffness, and reduced spinal mobility (100). SPARC knockout results in elevated levels of inflammatory mediators and vascular endothelial growth factor, which can be mitigated by interventions like exercise and treatment with TAK-242 (a TLR-4 antagonist) or reparixin (an inhibitor of CXC chemokine receptors [CXCR1/2]) (80, 101–103). Therefore, SPARC is a promising target for preventing IVDD in modulating cell–matrix interactions and governing neural, immune, and inflammatory pathways (79, 104, 105).

3.6 Periostin

Periostin is a bone turnover-related osteokine that is highly expressed in collagen-rich tissue—including periosteum—and mediates tissue remodeling through binding to integrin receptors and proteoglycans. In human and rat discs, periostin levels gradually decrease from the outer AF to the central NP and increase with degeneration development (106–108). Mechanistically, periostin promotes NP cell apoptosis via the Wnt/β-catenin signaling pathway and cellular senescence via the NF-κB pathway, contributing to the development of IVDD (109, 110). Considering its role as a matricellular protein, further investigation is needed to elucidate whether periostin participates in the regulation of disc cell–matrix interactions (111).

3.7 Other potential osteokines

Lipocalin-2 (LCN-2), a glycoprotein secreted by osteoblasts and adipocytes, functions as a pro-inflammatory factor in obesity-related metabolic disorders, despite our limited understanding of the potential LCN-2 receptors (112, 113). A recent study suggested a correlation between LCN-2 and the expression of inflammation-related genes in human discs (114). Moreover, upregulated expression of LCN-2 has been validated to increase MMP-9 activity in AF cells (115). Considering that LCN-2 could function as a biomechanical and inflammatory sensor in bone–cartilage crosstalk, its specific role in IVDD needs to be elucidated (9).

Fibroblast growth factor-23 (FGF-23) is the first identified osteokine that can bind to the tyrosine kinase FGF receptors (FGFRs) to regulate phosphate and vitamin D metabolism (116). However, direct evidence linking FGF-23 to IVDD is currently lacking. Klotho, a crucial cofactor for FGF-23 in the activation of FGFRs, mitigates inflammation in NP cells and counteracts extracellular matrix degradation in IVDD (117, 118). Accordingly, the role of FGF-23 in IVD homeostasis, potentially analogous to that of Klotho, presents an intriguing avenue for further investigation.

4 Myokines and cardiokines

Similar to adipose tissue and bone, skeletal muscle and cardiac muscle can function as endocrine organs and secrete tissue-specific hormones, termed myokines and cardiokines, respectively (119) (Figure 4, Table 3). It is well recognized that these molecules mediate cross-organ crosstalk beyond the muscle tissue itself and orchestrate the multi-tissue response to physical activity and other stress (112, 113, 119, 136). Given the emerging link between muscle activity and IVDD progression, the roles of myokines and cardiokines in IVDD deserve more attention and in-depth investigation.

4.1 Irisin

Irisin is a well-characterized myokine derived from fibronectin type III domain-containing protein 5 (FNDC5). It mediates the health benefits of exercise by binding with integrins. Exercise elevated irisin levels in plasma and NP tissue and FNDC5/irisin knockout abolished the protective effects of exercise against IVDD in a murine model (114). By activating autophagy or large tumor suppressor kinase (LATS)/yes-associated protein (YAP)/connective tissue growth factor (CTGF, also known as CCN2) signaling, irisin

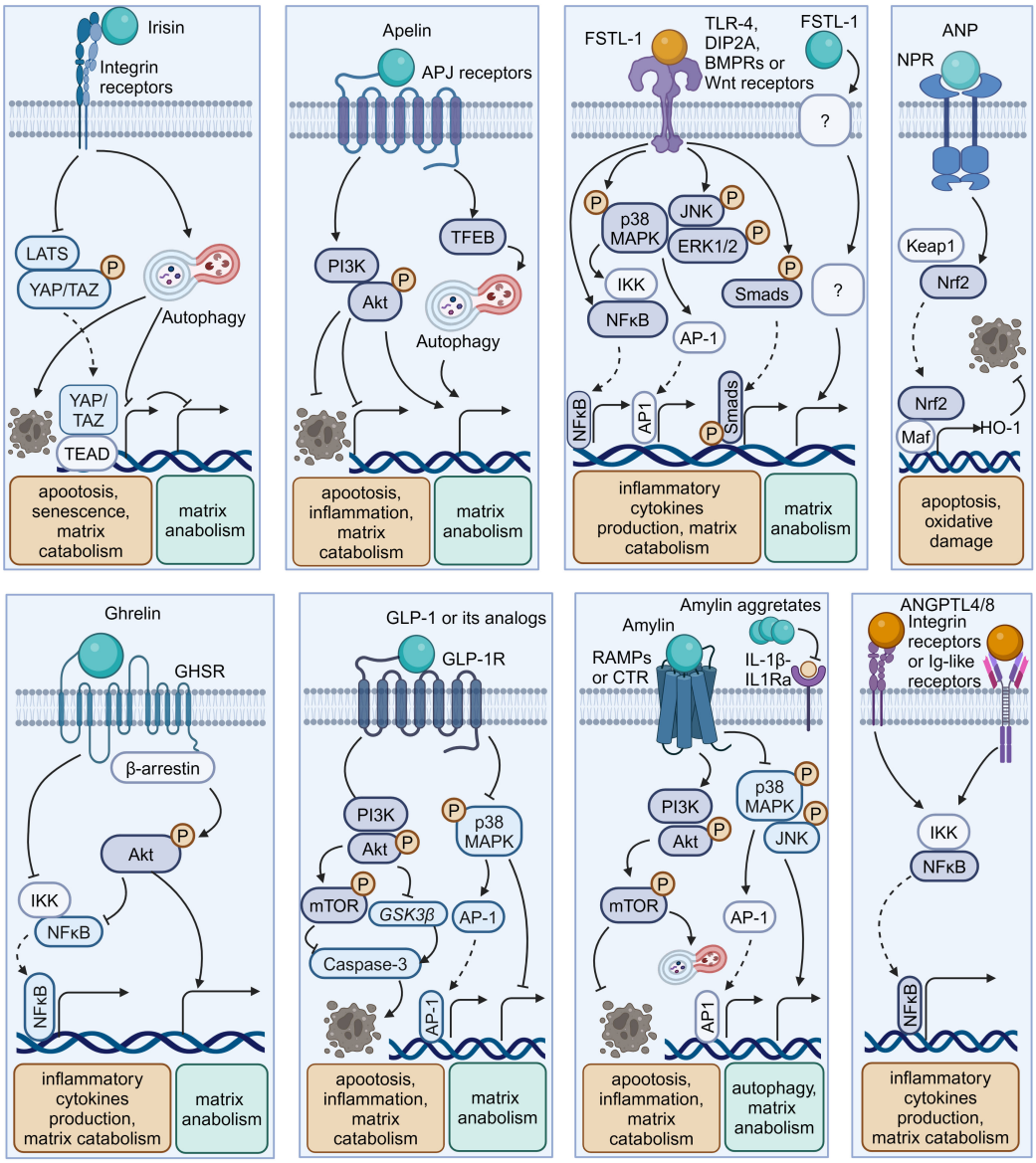

FIGURE 4
Schematic plots illustrate the signaling mechanism of other organokines in intervertebral disc cells. Irisin binds Integrin receptors to activate autophagy and inhibit LATS/YAP pathway to protect disc from degeneration. Apelin, binding APJ receptors to activate PI3K/Akt pathway and induce TFEB-mediated autophagy to protect disc from degeneration. FSTL-1, binding TLR-4, DIP2A, BMPRs or Wnt receptors, could activate MAPK, JNK, ERK1/2, NF-κB and Smad pathway to promote disc degeneration. Additionally, the FSTL-1 deficiency inhibit the maintenance of disc homeostasis. ANP binds NPR to protect cell from apoptosis and oxidative damage by activating NRF2/HO-1 pathway. Ghrelin binds GHSR to activate Akt and inhibit NF-κB pathways to protect disc from degeneration. GLP-1 or its analogs, binding to GLP-1R, activate PI3K/Akt/mTOR, PI3K/Akt/GSK3β, as well as inhibiting MAPK/AP-1 pathways to protect disc from degeneration. Amylin binds RAMPs or CTR to activate PI3K/Akt/mTOR pathway and inhibits MAPK/AP-1 pathways to protect disc from degeneration. Meanwhile, Amylin aggregates could inhibit IL-1β/IL1Ra interactions. ANGPTL4/8 could bind Integrin receptors or Ig-like receptors to activate NF-κB pathway to promote disc degeneration. Graphic elements were created using biorender.com.

can help maintain cellular activity and matrix metabolism balance and inhibit inflammatory effects, thereby decelerating the progression of IVDD (115, 117).

4.2 Myostatin

Myostatin (also known as GDF8) functions as a negative regulator of skeletal muscle growth. It binds to activin receptors (ACVRs) and can be expressed in back muscles after IVD injury (118, 137). However, the role of myostatin in IVDD is incompletely understood. Myostatin plays an inhibitory role in cartilage formation and chondrocyte proliferation, and its serum levels exhibit a positive correlation with the severity of conditions such as osteoarthritis and rheumatoid arthritis (121, 138). Additionally, ACVR1 silencing reversed lipopolysaccharide-induced inflammation and matrix degradation in NP cells, implying the potential unfavorable impacts of ACVR1 activation by myostatin

TABLE 3 Main characteristics of other organokines modulating IVD homeostasis and degeneration.

Organokines (Receptors)	Target	Model	Signaling pathway	Cellular behavior or phenotype induced by organokines	Citation
Periostin (Wnt, Integrins)	NPC NP	Exposure[b]; KD[b]; Inhibitor[a]	Wnt/β-catenin↑; NF-κB↑	Apoptosis (Caspase-9, cleaved-caspase-3, Bcl-2, Bax)↑; Senescence (β-Gal, IL-1β, IL-6, IL-8)↑; Anabolism (Acan, Col II)↓; Catabolism (MMP-13)↑; Pfirrmann grade↑	(109, 110)
Irisin/FNDC5 (Integrin receptors)	NPC	FNDC5 KO[b]; OE[a,b]	AMPK/mTOR↑	Autophagy (p62, LC3B)↑; Senescence (SA-β-gal, p16)↓; Apoptosis (C-caspase-3)↓; Histological grades↓; DHI↑	(114)
	NPC	Exposure[a]	LATS/YAP/CTGF↑	Anabolism (Acan, Col II)↓; Catabolism (MMP-9, 13↑, ADAMTS-4, 5↑, TIMP-1, 3↓)↑	(115, 117)
Myostatin/ GDF8 (ACVR1)	NPC	Receptor KD[a]	NA	Apoptosis↑; Inflammation (NF-α, IL-1β, IL-6)↑; Anabolism (Acan, Col II)↑	(120)
	IVD	Myostatin KO[b]	NA	Chondrogenesis (Col II, SOX-9, proteoglycan)↓; Endplate ossification↓	(121, 122)
Apelin (APJ)	NPC	Exposure[a]	TFEB↑	Autophagy (LC3B, p62)↑; Anabolism (Acan, Col II)↑	(123)
	NPC	Exposure[a]	PI3K/Akt↑	Apoptosis↓; Inflammation (IL-6, TNF-α)↓; Anabolism (Acan, Col II, SOX9)↑; Catabolism (MMP-3, 13)↓	(124)
FSTL-1 (TLR-4, etc.)	NPC	Exposure[a]	MAPK/ERK1/2↑; JNK↑; NF-κB↑	Inflammation (TNF-α, IL-1β, IL-6, COX-2, iNOS)↑; Catabolism (MMP-13)↑	(125, 126)
	NP	KD[b]	Smad1/5/8↑; ERK1/2↑; NF-κB↑	Inflammation (COX-2, iNOS, MMP-13, ADAMTS-5)↓; Cartilage area mean density↑	(127)
ANP (NPR)	CEPC	Exposure[a]	Nrf2/HO-1↑	Apoptosis (Bcl-2, Bax, C-caspase-3)↓; Oxidative Stress (MDA, SOD, NO)↓	(128)
Amylin/IAPP (RAMPs or CTR)	Disc cell IVD	Exposure[a]; KD[a]	IL-1β/IL-1Ra; PI3K/Akt/mTOR↑; MAPK/JNK↑	Apoptosis (Caspase-3↓, Fas/FasL↓, VDAC-1↓, cyto-C↓, Bax↓; Bcl-2↑)↓; Anabolism (Acan, Col II, SOX9)↑; Catabolism (MMP3, 9, 13; ADAMTS5)↓; Histological grades↓	(129–131)
Ghrelin (GHSR)	NP	Exposure[a,b]	NF-κB↓ Akt↑	Inflammations(MMP13, ADAMTS-5, TNF-α, iNOS)↓; Anabolism(Acan, Col II, SOX-9)↑; Pfirrmann grade↓	(132)
GLP-1 (GLP-1R)	NPC	Receptor activation[b]	MAPK/AP-1↓	Anabolism(Acan, Col II, SOX9)↑; Catabolism (ADAMTS5, MMP3, 13)↓; Histological scores↓; Pfirrmann grade↓	(133)
	NP	Receptor activation[a]	PI3K/Akt/ mTOR& GSK3β↑	Apoptosis (Caspase-3)↓	(134, 135)

↑, increase; ↓, decrease; NA, not available; Exposure[a], exposure in vitro; Exposure[b], exposure in vivo; KD[a], konock down in vitro; KD[b], knock down in vivo; KO[b], knock out in vivo; Receptor activation[a], receptor activation in vitro; Receptor activation[b], receptor activation in vivo; ACVR1, Activin receptors-1; NPR, Natriuretic peptide receptors; RAMPs, Receptor activity modifying proteins; CTR, C-terminal peptide; AP-1, activator protein 1; GSK3β, Glycogen synthase kinase-3 beta; VDAC-1, Voltage-dependent anion channel-1; Bcl-2, B cell CLL/lymphoma-2; Bax, Bcl-2-associated X protein.

upon discs (120). However, earlier studies indicated the fundamental role of myostatin in disc homeostasis. Myostatin deficiency in mice resulted in increased muscle weight, accompanied by endplates ossification at the L4–L5 level and a notable reduction in proteoglycan content in the endplates and inner AF (122, 139). Therefore, more comprehensive research is needed to elucidate the potential mechanism underlying the multi-faceted role of myostatin–ACVRs interactions in IVDD.

4.3 Apelin

Apelin, identified as the endogenous ligand for the G-protein coupled receptor APJ, plays a regulatory role across diverse tissues including skeletal muscle and the cardiovascular system. Apelin and its receptor APJ are downregulated in degenerative NP tissue (123, 124). Moreover, administration of apelin results in suppressed matrix degradation, apoptosis, and inflammation in the presence of IL-1β and increased matrix anabolism in the presence of the oxidative stress inducer H_2O_2 (123, 124). Mechanistically, apelin enhances the PI3K/Akt pathway and transcription factor EB (TFEB)-mediated autophagy flux in NP cells (123, 124). Considering the significant role of apelin in exercise-induced benefits, exploring whether and how apelin participates in muscle–disc crosstalk is valuable (140).

4.4 Follistatin-like-1

Follistatin-like-1(FSTL-1) is a kind of myokine and cardiokine modulating immune responses, cell proliferation, and differentiation through binding to TLR-4, Wnt receptors, and various growth factors. FSTL-1 has an adverse effect on disc homeostasis, accompanied by increased concentrations in the

serum of IVDD patients, discs of rats with IVDD, and the cerebrospinal fluid of dogs with IVDD (125, 141). Mechanistically, FSTL-1 promotes NP cell inflammation by activating the MAPK, Smads, or NF-κB signaling pathway (125, 141). Interestingly, the knockout of FSTL-1 during embryonic development leads to a decrease in vertebral cartilage and matrix anabolism, indicating its fundamental role in early IVD formation (142). Moreover, FSTL-1 may play diverse roles in disc development and maturation, given that it could mediate the differentiation of pre-cartilaginous stem cells into NP-like cells (143).

4.5 Atrial natriuretic peptide

As a typical cardiokine, ANP binds to natriuretic peptide receptors (NPRs) to induce diuretic, natriuretic, and vasodilating effects and regulate the renin–angiotensin–aldosterone system (144). NPR mutations can result in impaired cartilage development, potentially leading to secondary degenerative changes and suboptimal joint development (145, 146). Recent studies indicated that ANP inhibited oxidative damage and cell death in endplates by activating the nuclear factor erythroid 2-related factor 2 (Nrf2)/Heme oxygenase-1 (HO-1) signaling pathway (128). Additionally, given the presence and adverse effects of the local tissue renin–angiotensin system (tRAS) in discs, whether ANP has a protective impact via suppressing the tRAS in IVDD is an interesting avenue for future research (147–149).

5 Gastrointestinal hormones and hepatokines

In recent years, the interplay between the digestive system and disc homeostasis has received increasing attention, with a special focus on the role of the gut microbiota in the gastrointestinal endocrine system (150–153). It is worth noting that the endocrine functions of the digestive system facilitate complex inter-organ communication through various gastrointestinal hormones (such as Ghrelin and Amylin) and hepatokines (Figure 4, Table 3) (154, 155). Insight into how these endocrine factors influence disc physiology can expand our understanding of IVDD.

5.1 Ghrelin

Ghrelin is a circulating brain–gut peptide hormone that promotes growth hormone secretion via binding to the growth hormone secretagogue receptor (GHSR) and participates in the regulation of insulin resistance, obesity, and inflammation. Ghrelin was found present in the NP tissue, and ghrelin administration demonstrated a protective effect in a rabbit IVDD model (132). Mechanistically, ghrelin suppresses IL-1β-induced catabolism and inflammatory cytokine production by inhibiting the NF-κB pathway, while promoting anabolism via Akt signaling (132).

5.2 Glucagon-like peptide-1

Glucagon-like peptide-1(GLP-1), a peptide hormone secreted by intestinal L-cells, has broad pharmacological potential for managing type 2 diabetes mellitus and metabolic syndrome-related disorders by binding to its receptor -GLP-1R. GLP-1R activation leads to the inhibition of inflammation and apoptosis through downstream pathways including the PKA, PKC, and extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathways (156, 157). Notably, GLP-1R activation by liraglutide (a long-acting GLP-1 analog) has been shown to protect NP cells against hyperglycemia-induced apoptosis via the PI3K/Akt signaling pathway (134, 135). Administration of another GLP-1R agonist, exenatide, in discs promotes matrix synthesis and mitigates oxidative stress-induced matrix catabolism via inhibiting the activation of MAPK and activator protein-1 (AP-1) activity (133). Considering the therapeutic potential of GLP-1 activation, there is a need to further elucidate the role of endogenous GLP-1 in IVDD.

5.3 Amylin

Amylin, also known as islet amyloid polypeptide (IAPP), is a peptide that is predominantly secreted by pancreatic islet β-cells and participates in the development of diabetes through receptor activity-modifying proteins (RAMPs) or the calcitonin receptor (CTR) to inhibit insulin and glucagon secretion. During IVDD progression, amylin and its receptors are downregulated in the NP and AF cells, while amylin aggregates accumulate in NP tissues (129–131). Amylin overexpression in NP cells can maintain matrix metabolism balance and control the autophagy–apoptosis crosstalk by the PI3K/Akt/mTOR and MAPK signaling pathways (130). Meanwhile, these protective effects could be augmented by neutralizing IL-1β/IL-1 receptor antagonist (IL-1Ra) signaling induced by amylin aggregation (129). Furthermore, the amylin analog pramlintide showed the ability to relieve matrix metabolism impairment and enhance cell survival via a mitochondrial-dependent apoptotic pathway in NP cells (158). Additionally, amylin activates Akt/mTOR signaling to protect AF cells from death through the death receptor Fas/FasL and the mitochondrial-dependent apoptotic pathway (131).

5.4 Hepatokines

Hepatokines, such as angiopoietin-like proteins (ANGPTLs) and fetuin-A (also known as α2-HS-glycoprotein), are hormone-like proteins secreted by hepatocytes (159). ANGPTLs act as modulators of lipid metabolism, angiogenesis, and inflammation via binding to integrin receptors and immunoglobulin-like receptors. However, their roles in regulating disc homeostasis are poorly understood. Recent research illustrated the correlation between the upregulation of ANGPTL4/8 and the severity of disc degeneration (160, 161). Mechanistically, ANGPTL4/8 appears to promote matrix degradation and the production of inflammatory cytokines like TNF-α through the activation of the NF-κB signaling pathway (161, 162).

Fetuin-A functions as an indirect inhibitor of ectopic mineralization and inflammation. Recent studies demonstrated that intra-articular injection of fetuin-A derivatives leads to improved osteoarthritis scores and mobility in a rat osteoarthritis model (163). Thus, it is worthwhile to explore the role of fetuin-A in IVDD.

6 Regulatory factors of organokines

Organokines, serving as the potential communicator between the extra discal tissues and the disc, participate in pathological processes such as cell death, inflammation, and matrix loss, thereby contributing to IVDD onset and progression. Current evidence suggests that the release and interactions of organokines could be regulated by multiple factors, making their impact challenging to quantify. Lifestyle factors such as exercise, diet, stress, sleep, and microbiome profoundly influence organokines production, affecting disc homeostasis and susceptibility to IVDD-related diseases (6, 164–166). Exercise, known as beneficial for IVD homeostasis, can modulate the release and activity of organ factors like irisin, ANGPTL4, osteocalcin, and adiponectin (113, 167). Notedly, acute exercise can fast change levels of myokines, hepatokines, osteokines, and immune cytokines, while long-term training alters baseline adipokines (113, 167). For instance, exercise normalizes leptin and lowers resistin, reducing inflammation and insulin resistance, which may help protect against IVDD (113). Considering the individual variability in response, further research is essential to explore pharmacological mimics of exercise on organokines modulation for IVDD treatments.

Diet type or pattern have potential protective effects on disc homeostasis and degeneration, taking Dietary supplements such as n-3 fatty acids (FAs) and bioactive dietary polyphenol preparations (BDPP) for example (168, 169). Interestingly, recent studies indicate that dietary patterns and types are closely related to adipokine secretion (170). Mediterranean, low-fat, and low-carbohydrate diets have been found associated with decreased levels of leptin and vaspin and increased adiponectin (170). Leptin and vaspin may adversely affect disc homeostasis maintenance, while the role of adiponectin remains controversial. Therefore, future research may focus on identifying whether the secretion type, quantity, and activity of organokines underly the links between diet types, patterns, or nutritional supplements and disc homeostasis.

7 Conclusion and future directions

A variety of organokines from adipose, bone, muscle, or digestive tissues play an adverse or protective role in intervertebral disc homeostasis. Most studies have focused on the impact on cells or tissue of single origin and have not considered overall disc or extra discal dynamics. Functional studies using cell cultures and animal models are encouraged to comprehensively evaluate the role of organokines in IVDD, especially cross-organ communication. The impact and detailed mechanisms of organokines-mediated interactions warrant further investigation under both physiological and pathological conditions.

Future research should prioritize developing pharmacological agents or biologics designed to modulate organokines activity, agonists or antagonists for receptors of organokines and inhibitors for organokines signaling pathways for potential clinical applications. Current investigations into the regulation of organokines by exercise, diet, and stress predominantly rely on *in vitro* or animal models. Moreover, it is essential to elucidate which organokines paly dominant roles on disc cell homeostasis and matrix metabolism balance. Consequently, future studies need to be more holistic, examining the impact of specific lifestyle choices on the entire spectrum of organokines, ideally assessing both local disc tissue and systemic levels. Given that many aspects of these molecules in humans remain under-explored or contentious—such as their *in vivo* half-life, protein binding in circulation, effective concentration in disc tissues, receptor interactions, and overall impact on disc health—clinical trials face a considerable journey ahead.

Author contributions

YH: Data curation, Investigation, Visualization, Writing – original draft, Writing – review & editing. SL: Investigation, Visualization, Writing – original draft, Writing – review & editing. HL: Funding acquisition, Investigation, Resources, Writing – review & editing. FD: Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Project administration. ZS: Conceptualization, Project administration, Resources, Supervision, Writing – review & editing. LX: Conceptualization, 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.

Publisher's note

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Glossary

AdipoRs	Adiponectin receptors
ACVRs	Activin receptors
AdipoR-2	Adiponectin receptor-2
AdipoR-1	Adiponectin receptor-1
Adipo-sMV	Small extracellular vesicles derived from adipocytes
AF	Annulus fibrosus
AMPK	AMP-activated protein kinase
ANGPTLs	Angiopoietin-like proteins
ANP	Atrial natriuretic peptide
AP-1	Activator protein-1
BMPs	Bone morphogenetic proteins
CEP	Cartilage endplates
CMKLR1	Chemokine-like receptor 1
CTGF	Connective-tissue growth factor
CTR	Calcitonin receptor
DKK-1	Dickkopf-1
DM	Diabetes mellitus
ECM	Extracellular matrix
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
FGFRs	FGF receptors
FNDC5	Fibronectin type III domain-containing protein 5
GDF5	Growth differentiation Factor 5
GHSR	growth hormone secretagogue receptor
GLP-1	Glucagon-like peptide-1
GLP-1R	Glucagon-like peptide-1 receptor
HO-1	Heme oxygenase-1
IL-17	Interleukin-17
IL-6	Interleukin-6
IVD	Intervertebral disc
IVDD	Intervertebral disc degeneration
JNK	C-Jun N-terminal kinse
LATS	Latency-associated transcript
LCN-2	Lipocalin-2
lepR	Leptin receptor
LRPs	Lipoprotein receptor-related proteins
MAPK	Mitogen-activated protein kinase
MMP	Matrix metalloproteinase
MMP-13	Matrix metalloproteinase-13
MRI	Magnetic resonance imaging
mTOR	Mammalian TOR
NAD	Nicotinamide adenine dinucleotide
NAMPT	Nicotinamide-phosphate ribosyl transferase
NF-κB	Transcription factor-Kb
NLRP3	NLR family pyrin domain containing 3
NP	Nucleus pulposus
NPR	Natriuretic peptide receptors
Nrf2	Nuclear factor E2-related factor 2
OA	Osteoarthritis
OPG	Osteoprotegerin
PGRN	Progranulin
PI3K	Phosphoinositide 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PTH	Parathyroid hormone
PTH1R	Parathyroid hormone 1 receptor
PTHrP	Parathyroid hormone Related Protein
RAMPs	Receptor activity modifying proteins
rhGDF-5	Recombinant human growth differentiation factor 5
SPARC	Secreted protein acidic and rich in cysteine
TGF-β	Transforming growth factor-β
TLR-4	Toll-like receptor 4
TNF	Tumor necrosis Factor
TNFR1	tumor necrosis factor receptors 1
TNFR2	tumor necrosis factor receptors 2
TNFRs	tumor necrosis factor receptors
TNF-α	Tumor necrosis Factor Alpha
tRAS	Tissue renin–angiotensin system

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The association between diabetes mellitus and musculoskeletal disorders: a systematic review and meta-analysis

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Background: Despite the fact that DM patients are living longer, research on the prevalence of MSDs and other related illnesses is still lacking compared to that of other comorbidities. This study systematically reviewed and meta-analyzed cohort studies to determine the association between diabetes mellitus (DM) and musculoskeletal disorders (MSDs).

Methods: A comprehensive search of international databases, including Medline (PubMed), Web of Science, Scopus, and Embase, was conducted up to June 2023 to identify relevant studies investigating the association between MSDs and DM.

Results: The meta-analysis included ten cohort studies with a total of 308,445 participants. The pooled risk ratio (RR) estimate for the association between MSDs and DM was 1.03 (95% CI 1.00-1.06). Based on subgroup analysis, the association between longer duration (more than 7), European, below the age of 70, and female patients was higher than the others.

Conclusion: In conclusion, the results of this meta-analysis suggest that there may be an association between MSDs and diabetes in people with diabetes. These findings add to the existing knowledge on this topic and highlight the importance of recognition and management of MSDs in people with DM. There is a need for further research to investigate the underlying mechanisms and to develop targeted interventions for the prevention and management of MSDs in this population.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=381787, identifier CRD42022381787.

KEYWORDS

musculoskeletal disorders, diabetes mellitus, complications, evidence synthesis, meta-analysis

Introduction

Diabetes mellitus (DM) is a chronic disorder defined as persistent hyperglycemia (1). The prevalence of DM in 2011 was 366 million worldwide, which will rise to 552 million by 2030 (2). DM is associated with a range of complications, including both microvascular and macrovascular conditions. Microvascular complications of DM involve damage to the small blood vessels, particularly in the eyes (retinopathy) and nerves (neuropathy). Retinopathy can lead to vision impairment or even blindness, while neuropathy can result in numbness, tingling, or pain in the extremities. In severe cases, it may lead to foot ulcers or amputation. On the other hand, macrovascular complications of DM are related to large blood vessels and can affect various organs. Ischemic heart disease, in which blood flow to the heart muscles is reduced, and stroke, in which blood flow to the brain is interrupted, are two of the most common macrovascular problems that can happen because of DM. These conditions increase the risk of heart attacks and strokes in individuals with diabetes. Both microvascular and macrovascular complications contribute significantly to the morbidity and mortality associated with DM. Therefore, managing and preventing these complications are essential aspects of diabetes care and require comprehensive strategies targeting blood glucose control, blood pressure management, lipid control, and lifestyle modifications (3). Signs of MSDs associated with DM include muscle pain, joint pain or stiffness, reduced joint mobility, joint swelling, deformities, and a sensation of pins and needles in the arms or legs. Some MSDs are unique to individuals with diabetes. These complications significantly impact the quality of life and life expectancy of diabetic patients. Despite the increasing life expectancy of DM patients due to the availability of new antidiabetic drugs, the prevalence of MSDs and related disorders remains understudied compared to other complications (4–7).

The exact mechanism of MSDs in DM is unclear, but changes in collagen deposition and progressive non-enzymatic glycosylation in the connective tissue may be the cause (8, 9). MS complications affect different parts of the body. Soft tissue disorders such as cheiroarthropathy, carpal tunnel syndrome, trigger finger, Dupuytren's contracture, and frozen shoulder can occur. Charcot arthropathy and gouty arthritis are examples of joint disease in people with diabetes. Bone involvement, such as osteoporotic and non-osteoporotic fractures and idiopathic skeletal hyperostosis, is seen (10, 11). Different places in patients with DM, such as the wrist, neck, spine, and knee, are involved (12). Diabetes duration, glucose level control, sex, and age are some risk factors for musculoskeletal complications (13, 14). Various studies, such as case-control or cohort studies, have been conducted around the world, but the results of these studies are controversial. This condition has implications for clinical and public health decision-making worldwide, particularly in developing countries. Determining the exact association between DM and MSDs may help clinicians and specialists reduce the impact and improve the quality of life of people with DM. As well as the results of this meta-analysis help develop and update clinical guidelines and improve evidence-based medicine (EBM) knowledge and policy in this field. Also, based on this information and the results of previous studies, DM is associated with musculoskeletal disorders (MSDs) and is often clinically underdiagnosed and undertreated. There have also been no systematic reviews of the literature to determine the association between DM and MSDs in the general population. Therefore, this study aimed to review systematically and meta-analyses the association between DM and MSDs by combining cohort studies.

Materials and methods

This systematic review and meta-analysis were based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (15). The study protocol was registered in PROSPER with the code CRD42022381787 (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=381787).

Search terms and search strategy

A comprehensive search strategy was used to identify relevant trials for this meta-analysis. International databases, including Scopus, Web of Science, PubMed (Medline), and Embase, were searched using specific search terms and MeSH terms for 'diabetes mellitus' and 'musculoskeletal disorders'. Supplementary Table 1 provides detailed information on the systematic search. The search strategy for this meta-analysis covered the period up to July 2023. To ensure a comprehensive search, synonyms, and additional terms were included by reviewing other studies in the field. The first ten pages of Google Scholar were also searched, and related articles were selected. In addition, a manual search was conducted by reviewing the references to relevant articles. The collected articles were managed using Endnote software version 9. Duplicate articles were identified and removed based on the software's default settings. Considering the predefined inclusion criteria, the remaining articles were evaluated based on their title, abstract, and full text. Two authors (MA and AM) independently and separately screened the articles based on their titles, abstracts, and full text. In cases of disagreement, the supervisor (YM) reviewed the results to reach a consensus.

Inclusion and exclusion criteria

In this meta-analysis, we specifically included cohort studies that examined the association between DM and the occurrence of MSDs. The PECO structure was used to define the inclusion criteria:

Population: All patients with DM.

Exposure: Presence of MSDs.

Comparison: DM patients without MSDs.

Outcomes: Occurrence of MSDs and associated risk factors.

Cohort studies were selected for this meta-analysis because they can investigate causal associations in observational research. To

maintain the focus on relevant studies, we excluded review articles, case reports, case series, clinical trials, other interventional studies, and letters to the editor. This exclusion criterion ensured that the included studies were cohort designs explicitly examining the relationship between DM and MSDs. By employing these inclusion and exclusion criteria, we aimed to conduct a meta-analysis using cohort studies to provide valuable insights into the association between DM and the occurrence of MSDs and their related risk factors.

Data extraction

A checklist was developed to guide the data extraction process for this meta-analysis. The checklist included the first author's name, country where the study was conducted, type of study (cohort study), study population, sample size, race/ethnicity of the study population, type of diabetes mellitus (DM), age of participants (mean and dispersion, if available), gender distribution (number of males), duration of DM, HbA1c levels, site(s) of involvement, type of MSDs, effect size (RR). Based on the checklist, two authors (MA and AM) independently performed the data extraction process. In cases where conflicts or disagreements arose, a third person (YM) was involved to resolve them and reach a consensus. This systematic data extraction approach collected the relevant information from each included study consistently and comprehensively, ensuring accuracy and minimizing bias in the subsequent analysis.

Quality assessment

Two authors (MA and YM) assessed the quality of the included studies using the Joanna Briggs Institute (JBI) checklist for cohort and case-control studies. The JBI checklist is a validated tool that assesses the methodological quality of studies and the potential for bias in their design, conduct, and analysis. The JBI checklist consists of eleven cohort and ten case-control study questions. Each question is answered with 'yes,' 'no,' 'not applicable,' or 'unclear' to indicate whether the study adequately addressed the specific methodological criteria. Assessing the studies using the JBI checklist helped the authors determine the quality of the studies and decide whether to include or exclude them based on their quality assessment. It is worth noting that the JBI checklist has undergone extensive peer review and has been endorsed by the JBI Scientific Committee, further validating its usefulness for assessing the methodological quality of research studies (16, 17).

Statistical analysis

The effect size in this meta-analysis was based on the risk ratio (RR) with a 95% confidence interval. Meta-set commands were used to assess the logarithm and log standard deviation of the RR. Cochrane I2 and Q tests were used to assess heterogeneity between trials. The Egger test was used to calculate publication bias. Subgroup analyses were performed based on the duration of DM, continent, fracture site, type of MSDs, age, and sex. Meta-regression analysis was also performed to determine the effect of age and BMI on the association of interest. Statistical analysis was performed using STATA 17.0, and a P value < 0.05 was considered.

Results

Study characteristics

Based on the completion of the international database search, 4,095 studies were retrieved from PubMed, Scopus, Web of Science, and Embase databases. Following the removal of 538 duplicate studies, 3,557 studies remained. Subsequently, a screening process based on titles and abstracts resulted in 37 studies that met the eligibility criteria. Finally, after a thorough review of the full texts and consideration of inclusion and exclusion criteria, ten studies were included in our meta-analysis (Figure 1). The characteristics of the studies included in this meta-analysis have been reported in Table 1.

Association of DM with MSDs

The present meta-analysis included 10 cohort studies comprising 69,979 patients with diabetes mellitus (DM) and 236,435 healthy controls. These studies aimed to assess the association between diabetes and MSDs. Gue et al. reported the highest RR of 1.26 (95% CI 1.05–1.51), while Schwartz et al. reported the lowest RR of 0.69 (95% CI 0.62–0.76). Upon combining these findings, the pooled RR estimate was 1.03 (95% CI 1.00–1.06), as depicted in Figure 2. The analysis revealed a substantial heterogeneity rate of 91.31 percent. Furthermore, an evaluation of publication bias in the included studies was conducted. The funnel plot showed significant publication bias across studies. However, the Egger's test results did not (B=-4.31; SE=3.645; P value=0.237), as shown in Figure 2.

Subgroups analysis

Additionally, a subgroup analysis was performed in our study, considering various factors such as the duration of DM, continent, place of fracture, type of musculoskeletal disorder, age, and sex. The results of this subgroup analysis are summarized in Table 2.

Duration of DM

We divided the studies into two categories: more than seven years and less than seven years. Six trials were assessed, and each group contained three trials. In the more than seven-years group, the pooled estimate of RR was 1.09 (95% CI 1.05-1.14), but in the less than seven-years group, it was 1.03 (95% CI 0.97-1.10; P-value=0.00, 0.22) with heterogeneity (I2) of 88.48% and 33.80%, respectively (Table 2).

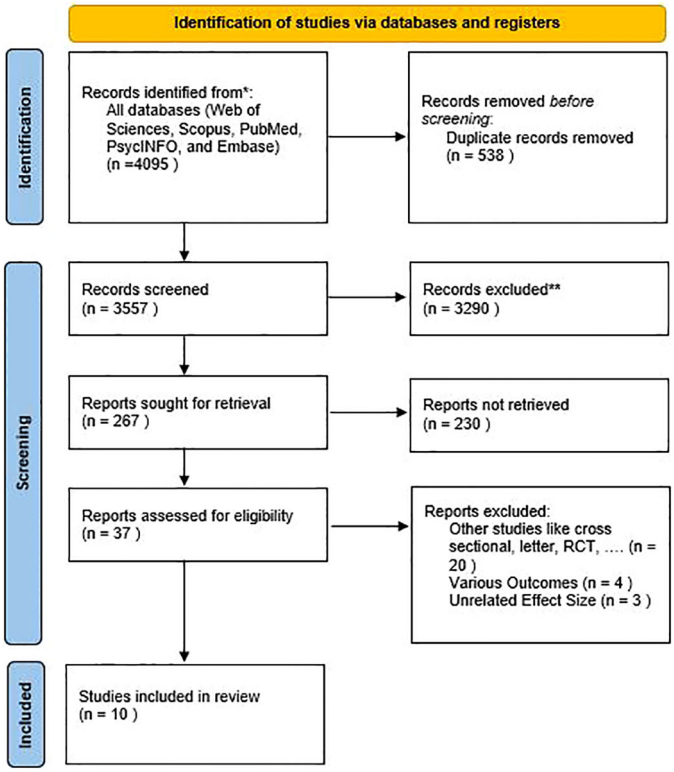

FIGURE 1
PRISMA 2020 flow diagram for new systematic reviews, which included searches of databases.

Continent

Studies have been conducted on three continents: six in America, two in Asia, and two in Europe. Our findings revealed that the combined RR estimate was 1.02 (95% CI 0.98–1.06; P-value=0.00) in America, 1.04 (95% CI 0.94–1.16; P-value=0.01) in Asia, and 1.07 (95% CI 0.99–1.15; P-value=0.76) in Europe, accompanied by heterogeneity (I2) rates of 94.78%, 84.17%, and 0.00% respectively. Notably, the risk of MSDs in patients with diabetes mellitus (DM) was found to be higher in Europe compared to America. At the same time, no significant association was observed in Asia (Table 2).

Age and gender

We categorized the studies based on age, specifically comparing those below 70 with those above it. Three articles were analyzed for the lower age group, revealing a pooled RR estimate of 1.15 (95% CI 1.10–1.21; P-value=0.03, I2 = 71.73%). However, in the higher age group comprising six articles, the pooled RR estimate was 1.02 (95% CI 0.97–1.07; P-value=0.26, I2 = 23.03%). These findings indicate that the risk of MSDs is higher in individuals below the age of 70 (Table 2).

Furthermore, we observed differences based on sex. Four studies were included in the analysis of the pooled RR estimate for females, resulting in a value of 1.15 (95% CI 1.08–1.21; P-value=0.01). Conversely, only one study evaluated the RR for males, which was 1.00 (95% CI 0.94–1.07) (Table 2).

Place of the fracture

Among the articles that examined fractures, they were categorized into four groups based on the specific location. Six of these articles specifically investigated hip, pelvis, and upper leg fractures, resulting in a pooled RR estimate of 1.53 (95% CI 1.42–1.65). No significant differences were observed in other locations (Table 3). In six of the articles, the focus was specifically on hip disorders. The study by Dobing et al. reported the lowest RR of 1.07 (95% CI 0.79-1.45), whereas the study by Schwartz et al. reported the highest RR of 2.01 (95% CI 1.79-2.25). The pooled RR estimate for hip involvement was 1.53 (95% CI 1.42–1.65; P-value=0.00, I2 = 88.73%) (Table 3). In addition to these results, 2 studies reported the outcomes of interest based on the inclusion criteria for arm/wrist/hand fracture and 2 studies also reported lower leg/ankle/knee fracture. After combining these results, the meta-analysis showed that the association between the presence of diabetes and arm/wrist/hand fracture was 0.96 (% 95 CI 0.83 - 1.10) and for lower leg/ankle/knee fracture was 1.22 (% 95 CI 1.07 - 1.38). Similarly, the association between diabetes and foot fracture was 1.28 (% 95 CI 1.11 - 1.48) (Table 3).

TABLE 1 The characteristics of included cohort studies.

Author (year)	Country	Type of study	Study population	Sample size	Age	Type of diabetes	Duration of DM	HbA1c	BMI	Place of involvement	Type of MS	Outcome
Strotmeyer et al. (2005) (18)	USA	Cohort	Older adults	2979	DM: 74 No DM: 73	Type 2 DM, IFG	DM and Fracture: 6.5	6.0 ± 0.5	NR	Radius/ Ulna (21%)	Fracture	DM: 1.64 (1.07-2.51)
										Spine (18%)		
							DM and No Fracture: 6			Hip (18%)		IFG: 1.34 (0.67-2.67)
										Tibia/Fibula/ Ankle (10%)		
										Foot (9%)		
Schwartz et al. (2001) (19)	USA	Cohort	Women aged 65 years and older	9654	72.0 ± 5.1	Type 2 Diabetes	9.2 ± 7.9	NR	28.8 ± 5.5	Hip (DM:48, No DM:501)	Fracture [Osteoarthritis: (DM:25, No DM:11)]	1.22 (1.06 -1.41)
										Knee (DM:24, No DM:258)		
										Foot (DM:23, No DM:264)		
Dobnig et al. (2006) (20)	Austria	Cohort	Female patients above 70 recruited in 95 nursing homes	1664	82.8 ± 5.9	Type 2 Diabetes	NR	6.5 ± 0.9	26.4 ± 4.6	Hip DM: 41 (7.0%) CTR: 69 (6.3%)	Fracture [Osteoarthritis: (DM:116, No DM:395]	0.90 (0.60 -1.34)
										Hand DM: 14 (2.4%) CTR: 21 (1.9%)		
										Other no vertebral fractures DM: 100 (9.2%) CTR: 48 (8.2%)		
Bonds et al. (2006) (21)	USA	Cohort	Postmenopausal women	93402	64.9 ± 7.0	Type 2 Diabetes	9.3 ± 10.0	NR	NR	Hip (DM:128, No DM:1531)	Fracture [Osteoarthritis: (DM:444, No DM:776]	1.20 (1.11–1.30)
										Hand (DM:117, No DM:3161)		

(Continued)

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TABLE 1 Continued

Author (year)	Country	Type of study	Study population	Sample size	Age	Type of diabetes	Duration of DM	HbA1c	BMI	Place of involvement	Type of MS	Outcome
										Knee (DM:207, No DM:2828)		
										Foot (DM:153, No DM:1940)		
Ottenbacher et al. (2002) (22)	USA	Cohort	Mexican American Older Adults ≥ 65 years	2884	71.8 ± 5.7	NR	1213	NR	NR	Hip (DM:23, No DM:20)	Fracture	1.57(1.3-2.39)
Schwartz et al. (2011) (23)	USA	Cohort	–	16885	NR	Type 2 Diabetes	7436	NR	NR	Hip (DM:346, No DM:1275)	Fracture	Women/hip:1.88 (1.43-2.48)
												Women/non-spine:1.52 (1.31-1.75)
												Men/hip:5.71 (3.42-9.53)
												Men/non-spine:2.17 (1.75-2.69)
												Women/ Osteoporotic:1.04 (1.02-1.05)
												Men/ Osteoporotic:1.09 (1.04-1.14)
Napoli et al. (2014) (24)	USA	Cohort	men (≥65 years)	5994	73.3 ± 5.1	Type 2 Diabetes	5994	9.1 (2.7)	NR	Non-vertebral	Fracture	1.12 (0.94–1.34)
Martinez-Laguna et al. (2015) (25)	Spain	Cohort	NR	171931	62.71 ± 11.90	Type 2 Diabetes	97226	6 (1.69)	NR	Hip	Osteoporotic fractures T2DM: 444 Control:776	1.11 (0.99-1.24)
Guo et al. (2020) (26)	China	Cohort	Over 60 Years Old	3430	69:09 ± 6:53	Type 2 Diabetes	1229	NR	NR	–	Fracture (201) T2DM (78) Non-T2DM (123)	1.35 (1.00-1.82)
Sosa et al. (2009) (27)	Spain	Cohort	women aged more than 65 years old	203	71.7 ± 5.0	Type 2 Diabetes	All female	NR	NR	Hip (DM:1, No DM:0) Lumbar spine and femur	Fracture	1.04 (0.53-2.05)

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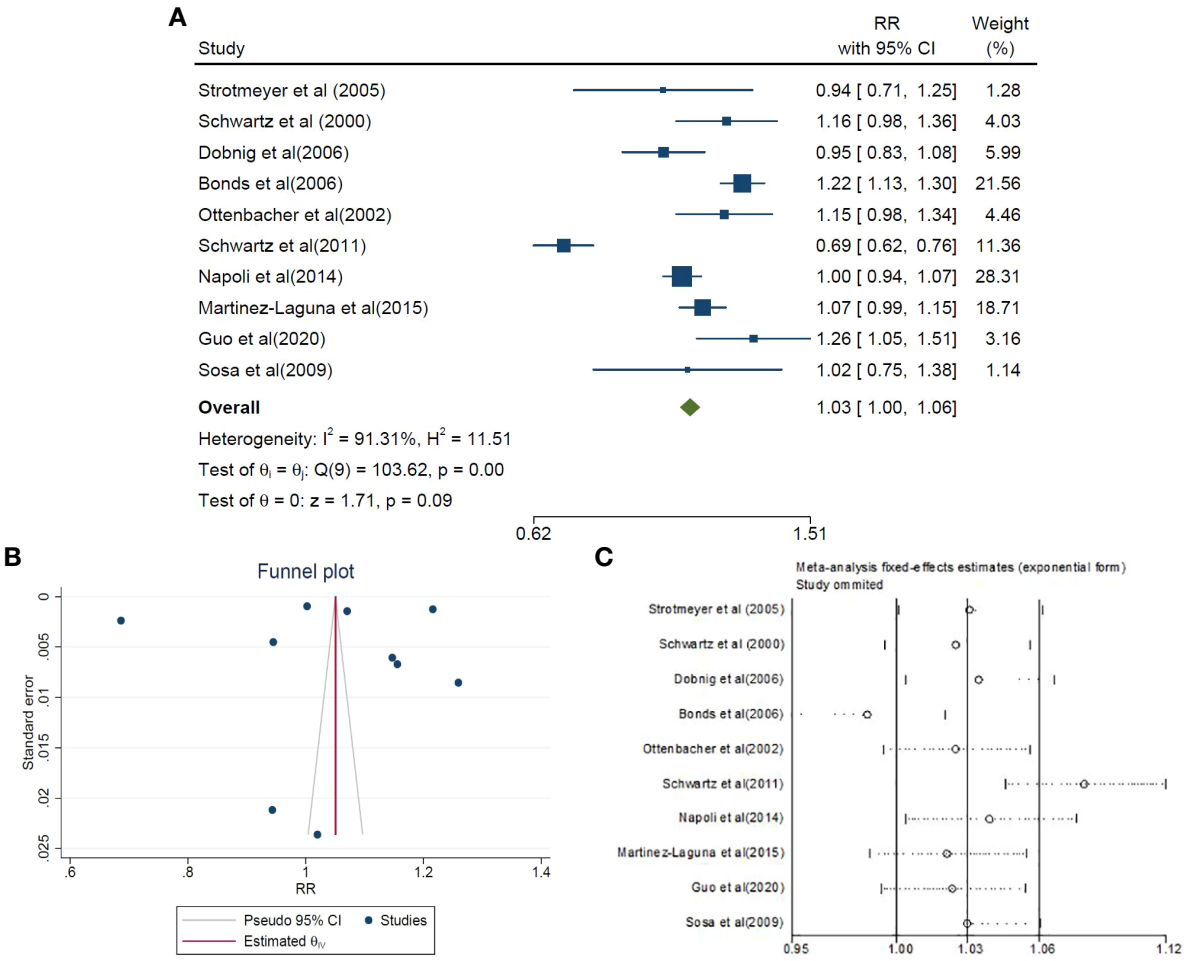

FIGURE 2
The risk ratio (RR) between DM and MSDs, (**A**: forest plot) sensitivity analysis **(C)** and publication bias **(B)** using a combination of the results of cohort studies (CI, Confidence Interval).

Types of MSDs

Osteoarthritis was among the disorders examined in several studies. The combined RR estimate for the three studies was 4.88 (95% CI 4.55–5.22; P-value=0.00, I2 = 99.72%). This finding indicates that DM could be a significant risk factor for osteoarthritis (Table 3).

Meta-regression results

This analysis examined the relationship between our results and three variables: age, BMI, and year. The regression coefficients and statistical significance for each variable were as follows: age (B: -0.009, SE: 0.005, P: 0.096), BMI (B: -0.007, SE: 0.022, P: 0.746), and year (B: -0.001, SE: 0.01, P: 0.890). The age variable demonstrated a weakly decreasing relationship but did not reach statistical significance. The variables of BMI and year did not show any significant associations with our results.

Quality assessment

The quality of 10 cohort studies was evaluated using the Joanna Briggs Institute (JBI) critical appraisal tools. The assessment revealed that the majority of the cohort studies received high-quality scores, indicating a high level of methodological rigor (Table 4).

Discussion

This meta-analysis aimed to evaluate the association between DM and MSDs. Various categories were considered in the analysis, including the duration of DM, continent, place of fracture, type of MSDs, age, and sex. The results consistently indicated that individuals with DM have a higher risk of developing musculoskeletal complications than the general population. These findings align with previous studies and further support the association between DM and MSDs. Indeed, studies have

TABLE 2 The subgroups analysis of association between DM and MSDs by combining cohort studies based on duration of DM, continents, age, and gender.

Variables	cat	No. study	Pooled RR (% 95 CI)	Heterogeneity Assessment between studies			Heterogeneity Assessment between subgroup	
				I2	P-value	Q	Q	P-value
Over all		10	1.03 (1.00 – 1.06)	91.31%	0.00	103.62	–	–
Duration of DM	<7	3	1.03 (0.97 – 1.10)	33.80%	0.22	3.02	2.12	0.15
	>7	3	1.09 (1.05 – 1.14)	88.48%	0.00	17.36		
Continent	America	6	1.02 (0.98 – 1.06)	94.78%	0.00	95.73	1.48	0.48
	Asia	2	1.04 (0.94 – 1.16)	84.17%	0.01	6.32		
	Europe	2	1.07 (0.99 – 1.15)	0.00%	0.76	0.09		
Age	<70	3	1.15 (1.10 – 1.21)	71.73%	0.03	6.99	12.57	0.00
	>70	6	1.02 (0.97– 1.07)	23.03%	0.26	6.50		
Sex	Female	4	1.15 (1.08 – 1.21)	74.19%	0.01	11.62	10.17	0.00
	male	1	1.00 (0.94 – 1.07)	–	0.00	0.00		

demonstrated that DM affects the human body in various ways, although the exact mechanisms are not fully understood. One proposed mechanism involves increased levels of advanced glycation end-products (AGEs), which can trigger fibroproliferative complications. When the receptor for AGEs (RAGE) is turned on, it activates several signaling pathways, such as the MAPK/ERK, TGF-β, JNK, and NF-κB pathways (28). This activation leads to two main complications. Firstly, it induces oxidative stress in the endoplasmic reticulum (ER) (29). Secondly, it upregulates the expression of inflammatory cytokines such as IL-1β, IL-6, and TNFα (28, 30–32). These pathways can influence proteins like collagen, making them more susceptible to glycation (33). Histologic studies have revealed that this mechanism affects individuals with diabetes, as changes in AGE pathways can lead to decreased elasticity and load-bearing capacity of tendons (34, 35). Additionally, chondrocytes, the cells responsible for cartilage maintenance, can adapt their expression of glucose transporters (GLUT-1, GLUT-3, and GLUT-9) in response to blood glucose levels. However, this adaptive mechanism may be absent in MSDs such as osteoarthritis. TNFα and IL-1β can make IL-6, PGE2, and phosphokinase C work, which controls the amount of expressed GLUT transporters (36–38). Therefore, hyperglycemia and insulin resistance in diabetes can alter these pathways and impact the musculoskeletal system.

In the subgroup analysis, the association between DM and MSDs was found to vary across different categories. Specifically, diabetic patients with a longer duration of DM were observed to have a higher risk of developing musculoskeletal complications. This finding can be attributed to the cumulative effects of prolonged exposure to diabetes, which can lead to both microvascular and macrovascular complications. Over time, diabetes can adversely affect the small blood vessels (microvascular) and larger blood vessels (macrovascular) throughout the body. These vascular complications can impair blood flow, nutrient delivery, and oxygen supply to the musculoskeletal system, increasing the risk of musculoskeletal problems. It is important to note that the longer

TABLE 3 The subgroups analysis of association between DM and MSDs by combining cohort studies based on place of fracture, type of MSDs.

Variables	cat	No. study	Pooled RR (% 95 CI)	Heterogeneity Assessment between studies			Heterogeneity Assessment between subgroup	
				I2	P-value	Q	Q	P-value
Fracture	hip/pelvis/ upper leg	6	1.53 (1.42 – 1.65)	88.73%	0.00	44.38	11.14	0.00
	lower arm/ wrist/hand	2	0.96 (0.83 – 1.10)	0.00%	0.36	0.83	-0.64	0.52
	lower leg/ ankle/knee	2	1.22 (1.07 – 1.38)	0.00%	0.86	0.03	3.05	0.0
	foot	2	1.28 (1.11 – 1.48)	0.00%	0.67	0.19	3.40	0.00
Type of MSDs	Osteoarthritis	3	4.88 (4.55 – 5.22)	99.72%	0.00	709.16	45.17	0.00

TABLE 4 Quality assessment of cohort studies based on the JBI critical appraisal checklist.

Studies	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Total Score
Strotmeyer et al. (2005) (18)	Y	Y	Y	Y	Y	Y	Y	N	Y	UC	Y	9
Schwartz et al. (2001) (19)	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	10
Dobnig et al. (2006) (20)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	10
Bonds et al. (2006) (21)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
Ottenbacher et al. (2002) (22)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
Schwartz et al. (2011) (23)	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	9
Napoli et al. (2014) (24)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11
Martinez-Laguna et al. (2015) (25)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	10
Guo et al. (2020) (26)	Y	Y	Y	Y	Y	Y	Y	N	UC	Y	Y	9
Sosa et al. (2009) (27)	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	10

Q1: Were the two groups similar and recruited from the same population?
Q2: Were the exposures measured similarly to assign people to both exposed and unexposed groups?
Q3: Was the exposure measured in a valid and reliable way?
Q4: Were confounding factors identified?
Q5: Were strategies to deal with confounding factors stated?
Q6: Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?
Q7: Were the outcomes measured in a valid and reliable way?
Q8: Was the follow up time reported and sufficient to be long enough for outcomes to occur?
Q9: Was follow up complete, and if not, were the reasons to loss to follow up described and explored?
Q10: Were strategies to address incomplete follow up utilized?
Q11: Was appropriate statistical analysis used?
Y, YES; N, NO; UC, UNCLEAR; NP, Not applicable.

the duration of DM, the higher the likelihood of developing microvascular complications such as diabetic neuropathy, retinopathy, and nephropathy. These complications can contribute to the development of musculoskeletal issues by affecting nerve function, blood supply, and overall tissue health. As a result, the subgroup analysis shows that the length of DM is strongly related to the risk of musculoskeletal complications. The longer the DM, the higher the risk, as the microvascular and macrovascular complications increase over time (39). Our results were confirmed in the study of Bonds et al. in 2006 (21). Indeed, age and sex differences have been identified as potential risk factors for MSDs in diabetic patients. Their study found musculoskeletal complications were more prevalent in younger and female individuals. This observation aligns with the existing literature and can be explored from different perspectives, including the influence of sex hormones. Sex hormones, such as estrogen, play a role in regulating the health and function of tendons and ligaments. Estrogen has been shown to have a protective effect on these connective tissues, promoting their strength and elasticity. In females, the decline in estrogen levels during menopause can contribute to changes in tendon and ligament properties, making them more susceptible to injury or degeneration. Moreover, hormonal differences between males and females can also impact the inflammatory response and immune function, which may contribute to the development and progression of MSDs. Additionally, differences in body composition, muscle mass, and biomechanics between sexes may influence the distribution of forces and loading patterns on the musculoskeletal system, potentially increasing the risk of certain conditions. Considering age, it is essential to note that younger individuals may be more susceptible to musculoskeletal complications due to longer exposure to diabetes-related metabolic abnormalities and prolonged disease duration. Furthermore, age-related factors such as decreased tissue regeneration capacity, increased oxidative stress and cumulative damage over time can contribute to a higher risk of MSDs in older individuals. By considering these age and sex-related factors, a more comprehensive understanding of the underlying mechanisms and risk factors associated with MSDs in diabetic patients can be achieved (40). Second is diversity in muscle fibers; for example, women have more type I fibers than others (41). Third, lower pain thresholds in females can be the other cause (42). One of the specific diseases investigated in the diabetic population was osteoarthritis, and our study revealed a heightened risk of its occurrence. This increased risk can be attributed to elevated levels

of pro-inflammatory cytokines, oxidative stress, obesity, and higher secretion of adipokines in individuals with diabetes. These factors play significant roles in the development and progression of osteoarthritis (43, 44). Our findings approved Courties et al.'s previous study 2016 (45).

Future research should focus on identifying additional factors contributing to the increased risk of MSDs in diabetic individuals, such as specific treatment modalities, comorbidities, lifestyle factors, or genetic predisposition. By expanding our knowledge of the risk factors and underlying mechanisms, healthcare providers can develop more targeted strategies for prevention, early detection, and effective management of musculoskeletal complications in individuals with diabetes. This will ultimately improve patient outcomes and contribute to the development of evidence-based guidelines for clinical practice.

The study possesses several strengths that contribute to its significance. Firstly, it stands as the first systematic review and meta-analysis to investigate and analyze the association between DM and MSDs comprehensively. This approach enhances the overall understanding of the topic and provides a valuable synthesis of existing evidence. Secondly, the study employed diverse studies that investigated various outcomes related to MSDs. This allowed for a subgroup analysis based on different specifications, enabling a more nuanced association examination. The high homogeneity observed in the results indicates that the included articles were appropriately chosen, further strengthening the validity of the findings. Thirdly, using cohort studies for meta-analysis provides a foundation for exploring causal associations between DM and MSDs. This approach allows for incorporating genetic and molecular science studies, which can shed light on underlying mechanisms and potential pathways. However, it is important to acknowledge certain limitations of the study. One such limitation is the lack of analysis based on confounding variables. MSDs can develop and advance as a result of factors like the type of treatment diabetic patients receive and the presence of other underlying diseases. The absence of consideration for these confounders restricts the ability to explain the study results fully and may leave room for alternative interpretations. Despite these limitations, the study's strengths, including its systematic and comprehensive approach, subgroup analysis, and utilization of cohort studies, contribute to its valuable insights into the association between diabetes and musculoskeletal disorders. Despite study limitations, further research is crucial to understand the underlying mechanisms, potentially informing targeted interventions that can mitigate MSDs prevalence and impact in the diabetic population. This underscores the importance of an integrated healthcare approach, ultimately enhancing the overall quality of life for affected individuals.

Conclusion

In conclusion, our findings shed light on an understudied aspect of diabetes-related comorbidities, emphasizing the nuanced nature of this relationship, particularly in specific subgroups. Importantly, they highlight the need for a holistic approach to diabetes management, addressing both glycemic control and musculoskeletal health.

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.

Author contributions

MAz: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. AM: Data curation, Writing – original draft. MAf: Formal analysis, Methodology, Writing – original draft. LS: Data curation, Investigation, Writing – original draft. MT: Data curation, Writing – original draft. KK: Data curation, Investigation, Writing – original draft. SK: Conceptualization, Data curation, Investigation, Project administration, Writing – original draft, Writing – review & editing. YM: Conceptualization, Data curation, Investigation, Methodology, Software, 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1320468/full#supplementary-material

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Causal relationship between intervertebral disc degeneration and osteoporosis: a bidirectional two-sample Mendelian randomization study

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Introduction: The relationship between intervertebral disc degeneration (IVDD) and osteoporosis (OP), diagnosed primarily using bone mineral density (BMD), remains unclear so far. The present study, therefore, aimed to investigate the potential relationship between osteoporosis and intervertebral disc degeneration using Mendelian randomization and genome-wide association analyses. Specifically, the impact of bone mineral density on the development of intervertebral disc degeneration was evaluated.

Materials and methods: The genome-wide association studies (GWAS) summary data of OP/BMDs and IVDD were collected from the FinnGen consortium, the GEFOS consortium, and MRC-IEU. The relationship between IVDD and OP was then explored using TSMR. The inverse-variance weighted (IVW) method was adopted as the primary effect estimate, and the reliability and stability of the results were validated using various methods, including MR-Egger, weighted median, simple mode, weighted mode, and MR-PRESSO.

Results: No significant causal relationship was observed between OP and IVDD (IVW, P > 0.05) or between femoral neck BMD (FA-BMD) and IVDD when OP and FA-BMD were used as exposures. However, increased levels of total body BMD (TB-BMD) and lumbar spine BMD (LS-BMD) were revealed as significant risk factors for IVDD (TB-BMD: IVW, OR = 1.201, 95% CI: 1.123–1.284, $P = 8.72 \times 10^{-8}$; LS-BMD: IVW, OR = 1.179, 95% CI: 1.083–1.284, $P = 1.43 \times 10^{-4}$). Interestingly, both heel BMD (eBMD) and femur neck BMD (FN-BMD) exhibited potential causal relationships (eBMD: IVW, OR = 1.068, 95% CI: 1.008–1.131, $P = 0.0248$; FN-BMD, IVW, OR = 1.161, 95% CI: 1.041–1.295, $P = 0.0074$) with the risk of IVDD. The reverse MR analysis revealed no statistically causal impact of IVDD on OP and the level of BMD ($P > 0.05$).

Conclusion: OP and the level of FA-BMD were revealed to have no causal relationship with IVDD. The increased levels of TB-BMD and LS-BMD could promote the occurrence of IVDD. Both eBMD and FN-BMD have potential causal

relationships with the risk of IVDD. No significant relationship exists between IVDD and the risk of OP. Further research is warranted to comprehensively comprehend the molecular mechanisms underlying the impact of OP and BMD on IVDD and vice versa.

KEYWORDS

osteoporosis, bone mineral density, intervertebral disc degeneration, Mendelian randomization, genome-wide association studies

1 Introduction

Intervertebral disc degeneration (IVDD) serves as the pathological basis for various spinal degenerative diseases that contribute to disability and reduce the quality of life of the affected patients (1). The intervertebral disc comprises the nucleus pulposus, the cartilage endplate, and the annulus fibrosus. Each of these three main components of the intervertebral disc is mostly composed of collagen and proteoglycan, both of which are crucial for imparting critical qualities to the disc (2). Intervertebral disc degeneration (IVDD) is a pathological condition characterized by a progressive decline in the levels of proteoglycans and the water content inside the nucleus pulposus. This degenerative condition is well-recognized as the primary contributor to the development of lower back pain (3). In severe cases, the destroyed discs compress the spinal nerve roots located at L4-S2, which often results in sciatica (4).

Osteoporosis, often referred to as OP, is a multifaceted skeletal disorder characterized by a loss in bone mass and impairment of bone microarchitecture, which results in heightened vulnerability to fractures and increased fragility of the bones (5). A key feature of osteoporosis is the reduction in bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) is currently the primary method of establishing the clinical diagnosis of osteoporosis (6). OP is prevalent globally, affecting nearly 200 million individuals, particularly postmenopausal women (7). The important risk factors reported for OP include genetics, living habits, and medical history (5). The timely identification and management of osteoporosis (OP) is important as it would significantly contribute to preventing bone fractures and enhance the overall quality of life of the affected patients.

The correlation between osteoporosis and disc degeneration remains debatable in the scientific community to date. Liang et al. (8) demonstrated through a retrospective study that reduced vertebral bone mineral density (BMD), osteoporosis, and the exacerbation of disc degeneration were correlated. On the other hand, Kaiser et al. (9) demonstrated, through a prospective study, that higher trabecular BMD is negatively associated with disc height loss. Interestingly, certain retrospective and prospective studies have also reported no correlation between BMD and the progression of IVDD (10–13). These inconsistent conclusions could be originating from the confounders or covariates, which impact the association between BMD and IVDD.

IVDD and osteoporosis share a few genetic mechanisms and signaling pathways. Inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α), influence the development, progression, and severity of intervertebral disc degeneration (IVDD) (14–16) and OP (17–19). The matrix metallopeptidase 9 (MMP-9), which is an important proteolytic enzyme, is also closely associated with IVDD and OP. In addition, several same signaling pathways, including PI3K-AKT (20, 21), WNT/β-catenin (22, 23), and NF-κB (24, 25), are associated with intervertebral disc degeneration (IVDD) and also reported to significantly affect the pathogenesis of osteoporosis.

While numerous studies have reported the association between IVDD and OP, the direct causal relationship remains obscure. Mendelian randomization (MR) is a novel methodology that is being increasingly utilized to explore the causal associations between modifiable exposures and various illnesses or features (26, 27). MR allows for effectively overcoming certain limitations, especially unfeasible causality in the randomized controlled trials (RCT) and inevitable confounding bias or reverse causality in observational studies (26). MR also aids in distinguishing causal pathways from risk factors that are challenging to randomize or prone to measurement errors (28).

Our study indicates no discernible causal association between OP and the risk of intervertebral disc degeneration (IVDD) or between FA-BMD and the risk of IVDD. The elevated levels of TB-BMD and LS-BMD could, however, contribute to the development of intervertebral disc degeneration (IVDD). In addition, both eBMD and FN-BMD were revealed to have a putative causal relationship with the risk of intervertebral disc degeneration (IVDD). Notably, no substantial correlation was revealed between intervertebral disc degeneration (IVDD) and osteoporosis (OP) or

Abbreviations: GEFOS, the Genetic Factors for Osteoporosis; MRC-IEU, The Medical Research Council Integrative Epidemiology Unit; IVDD, intervertebral disc degeneration; OP, osteoporosis; TB-BMD, total body bone mineral density; FN-BMD, femoral neck bone mineral density; FA-BMD, forearm bone mineral density; LS-BMD, lumbar spine bone mineral density; eBMD, heel bone mineral density; NA, not available; IVW, inverse variance weighted; nsnp, number of single-nucleotide polymorphism; OR, odd ratios; CI, confidence interval.

among the bone mineral density across various anatomical locations and different age cohorts.

2 Methods

2.1 Data resources and sample information

The study design is depicted in Figure 1. The study was conducted by following the STROBE-MR guidelines (29). The data used in the present study were the summary genome-wide association studies (GWAS) data of intervertebral disc degeneration (IVDD) and osteoporosis, which were obtained from the FinnGen collaboration. The IVDD dataset comprised 20,001 cases and 164,692 controls, whereas the osteoporosis dataset comprised 3,203 cases and 209,575 controls (30). The genome-wide association study (GWAS) data on lumbar spine bone mineral density (LS-BMD), femur neck bone mineral density (FN-BMD), and forearm bone mineral density (FA-BMD) collected by the Genetic Factors for Osteoporosis (GEFOS) consortium were also used. The dataset comprised 25,509 cases for FN-BMD, 12,906 cases for LS-BMD, and 563 cases for FA-BMD (31, 32). In addition, heel bone mineral density (eBMD) data were obtained from the MRC-IEU, and the dataset comprised 265,627 individuals. The data for TB-BMD for different age groups were obtained from the reports of a large GWAS meta-analysis study (32). The TB-BMD, LS-BMD, FN-BMD, and FA-BMD assessments were conducted using dual-energy X-ray absorptiometry (DXA) (6). The eBMD assessments were conducted using ultrasonography (33). A reverse analysis was conducted to obtain the genetic instruments for OP and bone mineral density (BMD) at different anatomical sites, and their impact on intervertebral disc degeneration (IVDD) was determined. IVDD was diagnosed using the International Classification of Diseases 10th Revision (ICD-10) M51, ICD-9 722, and ICD-8 275, with removed ICD-9 7220|7224|7227|7228Av and ICD-8 7250. Detailed information of these GWAS summary data is provided in Supplementary Table 1.

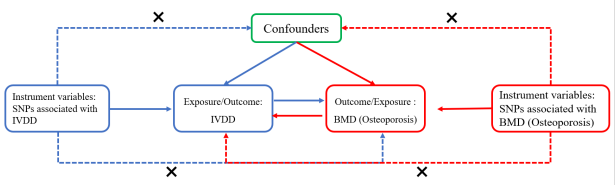

FIGURE 1
The flowchart for the two-sample bidirectional Mendelian randomization analysis. The blue line represents the Mendelian randomization analysis of the causal relationship of intervertebral disc degeneration (IVDD) with bone mineral density (BMD) or osteoporosis. The red line represents the Mendelian randomization analysis of the causal relationship of bone mineral density or osteoporosis with IVDD. The following three assumptions had to be considered: the selected instrument variants (SNPs) should be significantly associated with the exposure; the variants should be independent of any exposure–outcome relationship confounders; the variants affect the outcome only via the exposure.

2.2 MR analysis

2.2.1 Selection of genetic variants

In order to address the issue of weak instrument bias, a genome-wide significance threshold of $P < 5 \times 10^{-8}$ was used as the default criterion for identifying the single-nucleotide polymorphisms (SNPs), whereas the threshold of minor allele frequency (MAF) > 0.01 was used as the genetic instruments for osteoporosis (OP), bone mineral density (BMD), and intervertebral disc degeneration (IVDD). Next, the clumping threshold (the genetic distance = 10,000 kb and the SNP linkage disequilibrium value $r^2 < 0.001$) was established using the data from the 1000 genomes project for European ancestry (34) to resolve the linkage disequilibrium (LD) issue based on the identified SNPs.

Diabetes, BMI, and smoking are significant prognostic factors for both OP and IVDD. Several scholars have proposed that the risk factors for diabetes mellitus (35, 36) and body mass index (BMI) are associated with IVDD (37). Moreover, a retrospective study indicated that cigarette smoking accelerates the development of cervical disc degeneration (38). Interestingly, a higher level of education was reported as a risk factor for cervical disc degeneration, regardless of age differences among the respondents (39). OP is also reported to be closely associated with diabetes mellitus, BMI (40), smoking (41, 42), and the level of education (43). BMI and smoking are considered to have significant effects on OP, particularly in postmenopausal women. Accordingly, to ensure obtaining statistically meaningful outcomes, the present study used *PhenoScannerV2* to identify and exclude certain confounding single-nucleotide polymorphisms (SNPs) associated with diabetes, body mass index (BMI), smoking, and social status in our study.

The strength of the IVs was assessed by computing the F-statistics using the formula $F = R^2 \times (N - 2)/(1 - R^2)$. In the formula, R^2 denotes the proportion of variation in the exposure variable explained by the IV, and N denotes the sample size of the original GWAS used as the outcome variable (44). The R^2 for each IV was computed using the formula $R^2 = [2 \times EAF \times (1 - EAF) \times beta^2)/[(2 \times EAF \times (1 - EAF) \times beta^2] + [2 \times EAF \times (1 - EAF) \times N \times (SE \times beta^2)]$. In this formula, EAF denotes the effect of allele frequency, beta represents the estimated genetic effect on the outcome, N denotes the sample size of the genome-wide association study (GWAS), and SE denotes the standard error of the genetic effect (45). The IVs with the F statistics over 10 were considered credible instrumental variables and selected for the subsequent Mendelian randomization study.

2.2.2 Sensitivity analysis and statistical analysis

The potential causal relationship between intervertebral disc abnormalities and osteoporosis was investigated in the present study primarily using the inverse variance weighted (IVW) method, as described in a previous report (46). The Cochrane's Q test was conducted to evaluate the heterogeneity among the single-nucleotide polymorphisms (SNPs). Furthermore, the strength and reliability of the primary results obtained in the previous steps were assessed using various sensitivity analyses, including the weighted-median approach and the IVW method. Moreover, the possible presence of directional pleiotropy was evaluated using MR-Egger regression (47). The slope of MR-Egger regression indicates the

causal estimates that have been adjusted for pleiotropy, whereas the intercept's value provides an estimation of the extent of pleiotropy. In addition, the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test was conducted as a supplementary approach to identify and address the horizontal pleiotropic outliers (48). A global test of heterogeneity was conducted through a regression analysis of the relationships between the single-nucleotide polymorphisms (SNPs) and the outcomes while considering the connections between the SNPs and the exposures. The observed distance of each SNP from the regression was then compared with the anticipated distance according to the null hypothesis of no pleiotropy. The robustness of the obtained results was assessed using "leave-one-out" analyses, which involved systematically eliminating one single SNP at a time. This was followed by a reanalysis of the Mendelian randomization (MR) results using the IVW approach with the remaining instrumental variables (IVs).

2.3 Calculation of statistical power

The statistical power was evaluated using the mRnd website (https://shiny.cnsgenomics.com/mRnd/) (49). The key determinants of statistical power were the size of the sample for the result and the extent to which the genetic instrument accounted for the variance in the exposure variable.

All analyses were performed using R (version 4.1.2) and the related R packages (TwoSampleMR and forestplot). A *P*-value of <0.005 (0.05/10) indicated strong evidence of a causal association after the Bonferroni correction threshold was applied. A *P*-value ranging between 0.05 and 0.005 was considered suggestive evidence for a potential causal association.

3 Results

3.1 Causal effects of osteoporosis and bone mineral density on IVDD

All the IVs that were ultimately selected exhibited F-statistic values of over 10. Details of the method adopted to screen out the single-nucleotide polymorphisms (SNPs) are provided in Supplementary Table 2. In the analysis of the effect of eBMD on the risk of IVDD, rs7816131 was excluded because of its robust association with IVDD ($P = 8.3 \times 10^{-9}$), which is not in accordance with the third assumption of MR analysis. Finally, 2 SNPs of OP, 80 SNPs of TB-BMD, 22 SNPs of LS-BMD, 20 SNPs of FN-BMD, 3 SNPs of FA-BMD, and 331 SNPs of eBMD were used as IVs to analyze the relationship between OP/BMDs and the risk of intervertebral disc degeneration (IVDD). Comprehensive data on the IVs for BMD are provided in Supplementary Table 3. The variation explained by these IVs was 0.02% for OP, 9.05% for TB-BMD, 2.1% for FN-BMD, 1.65% for FA-BMD, 2.27% for LS-BMD, and 14.27% for eBMD. The statistical power values determined in the Mendelian randomization analyses of OP and BMD in terms of their effects on IVDD are presented in Supplementary Table 5.

The MR analysis did not yield statistically significant evidence of the causal effects of OP (IVW, $P > 0.05$) and FA-BMD (IVW, $P > 0.05$) on IVDD (Figure 2). However, positive correlations of the BMDs (TB-BMD, LS-BMD, and eBMD) with the risk of IVDD were revealed. The results indicated a significant association between the genetically enhanced BMD levels and a higher susceptibility to IVDD (TB-BMD: IVW, OR = 1.201, 95% CI: 1.123–1.284, $P = 8.72 \times 10^{-8}$; LS-BMD: IVW, OR = 1.179, 95% CI: 1.083–1.284, $P = 1.43 \times 10^{-4}$; Figure 2). The different results may be attributed to too few amounts of SNPs associated with OP and FA-BMD which served as exposures. Thus, the results of the MR-Egger and (or) MR-PRESSO test are not available. Moreover, heterogeneity (P for Cochrane's Q in IVW = 0.049 or 0.005 when OP and FA-BMD were served as exposures, Table 1) may exert an influence on this difference (Table 1).

MR sensitivity analysis of TB-BMD and IVDD exerted heterogeneous (P for Cochrane's Q in IVW = 0.007, Table 1) and no horizontal pleiotropy (P for MR-Egger intercept = 0.171, Table 1) when TB-BMD was served as the exposure. The MR-Egger analysis exhibited less statistical power, as seen by the lack of a significant P value and broader confidence intervals when compared with the IVW technique (50). Therefore, the MR-PRESSO Outlier Test, another horizontal pleiotropy test, was performed. The SNP rs4846580 was identified as an outlier using the MR-PRESSO Outlier Test, with an observed residual sum of squares (RSSobs) value of 1.92×10^{-3} ($P < 0.08$). Therefore, this SNP was removed during the analysis of the relationship between TB-BMD and the risk of IVDD. The results of the MR analysis indicated no statistically significant association between TB-BMD and IVDD (IVW, OR = 1.073, 95% CI: 1.014–1.135, $P = 1.47 \times 10^{-2}$) when the analysis was repeated with the remaining five single-nucleotide polymorphisms (SNPs) (Supplementary Figure 5). Notably, neither heterogeneity nor horizontal pleiotropy was indicated (P for Cochrane's Q in IVW = 0.349; P for MR-Egger intercept = 0.016, P for MR-PRESSO Global Test = 0.37, Table 1) in the MR sensitivity analysis of LS-BMD with IVDD.

Interestingly, both eBMD and FN-BMD were revealed to have potential causal relationships (eBMD: IVW, OR = 1.068, 95% CI: 1.008–1.131, $P = 0.0248$; FN-BMD, IVW, OR = 1.161, 95% CI: 1.041–1.295, $P = 7.40 \times 10^{-3}$; Figure 2) with the risk of IVDD according to statistical significance standard (P value threshold) defined in the Method section. Neither heterogeneity nor horizontal pleiotropy was indicated in the directional pleiotropy in the MR sensitivity analysis of FN-BMD with IVDD (P for Cochran's Q in IVW = 0.118, MR-Egger intercept = −0.029, P = 0.097; P for MR-PRESSO Global Test = 0.37, Table 1). However, horizontal pleiotropy and heterogeneity (P for MR-PRESSO Global Test <0.001, P for Cochrane's Q in IVW <0.0001, Table 1) were indicated in the MR sensitivity analysis of eBMD with IVDD, although P for the MR-Egger intercept is more than 0.05 (Table 1). This may be attributed to too many amounts of SNPs associated with eBMD that served as the exposures. The scatter plots for the effect sizes of the SNPs for OP and TB-BMD at different anatomical sites on IVDD are presented in Supplementary Figure 1. The forest plots, leave-one-out analysis plots, and funnel plots for the causal effect of BMD on IVDD are depicted in Supplementary Figures 2-4. When the leave-one-out analysis was

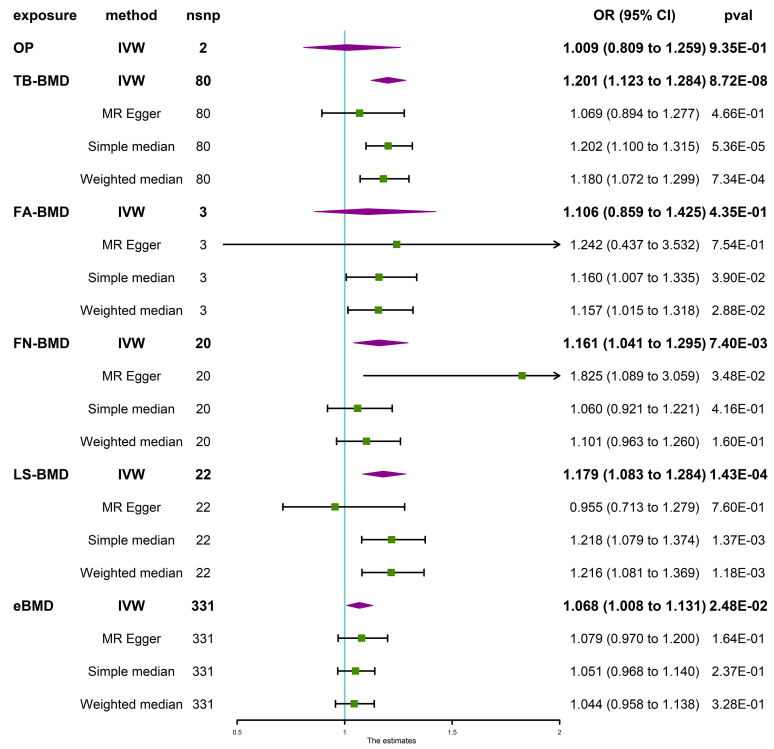

FIGURE 2
Causal effect of OP and BMD at different anatomical sites on IVDD. IVDD, intervertebral disc degeneration; OP, osteoporosis; TB-BMD, total body bone mineral density; FN-BMD, femoral neck bone mineral density; FA-BMD, forearm bone mineral density; LS-BMD, lumbar spine bone mineral density; eBMD, heel bone mineral density; IVW, inverse variance weighted; nsnp, number of single nucleotide polymorphisms; CI, confidence interval.

conducted using the IVW method, most of the determined correlations remained unchanged even when considering a single SNP associated with bone mineral density (BMD).

3.2 Causal effect of IVDD on BMD at different anatomical sites or in different age groups

Six SNPs (rs3010043, rs4473430, rs3135840, rs6470763, rs4284332, and rs17487277) were selected as IVs for determining the causal effect of IVDD on the risk of OP, TB-BMD, FN-BMD, FA-BMD, and LS-BMD. Notably, rs6470763 was directly associated with heel BMD (beta = 0.021, $P = 7.60 \times 10^{-11}$), which is not in accordance with the third assumption of MR analysis. Therefore, this SNP was removed from the analysis of the relationship between intervertebral disc degeneration (IVDD) and the risk of eBMD. All IVs that were finally selected for the analysis had F-statistic values over 10. Detailed information on the IVs strongly associated with IVDD is provided in Supplementary Table 4. These IVs accounted for a variance of 0.11% or 1.20% in the analysis of the effect of IVDD on BMD.

The MR analysis revealed that IVDD was not associated with BMD at different anatomical sites (Figure 3) and in different age groups (Figure 4). Neither heterogeneity nor horizontal pleiotropy (all P for Cochran's Q in IVW, MR-Egger intercept, and MR-PRESSO Global Test are more than 0.05) was indicated in the MR sensitivity analysis of IVDD with BMD at different sites, and in different age groups, excluded heterogeneity was found in the analysis of IVDD with TB-BMD and TB-BMD (age 0–15) (P for Cochrane's Q in IVW is less than 0.05). Otherwise, the scatter plots for the effect sizes of SNPs for IVDD's relationship to OP and TB-BMD at different anatomical sites are depicted in Supplementary Figures 6, 7. The forest plots, leave-one-out analysis plots, and funnel plots for the causal effect of IVDD on BMDs are depicted in Supplementary Figures 8, 9.

4 Discussion

The present study revealed no significant evidence to support the causal effect of OP and FA-BMD on the risk of IVDD. Moreover, the positive relationships of TB-BMD and LS-BMD with the risk of IVDD were revealed. In addition, both eBMD and FN-BMD exhibited potential causal relationships with the risk of IVDD. In reverse MR analysis, no causal effects of IVDD on the risk of OP and the change in bone mineral density were revealed.

The scientific community continues to debate whether bone mineral density (BMD) affects IVDD and, if so, how. While certain studies have reported osteoporosis as a causal factor of IVDD, others have considered it a protective factor against IVDD. Otherwise, several studies demonstrate there is no association between OP and IVDD (12, 51).

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TABLE 1 MR sensitivity analyses of IVDD and BMD at different sites and in different age groups.

Exposure	Outcome	Inverse variance weighted			MR-Egger			MR-PRESSO Global Test
		Cochran Q	Q_df	Q_Pval	Intercept	Se	P value	P value
OP	**IVDD**	3.9	1	0.049	NA	NA	NA	NA
TB-BMD	**IVDD**	113.4	79	0.007	0.007	0.005	0.171	0.011
FA-BMD	**IVDD**	10.5	2	0.005	−0.016	0.068	0.856	NA
FN-BMD	**IVDD**	26.5	19	0.118	−0.029	0.016	0.097	0.097
LS-BMD	**IVDD**	22.9	21	0.349	0.016	0.011	0.156	0.37
eBMD	**IVDD**	515.2	330	<0.0001	−0.0004	0.002	0.829	<0.001
IVDD	**OP**	6.6	5	0.250	0.038	0.119	0.764	0.277
IVDD	**FN-BMD**	3.4	5	0.639	0.021	0.027	0.486	0.67
IVDD	**FA-BMD**	2.9	5	0.711	0.049	0.056	0.434	0.747
IVDD	**eBMD**	3.2	4	0.53	−0.001	0.011	0.931	0.545
IVDD	**LS-BMD**	7.3	5	0.198	−0.002	0.043	0.973	0.239
IVDD	**TB-BMD**	11.5	5	0.042	0.054	0.021	0.064	0.076
IVDD	**TB-BMD (age 0–15)**	11.9	5	0.036	−0.022	0.076	0.782	0.061
IVDD	**TB-BMD (age 15–30)**	8.1	5	0.150	0.193	0.080	0.074	0.177
IVDD	**TB-BMD (age 30–45)**	2.2	5	0.815	0.049	0.052	0.397	0.814
IVDD	**TB-BMD (age 45–60)**	3.1	5	0.692	0.012	0.038	0.776	0.657
IVDD	**TB-BMD (age over 60)**	9.7	5	0.084	0.084	0.035	0.075	0.11

IVDD, intervertebral disc degeneration; OP, osteoporosis; TB-BMD, total body bone mineral density; FN-BMD, femoral neck bone mineral density; FA-BMD, forearm bone mineral density; LS-BMD, lumbar spine bone mineral density; eBMD, heel bone mineral density; NA, not available.

It has long been suggested that diminished bone quality is associated with the gradual deterioration of the endplates and the development of spondylosis, which ultimately results in heightened disc degeneration (52). Nevertheless, the incidence of intervertebral disc degeneration (IVDD) is lower among individuals with a low bone mineral density (BMD), even though these people are more susceptible to vertebral body fractures (53–55). According to this concept, it is postulated that osteoporosis, a degenerative and incapacitating disorder associated with aging and affecting a significant population globally (56), potentially delays the onset of intervertebral disc degeneration (IVDD). Functional investigations aimed at elucidating the correlation between the genetic factors influencing bone mineral density (BMD) and intervertebral disc degeneration (IVDD) are expected to provide valuable insights into this association and facilitate the discovery of novel treatment approaches.

Mechanistically, some scholars believe that osteoporosis leads to lower vertebral BMD, aggravates disc load, reduces the supply of nutrients to the intervertebral disc (57), and increases inflammatory factors that cause disc degeneration (15, 58). Others consider osteoporosis to delay disc degeneration that the vertebral body with lower BMD has a loose bony microstructure, which allows the vascular buds that nourish the cartilage endplate to grow better, and the increase in the number of vascular buds to enrich the blood supply of the cartilage endplate and can better provide nutrients to the intervertebral discs, thereby delaying IVDD (59, 60). In terms of genetic predictions, our results support the latter opinion that increased levels of TB-BMD and LS-BMD are the risk factors for IVDD, whereas eBMD and FN-BMD were revealed as the potential risk factors for IVDD. On the other hand, the results of the present study revealed no causal effect of OP on IVDD when just two SNPs were selected as IVs. These different results could be attributed to the different number of SNPs associated with the exposure and types of exposure variables. Furthermore, no statistically significant correlation was observed between intervertebral disc degeneration (IVDD) and osteoporosis (OP) or among the bone mineral density at different anatomical locations and in different age groups.

The present MR study offers several advantages. The use of Mendelian randomization effectively mitigated the potential influences of confounding bias and reverse causation. In addition, several sensitivity analyses were conducted to assess the robustness of the three Mendelian randomization assumptions, which reduced the likelihood of spurious findings. The instrumental variable weighted (IVW) method, which was adopted as the principal approach in the present study, resulted in a superior statistical

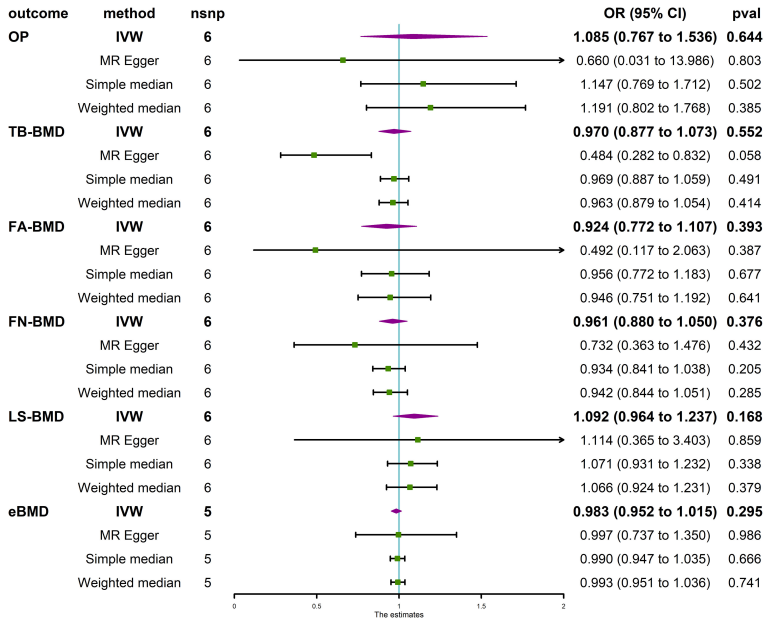

FIGURE 3
Causal effect of IVDD on OP and BMD at different anatomical sites. IVDD, intervertebral disc degeneration; OP, osteoporosis; TB-BMD, total body bone mineral density; FN-BMD, femoral neck bone mineral density; FA-BMD, forearm bone mineral density; LS-BMD, lumbar spine bone mineral density; eBMD, heel bone mineral density; IVW, inverse variance weighted; nsnp, number of single-nucleotide polymorphisms; CI, confidence interval.

power compared with other Mendelian randomization (MR) approaches, particularly the MR-Egger approach (50). Consequently, the MR-Egger analysis led to lower statistical power, as evident in the lack of a significant P value and broader confidence intervals compared with the IVW technique. The findings of the present study provided additional support for the necessity of maintaining a constant beta direction across all magnetic resonance (MR) procedures. Furthermore, harmonized data were consistently identified and rectified using the MR-PRESSO method (48). This ensured the absence of horizontal pleiotropy throughout the Mendelian randomization (MR) analysis and enhanced the reliability of the obtained findings.

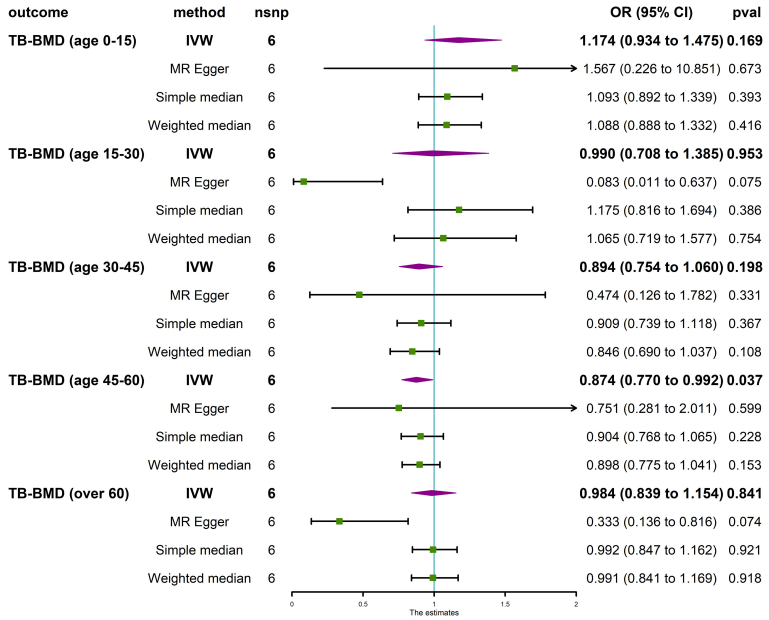

FIGURE 4
Causal effect of IVDD on BMD in different age groups. TB-BMD, total body bone mineral density; IVW, inverse variance weighted; nsnp, number of single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

Nonetheless, similar to other studies, the present study also had certain limitations. The first limitation is that the study participants were exclusively of European origin, which may cause the findings of this study not to be generalizable to individuals of other nations, such as those of African or East Asian descent. Furthermore, the comprehensive elimination of pleiotropy was challenging due to the limited understanding of the overall biological functionality of these instrumental variations. In addition, while the findings of the present study indicated the existence of potential causal relationships between bone mineral density at various anatomical sites and the risk of intervertebral disc degeneration (IVDD), a further comprehensive investigation of the intricate underlying processes is nonetheless warranted. Lastly, it is difficult to validate the findings of the present study in wet lab assays.

In summary, the present study revealed no substantial causal relationship, either directly from the causative impact of OP or indirectly via FA-BMD, on IVDD. The elevation in the levels of TB-BMD (total body bone mineral density) and LS-BMD (lumbar spine bone mineral density) could, however, contribute to the development of intervertebral disc degeneration (IVDD). Both eBMD and FN-BMD exhibited a putative causal relationship with the risk of intervertebral disc degeneration (IVDD). Furthermore, the association of intervertebral disc degeneration (IVDD) with osteoporosis (OP) and bone mineral density (BMD) was not statistically significant. Therefore, further investigation is required to elucidate the molecular mechanisms underlying the impact of bone mineral density (BMD) on intervertebral disc degeneration (IVDD), which will facilitate the precise treatment of these two bone degenerative diseases by orthopedic surgeons.

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

GL: Investigation, Validation, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Writing – original draft, Visualization, Software, Project administration, Methodology. HZ: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. MC: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. WC: Funding acquisition, Supervision, Writing – review & editing, Investigation, Validation.

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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/fendo.2024.1298531/full#supplementary-material

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Correlation between degeneration of cervical intervertebral disc and degeneration of paravertebral muscle

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Objective: To investigate the relationship between degeneration of cervical intervertebral disc and degeneration of paravertebral muscles[multifidus (MF), cervical semispinalis (SCer), semispinalis capitis (SCap) and splenius capitis (SPL)].

Methods: 82 patients with chronic neck pain were randomly selected, including 43 males and 39 females, with 50.73 0.7.51 years old. All patients were scanned by 3.0T MRI Philips Ingenia performed conventional MRI sequence scanning and fat measurement sequence mDIXON-Quant scanning of cervical. Fat infiltration (FI) and cross-sectional area (CSA) of cervical paravertebral muscle (MF, SCer, SCap and SPL) at central level of C5–6 disc were measured by Philips 3.0T MRI image post-processing workstation. According to Pfirrmann grading system, there was no grade I in the included cases. The number of grade IIr IV cases were n=16, 40, 19 and 7 respectively. CSA and FI of cervical paravertebral muscles were compared with t test or one-way ANOVA, Spearman correlation analysis was used to evaluate the correlation between age, disc degeneration, and CSA, FI of cervical paravertebral muscles, and multiple linear regression analysis was used to analyze the independent influencing factors of CSA and FI.

Results: CSA of cervical paravertebral muscles in male patients was significantly higher than that in female patients (all P<0.001), but there was no significant difference in FI (all P>0.05). Age was weakly correlated with CSA of MF+SCer, moderately correlated with CSA of SCap and SPL (r=-0.256, -0.355 and -0.361, P<0.05), weakly correlated with FI of SCap and SPL (r= 0.182 and 0.264, P<0.001), moderately correlated with FI of MF+SCer (r=0.408, P<0.001). There were significant differences in FI with disc degeneration (P<0.001, P=0.028 and P=0.005). Further correlation analysis showed that disc degeneration was strongly correlated with FI of MF+SCer (r=0.629, P<0.001), and moderately correlated with FI of SCap and SPL (r=0.363, P=0.001; r=0.345, P=0.002). Multiple linear regression analysis showed that sex and age were the influencing factors of CSA of SCap and SPL, sex was the independent influencing factor of CSA of MF+SCer, and disc degeneration was the independent influencing factor of FI.

Conclusions: Age is negatively correlated with CSA and positively correlated with FI. Disc degeneration was correlated with FI of paravertebral muscles, especially with FI of MF and SCer. Sex and age were the influencing factors of CSA, while disc degeneration was the independent influencing factor of FI.

KEYWORDS

paraspinal muscle degeneration, intervertebral disc degeneration, mDIXON-Quant, fat infiltration, cervical

Introduction

Chronic neck pain and low back pain are common symptoms in the general population, mostly in middle-aged and elderly people, and increase with age (1). The degeneration of paravertebral muscles may be one of the important causes of chronic neck pain and low back pain (2). One study has reported reduced muscle CSA in patients with low back pain compared to asymptomatic controls (3). Similarly, related studies have found that paraspinal muscle atrophy is more pronounced in patients with low back pain than in those without low back pain, and that paraspinal muscle atrophy is more pronounced on the symptomatic side than on the asymptomatic side (4–6). Therefore, many studies have confirmed that lumbar paravertebral muscle degeneration is closely related to the occurrence, development, clinical efficacy and prognosis of lumbar degenerative diseases. Paracervical musculature plays an important role in maintaining the level and neutrality of the cervical spine and in distributing head loads through flexion, extension, and translation movements (7, 8). It is estimated that neck muscles provide about 80% of total stability, while bony ligament structures contribute the remaining 20%. Degeneration of the deep neck extensors, which support neck movement and provide neck stability, has been associated with neck pain.

Spinal degeneration begins at the intervertebral disc. The early imaging signs of cervical disc degeneration show changes in disc signal, but few scholars have evaluated the FI of paravertebral muscles and cervical disc degeneration. Therefore, the correlation between disc signal and paravertebral muscle degeneration can better reflect the role of paravertebral muscle in the occurrence and development of cervical degenerative diseases. However, there are no relevant research reports. Meanwhile, most of the studies are subjective evaluation or semi-quantitative analysis of paravertebral muscle degeneration, and there are observation data bias and lack of reliability of experimental data to some extent (9, 10). The mDIXON-Quant technology is a new magnetic resonance scanning technology for quantitative fat measurement based on the principle of water-fat separation technology based on chemical shift introduced in recent years (11–13). At present, it is mainly applied to quantitative measurement of liver fat, but there is no report on FI evaluation of paravertebral muscle (11). Therefore, this study intends to use mDIXON-Quant technique to quantitatively measure FI of paravertebral muscles and evaluate the correlation between FI of paravertebral muscles and cervical disc degeneration.

Methods

Patient population

82 patients with chronic neck pain were randomly selected from outpatient or inpatient department. In order to exclude the influence of different MRI machines on the results, we uniformly selected the cases scanned by Philips Ingenia 3.0T MRI scanner in our hospital. Inclusion criteria were as follows: (1) Patients with chronic neck pain; (2) mDIXON-Quant sequence for 3.0T MRI and fat measurement; (3) aged from 30 to 75 years old; (4) Body Mass Index (BMI) between 18.5 and 23.9; (5) No physical therapy, acupuncture and other treatment measures affecting paravertebral muscles. Exclusion criteria were as follows: (1) Patients with a history of cervical spine surgery or cervical spine trauma; (2) Patients with concomitant diseases of important organs or serious systemic diseases; (3) MRI images were poor, and the range and boundary of muscles could not be distinguished and recognized well;④ Patients with MRI contraindications exist.

MRI scanning methods

Conventional MRI sequences (T1WI, T2WI, T2WI-mDIXON) and fat-measuring sequences (mDIXON-Quant) of cervical spine were performed with Philips Ingenia in supine position. mDIXON-Quant parameters: flip angle, 3°; TR, 5.8 ms; TE, 1.02 ms; voxel, 2.5 mm×2.5 mm×6mm; slice thickness, 6mm; field of view, 400mm×350mm×210mm; matrix, 160×40×70; and number of excitations, 1. The relevant scanning parameters are shown in Table 1.

Pfirrmann grade

We graded the degree of cervical disc degeneration by using the internationally common Pfirrmann classification system on the median sagittal position of 3.0T MRI with the help of Picture

TABLE 1 List of scanning parameters of MRI sequences.

Scan parameters	T1WI	T2WI	T2WI-mDIXON	mDIXON-Quant
TR	454	2500	2500	5.8
TE	8	110	100	1.02
FOV	160×254	200×162	160×250	400×350
SNR	1	1.8	1	1
NSA	1	1	1	1
Layer thickness/spacing	3.0/0.3	3.0/0.3	3.0/0.3	6.0/-3
scanning time, s	2:00	2:25	2:25	0:14

Archiving and Communications Systems (PACS) (14). Pfirrmann classification system divides disc degeneration into 5 grades. Pfirrmann grade I is mainly found in children, so only Pfirrmann grade II~IV disc degeneration is studied in this study. C5–6 is the most common segment for cervical disc degenerative disease, so this study will analyze this segment. The cervical disc grading results were assessed independently by 3 spine surgeons, and the results with different opinions were discussed and decided together. Finally, all the results were summarized and collected.

CSA and FI determination of cervical paravertebral muscles

The axial scan images of mDIXON-Quant sequence were transmitted to Philips 3.0T MRI image post-processing workstation. Two doctors with more than 5 years of experience in skeletal muscle imaging delineated the region of interest (ROI) of all C5–6 disc center layers on fat fraction images respectively. The ROI of cervical paravertebral muscles was set at multifidus (MF), cervical semispinalis (SCer), semispinalis capitis (SCap) and splenius capitis (SPL) on both sides respectively. Along the muscle contour, the system automatically generates ROI fat fraction (FF), i.e. FI and CSA, as shown in Figure 1. The left and right sides of each paravertebral muscle were measured three times and the average value was taken. The CSA representative value of the muscle at the same level was taken as the average value of CSA of the muscle at the same level. Similarly, the average FI value of the left and right muscles at the same level was taken as the FI representative value of the muscle at that level. In previous studies, MF and SCer muscles were not clearly demarcated on MRI in some cases, and both muscles belonged to deep neck extensors. Therefore, MF and SCer muscles were analyzed as a whole in this study.

Consistency evaluation of CSA and FI measurements

Twenty patients were randomly selected for repeated measurement. CSA and FI of paracervical muscles were measured independently and without interference by two observers. Inter-observer consistency of measurements was evaluated using intra-class correlation coefficients (ICC) between two observers measuring one week apart; in addition, intra-observer consistency of measurements was evaluated using ICC when one of the observers repeated measurements one week later. ICC>0.75 is considered to have good measurement consistency.

Statistical analysis

Data were analyzed statistically using SPSS 26.0 software (SPSS Inc., Chicago, IL, USA) and presented as mean ± standard deviation. For quantitative data conforming to normal distribution, t test was used for comparison between two groups, variance analysis was used for comparison between multiple groups, and rank sum test was used for non-normal distribution. Spearman correlation analysis was used to analyze the correlation between variables, and multiple linear regression analysis was used to analyze the independent influencing factors of paravertebral muscle degeneration. Correlation coefficient r≥0.7 was considered significant correlation; r=0.5~0.7 was strong correlation; r=0.3~0.5 was moderate correlation; r ≤ 0.3 was weak correlation. P<0.05 was statistically significant.

Results

The ICC of CSA and FI of cervical paraspinal muscles was greater than 0.75 within and between observers, indicating good consistency (Table 2). A total of 82 patients were included in this study, 43 males and 39 females, with an average age of 50.73 0.7.51 years, of which 40 - 50 years and 50 - 60 years were the main population, accounting for 30.49% (25/82) and 35.37% (29/82) respectively. In this study, C5–6 was selected as the most common cervical segment degeneration. 16 cases (19.51%), 40 cases (48.78%), 19 cases (23.17%) and 7 cases (8.54%) of C5–6 were grade II, III, IV and V, respectively (Table 3).

CSA in MF+SCer, SCap, and SPL were significantly higher in males than in females (all P<0.001), but not in FI (all P>0.05) (Table 4). In CSA of cervical paravertebral muscles, there were statistically significant differences in SCap and SPL between age (P=0.007 and P=0.005); in FI of cervical paravertebral muscles, there were statistically significant differences in MF+SCer and SPL

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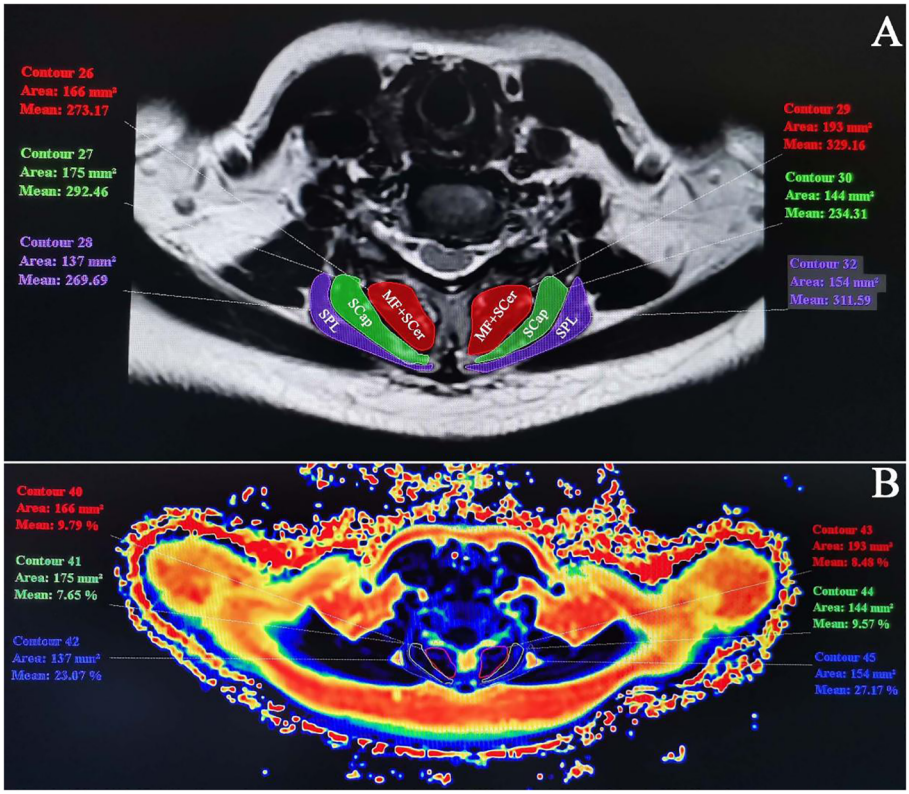

FIGURE 1
CSA and FI measurements of cervical paravertebral muscles at C5–6. **(A)** C5–6 T2WI axial sequence. CSA was measured along the outline of the muscles: MF+SCer, SCap and SPL. **(B)** Fat pseudo-color map. Measure ROI muscle FI in FF image.).

between age (P=0.015 and P=0.019) (Table 5). Further Spearman correlation analysis showed that age was positively correlated with CSA of MF+SCer, SCap and SPL, and weakly correlated with CSA of MF+SCer, and moderately correlated with CSA of SCap and SPL (r =-0.256,-0.355 and-0.361, respectively, P<0.05); age was positively correlated with FI of MF+SCer, SCap and SPL, and weakly correlated with FI of SCap and SPL (r = 0.182 and 0.264, respectively), and moderately correlated with FI of MF+SCer (r=0.408, P<0.001) (Figure 2).

FI of cervical paravertebral muscles was significantly different in patients with different degree of disc degeneration (P<0.001, P=0.028 and P=0.005) (Table 6). Spearman correlation analysis showed that the degree of disc degeneration was strongly correlated with FI of MF +SCer (r=0.629, P<0.001), and moderately correlated with FI of SCap and SPL (r=0.363, P=0.001;r=0.345, P=0.002) (Figure 3).

Multivariate linear regression analysis was used to evaluate whether sex, age and disc degeneration grade were independent factors of CSA and FI. The results showed that sex and age were independent factors of CSA and SPL of paravertebral muscles; sex was independent factor of MF+SCer CSA; disc degeneration grade was independent factor of FI of paravertebral muscles (P<0.001, P<0.001, P=0.01, P=0.006) (Tables 7–9).

TABLE 2 Intra-and inter-observer consistency of CSA and FI measurements of cervical paravertebral muscles.

Measurement parameters	Intra-observer		Inter-observer	
	ICC	95% *CI*	ICC	95% *CI*
C5-6 CSA (MF+SCer)	0.907	0.784-0.962	0.905	0.751-0.963
C5-6 FI (MF+SCer)	0.932	0.267-0.983	0.925	0.822-0.970
C5-6 CSA (SCap)	0.863	0.686-0.944	0.876	0.714-0.949
C5-6 FI (SCap)	0.881	0.723-0.951	0.872	0.701-0.948
C5-6 CSA (SPL)	0.921	0.814-0.968	0.901	0.769-0.960
C5-6 FI (SPL)	0.852	0.665-0.939	0.802	0.570-0.916

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TABLE 3 General information of patients with cervical degenerative diseases.

General information	Number of cases/average
Average age, years	50.73 ± 9.51
Age, years	
30~40	17 (20.73%)
40~50	25 (30.49%)
50~60	29 (35.37%)
>60	11 (13.41%)
Sex, n (%)	
Male	43 (52.44%)
Female	39 (47.56%)
Pfirrmann grade, n (%)	
II	16 (19.51%)
III	40 (48.78%)
IV	19 (23.17%)
V	7 (8.54%)

Discussion

Cervical hypermobility and overload is an important factor causing cervical spine degeneration (15–17). Teraguchi et al. (18) surveyed 975 participants aged 21 - 97 years and found that the highest prevalence of disc degeneration was at the C5–6 level, 51.5% in males and 46% in females. Therefore, in this study, C5–6 segment with the most obvious and representative cervical degeneration was selected for the study of intervertebral disc degeneration. At present, a large number of studies have confirmed that the number of muscle fibers in muscle decreases gradually with the increase of age, and muscle fiber degeneration occurs gradually, resulting in muscle atrophy and mass decline (19). A 10-year MRI study of cervical posterior extensor CSA in asymptomatic subjects showed a gradual increase in muscle CSA in subjects aged 10 to 30 years and a gradual decrease in muscle CSA in subjects aged 40 years and older (11). Therefore, the study controlled the age of the population to be included before the reduction of muscle CSA, i.e., 30 years. Valera-Calero et al. (20) analyzed cervical extensor CSA in healthy subjects using panoramic ultrasound and found that males had greater cervical extensor CSA than females. Sasaki et al. (21) reported that CSA in men was greater than that in women, while FI in paravertebral muscles in women was higher than that in men. The results of our study showed that at C5–6 level, CSA of paracervical muscles in male patients was significantly greater than that in female patients. This result is consistent with the study described above.

At present, the evaluation of paravertebral muscle degeneration is divided into quantitative evaluation and visual semi-quantitative evaluation (9, 10). The most widely used semi-quantitative assessment is the Goutallier grading system (22, 23). However, visual semi-quantitative assessment methods are affected by inter-observer differences, which will affect the results of analysis to some extent. The mDIXON-Quant sequence is a 3-dimensional Fast Field Echo (3D-FFE) sequence that uses multiple acquired echoes to generate water, fat, in-phase, and inverted images synthesized from water-fat images (24–26). Because there is almost no limit on echo time, mDIXON-Quant has the advantage of being more efficient and accurate than other MR fat quantification techniques. However, only a few studies have reported quantitative measurements of paravertebral muscle fat based on the mDIXON-Quant sequence. Zhang et al. (27) evaluated the reliability of measuring fat content in lumbar bone marrow and paravertebral muscle using mDIXON-Quant sequence, and found that mDIXON-Quant imaging has high reliability in measuring fat content in lumbar bone marrow and paravertebral muscle, which is suitable for clinical use. However, quantitative measurements of fat content in paravertebral muscles using the mDIXON-Quant sequence have not been reported. In addition, in order to ensure the reliability of the results, we used ICC to evaluate the consistency of the measurements within and between observers. The correlation coefficients were all>0.75, indicating that the measurement results were consistent and reproducible.

The relationship between muscle atrophy and fatty infiltration due to degeneration of the cervical paracervical muscles and the degree of cervical disc degeneration is unclear. There was no statistical difference between the degree of disc degeneration and CSA (P>0.05), or between the degree of disc degeneration and CSA (P>0.05). The possible reason is that muscle morphology and CSA are affected by many factors, and when CSA does not change, FI of neck muscle has increased, and CSA of paravertebral muscle is not correlated with FI (28). FI was more pronounced in MF and erector

TABLE 4 Comparison of CSA and FI of Paracervical muscle between sex($\bar{x} \pm s$).

Sex	Number	MF+SCer		SCap		SPL	
		CSA (mm^2)	FI (%)	CSA (mm^2)	FI (%)	CSA (mm^2)	FI (%)
Male	43	338.35 ± 66.15	23.85 ± 10.14	283.67 ± 64.90	15.65 ± 5.62	278.70 ± 71.63	14.60 ± 5.28
Female	39	256.82 ± 48.45	23.82 ± 9.77	199.23 ± 44.37	15.99 ± 6.75	186.13 ± 44.95	14.89 ± 5.60
T value		6.312	0.015	6.808	-0.253	6.926	-0.239
P value		<0.001	0.988	<0.001	0.801	<0.001	0.812

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TABLE 5 Comparison of CSA and FI of cervical paravertebral muscles between age ($\bar{x} \pm s$).

Age	Number	MF+SCer		SCap		SPL	
		CSA (mm^2)	FI (%)	CSA (mm^2)	FI (%)	CSA (mm^2)	FI (%)
30-40	17	323.00 ± 71.62	19.39 ± 8.32	270.88 ± 49.92	14.05 ± 5.40	281.00 ± 76.29	11.99 ± 3.41
40-50	25	315.16 ± 67.17	21.68 ± 9.36	267.96 ± 69.36	16.32 ± 5.84	246.68 ± 91.30	15.43 ± 5.58
50-60	29	274.93 ± 68.49	26.02 ± 9.62	217.03 ± 71.97	15.31 ± 6.62	206.03 ± 50.62	14.41 ± 5.52
>60	11	292.91 ± 73.32	29.84 ± 10.77	215.45 ± 63.66	18.70 ± 6.28	211.27 ± 57.18	18.29 ± 5.45
F value		2.320	3.684	4.322	1.414	4.571	3.527
P value		0.082	0.015	0.007	0.245	0.005	0.019

spinae in patients with severe disc degeneration (29). Cloney et al. (30) found that FI of cervical MF was associated with decreased sensation and function in CSM patients. Meanwhile, extensive FI was observed in the cervical extensors of patients with chronic neck pain-related diseases, but the maximum FI was observed in the deep neck extensors such as MF and SCer compared with the superficial tissues (such as SCap, SPL) (31). Our study found that there was a difference in FI of paracervical muscles among patients with disc

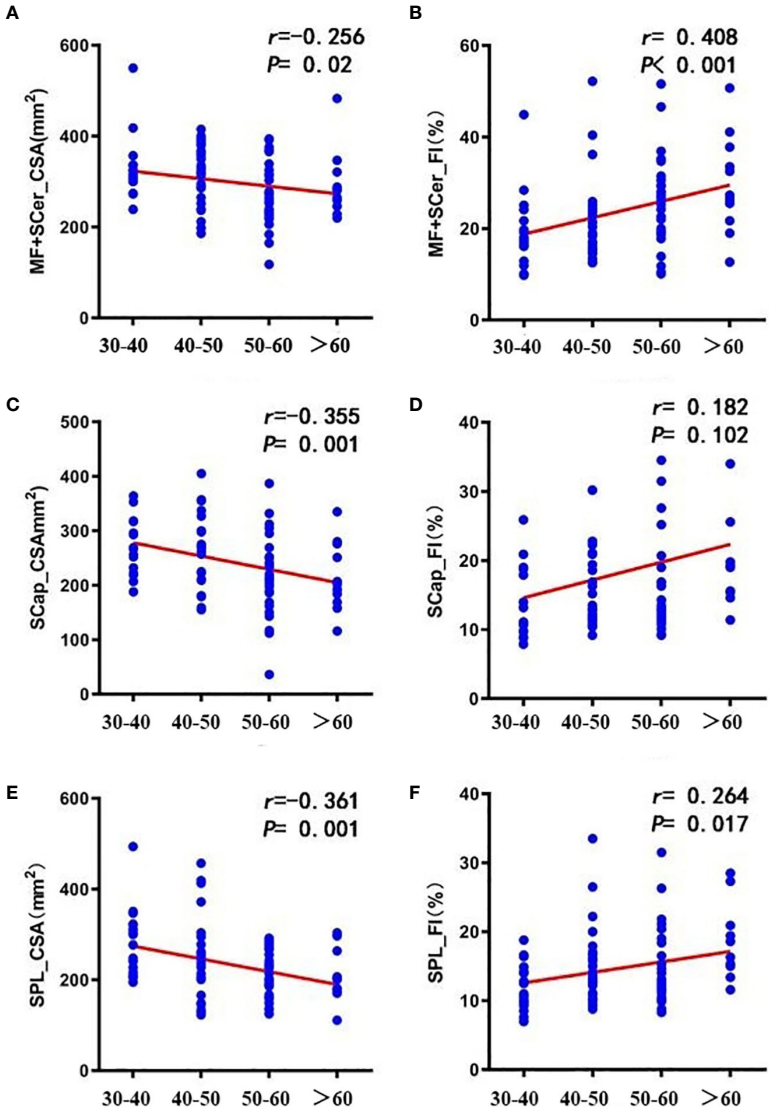

FIGURE 2
Correlation between age and CSA **(A, C, E)** and FI **(B, D, F)** of cervical paravertebral muscles. [**(A, B)** MF+SCer; **(C, D)** SCap; **(E, F)** SPL].

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TABLE 6 Differences in CSA and FI of cervical paravertebral muscles in patients with different degrees of cervical disc degeneration ($\bar{x} \pm s$).

Pfirrmann grade	Number	MF+SCer		SCap		SPL	
		CSA (mm^2)	FI (%)	CSA (mm^2)	FI (%)	CSA (mm^2)	FI (%)
II	16	303.31 ± 56.63	14.94 ± 4.02	241.75 ± 61.91	13.14 ± 4.68	224.06 ± 61.36	12.51 ± 3.53
III	40	293.65 ± 77.03	22.92 ± 8.45	239.33 ± 75.55	15.09 ± 5.63	246.78 ± 82.12	13.94 ± 4.90
IV	19	296.68 ± 76.82	29.51 ± 9.61	241.74 ± 70.30	18.37 ± 7.10	217.37 ± 83.34	16.26 ± 5.09
V	7	332.71 ± 47.79	34.00 ± 10.22	276.29 ± 58.05	19.10 ± 6.52	236.71 ± 40.02	20.26 ± 8.31
F value		0.617	12.886	0.556	3.198	0.766	4.699
P value		0.606	<0.001	0.645	0.028	0.517	0.005

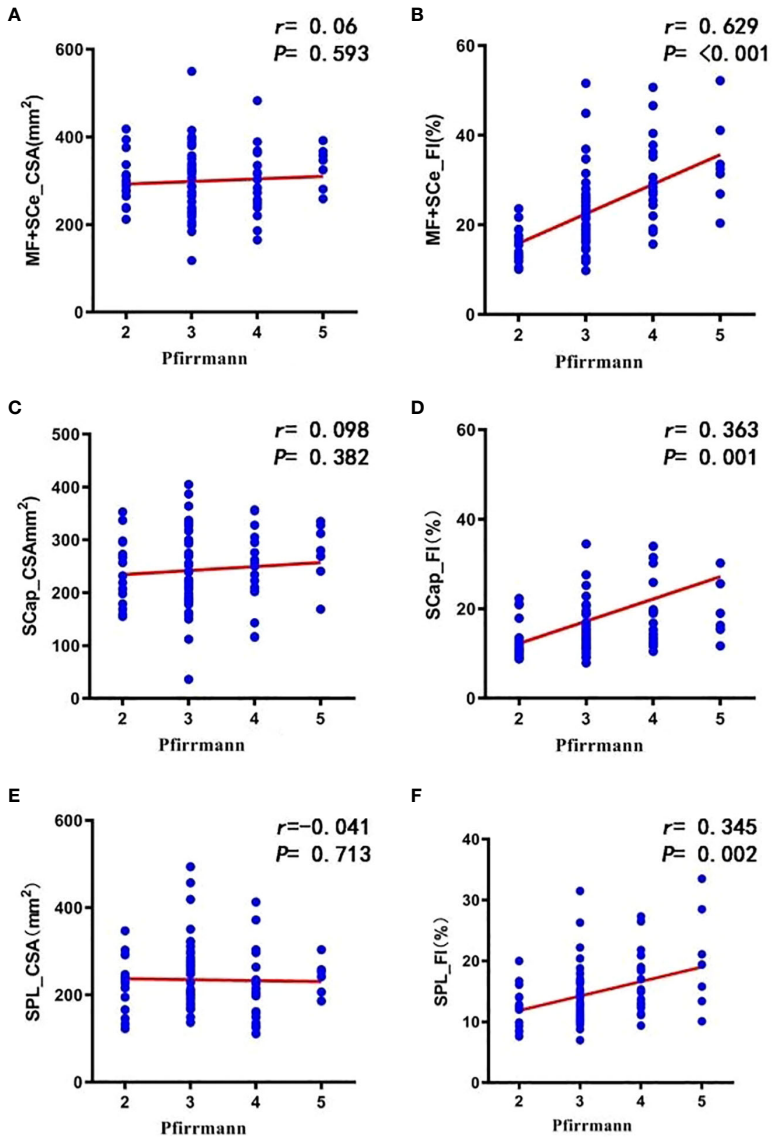

FIGURE 3
Correlation between the degree of disc degeneration and total CSA **(A, C, E)** and total FI **(B, D, F)** of cervical paravertebral muscles.

TABLE 7 Multiple linear regression analysis of independent influencing factors of MF+SCer CSA and FI in cervical paravertebral muscles.

	CSA					FI				
	B	SE	β	t	P	B	SE	β	t	P
Constant	410.86	32.554	–	12.621	<0.001	1.474	4.55	–	0.324	0.747
Sex	-76.272	13.592	-0.539	-5.612	<0.001	-0.145	1.9	-0.007	-0.076	0.939
Age	-9.357	7.688	-0.127	-1.217	0.227	1.488	1.075	0.145	1.384	0.17
Pfirrmann grade	7.438	8.426	0.09	0.883	0.38	5.918	1.178	0.512	5.026	<0.001

After multiple regression analysis by SPSS software, the standard coefficient of constant is –.

TABLE 8 Multiple linear regression analysis of independent influencing factors of CSA and FI in cervical paravertebral muscles.

	CSA					FI				
	B	SE	β	t	P	B	SE	β	t	P
Constant	354.826	30.034	–	11.814	<0.001	7.452	3.293	–	2.263	0.026
Sex	-73.472	12.54	-0.527	-5.859	<0.001	0.489	1.375	0.04	0.356	0.723
Age	-19.85	7.093	-0.274	-2.798	0.006	0.179	0.778	0.028	0.23	0.819
Pfirrmann grade	14.04	7.773	0.172	1.806	0.075	2.247	0.852	0.313	2.636	0.01

After multiple regression analysis by SPSS software, the standard coefficient of constant is –.

TABLE 9 Multivariate linear regression analysis of independent influencing factors of SPL CSA and FI in cervical paravertebral muscles.

	CSA					FI				
	B	SE	β	t	P	B	SE	β	t	P
Constant	391.838	32.758	–	11.962	<0.001	6.15	2.806	–	2.192	0.031
Sex	-83.368	13.677	-0.551	-6.096	<0.001	0.12	1.172	0.011	0.102	0.919
Age	-18.457	7.736	-0.235	-2.386	0.019	0.78	0.663	0.14	1.178	0.242
Pfirrmann grade	3.248	8.478	0.037	0.383	0.703	2.035	0.726	0.323	2.803	0.006

After multiple regression analysis by SPSS software, the standard coefficient of constant is –.

degeneration degree, and multiple regression analysis showed that disc degeneration degree was an independent factor affecting FI of paracervical muscles, indicating that it was not affected by other factors. Further Spearman correlation analysis showed that the degree of disc degeneration was moderately or strongly correlated with FI, and the degree of disc degeneration at C5–6 was most closely correlated with FI of deep cervical extensor (MF+SCer), showing a strong correlation. Among the cervical paravertebral muscles, the deep cervical extensor group, dominated by MF and SCer, attaches directly to the cervical spine and is considered to play a key role in maintaining stability and biomechanics, and these muscles may be sensitive to changes in neck function and pain (31). It may be related to the distribution, morphology and density of muscle spindles of different muscles in the neck, which is one of the factors for proprioceptive regulation of skeletal muscles (32). It has been found that the relative CSA of the most superficial extensors of the neck (SCap, SPL) is not reduced compared to the rectus capitis posterior minor, because muscles with high muscle spindle density (e.g., rectus capitis posterior minor and rectus capitis posterior major) may be more sensitive than those with low spindle density (SCap, SPL) (33). Our results show that the more severe the disc degeneration, the more likely the fat infiltration of the deep neck extensors (MF+SCer) is, similar to the results previously reported in patients with low back pain, and muscle degeneration is more common in lumbar MF.

Limitations

There are a few limitations of the present study. Our study only selected C5–6, the most common cervical disc degeneration, and did not include more segments (such as C4–5 and C6–7) for comparative study. The Pfirrmann classification system for evaluating disc degeneration in this study is not perfect, and other signs of disc degeneration such as spinal cord compression, disc herniation, intervertebral foraminal stenosis, Schmermer's node,

etc. cannot be considered. It is impossible to accurately identify and isolate individual muscles in the deep neck extensors due to MRI images. In the future, it is hoped that higher resolution images and more advanced MRI sequences will better identify and differentiate individual muscles in the cervical paravertebral muscle group.

Conclusions

There is a correlation between disc degeneration and paravertebral muscle degeneration, especially in deep neck extensors (MF and SCer). Therefore, patients with cervical disc degeneration should exercise paraspinal muscles more actively in the early stage, which is helpful to restore paraspinal muscle degeneration function and delay degeneration process.

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 the People's Hospital of Ningxia Hui Autonomous Region. 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

QL: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. XL: Data curation, Writing – original draft, Writing – review & editing. RW: Conceptualization, Data curation, Software, Writing – review & editing. PN: Conceptualization, Methodology, Software, Writing – review & editing. LC: Conceptualization, Methodology, Software, Supervision, Writing – review & editing. LW: Conceptualization, Funding acquisition, Methodology, Supervision, Visualization, Writing – review & editing. YS: Conceptualization, Funding acquisition, Methodology, 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.

Publisher's note

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Advances in the interaction between lumbar intervertebral disc degeneration and fat infiltration of paraspinal muscles: critical summarization, classification, and perspectives

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More than 619 million people in the world suffer from low back pain (LBP). As two potential inducers of LBP, intervertebral disc degeneration (IVDD) and fat infiltration of paraspinal muscles (PSMs) have attracted extensive attention in recent years. So far, only one review has been presented to summarize their relationship and relevant mechanisms. Nevertheless, it has several noticeable drawbacks, such as incomplete categorization and discussion, lack of practical proposals, etc. Consequently, this paper aims to systematically summarize and classify the interaction between IVDD and fat infiltration of PSMs, thus providing a one-stop search handbook for future studies. As a result, four mechanisms of IVDD leading to fat infiltration of PSMs and three mechanisms of fat infiltration in PSMs causing IVDD are thoroughly analyzed and summarized. The typical reseaches are tabulated and evaluated from four aspects, i.e., methods, conclusions, benefits, and drawbacks. We find that IVDD and fat infiltration of PSMs is a vicious cycle that can promote the occurrence and development of each other, ultimately leading to LBP and disability. Finally, eight perspectives are proposed for future in-depth research.

KEYWORDS

low back pain, intervertebral disc degeneration, paraspinal muscles, fat infiltration, interplay mechanisms

1 Introduction

Low back pain (LBP) has been identified as a major global public health problem (1) and the leading cause of the disease burden worldwide (2). According to statistics, up to 2020, approximately 619 million people around the world suffer from LBP (3). Given its prevalence and burden rise with age (4), as well as the increasing population aging and the modern lifestyle changes, LBP should be urgently addressed in the 21st century.

The pathogenesis of LBP is diverse, including intervertebral disc degeneration (IVDD), radicular pain, facet arthropathy, myofascial pain, sacroiliac joint pain, spondyloarthropathies, etc. More details are available in the review by Knezevic et al. (5). Among them, IVDD is the main pathogenic factor of LBP (6, 7). It generally exhibits disc cell apoptosis, annulus fibrosus (AF) tears, and nucleus pulposus (NP) protrusion, structural changes in the cartilaginous endplates (CEP), disc collapse, and metabolic changes (8, 9). These degenerative changes contribute to the loss of normal structure and function of the intervertebral disc (IVD), giving rise to disc-related pain and inflammation, along with decreased spinal stability (10, 11). Additionally, during disc degeneration, structure remodeling of paraspinal muscles (PSMs) usually occurs, e.g., fat infiltration, muscle asymmetry, and size reduction (12). Notably, fat infiltration of PSMs increases with the progression of IVDD and is actively associated with the severity and dysfunction of LBP (13, 14).

In fact, there is a close interaction between fat infiltration in PSMs and IVDD (12, 15). On the one hand, IVDD can induce fatty infiltration of PSMs by affecting their loading (16) and innervation (17, 18), as well as releasing inflammatory mediators (19, 20). On the other hand, fat-infiltrated PSMs can promote IVDD via increasing mechanical stress on the disc (21) and releasing inflammatory (22) and adipokine factors (23).

In recent years, a growing number of studies have reported the interaction between IVDD and fat infiltration of PSMs. To further understand the mechanism of their interaction, relevant literature from 2014 to Apr. 2024 is investigated and counted based on the search keywords of 'intervertebral disc degeneration, paraspinal muscles, and fatty infiltration'. The specific search process and results are shown in Figure 1. From Figure 1B, it can be seen that the research on their interaction is generally at a relatively high level, especially in the recent three years.

Until now, only Wang et al. (24) have published a review on the relationship between IVDD and fat infiltration of PSMs, which has the following shortcomings: (i) Incomplete investigation and analysis on the interaction between IVDD and fat infiltration of PSMs; (ii) Lack of detailed summaries and perspectives. In order to overcome the above shortcomings, this paper offers a comprehensive and in-depth investigation of the interaction between IVDD and fat infiltration of PSMs, aiming to offer a state-of-the-art, one-stop manual for present and further research. Then, the screening process for the review is depicted in Figure 2. Inclusion criteria encompassed studies in English focusing on the IVDD and PSMs relationship, with excluded material such as reviews, case reports, and conference abstracts. Finally, a total of 72 papers were included in the investigation. Its main contributions are summarized as follows:

- Four mechanisms of IVDD involved in the fatty infiltration of PSMs and three mechanisms of fatty infiltration of PSMs engaged in IVDD are systematically classified and summarized, upon which the related studies are systematically tabulated and evaluated from four aspects, i.e., methods, conclusions, benefits, and drawbacks.
- In the part of summarizations and discussions, five key findings are put forward from three aspects: research content, methods and results, and their reasons are analyzed.
- Eight perspectives in four main aspects (i.e., promising research, experimental methods, models and samples) are proposed for future research.

The rest of this article is organized as follows: Section 2 summarizes four mechanisms of IVDD involved in fat infiltration of PSMs. Three mechanisms of fat infiltration of PSMs participated in IVDD are concluded in Section 3. Section 4 gives comprehensive

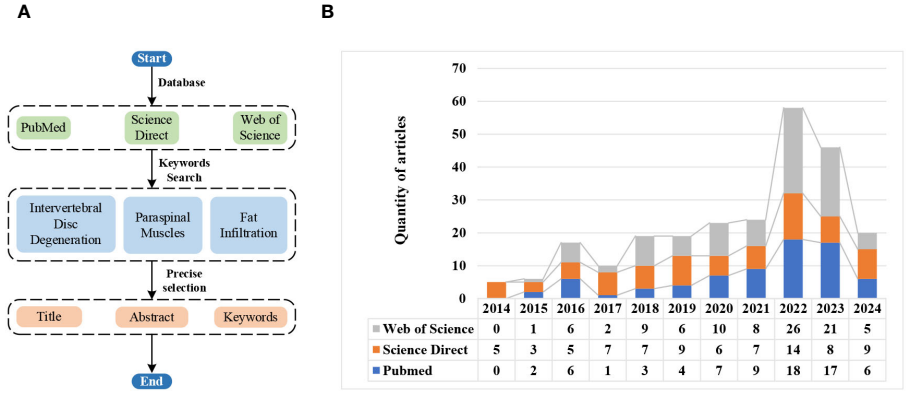

FIGURE 1
Literature search flowchart and results via different databases. **(A)** Search flowchart. **(B)** Search results.

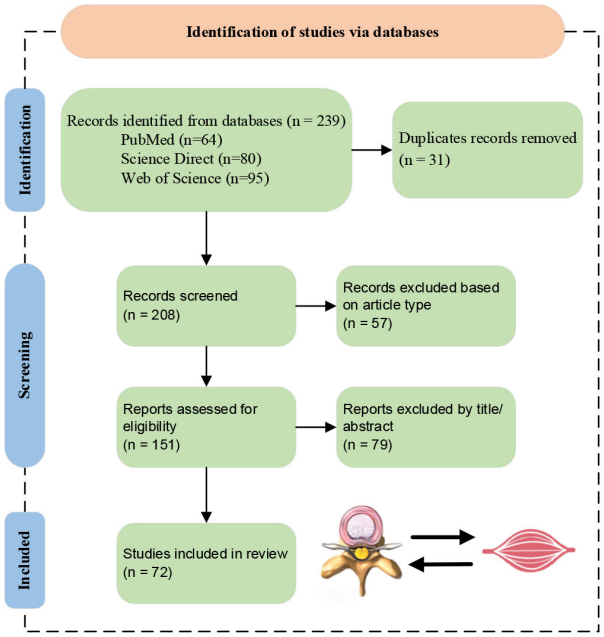

FIGURE 2
Literature screening flowchart.

summarizations and discussions of some findings. In Section 5, four conclusions are presented. Finally, Section 6 offers several proposals and perspectives for further research.

2 Mechanisms of IVDD involved in fat infiltration of PSMs

There are four main mechanisms of IVDD causing fatty infiltration of PSMs, particularly the multifidus muscle (MM): inflammatory response, muscle denervation, muscle chronic disuse, and biomechanical imbalance.

2.1 Inflammatory response

Both human and animal studies have consistently demonstrated that dysregulation of local inflammatory activity is a significant contributing factor to the fatty infiltration of PSMs associated with IVDD (12, 15, 19, 20, 25). The summarizations of recent studies exploring this mechanism are presented in Table 1. Inflammatory factors play a crucial role in the occurrence and development of IVDD (26, 27). Specifically, tumor necrosis factor-α (TNF-α)and interleukin-1beta (IL-1β), as the most pivotal inflammatory cytokines, exhibit strong pro-inflammatory activity and can stimulate the release of various proinflammatory mediators. Their upregulated expression in degenerative IVD is closely linked to multiple pathological processes of IVDD (28).

IL-1β mediates the production of reactive oxygen species (ROS), and the excessive accumulation of ROS can cause oxidative stress (28). Increased production of ROS is associated with the differentiation of preadipocytes to adipocytes and the accumulation of adipose tissue (29). Persistent oxidative stress can lead to muscle damage, significantly affecting the contractility of skeletal muscle and inducing fibrotic degeneration as well as muscle fatty changes (30). TNF-α is a key player in muscle atrophy, which can induce the upregulation of inflammatory cytokine gene expression and activate the nuclear factor-kappa B (NF-κB) pathway in muscle atrophy (31). Furthermore, TNF-α is related to the disorder of lipid metabolism, and it is potentially an important factor in promoting white adipogenesis in skeletal muscle (22, 28, 32). Interestingly, the relationship between adiposity and pro-inflammatory cytokines is two-way; TNF-α is produced not only by adipose tissue but also regulates adipogenesis (19, 20). In animal models of IVDD or injury, despite no muscle damage, higher expression of proinflammatory cytokine genes (e.g., TNF and IL-1β) and fat infiltration were observed in MM (25, 33–35). The mechanism of fat infiltration in MM is related to the local inflammatory response induced by TNF, which originates from the degenerated disc rather than the PSMs (12). In addition, TNF expression in MM after IVDD is mediated by M1 macrophages in adipose tissue (25).

Fibroblasts, preadipocytes, and satellite cells are existent in the perimuscular connective tissue. These cells can transdifferentiate into adipose tissue under the stimulation of pro-inflammatory cytokines, ultimately leading to adipocyte proliferation (30). A large number of fibro-adipogenic progenitors (FAPs) and satellite cells (SCs) were found in the MM of patients with lumbar disc herniation (LDH) (36). FAPs are an inherent muscle stem cell population that differentiate into adipocytes and fibroblasts and contribute significantly to muscle fat infiltration and fibrosis (37, 38). FAPs appear as a brown/beige fat (BAT) phenotype, whereas

TABLE 1 Summary of inflammatory response.

Literature	Year	Methods	Main conclusions	Benefits	Drawbacks
James et al. (25)	2018	Animal experiment	a) During IVDD, macrophages and TNF actively participate in the subacute/early chronic stage of remodeling in muscle, adipose and connective tissues of the MM.	a) Save cost; b) Presents a novel target for treatment.	a) Unknown interference factors may exist in the previous handling of animals; b) Sheep may not be the best animal model for this experiment.
James et al. (20)	2021	Cross-sectional study	a) Increased fatty infiltration of MM is associated with inflammatory dysregulation; b) TNF induces pain and muscle alterations through inflammatory processes in epidural adipose tissue.	a) Provide support for translating observations from animal studies to humans; b) Provide a new mechanism for multifidus muscle changes.	a) Poor sample representativeness; b) Effect of age and BMI on results not excluded; c) Lack of comparison tissue samples; d) Small sample size.
Shi et al. (12)	2022	Retrospective study	a) Fat infiltration of MM may be associated with inflammatory reaction induced by TNF coming from the degenerated IVD; b) Optimal correlation between fatty infiltration of MM and IVDD.	a) Keep consistent baseline demographic characteristics; b) Select patients with NCLBP as control subjects.	a) Select patients with LDH only at L4/L5; b) Small tissue sample size; c) Lack of tissue samples of the pain-free control group.
Huang et al. (15)	2022	Animal experiment and controlled clinical trial	a) Fat infiltration of PSMs and IVDD are causally related; b) Fat infiltration of PSMs is closely related to inflammation, after IVDD.	a) Simple and easy-to-use experimental method; b) Develop a new rat model of DLBP; c) Set up a sham group to exclude puncture interference.	a) Only punctured the L4–6 rat IVD; b) Exist impact on the results from the movement disorder after rat IVD puncture; c) Extraordinary difference between the physiology of humans and rats.
Chen et al. (19)	2023	Prospective cohort study	a) Differences between the ipsilateral and unilateral PSMs were influenced by IVDD and fat infiltration; b) Inflammatory dysfunction is correlated with high-fat filtration and decreased CSA of MM.	a) Provide quantitative data; b) Compare with clinical outcomes.	a) Lack of control group; b) Possible bias and error in tissue sampling; c) Lack of protein expression testing; d) Small sample size.

IVDD, intervertebral disc degeneration; TNF, tumor necrosis factor; MM, multifidus muscle; BMI, body mass index; IVD, intervertebral disc; LDH, lumbar disc herniation; NCLBP, nonspecific low back pain; DLBP, discogenic low back pain; PSMs, paraspinal muscles; CSA, cross sectional area.

white adipose tissue makes up the majority of muscle fat infiltration (39, 40). BAT has demonstrated positive effects on myogenesis (41). Therefore, a potential avenue for mitigating fat infiltration (FI) and muscle atrophy in the future involves directing FAPs toward a BAT phenotype.

Figure 3 shows the current lineage of research on inflammatory response.

2.2 Muscle denervation

IVDD is a complex process involving molecular and structural alterations, which causes the compression of both the dorsal root ganglia and the nerve root (42), with atrophy and fat infiltration in the PSMs they innervate (17, 18, 43, 44). Recent studies on this mechanism are summarized in Table 2. Muscle denervation usually undergoes three stages: 1) immediate loss of function, followed by rapid weight loss and muscle fiber atrophy, 2) more severe muscle atrophy, including loss of most sarcomeric tissue, and 3) interstitial fibrosis and fat deposition (45). Additionally, denervated muscles experience alterations in glucose metabolism and protein hydrolysis, which are associated with reduced muscle fiber diameter and increased fat content. A review of the MRI appearance of muscle denervation by Kamath et al. reveals that progressive muscle atrophy and fat infiltration can be observed a few weeks after denervation (46). Consequently, in the absence of neural innervation, skeletal muscles inevitably undergo a process of atrophy, eventually largely replaced by fibrous connective and adipose tissue. Similarly, continuous compression of a nerve root by disc herniation also leads to muscle atrophy and fat replacement in muscles supplied by that nerve (47, 48). The MM is particularly susceptible because of being innervated only by the medial branch of the dorsal root of the spinal nerve, with no intersegmental nerve supply like other PSMs (44). Early studies demonstrated that denervated rabbit muscles can transform into white adipose tissue and express specific genes, especially the adipose obese (ob) gene (49). The expression of the ob gene is specific to adipose tissue in various animal species, including humans. Moreover, denervated skeletal muscles of most animals exhibit a dramatic increase in DNA synthesis, since the proliferation of all kinds of cells such as satellite cells, macrophages, mast cells, etc. Most of these cells secrete various cytokines to stimulate the proliferation and differentiation of macrophages, mast cells, fibroblasts, preadipocytes, and muscle precursor cells, thereby promoting muscle repair or fat degeneration (49). Additionally, it has been shown that TNF-α influences myoblast differentiation and fiber

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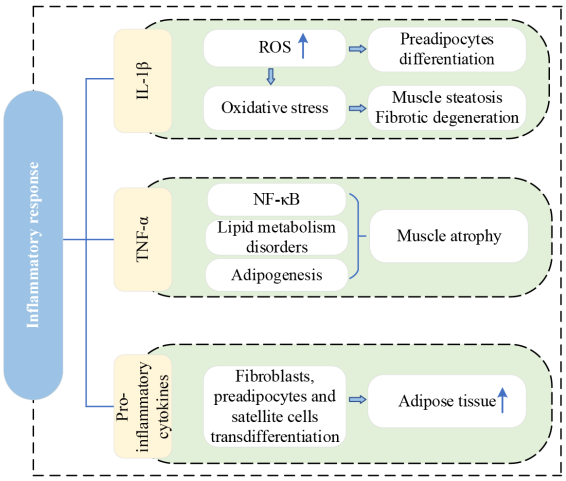

FIGURE 3
Research lineage of inflammatory response. IL-1β, interleukin-1beta; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-kappa B.

TABLE 2 Summary of muscle denervation.

Literature	Year	Methods	Main conclusions	Benefits	Drawbacks
Hodges et al. (17)	2006	Animal experiment	a) The cross-sectional area of MM decreases and adipocytes enlarge after disc and nerve lesions; b) Changes after disc lesion affect one level, which may be due to disuse following reflex inhibitory mechanisms.	a) Set up a sham group to exclude operative procedure interference; b) A new reliable method of measuring muscle cross-sectional area.	a) The anterolateral annular lesion is not the best model to study the effects of disc injury; b) Differences in muscle response and mechanisms to injury between pigs and humans.
Battie et al. (43)	2012	Cross-sectional study	a) Within 6 weeks of the appearance of radicular symptoms related to disc herniation, the MM shows fatty infiltration rather than CSA asymmetry.	a) Quantitative measurements of muscle CSA and signal using MRI; b) Minimize the impact of confounding factors.	a) Cannot exclude the possibility that muscle asymmetry existed before the onset of symptoms; b) Lack of evidence to confirm nerve root involvement and denervation; c) Possible measurement bias.
Yaltirik et al. (44)	2018	Case-control study	a) Long-term pressure of nerve roots by disc herniation can cause muscle atrophy and degeneration; b) Muscle degeneration can be detected by decreased cross-sectional area and increased fat deposition.	a) Considered gender and age; b) Independent observer measurement.	a) Not exclude the effect of potential factors such as body weight on the cross-sectional area of PSMs.
Boström et al. (18)	2022	retrospective study	a) Increased fat infiltration in MM and longissimus muscle in dogs with compressive IVDD relative to non-compressive nucleus pulposus extrusion, likely resulting from denervation and secondary disuse caused by spinal cord compression and pain.	a) Reliable measurement method; b) Measurement of several slices from the same segments.	a) Impossible to compare with healthy dogs; b) May have clinical signs in IVDD compression dogs before the scan; c) Lack of information on exercise level and parameters; d) Influence of transverse slice in cross-sectional area measurement.

MM, multifidus muscle; CSA, cross sectional area; PSMs, paraspinal muscles; IVDD, intervertebral disc degeneration.

degradation and its expression increases with disc injury and affects axon conduction (50).

Figure 4 illustrates the current vein of research on muscle denervation.

2.3 Muscle disuse

IVDD is the main cause of LBP and significantly affects the stability and motor function of the spine. Pain and discomfort from IVDD often make individuals reduce exercise and activity, causing the disuse of PSMs – a lack of exercise. Short-term disuse promotes fatty acid infiltration into skeletal muscle cells and is associated with the development of intermuscular adipose tissue(IMAT) (51). Table 3 provides a summary of recent studies on this mechanism. When PSMs lack exercise, their capillary reactivity decreases, resulting in the muscle blood supply being inadequate and leaving the muscle in a hypoxic state (56). Persistent hypoxia causes incomplete utilization of muscle glycogen and accumulation of lactic acid and various metabolites, which induces muscle edema, stimulating or exacerbating nerve pain (57, 58). The long-term effect makes the muscle gradually thin and degenerate, eventually replaced by adipose tissue (59). Previous studies have indicated a higher accumulation of adipose tissue in PSMs is related to physical inactivity (54, 60). Movement and gravitational loading are essential for maintaining a healthy spine. When the axial load of the human body decreases, such as during prolonged bedrest or spaceflight, the active and passive elements of the lumbar spine are affected (61). In response to decreased use, the lumbar spine undergoes adaptive remodeling (62). Adverse effects include lumbopelvic muscle atrophy, fat accumulation in PSMs, reduced lumbar lordosis, and altered disc hydration status (54, 61). Surprisingly, Hides et al. (63) observed a significant increase in the CSA of the MM during the last magnetic resonance scan of bed rest and the first scan of re-loading. They suggested that this alteration might be attributed to fluid transfer of the atrophic muscle during reloading or fiber and connective tissue damage (subsequent swelling) after walking to mask muscle atrophy. The double S-curve of the spine enables the body to slow down the vertical gravitational loads and spread forces horizontally. However, this requires the action of muscles in the lumbopelvic region to maintain the anterior kyphosis of the spine, especially the MM (64). Bed rest eliminates axial gravitational loading from the spine, potentially depriving the necessary stimulus for the normal functioning of the MM (63). In addition, disc degeneration itself can cause the physiological curve of the spine to become shallower and disappear.

Brisk walking (65), aerobic training (66), resistance training (67–69), and other forms of exercise can effectively prevent fat infiltration or delay its progression speed. Additionally, exercise not only prevents but also improves the inflammatory response in muscles secondary to IVDD (52). This positive effect is attributed to exercise promoting the release of immunomodulatory factors in the blood, which exerts anti-inflammatory effects. Interestingly, Wesselink et al.'s review shows that fat infiltration of PSMs in patients with LBP is irreversible through exercise (55). However, it's important to note that their conclusions are based on studies with low statistical power and methodological quality, and only provide moderate-quality evidence. In the future, more and higher quality research is needed to draw definitive conclusions.

Figure 5 provides the current research network on muscle disuse.

2.4 Biomechanical imbalance

The IVD and PSMs are not only surface adjacent but also biomechanical and biochemical interconnected (70). Serving as a crucial structure in the spine, the IVD offers buffering and support between adjacent vertebrae but is susceptible to degeneration influenced by factors such as age, lifestyle, and genetics. After degeneration, IVD loses its normal structure and function, which leads to the narrowing of the disc space and settling of the motion segment (71). Subsequently, the ligamentum flavum buckles, eventually leading to instability of the lumbar spine. The main function of PSMs is to maintain the stability and movement of the spine. Under normal circumstances, the load on PSMs in humans is

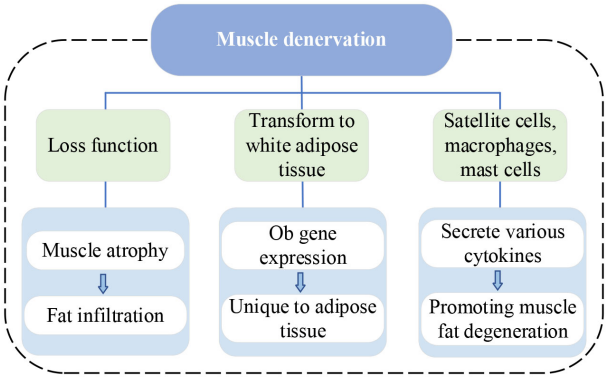

FIGURE 4
Research vein of muscle denervation. ob, adipose obese.

TABLE 3 Summary of muscle disuse.

Literature	Year	Methods	Main conclusions	Benefits	Drawbacks
James et al. (52)	2018	Longitudinal case-control animal model	a) The sub-acute/early chronic phase of remodeling in the multifidus and muscle hyperalgesia are driven by inflammatory processes; b) The inflammatory response (i.e. expression of IL-1β and adiponectin) in muscle secondary to IVDD was prevented by exercise.	a) Provide four new insights about IVDD in regulating inflammatory pathways in MM; b) Offer a new chance to study the inflammatory profile of MM near spontaneous IVDD.	a) Lack the time course information from the onset of IVDD; b) Differences in the pathological mechanisms between humans and rats.
Maurer et al. (53)	2020	Cross-sectional case-control study	a) The degree of physical inactivity observed over 14 14-year period showed a strong correlation with IVDD of the thoracic and lumbar spine.	a) Large sample size; b) A 14-year observation period.	a) Limited MRI dates with no earlier images for comparisons; b) Exclusion of chronic back pain patients; c) Only correlation without a causal relationship was investigated; d) Lack of physical activity details.
De Martino et al. (54)	2022	Prospective longitudinal study	a) Accumulation of ILC and inhomogeneous spatial distribution were found in the lumbar musculature after 60 days of bed rest; b) Provide a new target for lumbar muscle repair for those exposed to prolonged extreme physical inactivity, e.g., astronauts, the elderly, or individuals with chronic LBP.	a) Quantify the spatial pattern of ILC accumulation.	a) Small sample size; b) Complex and costly research; c) Limitations of muscle segmentation methods.
Wesselink et al. (55)	2023	Review article	a) Moderate-quality evidence suggests that paraspinal fat infiltration cannot be reversed by exercise in patients with LBP; b) To draw definite conclusions, more studies with consistent, and consensus-driven, methodologies, larger sample sizes and longer treatment duration are required.	a) Compared with early reviews; b) Constructive perspectives for future tasks.	a) A small number of available studies; b) Limited to the pool and compare data from different studies; c) Provide moderate-quality evidence.

IL-1β, interleukin-1beta; IVDD, intervertebral disc degeneration; MRI, magnetic resonance imaging; ILC, intramuscular lipid concentration.

in balance. However, when IVDD causes changes in disc height and vertebral instability, the tension load on PSMs increases to maintain spinal stability. If instability persists or becomes severe, surpassing the stress limits of the PSMs, it can result in atrophy and fat infiltration (16, 71, 72).

The current context of research on biomechanical imbalances is shown in Figure 6.

3 Mechanisms of fat infiltration of PSMs involved in IVDD

Similar to the impact of IVDD on PSMs, fat-infiltrated PSMs also affect IVD. However, there are few studies on the impact of fat infiltration of PSMs in the IVDD. Here we summarize three

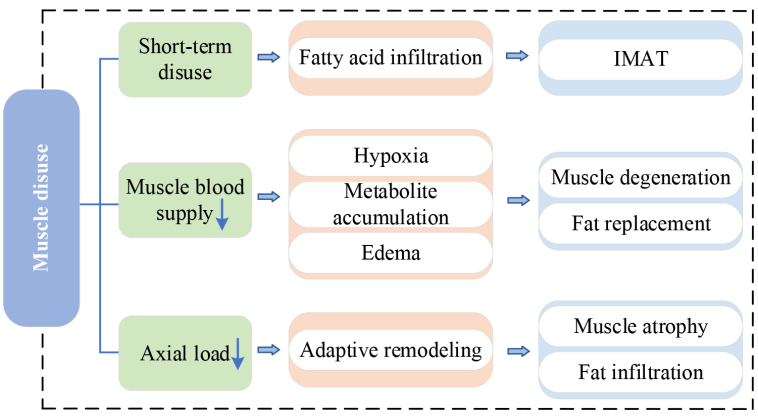

FIGURE 5
Research network of muscle disuse. IMAT, intermuscular adipose tissue.

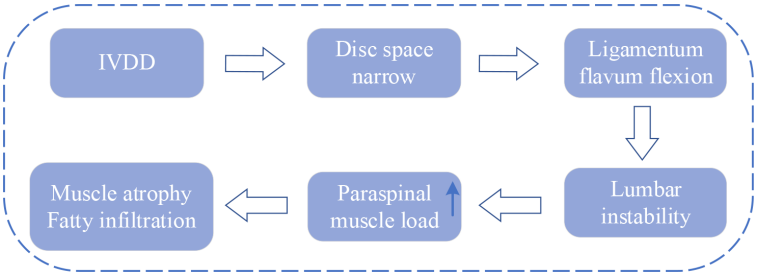

FIGURE 6
Research context of biomechanical imbalance. IVDD, intervertebral disc degeneration.

mechanisms, inflammation, lipid metabolism disorders, and abnormal mechanical load.

3.1 Inflammation mechanism

Adipose tissue is a primary source of numerous inflammatory cytokines, and an excess of adipocytes contributes to a state of systemic chronic low-grade inflammation. Previous studies have established a link between proinflammatory cytokines derived from adipose tissue(including TNF and IL-Iβ) and both obesity and osteoarthritis (73, 74). Obesity can cause fatty infiltration of PSMs (75). Furthermore, in obesity, paraspinal adipose tissue is considered to be a source of systemic pro-inflammatory cytokines (19). The fat infiltration of PSMs primarily consists of white adipose tissue, and the abnormal accumulation of fat leads to functional impairment of white adipose tissue, characterized by elevated levels of specific pro-inflammatory cytokines such as TNF (22). In addition, adipocytes in white adipose tissue recruit macrophages from the blood by synthesizing and secreting lactic acid, and the macrophages are polarized after infiltrating into fat, forming CD11c + M1 type, highly expressing pro-inflammatory cytokines such as IL-1β and TNF (76), which are key driving factors of IVDD and closely associated with various pathological processes of IVDD (28).

TNF-α primarily exerts its effects through activating the NF-κB signaling pathway, which causes the expression of type II collagen and proteoglycan to be downregulated, and the expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs)-4,5, matrix metalloproteinases (MMPs) to be upregulated. This cascade induces matrix degradation, cellular apoptosis, and precipitating degenerative changes in the lumbar vertebral discs (77). Simultaneously, the death domain of TNF receptors binds to other intracellular signaling proteins containing death domains, such as TNF receptor-associated death domain protein (TRADD) and Fas-associated death domain protein (FADD). This interaction triggers the cysteinyl-aspartate-specific protease (caspase) family of cascade reactions, which leads to caspase-3 activation, ultimately causing cell apoptosis (78). Additionally, TNF-α also directly inhibits NP cell activity and reduces the expression of growth differentiation factor 5(GDF-5) to accelerate IVDD.

IL-1β mainly activates mitogen-activated protein kinase(MAPK) and nuclear factor-kappa B(NF-κB) signaling pathways, upregulating MMPs-1, 2, 3, 4, 13, and ADAMTs-4, 5 (79). This leads to the degradation of type II collagen and proteoglycans, thereby accelerating the development of IVDD. Furthermore, IL-1β stimulates the expression of vascular endothelial growth factor(VEGF) and nerve growth factor (NGF), promoting the invasion of nerves and blood vessels into degenerated discs (80), eventually inducing LBP. Beyond these effects, IL-1β amplifies the inflammatory cascade by increasing the secretion of inflammatory cytokines (IL-6, 8, 17) and C-C Motif Chemokine Ligand chemokines (CCL-2, 3, 5, 7). This, in turn, attracts immune cells to accumulate at the site of inflammation, releasing inflammatory mediators that exacerbate IVDD (81–83).

Figure 7 portrays the present state of research on the inflammation mechanism.

3.2 Lipid metabolism disorder

Adipocyte-secreted adipokines, as biologically active substances with metabolic and immunomodulatory functions, play a significant role in the occurrence and progression of IVDD (23).

Leptin can stimulate the proliferation of AF and NP cells by inducing the expression of cell cycle protein D1 and activating JAK-STAT3, MEK-ERK and PI3K-Akt signaling pathways (84). However, despite this proliferative effect, the increased expression of extracellular matrix-degrading enzymes prevents the proper synthesis of extracellular matrix components by the proliferating cells. This imbalance promotes catabolism and contributes to disc cell senescence, eventually leading to IVDD (84, 85). Additionally, when leptin acts alone or in synergy with TNF-α, IL-1β, or IL-6 in NP cells, it significantly increases the production of NO and expression of inflammatory cytokines and MMPs, speeding up the process of IVDD (86). Leptin can also promote calcification of the CEP, interfering with the transport of nutrients to disc cells, and ultimately contributing to IVDD (87).

Adiponectin levels are related to IVDD, nevertheless, conflicting results have been reported in published literature. Khabour et al. discovered higher levels of circulating adiponectin in IVDD patients compared to healthy controls (88), while Yuan et al. verified a downregulation of adiponectin expression in IVDD patients, which is negatively correlated with IVDD severity (89). In addition,

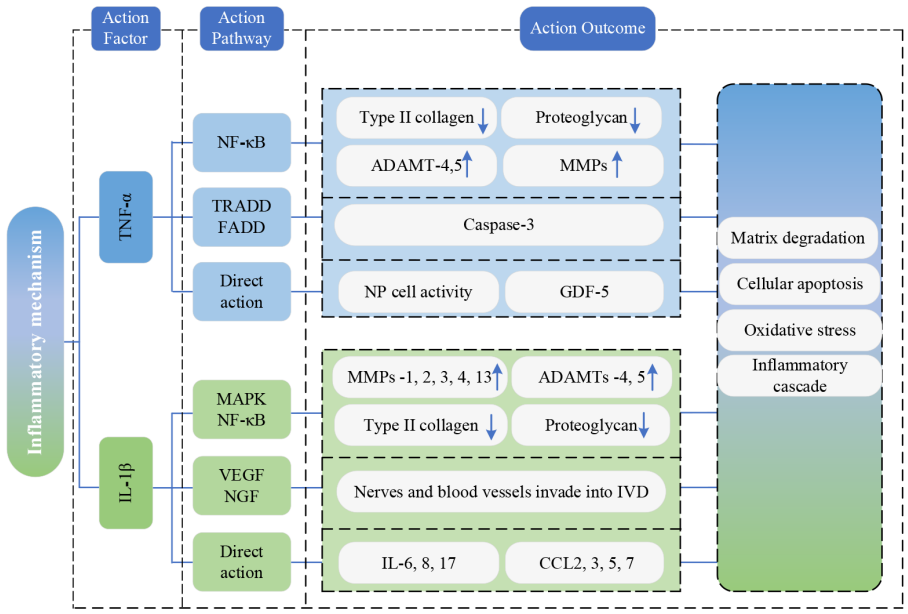

FIGURE 7
Research state of Inflammation mechanism. TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-kappa B; IL-1β, interleukin-1beta; ADAMTs, a disintegrin and metalloproteinase with thrombospondin motifs; MMP, matrix metalloproteinases; TRADD, TNF receptor-associated death domain protein; FADD, Fas-associated death domain protein; NP, nucleus pulposus; GDF-5, growth differentiation factor 5; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; MMPs, matrix metalloproteinases; ADAMTs, a disintegrin and metalloproteinase with thrombospondin motifs; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; IVD, Intervertebral disc; IL, interleukin; CCL, C-C Motif Chemokine Ligand.

adiponectin performs anti-inflammatory effects by inhibiting the expression of pro-inflammatory mediators (89). Therefore, the downregulation of adiponectin in IVD may lead to an imbalanced inflammatory response and contribute to disc degeneration (84).

Resistin is upregulated in IVDD and positively correlates with the Pfirrmann grade (90). Through activating p38-MAPK and NF-κB signaling pathways, resistin could bind to Toll-like receptor 4 (TLR4) and augment the expression of CCL4 in NP cells, promoting macrophage infiltration and inflammatory response in IVD tissue (90). Furthermore, resistin, by activating the p38-MAPK signaling pathway, induces metabolic disruption in NP cells and accelerates the progression of IVDD (91).

Visfatin is associated with cell differentiation, stress, apoptosis, and inflammation processes (92). In NP cells, IL-1β stimulates the expression of visfatin, increasing degradation-related proteins ADAMTs-4,5 and MMPs-3,13 and decreasing type II collagen and aggrecan. This process prompts the degradation of the extracellular matrix (22). Moreover, the expression level of visfatin is positively correlated with the degree of IVDD (93).

The current framework of research on adipokines is depicted in Figure 8.

3.3 Abnormal mechanical stress

Spinal instability is the premise of the main pathological mechanism leading to IVDD (94). PSMs are a major source of load-bearing for the spine and play a crucial role in its movement and stability. When the PSMs are replaced by fatty tissue, the contractility and load-bearing capacity of muscles are weakened. Consequently, more pressure is transferred to the discs and small joints (21). If the pressure load is excessively high or exits for too long, NP cells will age, apoptosis, and necrosis, resulting in lower activity and decreased numbers, which is one of the most important pathological processes in IVDD (95). Abnormal pressure load can also accelerate the calcification and vascular degeneration of the CEP, leading to a significant decrease in permeability coefficients and transport rates (42). This makes it more difficult for sugar, oxygen, and other macromolecular nutrients IVD cells require and metabolites such as lactic acid to diffuse or excrete. In the acidic and hypoxic microenvironment, the activity and numbers of NP cells decreased, with weakened extracellular matrix (ECM) anabolism capacity, as a result promoting the progress of IVDD (96, 97). Additionally, abnormal mechanical loads can induce increased expression of MMPs and ADAMTs genes, so that the catabolism of ECM exceeds the anabolism. This results in the content of type II collagen and proteoglycan decreasing significantly, leading to dehydration and fibrosis of NP tissue, a reduction in intervertebral space height, instability of the IVD structure, and an accelerated progression of IVDD (98).

The current lineage of research on abnormal mechanical stress is depicted in Figure 9.

4 Summary of the evidence and discussion

Figure 10 depicts the above mechanisms intuitively, illustrating more distinctly and visually the interaction between IVDD and fat

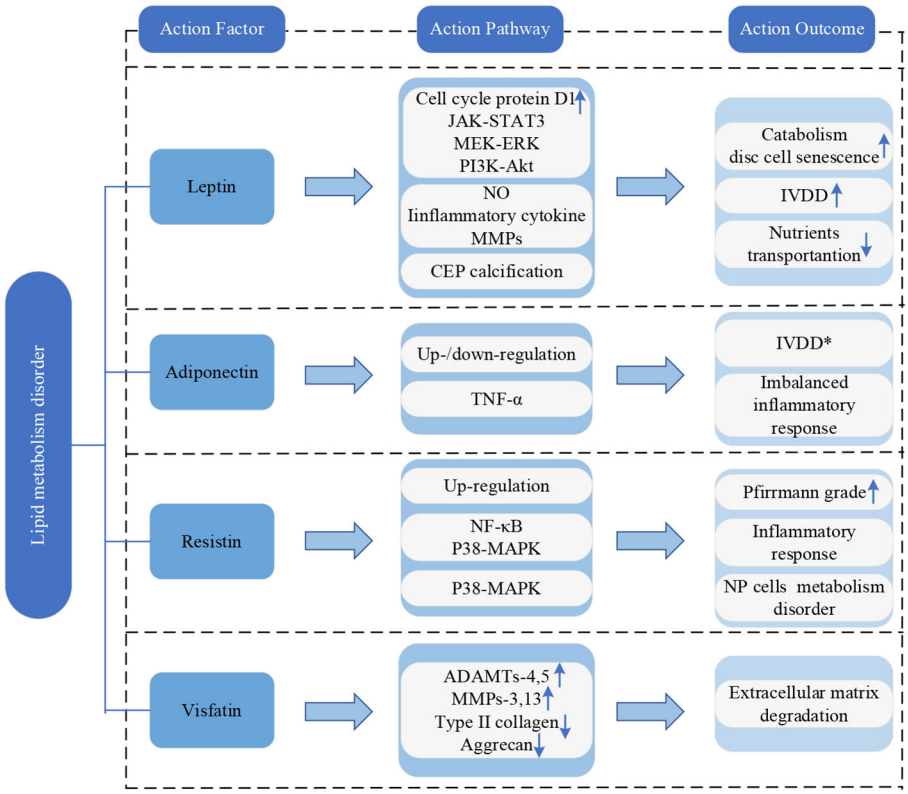

FIGURE 8
Research framework of lipid metabolism disorder. IVDD, intervertebral disc degeneration; CEP, cartilaginous endplate; TNF-α, tumor necrosis factor-α; NP, nucleus pulposus; NF-κB, nuclear factor-kappa B; MAPK, mitogen-activated protein kinase; ADAMTs, a disintegrin and metalloproteinase with thrombospondin motifs; MMPs, matrix metalloproteinases. * indicates controversy.

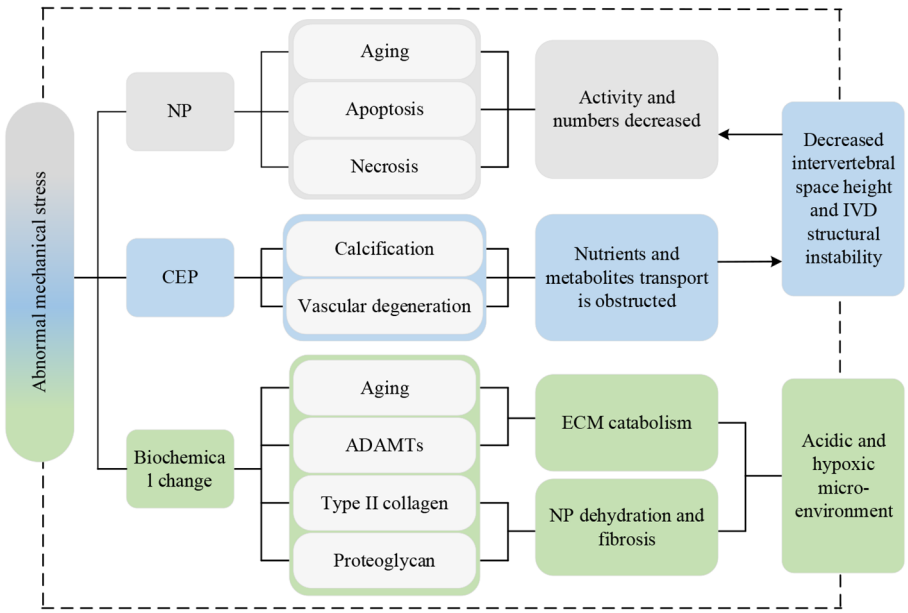

FIGURE 9
Research lineage of abnormal mechanical stress. Np, nucleus pulposus; CEP, cartilaginous endplate; IVD, intervertebral disc; ADAMTs, a disintegrin and metalloproteinase with thrombospondin motifs; ECM, extracellular matrix.

infiltration of PSMs. Here, several summaries and discussions are stated as follows:

a) Among all the above mechanisms, inflammation is the most extensively studied, because of the universality of the inflammatory response and the numerous types and functions of inflammatory factors.

b) Most studies concentrate on fatty infiltration of the MM. The reason is that MM has the largest attachment area and is only innervated by a single nerve, making it more susceptible and simple to study (99–101).

c) The degree and incidence of IVDD are higher at the L4-L5 and L5-S1 levels, since the proximity of the distal lumbar segments to the static sacrum, and their mobility and stresses are greater than the sacral segments, which is conducive to degeneration (102).

d) The research methods mainly focus on animal experiments and cross-sectional studies, it may be a choice that strategically considers factors such as time efficiency, cost-effectiveness, and the feasibility of repetition.

5 Conclusions

This paper systematically reviews the interaction between IVDD and fat infiltration of PSMs and classifies its mechanisms. Here, four main conclusions are drawn as follows:

a) IVDD and fat infiltration of PSMs can promote the occurrence and development of each other, forming a vicious cycle that ultimately leads to LBP and disability.

b) Four mechanisms of IVDD causing fatty infiltration of PSMs are summarized, i.e., inflammatory response, muscle denervation, muscle disuse, and biomechanical imbalance.

c) Three mechanisms of IVDD caused by fatty infiltration in PSMs are systematically reviewed: inflammation mechanism, lipid metabolism disorder, and abnormal mechanical stress.

d) Inflammation functions as a bridge between IVDD and fat infiltration of PSMs. Exercise can prevent and improve the inflammatory response.

6 Perspectives

Eight proposals and perspectives in four main aspects (i.e., promising research, experimental methods, models and samples) are proposed as follows:

a) The molecular-level interaction between intervertebral disc tissue and paraspinal muscle fat is not yet fully understood. Specifically, the molecular mechanisms of signal transduction and cytokine release between intervertebral disc cells and paraspinal muscle adipocytes still need to be further studied.

b) Muscles at a single spinal level do not represent the whole lumbar spine muscles, so future studies should measure the amount of fat infiltration in each spine-level muscle and collect its tissue samples.

c) Differences in the segmentation method of fat infiltration in PSMs (in- or excluding epimuscular fat) in different studies, thus clear quantification of lipid levels within and out of muscle cells is recommended for future research, ensuring more consistent and comparable outcomes in the studies assessing fat infiltration of PSMs.

d) Although rats are a commonly used experimental animal model, they cannot fully simulate humans due to the huge differences between them and humans. Hence, animal models closer to humans should be explored, or high-quality clinical cohort studies could be conducted.

e) Given the complexity, time, and cost of research, numerous studies, both animal experiments and clinical investigations often have relatively small sample sizes. Therefore, future studies could increase the sample size to obtain more powerful and reliable results.

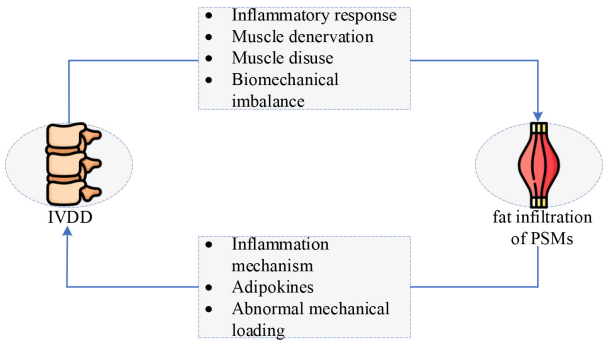

FIGURE 10
The interaction between IVDD and fat infiltration of PSMs: a vicious circle. IVDD, intervertebral disc degeneration. (The original image is from https://www.freepik.com/pricing.).

f) Considering the pivotal role of PSMs' health in spinal stability and functional outcomes, it is recommended to integrate information concerning the extent of fat infiltration and atrophy of PSMs into decision-making algorithms for Early Recovery After Surgery (ERAS) protocols. According to a systematic review by Zaed et al. (103), the importance of ERAS in spinal surgery is emphasized but still needs to be supported by high-quality clinical randomized controlled trials (RCT). Therefore, it is also advised that fat infiltration be taken into account in future RCT.

g) The treatment of LBP should pay more attention to the relationship between IVD and PSMs. Given the complex interplay between IVDD and fat infiltration of PSMs, a multimodal treatment targeting multiple mechanisms can be adopted in the future. Combining pharmacotherapy, exercise interventions, and neuromodulation techniques may offer synergistic benefits in managing LBP.

h) Considering that inflammation is a critical bridge between IVDD and fatty infiltration of PSMs, it may be possible in the future to disrupt the vicious cycle of IVDD and fatty infiltration and halt the progression of LBP with targeted anti-inflammatory drug therapy.

Author contributions

JJ: Writing – original draft, Writing – review & editing, Formal analysis, Investigation, Visualization. YH: Funding acquisition, Conceptualization, Writing – review & editing. BH: Funding acquisition, Supervision, Validation, 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

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LncRNAs as potential prognosis/diagnosis markers and factors driving drug resistance of osteosarcoma, a review

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Osteosarcoma is a common malignancy that often occurs in children, teenagers and young adults. Although the treatment strategy has improved, the results are still poor for most patients with metastatic or recurrent osteosarcomas. Therefore, it is necessary to identify new and effective prognostic biomarkers and therapeutic targets for diseases. Human genomes contain lncRNAs, transcripts with limited or insufficient capacity to encode proteins. They have been implicated in tumorigenesis, particularly regarding the onset, advancement, resistance to treatment, recurrence and remote dissemination of malignancies. Aberrant lncRNA expression in osteosarcomas has been reported by numerous researchers; lncRNAs have the potential to exhibit either oncogenic or tumor-suppressing behaviors and thus, to govern the advancement of this skeletal cancer. They are suspected to influence osteosarcoma cell growth, replication, invasion, migration, remote dissemination and programmed cell death. Additionally, they have been recognized as clinical markers, and may participate in the development of multidrug resistance. Therefore, the study of lncRNAs in the growth, metastasis, treatment and prognosis of osteosarcoma is very important for the active prevention and treatment of osteosarcoma. Consequently, this work reviews the functions of lncRNAs.

KEYWORDS

osteosarcoma, lncRNA, cancer cell, biomarkers, indicators

1 Introduction to the treatment and research of osteosarcoma

Osteosarcoma, which originates from mesenchymal cells, is one of the most frequently occurring bone tumor. It is responsible for a significant proportion of child and teenage mortalities occurring due to malignancy. Typical histological appearances of osteosarcoma include spindle cells and the abnormal generation of osteoid (1). The long-term survival rate of individuals who present without remote tumor dissemination is approximately 80%. Conversely, when metastatic deposits are detected at diagnosis, the likelihood of long-term survival is lower than 20%, even when aggressive treatment regimens are instituted (2). At present, some progress has been made in the treatment of osteosarcoma, including chemotherapy, radiotherapy, gene therapy, immunotherapy, molecular targeted therapy, and surgical treatment (3, 4) (Figure 1). Due to the limitations of early medical development, the early surgical treatment of osteosarcoma is mainly based on amputations to save lives. However, the appearance and function of residual limbs after amputations adversely affect patients' quality of life, causing long-term psychological distress. With the rapid development of medical science and technology, limb salvage treatment for osteosarcoma has replaced most amputations in clinical practice (5). Doxorubicin, methotrexate, cisplatin and, ifosfamide are known as classic drugs for osteosarcoma chemotherapy that have significantly improved the curative effect, survival rate, and quality of life, and reduced the harm to the human body caused by the side effects of treatment (6). The sensitivity of osteosarcoma to radiotherapy is relatively poor, and it is recommended to relieve the pain of patients and apply it to patients with no chance of surgery (7). Gene therapy, immunotherapy, and molecular targeted therapy are new methods for treating osteosarcoma, and their application prospects and values are incalculable. In the studies of whole genome sequencing and whole exome sequencing of osteosarcoma, some genes usually changed, including tumor suppressor genes *TP53*, *RB1*, *ATRX* and *DLG2* (8). Moreover, genomic instability of BRCA1/2-deficient tumors was observed in 80% of osteosarcomas (9). The combined genomic and transcriptome analysis of osteosarcoma samples also identified multiple fusion transcripts directly related to chromosome rearrangements. Although these fusion transcripts involve multiple loci in different cell lines and patient samples, the most common is affecting the *TP53* locus (10). Tailor-made therapies using biomarkers that predict responses to specific molecular targeted therapies are central concepts in precision medicine. Osteosarcoma mostly exhibits somatic changes in cell cycle and/or DNA damage repair pathways. Targeted therapy based on this abnormal prediction is a potential precise medical method. For example, abnormal expression of *TP53* impairs the G1 cell cycle checkpoint, thereby increasing the dependence of tumor cells on the G2 checkpoint to maintain DNA integrity and complete cell division (11). Therefore, drugs that disrupt the G2 checkpoint, such as WEE1 inhibitors, may enhance the activity of DNA damage agents and induce mitotic death in *TP53*-mutant osteosarcoma cells. However, most therapies are still in the stage of basic scientific research experiments, and only a few are used in clinical observation. In addition, the regulatory mechanism of osteosarcoma has yet to be clarified, so the therapeutic effect of targeted agents needs to be improved, which makes the use of contemporary treatments more challenging (12). Therefore, there is an urgent need to identify clinically relevant prognostic biomarkers for these patients.

Although several promising molecular markers have been detected in recent years, a diagnostic indicator particular to osteosarcoma has yet to be identified (13). Data are being

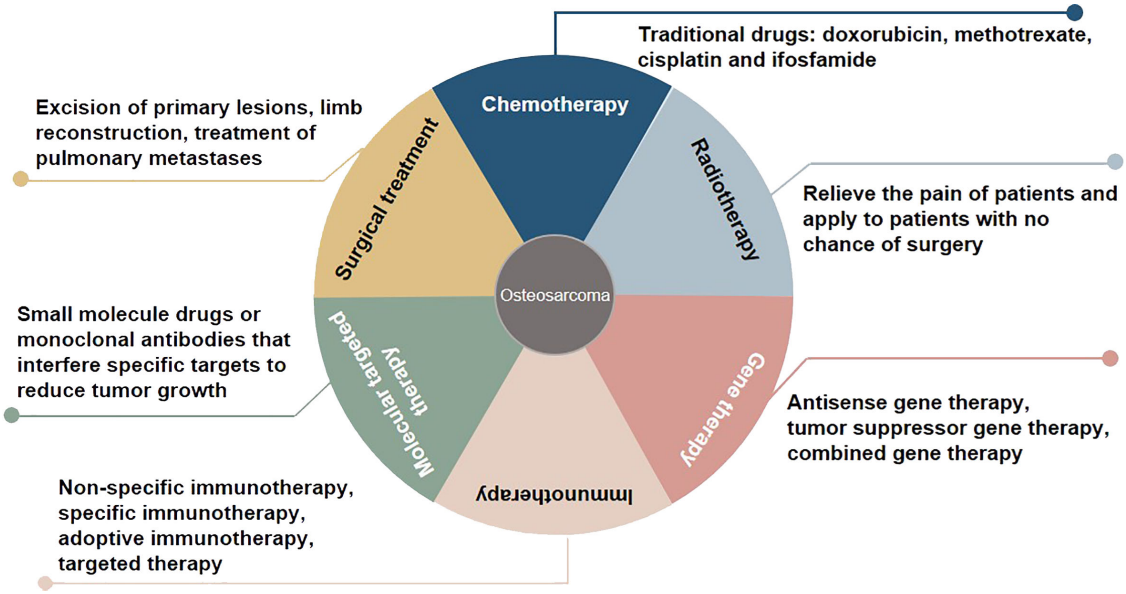

FIGURE 1
Overview of treatment methods for osteosarcoma.

accrued, which suggests that many malignancy-related genomic mutations occur in areas that, instead of encoding for proteins, frequently undergo transcription into lncRNAs. These are a cohort of non-protein transcripts of approximately 200 nucleotides in length (14).

LncRNAs contribute to the governance of gene expression via numerous mechanisms, for example, as epigenetic moderators, signals for the enhancement of transcription, transcription suppression decoys, or scaffolds for the generation of ribonucleoprotein complexes through engagement with proteins (15). The integrity and translation of mRNAs is influenced by lncRNAs; the latter also form antecedents to miRNAs and control their distribution. Overall, lncRNAs impact the expression of genes at both the transcriptional and post-transcriptional levels (15, 16). Additional pathways in which lncRNAs have been implicated encompass cell development, differentiation, and replication, together with the governance of the cell cycle and apoptosis (17, 18). They also play a significant part in the advancement and remote spread of a range of malignancies, for example, large intestine, hepatic, breast, bladder, and cervical neoplasias (19).

Even though research regarding the roles played by lncRNAs in osteosarcoma is still in its infancy, promising data imply that, in addition to their contribution to the control of numerous pathophysiological pathways, lncRNAs influence carcinogenesis as well as tumor advancement and remote dissemination (20, 21). They have also been utilized as biological markers, autonomous indicators of clinical prognosis and therapeutic targets, thus having notable diagnostic and therapeutic utility, as well as offering a method of tumor surveillance (22). Evidence suggests that the effect of chemotherapeutic drugs may be potentiated by lncRNAs, which influences patients' prognosis (23). Therefore, this study reviewed the role of lncRNAs in osteosarcomas, which includes their influence on tumorigenesis, remote dissemination, clinical outcomes, and resistance to chemotherapeutic agents.

2 Classification and biological function of lncRNAs

LncRNAs were first identified in murine transcripts (24). Typically, their length extend in excess of 200 nucleotides, and they have no capacity to encode proteins (24). They are categorized into the following types: bidirectional, intergenic, intronic, antisense, sense, and enhancer lncRNAs, depending on the configurational relationship between the lncRNAs and the genes for protein coding within the genome (Figure 2A) (25). RNA polymerase II is responsible for the transcription of most of these molecules, with a 5'cap and polyadenylated tail are common (26). Owing to the myriad transcripts, they were first deemed to be "noise" associated with the transcription process (27). However, more detailed contemporary work has demonstrated that lncRNAs have an abundance of physiological roles, such as the moderation of the epigenetic, transcriptional, and post-transcriptional expression of genes (Figure 2B). Additionally, they have considerable intracellular functionality, contributing to cell replication, differentiation, cell cycle advancement, growth, and programmed cell death (28, 29). The alteration and remodeling of chromatin and histone, together with the localization of the nuclear body, are mechanisms via which gene expression can be modified by lncRNAs (30). Chromosomal configurations can also be adjusted by lncRNAs in concert with the SWI/SNF complex, a process that immediately impacts gene expression (31). Although lncRNAs are not attributed to coding protein, several are suspected of involvement in silencing protein encoding genes during transcription through modification of the structure of chromatin and the conscription of complexes that impact this process (32).

In stem cells, the differentiation of cells is influenced by lncRNAs; in somatic and pluripotent stem cells, sizeable intergenic non-coding RNAs can promote cellular reprogramming (33). Furthermore, in addition to immediately influencing target expression, lncRNAs also have the ability to govern expression through secondary pathways, for example, acting as 'molecular sinks" for signaling molecules, such as RNA-binding proteins that moderate chromatin (32). A key mechanism for their ability to control gene expression is that they act as competing endogenous RNAs (ceRNAs) for miRNAs, reducing the bioavailability of miRNAs and enhancing target mRNA titers (34). Several studies have additionally demonstrated that lncRNAs work as key controlling molecules that behave as oncogenes or tumor-inhibiting agents. These are essential actors in numerous forms of malignancy, with the potential to promote the progression of carcinogenesis, remote tumor dissemination, and resistance to chemotherapeutic agents (35–38).

3 LncRNAs in osteosarcoma

3.1 Up-regulated lncRNAs in osteosarcoma

The potential oncogenic effects of lncRNAs in osteosarcoma described in previous studies are presented in Table 1. When samples from osteosarcomas were compared with neighboring normal tissues, the expression of the lncRNA TUG1, which is situated on chromosome 22q12.2 and is 7.1 kb in size, was noted to be amplified (2). This finding was related to adverse prognosis, forming an autonomous predictive indicator of overall survival (39). *In vitro* studies have demonstrated that cell replication is inhibited in the presence of TUG1 knockdown, which is associated with the arrest of the G0/G1 cell cycle and programmed cell death. Additionally, *in vivo* work has shown that TUG1 knockdown leads to a reduction in the growth of cancerous lesions (39). TUG1 can promote the proliferation of cancer cells. When TUG1 is inhibited, the proliferation of cancer cells decreases, and the replication efficiency of osteosarcoma cells decreases. Recent study has also found that TUG1 can act as a miR-26a-5p sponge and promote the progression of osteosarcoma by up-regulating ZBTB7C (40), so TUG1 can be used as a diagnostic marker for osteosarcoma, targeting TUG1 may be an effective strategy for the treatment of osteosarcoma.

Another lncRNA that exhibits excessive expression in osteosarcoma is metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which is also termed nuclear-enriched

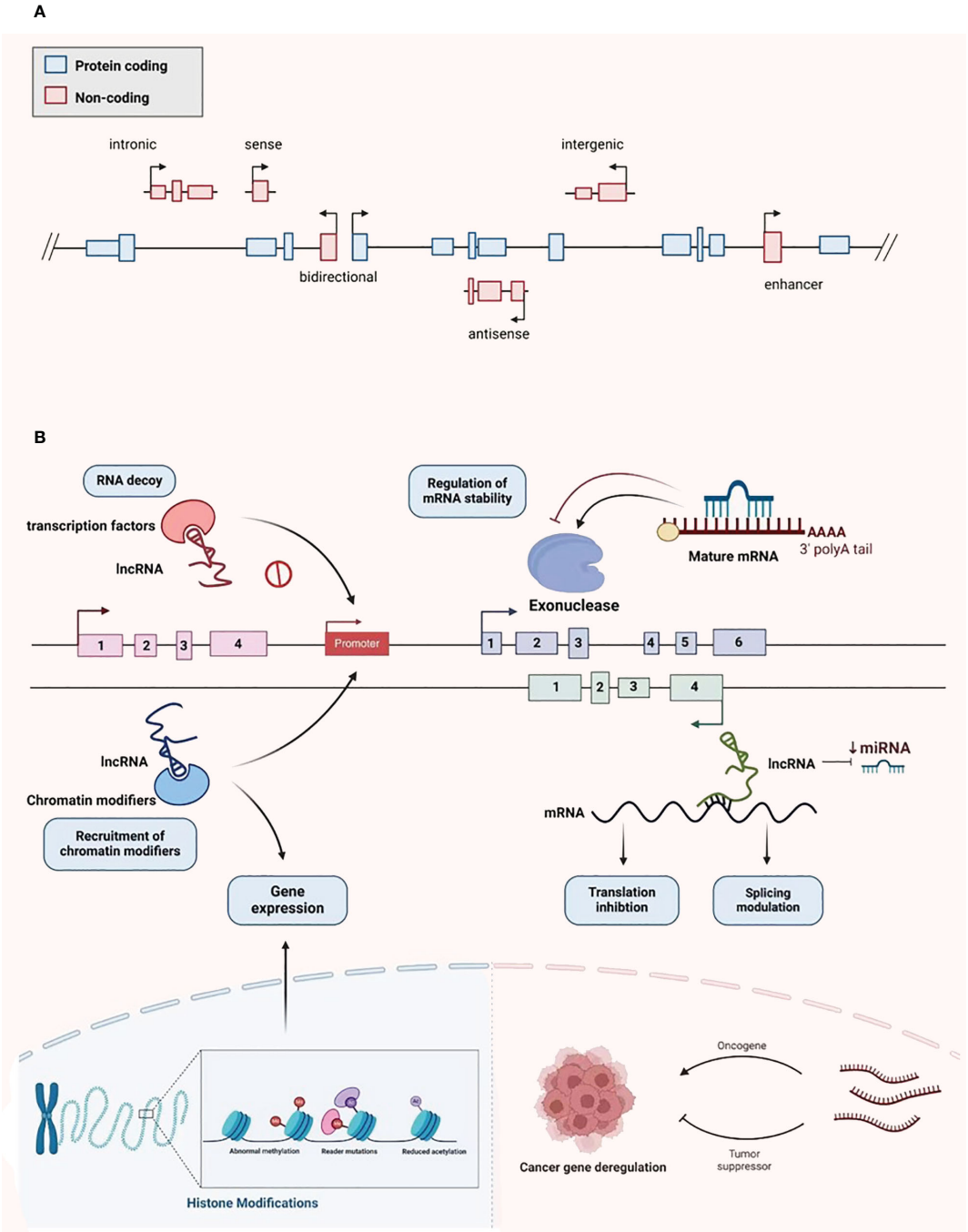

FIGURE 2
Classification and biological function of lncRNA. **(A)** LncRNA is divided into bidirectional, intergenic, intronic, antisense, sense, and enhancer lncRNA. **(B)** LncRNA plays various biological functions, including recruiting chromatin modifiers, regulating gene expression, participating in transcriptional silencing of protein coding genes, regulating mRNA stability, increasing mRNA expression, and being able to serve as key regulatory factors and oncogenes or tumor suppressors in tumor development and metastasis.

transcript 2 (NEAT2) and mascRNA. It comprises 8700 nucleotides and is present on a locus of chromosome 11q13 (41–43). MALAT1 is not polyadenylated but instead contains a highly unusual 3'-terminal structural motif designated as the stability element for nuclear expression (ENE), which is essential for the stability of mature transcripts and the subsequent accumulation and carcinogenic activity of MALAT1. Therefore, cells are free from the influence of cellular degradation pathways, leading to MALAT1's sustained carcinogenic activity in a variety of cancer types (44, 45). It was originally identified as a biological indicator of metastatic disease in the initial phases of non-small cell lung cancer (46). In osteosarcoma samples, MALAT1 expression was increased, a finding that has a positive association with metastatic deposits in the lungs (47). Furthermore, the expression of phosphorylated PI3Kp85α, matrix metallopeptidase 9 (MMP-9), proliferating cell nuclear antigen (PCNA), and Akt were significantly inhibited in

TABLE 1 Roles and function mechanisms of lncRNAs in osteosarcoma.

LncRNA	Expression	Function	First recognized	Role and mechanism
TUG1	Up	Oncogene	*in vivo*	Inhibits G0/G1 cell cycle arrest and apoptosis, and promotes cell proliferation by sponging miR-9-5p and miR-26a-5p
MALAT1	Up	Oncogene	*in vivo*	Promotes cell proliferation and metastasis by activating PI3K/AKT signaling pathway
H19	Up	Oncogene	*in vivo*	Induced by Hh signal transduction and Yap1 overexpression, thereby promoting the development of osteosarcoma
NEAT1	Up	Oncogene	*in vitro*	Enhances malignant features of osteosarcoma cells through sponging miR-579, and increase the EMT of osteosarcoma cells through sponging miR-438
DANCR	Up	Oncogene	*in vitro*	Enhances proliferation, migratory potential, invasiveness and autophagy of osteosarcoma cells by sponging miR-216a-5p
SPRY4- IT1	Up	Oncogene	*in vitro*	Enhances the osteosarcoma cell growth, migration and invasion through the inhibition of G1 arrest; Regulates the expression of ZEB1 and ZEB2 by sponge miR-101
LSINCT5	Up	Oncogene	*in vivo*	Plays a carcinogenic role by inhibiting the expression of adenomatosis polyposis coli; Promotes the proliferation, migration, and invasion of osteosarcoma cells
BCAR4	Up	Oncogene	*in vitro*	Promotes the progression of osteosarcoma by activating the GLI2 signaling pathway
UCA1	Up	Oncogene	*in vivo*	Enhances cell cycle progression through different mechanisms; interacts with various cancer-related signaling pathways such as mTOR, AKT, Wnt, Hippo and JNK pathways
ANRIL	Up	Oncogene	*in vivo*	Increases cell proliferation
Loc285194	Down	Tumor suppressor	*in vivo*	Inhibits the growth of tumor cells through inhibiting miR-211 expression
MEG3	Down	Tumor suppressor	*in vitro*	Reduces overall survival by regulating the expression of p53; Promotes osteosarcoma chemosensitivity by regulating anti-tumor immunity through the miR-21-5p/p53 pathway and autophagy
FER1L4	Down	Tumor suppressor	*in vivo*	Regulates EMT and cell apoptosis by serving as a sponge for miRNA-18a-5p
GAS5	Down	Tumor suppressor	*in vivo*	Suppresses osteosarcoma cell growth and invasion by affecting the PI3K/AKT pathway; Enhances the sensitivity of osteosarcoma cells to Cisplatin through the GAS5/miR-26b-5p/TP53INP1 axis
TRP53COR1	Down	Tumor suppressor	*in vivo*	Suppresses the proliferation of osteosarcoma cells through sponging miR-130b
SRA1	Down	Tumor suppressor	*in vitro*	Reduces cell proliferation, invasion, and migration via sponging miRNA-208a

MALAT1-deficient cells. The replication and migration of osteosarcoma cells was inhibited following MALAT1 knockdown. Additionally, in MNNG/HOS and U2OS cells, the generation of tubular network configurations was suppressed, and stress fibers were fractured (48). The up-regulated expression of MALAT1 is closely related to the presence of distal osteosarcoma deposits, and the inhibition of MALAT1 can reduce the migration and invasion of osteosarcoma cells. In addition, it has been shown that, METTL3 in osteosarcoma cells promotes the m6A modification of MALAT1, and enhances the carcinogenic function of MALAT1 (49). One of the key oncogenic pathways in osteosarcomas affecting humans is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (50, 51). It has several major functions relating to cancer cell growth, apoptosis, and migratory and invasive properties (52). Diminishing MALAT1 expression led to lower levels of phosphorylated PI3K p85α and Akt (47). MALAT1 exhibits competitive bonding to miRNA-129-5p and therefore prevents this molecule from promoting the breakdown of RET. Subsequently, the rise in RET concentrations enhances the activity of the PI3K/Akt signaling pathway (53). Additionally, MALAT1 demonstrated oncogenic functions, increasing osteosarcoma cell growth potentially through the stimulation of the PI3K/AKT and RhoA/ROCK pathway (47, 48).

Initially described as a molecule that could inhibit epithelial cell differentiation, differentiation antagonizing non-protein coding RNA (DANCR or ANCR), which has its locus on human chromosome 4q12, has also been shown to enhance the stemness properties of cell lines from hepatocellular carcinoma (54, 55). DANCR can promote the pathogenesis of osteosarcoma, and the silencing of DANCR has been demonstrated to promote programmed cell death, using functional assays, to inhibit the replication, migratory ability, invasiveness, and autophagic capability of cells from osteosarcomas. The expression of SOX5 and levels of the receptor tyrosine kinase AXL are amplified by DANCR owing to its sponge-like function in relation to miR-216a-5p and miR-33a-5p (56), respectively. Moreover, METTL3 promotes osteosarcoma progression by increasing the stability of DANCR mRNA through m6A modification, which means that METTL3 may be a promising therapeutic target for osteosarcoma treatment (57).

Another lncRNA, H19, is 2.3 kb in size and is near the telomeric region of human chromosome 11p15.5. It is implicated in the governance of the expression of IGF2 (58). In osteosarcomas, an abnormal degree of H19 expression has been detected, which can be promoted by amplified Hedgehog (Hh) signaling and upregulated yes-associated protein 1 (Yap1) (59). This observation substantiates the theory that erroneous Hh signaling expression in osteoblasts plays a key role in the osteosarcoma associated with the overexpression of H19 and Yap1. Gallic acid inhibits tumor growth in osteosarcoma cells through the H19-mediated Wnt/β-catenin signaling regulatory axis (60).

The familial tumor syndrome multiple endocrine neoplasia type 1 locus, situated on chromosome 11, gives rise to the transcription of the lncRNA NEAT1 (61). Its oncogenic properties have been demonstrated to augment the neoplastic characteristics of cells from osteosarcomas (62). By sponging miR-579 and upregulating MMP13, the role of NEAT1 in the epithelial-mesenchymal transition (EMT) is promoted (63). NEAT1 can also sponge miR-438, increase the expression of STAT3, and inhibit that of STAT1, and then increase the EMT of osteosarcoma cells (64).

Another lncRNA, SPRY4-IT1, which has a size of 708 bp, originates from a SPRY4 intron on chromosome 5q31.3, which contains the coding for an intrinsic receptor-transduced mitogen-activated protein kinase pathway inhibitor (65, 66). The expression of this lncRNA was observed to be amplified in cell lines and specimens from osteosarcomas, while the knockdown of it inhibited the invasive, migratory, and replicative properties of the malignant cells through the generation of G1 cell cycle arrest and heightened apoptosis (67). SPRY4-IT1 can also regulate the expression of ZEB1 and ZEB2 by sponge miR-101 activity, thereby promoting the progression of osteosarcoma (68).

Compared with adjacent normal tissues, long stress-induced non-coding transcript 5 (LSINCT5) was significantly up-regulated in osteosarcoma tissues. The abnormal expression of LSINCT5 is usually associated with cancer progression and poor prognosis, and high LSINCT5 expression is associated with advanced Enneking stage, large tumor volume, high histological grade, and current distant metastasis (69). The overexpression of LSINCT5 can promote the proliferation, migration, and invasion of osteosarcoma cells *in vitro*, while the inhibition of its expression has the opposite effect (69). The exploration of the mechanism displayed that LSINCT5 can interact with EZH2 to inhibit the expression of adenomatosis polyposis coli, a negative regulator of the Wnt/β-catenin pathway, to play a carcinogenic role (70). In general, LSINCT5 plays a carcinogenic role in osteosarcoma cells and may be a predictor of the clinical outcomes of osteosarcoma patients, and a promising candidate for osteosarcoma prognosis and treatment.

In addition, there are some other common lncRNAs that are up-regulated in osteosarcoma. For example, the expression of BCAR4 (71), HULC (72), UCA1 (73), and ANRIL (74) in osteosarcoma tissues is significantly higher than that in osteoblasts and adjacent tissues. After knocking down BCAR4, the proliferation, invasion and migration of osteosarcoma cells were inhibited. In addition, BCAR4 promotes the progression of osteosarcoma by activating the GLI2 signaling pathway (75). UCA1 can act as a sponge for many tumor suppressor miRNAs (73), enhance cell cycle progression through different mechanisms (76), and interact with various cancer-related signaling pathways such as mTOR, AKT, Wnt, Hippo and JNK pathways (77). Overexpression of ANRIL increases cell proliferation, and higher ANRIL expression is significantly associated with death and metastasis (78).

3.2 Down-regulated lncRNAs in osteosarcoma

Table 1 illustrates several cancer-suppressing lncRNAs that exhibit diminished expression in osteosarcoma.

The lncRNA, Loc285194, which is also named the LSAMP antisense RNA 3, has its locus on chromosome 3q13.3. It comprises 4 exons and is 2105 nucleotides long (79). Its tumor-suppressing activity is moderated via p53 due to the inhibition of the expression of miR-211 (80). In specimens and cell lines from primary osteosarcomas, a lack of Loc285194 expression has been demonstrated. When Loc285194 was depleted, the replication of normal osteoblasts was enhanced because of its effects on the cell cycle, apoptotic transcripts, and the expression of VEGF receptor 1 (79).

MEG3, is a member of the DLK1–MEG3 locus found on human chromosome 14q32.3 (81). Its functions include the accrual of protein p53, the transcription of p53-dependent promoters, and the exclusive moderation of the expression of p53 target genes (82, 83). When contrasted with neighboring benign tissue samples, there was an obviously reduced MEG3 expression in tissues from osteosarcomas. This observation was correlated with the disease stage and the presence of remote metastases ($P < 0.05$) (2). This gene is associated with the Notch, and TGF-β pathways (84); the amplified expression of MEG3 led to a suppression of TGF-β, Notch1, N-cadherin, and Hes1 expression, and the upregulation of the expression of E-cadherin (85). Mechanistically, MEG3 promotes osteosarcoma chemosensitivity by regulating anti-tumor immunity through the miR-21-5p/p53 pathway and autophagy, demonstrating that MEG3 may be a promising therapeutic target for osteosarcoma chemoresistance (86).

A range of malignant pathologies is influenced by the biological activities of FER-1 family member 4 (FER1L4), a lncRNA that has a size of 6.7 kb and is situated on chromosome 20q11.22 (87). Compared to normal tissues, titers of this molecule were notably reduced in samples and cell lines from osteosarcomas. Additionally, the osteosarcoma-enhancing miRNA, miRNA-18a-5p, which has been proposed to be the target molecule for FER1L4 in this malignancy, was recognized regarding the governance of programmed cell death and EMT (52).

Moreover, chromosome 1q25 is the location of the *de novo* tumor-suppressor, growth arrest-specific transcript 5 (GAS5). This lncRNA is a 5'-terminal oligopyrimidine RNA that comprises a dozen non-conserved exons and approximately 630 nucleotides (88). Its expression is diminished in breast, stomach, lung, and prostate tumors, as well as in additional neoplasias (89, 90). Its expression is also downregulated in osteosarcoma samples and cell lines in humans when judged against neighboring areas and normal

osteoblast cells. Functionally, it acts as a ceRNA, sponging miR-23a-3p to amplify the expression of PTEN and inhibit osteosarcoma cell replication and invasion through its effect on the PI3K/AKT pathway (91). In addition, GAS5 also enhances the sensitivity of osteosarcoma cells to Cisplatin through the GAS5/miR-26b-5p/TP53INP1 axis, which may be a potential indicator for the treatment of osteosarcoma (92).

In addition, the replication of osteosarcoma cells is subject to modification by lncRNA-p21, which is also referred to as TRP53COR1. This is associated with amplified PTEN expression due to the replication of osteosarcoma cells being subject to modification by lncRNA-p21, which is also termed TRP53COR1. This is associated with amplified PTEN expression because of sponging miR-130b, an oncomiR (64). As a principal gene dictating cancer suppression in humans, PTEN is responsible for the dephosphorylation of AKT at Thr 308 and Ser 473, which leads to the inhibition of the signaling potency of AKT and, consequently, a reduction in cell growth and the enhancement of apoptosis (65).

A further lncRNA, which operates as a coactivator of RNA, SRA1 has been proposed to be a major actor in myogenesis, steroidogenesis, breast carcinogenesis, and cardiac muscle disorders. Anomalous levels of expression of SRA1 have been identified in several malignancies in humans (93). An anti-cancer function of SRA1 was observed in osteosarcomas, in which SRA1 diminished the migratory, replicative, and invasive properties of the tumor cells and also acted as a sponge for miRNA-208a, thus promoting apoptosis (94).

4 LncRNAs regulate the cell proliferation, invasion and metastasis of osteosarcomas

Ongoing cell growth and replication without regulation is a pathognomonic sign of tumorigenesis; promoting the removal of malignant cells is deemed the definitive goal of therapy (95). Carcinogenesis can be facilitated by activating oncogenes and inhibiting tumor suppressor genes (96). Various lncRNAs display these functions in osteosarcomas, modifying either the cell cycle or apoptosis, and regulating cellular replication or migration (Figure 3).

4.1 LncRNAs regulate the cell proliferation and migration of osteosarcomas

In samples from osteosarcoma, a polymerase chain reaction was utilized to quantify the levels of hypoxia-inducible factor-2α (HIF2α) promoter upstream transcript (HIF2PUT). The results indicated that HIF2PUT inhibited tumor stem cells through its effects on the expression of HIF2α. Significant inhibitory influences were observed on cellular replication and migration in the presence of amplified HIF2PUT expression. The proportion of CD133-expressing cells was reduced and the MG63 cells evidenced a diminished capacity to form osteosarcoma stem spheres (19, 97).

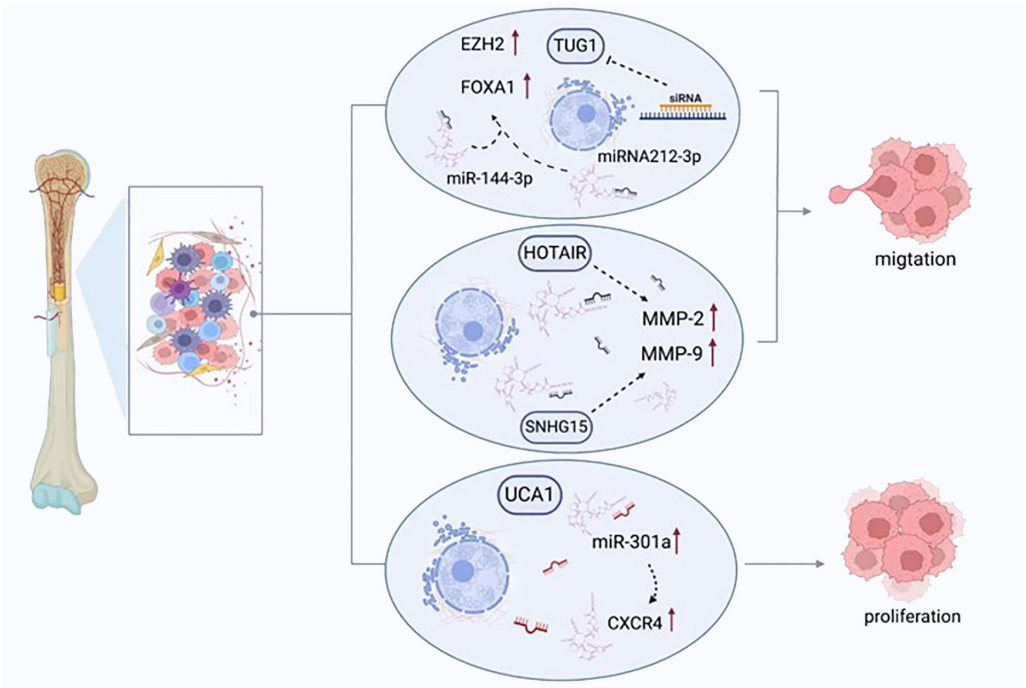

FIGURE 3
LncRNAs regulate the migration, proliferation, and apoptosis of osteosarcoma cells. TUG1 promotes the expression of FOXA1 and enhancer of zeste homolog 2 (EZH2) by competitively binding to miRNA-212-3p and miR-144-3p, thereby enhancing the proliferation of cancer cells. UCA1 enhances the migration of tumor cells by increasing the expression of miR-301a and chemokine receptor-C-X-C motif chemokine receptor 4 (CXCR4). HOTAIR and SNHG15 promote cell proliferation by regulating the expression of MMP-2/MMP-9.

Conversely, when HIF2PUT was depleted via siRNA, there was a marked enhancement of cell growth and migration (97).

It has also been determined that replication and colony generation in cells from osteosarcomas can be suppressed by tumor suppressor candidate 7 (TUSC7) (43). Its knockdown with siRNA caused increased cellular replication and the presence of cell colonies, together with a decrease in programmed cell death. However, there was no impact on the cell cycle (98). The amplification of the expression of the angiomotin (AMOT) gene in cells from human osteosarcomas by SNHG12 also has been reported to accelerate cellular replication and migration, while the knockdown of SNHG12 had the opposite consequence but failed to influence programmed cell death (99). When SNGH12 and AMOT underwent knockdown, attenuated cell migration and growth were detected (99). Notch signaling is a pathway that is extremely conserved, underpins many biological activities, and contributes to the pathogenesis of numerous malignancies (100). Notch signaling is responsible for the governance of a number of lncRNAs and is regulated by them simultaneously. SNHG12 stimulates the Notch signaling pathway and precipitates enhanced oncogenesis and osteosarcoma dissemination by sponging miR-195-5p (101). Research has observed that the growth and replication of osteosarcoma cells was impeded by atypical lncRNA expression. A lack of TUG1 diminishes the proliferation of cancerous cells and pauses the cell cycle at the stage, G0/G1 (102), additionally, it also acts as a ceRNA to sponging miRNA-212-3p and miR-144-3p (103). Subsequently, the FOXA1 and enhancer of zeste homolog 2 (EZH2) expression was increased, which are target genes for the transcription of oncogenes (103). The potency of osteosarcoma cell replication was diminished, and the apoptosis was enhanced with the suppression of TUG1 with siRNA (104). The arrest of the cell cycle and apoptosis are both triggered following MALAT1 knockdown, which also leads to the suppression of the growth of osteosarcoma and its dissemination (48).

The continuation of an undifferentiated cell type is influenced by a *de novo* recognized oncogenic lncRNA, DANCR (19). In U2OS and SAOS cells, the replication of the cells was markedly reduced by DANCR knockdown, which also reduced colony generation within U20S cells and paused the cell cycle in the latter at stage, G0/G1. The endogenous levels of proteins associated with the cell cycle, such as p21, CDK2, and CDK4, are governed by DANCR (105). miRNA-335-5p and miRNA-1972 expression are also promoted by DANCR, which accelerates replication that is induced via ROCK1 and ceRNA network transfer and consequently promotes the pathogenesis of osteosarcomas (106).

The amplified expression of TMPO antisense RNA 1 (TMPO-AS1) has been observed in osteosarcomas. Conversely, samples and cell lines from osteosarcomas have evidenced the downregulation of miR-199a-5p (107). The inhibition of cellular replication and enhanced programmed cell death were identified following TMPO-AS1 knockdown, and the regulation of WNT7B occurred via the immediate sponging of miR-199a-5p, which suppressed Wnt/β-catenin activity (108). Furthermore, the WNT7B knockdown salvaged the miR-199-5p inhibitor's suppressive effect on osteosarcomas, which could be eradicated by administering the Wnt pathway stimulator lithium chloride.

In individuals with osteosarcoma, a poor prognosis was suggested by the amplified expression of bladder cancer-associated transcript 1 (BLACAT1) (109). This was linked with promoted cellular replication and invasive properties, whereas a reduction in BLACAT1 expression was associated with the opposite effect. Interestingly, the enhancement of tumor cell replication and migratory function was achieved through an effect on STAT3 phosphorylation.

Another major influence on the onset and progression of osteosarcoma is the modified frailty index 2 (MFI2), which MMFI2 promotes the proliferation and migration of osteosarcoma cells by regulating the expression of forkhead box P4 (FOXP4) (110). Cellular replication within osteosarcomas has been demonstrated to be enhanced by P50-associated COX-2 extragenic RNA (PACER). The methylation of DNA has a regulatory influence on PACER, which arises through the NF-κB-dependent stimulation of the gene, COX-2 (111). Triggering ZEB1 transcription via ZEB1 Antisense 1 (ZEB1-AS1), which has oncogenic characteristics, also induces the replication of osteosarcoma cells (112). In cells from human osteosarcomas, oncogenic lncRNA SNHG12 additionally impacts cellular replication through angiomotin gene expression amplification (99). Osteosarcoma cell replication was suppressed by reduced HNF1A-antisense 1 (HNF1A-AS1) expression, an action mediated through Wnt/β-catenin cascade inactivation (113).

4.2 LncRNAs regulate the cell apoptosis of osteosarcomas

The interaction between EZHW and HOXD-AS1 reduces the expression of p57 and promotes the development of osteosarcoma (114). The most common arising Apo-ERα-regulated lncRNA (AER-lncRNA) in mammary tumors is down syndrome cell adhesion molecule anti-sense RNA 1 (DSCAM-AS1) (115), which has been found to have a high level of expression within cell lines from osteosarcomas. Apoptosis in osteosarcoma has been markedly accelerated through the DSCAM-AS1 knockdown and inhibition of the Wnt pathway (116).

4.3 LncRNAs regulate the invasion and metastasis of osteosarcoma

The main clinical issues relating to osteosarcomas are remote tumor dissemination and the likelihood of recurrence, which hinders the efficacy of therapies and leads to poor prognosis in individuals with this type of malignancy. Cancerous deposits distant to the primary lesion occur over several pathological stages via a complicated mechanism that comprises regional tumor invasion, intravasation, remote spread, extravasation, and colonization (22). At the original tumor site, the interplay between cells and the extracellular matrix (ECM) is changed by malignant cells. These cells break away from the primary location and extend into neighboring structures, and gain passage through the circulation to remote viscera, where they attach to the vasculature walls and

leak into their target tissues before replicating from microscopic colonies to generate secondary neoplastic deposits (98). Genetic and epigenetic abnormalities usually underlie this clinical process (117, 118). Metastatic tumors in osteosarcomas frequently occur in the lungs; these are challenging to address and give rise to the most common mode of death, respiratory failure (119). It is thought that about 85% of individuals with skeletal malignancy present with remote tumor deposits (120).

A group of proteolytic enzymes, MMPs, play a key role in the invasion and dissemination of cancerous cells through their capacity to breakdown the ECM and basement membrane, thus remodeling the microenvironment of the primary lesion and encouraging the formation of a malignant neovasculature. In many human malignancies, increased levels of MMP-2 and MMP-9 are released, an observation linked with an adverse clinical prognosis (121). Amplified HOTAIR expression accelerates osteosarcoma cell invasion through the elevated liberation of MMP-2 and MMP-9 (122). Conversely, reduced HOTAIR expression in cell lines from osteosarcomas, including U2OS, 143B, MNNG/HOS, and MG-63, suppresses malignant cell replication and invasion as well as inhibits the release of MMP-2 and MMP-9 (122). The growth of osteosarcoma can be inhibited in U20S cells with HOTAIR knockdown following implantation in xenograft models (122). SNHG15 can also promotes cell proliferation and invasion by regulating the expression of MMP-2/MMP-9. In addition, MMP-2 and MMP-9 expression can be enhanced by LINC00968 amplification, leading to ECM breakdown and encouraging migration and invasion by cancerous cells (123).

The evidence that lncRNAs are linked with signaling pathways, which are key actors in controlling osteosarcoma cell invasion, migration, and dissemination, is irrefutable. LncRNA plays a role in the development of osteosarcomas by affecting the wnt pathway. For example, when cell lines from osteosarcomas are contrasted with benign osteoblastic cells, the expression of the lncRNA gastric carcinoma proliferation enhancing transcript 1 (GHET1) is amplified. Knockdown of this molecule has been demonstrated to suppress the migration of osteosarcoma cells, together with their invasion and EMT. To some extent, these events are mediated through the control of the Wnt/β-catenin pathway. Compared with the control group, the expression levels of Wnt and β-catenin were decreased after GHET1 knockdown (124). Elevated levels of the lncRNA, CRNDE, have been identified in cell lines and tissue samples from osteosarcomas, with the knockdown of CRNDE leading to limited cellular invasion, the reduced expression of N-cadherin, vimentin, and snail, and amplified E-cadherin and ZO-1 expression (125). A potential mechanism for this process may be the activation of the Wnt/β-catenin signaling pathway via the increased phosphorylation of GSK-3β (125). The upregulation of MALAT1 expression strongly relates to the presence of remote osteosarcoma deposits. This lncRNA can bind to miR-144-3p and enhance ROCK1/2 levels which contribute significantly to the metastatic process (126). MALAT1 may inhibit tumor growth and metastasis through PI3K/AKT signaling pathway (47). The *in vivo* and *in vitro* lentivirus-mediated siRNA reduction of MALAT1 expression decreases in PCNA, MMP-9, p-PI3K, and RhoA/ROCKs expression, which then attenuates the growth, invasion, and dissemination of osteosarcoma cells (47, 48). MiR-184, as well as β-catenin, TCF4, and c-MYC, which are downstream Wnt signaling pathway factors, is negatively impacted by MEG3, which inhibits the replication and the migration of osteosarcoma cells *in vitro* and cancerous growth *in vivo* (127). The transition of epithelial cells into a mesenchymal phenotype is known as enforced EMT, which enhances tumor invasiveness and, clinically, leads to worse overall survival (128). This process can be recognized by a rise in mesenchymal indicators, including N-cadherin, Slug, Twist, Vimentin, and Fibronectin, and by a reduction in epithelial indicators such as E-cadherin (12). The Hh signaling exhibits anomalous activity in cell lines from osteosarcomas and samples from primary human osteosarcomas, which promotes migration and the onset of osteoblastic osteosarcoma (129). In mice with a heterozygous p53 background with amplified Hh pathway stimulation, skeletal cancers occurred freely, even at 7 months of age, in association with marked rises in the potent oncogenes Yap1 and H19 (59). Notably, cancer progression can be suppressed by silencing either of these genes (130).

Tumor cell migration and invasion can also be influenced by lncRNAs via miRNA. Cell survival, migration, and invasion were improved by urothelial carcinoma associated 1 (UCA1) (131), with the expression of this molecule having a positive correlation with chemokine receptor-C-X-C motif chemokine receptor 4 (CXCR4) and miR-301a. Additionally, the expression of miR-301a was amplified, which consequently heightened the expression of CXCR4 (107). Some studies have reported a robust link between the degree of CXCR4 expression and the invasive and metastatic properties of osteosarcoma (132, 133). Moreover, miR-301a has been demonstrated to display malignant functionality in osteosarcomas and additional human malignancies (134). Furthermore, amplified miR-301a expression can prevent the suppressive action of knockdown of UCA1 in cells from osteosarcomas, a process that can be reversed by inhibiting CXCR4. ATB has demonstrated an increase in cancer. In individuals with osteosarcomas, such raised levels lead to greater ZEB1 and SEB2 expression through the suppression of miRNA-200. These findings are associated with a late Enneking stage, remote tumor dissemination, and adverse survival statistics (135). When ATB is lacking, the use of shRNA has a marked inhibitory impact on the growth, migration, and invasion of osteosarcoma cells.

Augmented cancer dimensions, late Enneking stage, remote dissemination, and adverse survival rates were linked with FGFR3 antisense transcript 1 (FGFR3-AS1), which enhanced the stability of FGFR3 mRNA and amplified the expression of FGFR3 by coupling the antisense with FGFR3 3′-UTR (19). When FGFR3-AS1 underwent knockdown, the *in vitro* growth of osteosarcoma cells within a xenograft was inhibited, and similar findings were seen *in vivo* (136).

In contrast to the oncogenic function of many lncRNAs, some operate in a tumor-suppressive capacity. The depletion of NBAT1 accelerates tumor cell replication and invasion (137), while excessive NBAT1 expression suppresses these events and tumor cell migration by inhibiting the effects of miR-21 and impacting its targeted gene (138). Influencing the miR-34a-5p/Sirt1 network via the knockdown of CAMK2D-associated transcript 1 (C2dat1) has

also been demonstrated to diminish the invasive and migratory abilities of osteosarcoma cells (139).

5 LncRNAs' regulation of other signaling pathways in osteosarcoma

As a major contributor to cellular pathways, cell signaling has a significant influence on the modification of the expression of numerous genes in a spectrum of malignancies. A recent study has indicated that the control of many signaling cascades in osteosarcoma is impacted by lncRNAs, which results in changes to cell replication, differentiation, and programmed cell death (Figure 4). In advancing malignant disease in humans, Wnt signaling is a notable and well-recognized example (140). Changes in the constituents of this pathway, such as mutations, amplifications, deletions, hypermethylation of promoters, and changes in localization within the subcellular level, are considered some of the most significant mechanisms involved in the onset and development of osteosarcomas (141). In this malignancy, the regulation of several components, such as ligands, receptors, co-receptors, and antagonists, is disrupted, this finding suggests that this cascade plays a major role in promoting malignant characteristics and tumor metastasis (142). In individuals with osteosarcomas, there is marked enhancement of the activity of the Wnt signaling cascade, and the accrual of the downstream transcription factor, β-catenin, in tissue specimens (143). It has been suggested that this factor could be stabilized with the competitive blockade of miRNA in relation to mRNA binding, and through the conscription of RNA-binding protein to mRNA, processes facilitated by lncRNAs (144–146). There is further potential for lncRNAs to form a complex with DNA and to modify gene expression, such as amplifying the transcription of β-catenin and thus downregulating the expression of the Wnt pathway inhibitor (70). The amplified expression of the lncRNA, BE503655, has been identified in cell lines and samples from osteosarcomas (15), with the mRNA levels and protein expression of β-catenin markedly diminished in this tumor following BE503655 knockdown by regulating Wnt/β-catenin pathways (145). Equivalent findings are present for the lncRNA CAT 104, and the small nucleolar RNA host gene 1 (SNHG1), with a significant rise in their expression. They influenced the Wnt cascade by operating as ceRNAs sponging to miR-381 and miR-577, respectively (147, 148).

In addition to the signaling pathways mentioned above, there are some other signaling pathways associated with lncRNA in

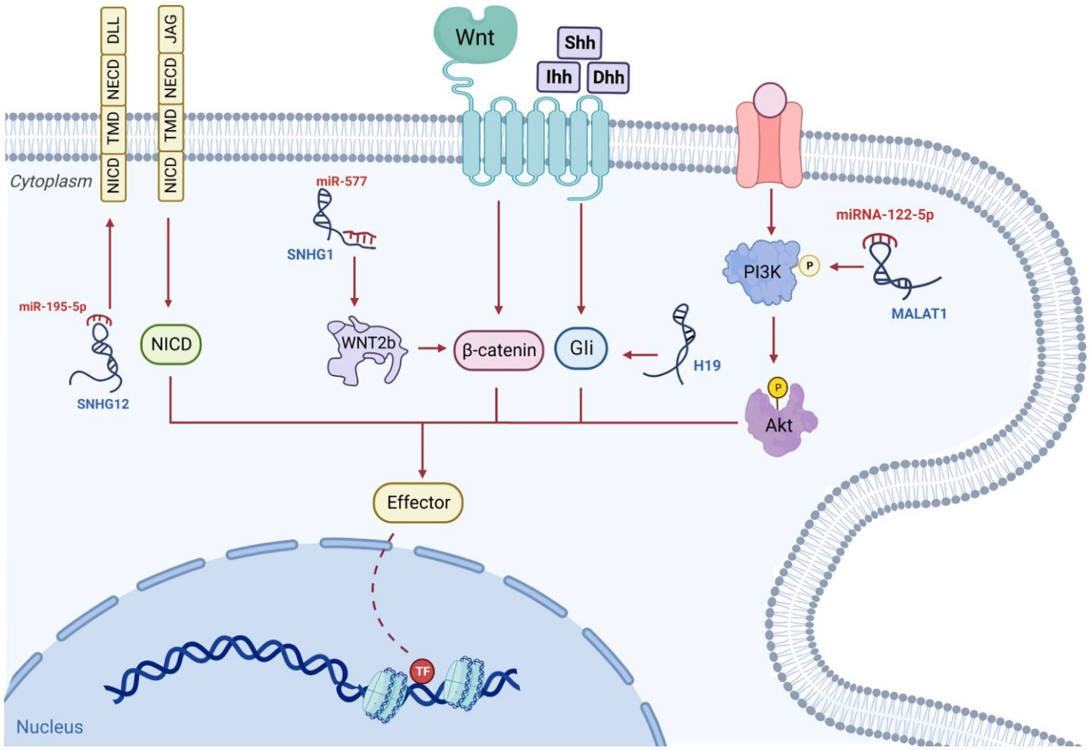

FIGURE 4
The signaling pathways associated with osteosarcoma are governed by lncRNAs. For example, the lncRNA, SNHG, behaves as a competitive miRNA blocker and increases the stability of β-catenin; subsequently, the triggered β-catenin is transported to the nucleus, where it instigates the transcriptive process. The competitive binding of MALAT1 to miRNA-122-5p can occur. This enhances phosphorylated PI3K and AKT levels which influence osteosarcoma progression. The hedgehog ligands, Shh, Ihh and Dhh, enhance Gli segregation from the microtube, after which Gli enters the nucleus to commence transcription. This mechanism may be enhanced by additional lncRNAs such as H19. NICD, TMD and NECD make up the lone transmembrane protein known as the Notch receptor. Ligands, including DLLs and JAGs, which are situated on adjacent cell walls, attach to their extracellular component, NECD, and switch on the Notch signaling cascade. The stimulated NICD is liberated into the cytoplasm to trigger transcription. Additional lncRNAs such as SNHG12, can increase Notch2 expression and facilitate this mechanism.

osteosarcoma. The NF-κB pathway plays a key role in cancer progression, which is regulated by a variety of mechanisms and controls cell growth, invasion and metastasis (149). The study found that the expression of NKILA, which reduced the proliferation, invasion and migration of osteosarcoma cells, was activated by the NF-κB pathway. NF-κB inhibitors can reverse the effect of knockdown of NKILA on cell migration and proliferation (150). In addition, targeting XIST can also inhibit cell proliferation and tumorigenesis by activating NF-kB and NF-kB-dependent PUMA signaling pathways (151). HIF-1α has been shown to regulate hypoxia gene expression through a signal transduction network. FOXD2-AS1 in the hypoxic tumor region can act as a miRNA sponge to regulate tumorigenesis. The expression of FOXD2-AS1 is up-regulated in osteosarcoma and is positively correlated with poor prognosis. HIF-1α can bind to the promoter region of FOXD2-AS, thereby increasing mRNA and protein levels (152). LINK-A may also act as an upstream activator of HIF1α and participate in the metastasis of osteosarcoma by up-regulating the HIF1α pathway (153).

6 Potential preventive and therapeutic effects of lncRNA in osteosarcoma

6.1 LncRNA in osteosarcoma diagnosis and prognosis

Although increasing numbers of current treatment alternatives are available for individuals with sarcomas, the efficacy of contemporary therapies has not changed over time. At present, the methods used to treat osteosarcoma include high-dose methotrexate, adriamycin and cisplatin before and after surgical resection. In addition, different combinations of etoposide, methotrexate, cisplatin, doxorubicin, and ifosfamide were also used to consolidate the treatment, but the results were not satisfactory (154, 155). Chemotherapy regimens consisting of ifosfamide and etoposide or gemcitabine and docetaxel have a certain effect on patients with unresectable recurrent diseases, but the overall event-free survival at 4 months is only 12% (156, 157). Adverse prognoses arise owing to the high risk of remote tumor dissemination and relapse. If malignancy were detected early and a precise prognosis was evident, there would be the potential for prompt targeted treatment and better overall survival statistics. Therefore, the presence of apposite clinical diagnostic or prognostic indicators would be extremely valuable. Numerous studies that have evaluated the role of lncRNAs in osteosarcoma have suggested that these molecules have promise for this application (Tables 2, 3).

Elevated MALAT1 in osteosarcoma tissue have been found to be associated with a late pathological stage, remote tumor deposits, and shortened overall survival, suggesting that this lncRNA could act as an autonomous prognostic factor for this tumor type (158). Significantly, MALAT1 levels within the serum could also be utilized in this regard. The degree of MALAT1 expression had a negative association with 5-year survival figures. In the cohorts with low and high expression, the 5-year survival statistics were 56.5% and 39.1%, respectively, a difference additionally shown with Kaplan-Meier survival curves (159).

An adverse clinical prognosis was robustly linked with amplified TUG1 expression, which was determined to be an autonomous marker of overall survival and progression-free longevity. When judged against preoperative values, serum TUG1

TABLE 2 Potential preventive and therapeutic effects of LncRNA in osteosarcoma.

LncRNA	Relevance	Role
MALAT1	Associated with a late pathological stage, remote tumor deposits and shortened overall survival	Acts as an autonomous prognostic factor for osteosarcoma
TUG1	Associated with adverse clinical prognosis	Acts as autonomous marker for overall survival and progression-free longevity
MEG3	Associated with individual survival	Acts as autonomous markers for a reduced overall survival time
FGFR3-AS1	Associated with elevated tumor dimensions, late Enneking stage and adverse clinical prognosis	Serves as a predict marker for metastasis potential and prognosis
HOTTIP	Associated with late-stage disease and the presence of remote tumor deposits	Acts as a potential prognostic marker and target for treatment in this clinical setting
FOXC2-AS1	Associated with adverse clinical prognosis	Acts as a possible treatment target
LUCAT1	Associated with drug resistance of chemotherapy drugs	Acts as a predictive biomarker in individuals with osteosarcoma
ODRUL	Associated with heightened osteosarcoma doxorubicin-resistance	Diminishes DXR susceptibility in osteosarcoma cells
LINC00161	Associated with apoptosis induced by cisplatin	Ameliorates the resistance of osteosarcoma cells
LINC00922	Associated with DXR resistance in osteosarcoma samples	The silence of LINC00922 inhibits osteosarcoma growth

TABLE 3 The way lncRNAs regulate drug resistance.

LncRNA	Ways to regulate drug resistance
FOXC2-AS1	Promotes ABCB1 expression, thereby increasing drug resistance in osteosarcoma
LUCAT1	Regulate drug resistance in osteosarcoma through the interaction between miR-200c and ABCB1
ODRUL	Diminishes DXR susceptibility in osteosarcoma cells owing to its ability to amplify ABCB1 expression
LINC00161	ameliorates the resistance of osteosarcoma cells by targeting the signaling axis comprising the miR-645-interferon-induced with tetratricopeptide repeats 2 (IFIT2)
SNHG12	Regulate drug resistance in osteosarcoma through its capacity to sponge miR-320a

levels were noted to be markedly diminished in individuals following surgery, with the increase of TUG1 in serum identified in those patients exhibiting advancing malignancy or recurrence (39). Moreover, TUG1 knockdown in the osteosarcoma cell lines, U20S and Saos-2, induced by selective siRNA, leads to a declining cell replication and colony generation, heightened programmed cell death, and the arrest of the cell cycle at the G1/S phase. It is also associated with reduced POU class 2 homeobox 1 (POU2F1) expression, indicating its influence on the TUG1/mir-p-5p/POU2F1-axis in these cell types (160). When these data are combined, TUG1 could be used as a marker for prognosis, treatment, diagnosis, and monitoring purposes.

Overall survival was less in individuals with reduced, as opposed to amplified MEG3 expression (161). In patients presenting with osteosarcomas, low levels of MEG3 expression, a late pathological stage, and the presence of remote tumor deposits were all determined to be autonomous markers of reduced overall survival time.

Elevated tumor dimensions, late Enneking stage, and adverse clinical prognosis have been linked to the amplified expression of FGFR3-AS1 (136). When identified within cells, FGFR3-AS1 promotes amplified FGFR3 expression through a mechanism involving the coupling of antisense with the 3'UTR section of FGFR3. Additionally, when the FGFR3-AS1 shRNA expression plasmid pGPU6/GFP/Neo is used to knockdown FDFR3-ASi in MG63 cell lines *in vitro*, advancement of the cell cycle and cell replication are inhibited (136). Correspondingly, prognosis may be predicted using FGFR3-AS1 in the clinical context of osteosarcoma.

The late-stage disease and the presence of remote tumor deposits have been linked with the amplified expression of HOTTIP in samples from osteosarcomas. Increased expression of HOTTIP has also been related to high mortality and is an autonomous prognostic indicator for overall survival in individuals with this tumor type. Hence, this lncRNA is a potential prognostic marker and target for treatment in this clinical setting (162).

Amplified MIR100HG, HOXD-AS1, EWSAT1, and LMCD1-AS1 expression, in addition to several further lncRNAs, have been determined by Kaplan-Meier curves to forecast adverse clinical outcomes in individuals with osteosarcoma (163, 164). Statistical analyses, such as univariate and multivariate Cox regression, have also indicated the prognostic value of lncRNAs, including LOXL1-AS1, EWSAT1, ILF3-AS1, CBR3-AS1, DLX6-AS1, UCA1, DICER1-AS1, and Ftx in this form of malignancy (164).

6.2 LncRNAs in osteosarcoma chemotherapy and chemotherapeutic drug resistance

The principal types of therapy applied to malignancy include operative resection, and treatment with either chemotherapy or radiation. However, many osteosarcomas fail to demonstrate significant regression following conventional chemotherapy or radiotherapy regimens and may develop resistance to therapies (165). Resistance to chemotherapeutic agents can be categorized as either acquired or primary (166). The latter is already present, whereas the former arises during treatment following the adaptation of malignant cells (167). This occurs via a complicated mechanism to which numerous factors contribute, together with impaired processes of programmed cell death and autophagy (168). In osteosarcoma, the lack of efficacy of chemotherapeutic endeavors predominantly arises owing to secondary resistance to drugs such as doxorubicin (DXR) and cisplatin (169). In some cases, using lncRNAs as targets for therapy has been shown to attenuate the issues of pharmaceutical resistance and enhance the susceptibility of the tumor to treatment (170) (Table 2).

An abundance of research has concentrated on utilizing genetic and molecular analytical techniques to investigate the mechanisms underpinning chemoresistance in osteosarcoma. The factors identified factors include a spectrum of intrinsic biological changes, including the heightened expression of members of the ATP-binding cassette (ABC) membrane transporter family, aberrant metabolic pathways, disruption to the governance of the cell cycle, and abnormal apoptotic processes (171, 172). These alterations can inactivate pharmaceutical agents, diminish the accrual of drugs within the cells, induce chemoresistance via the effects on malignant stem cells, and cause dysfunctional signal transduction pathways, the aberrant control of miRNAs and drug resistance due to aberrant cell death and autophagy (173, 174). The ABC transporter protein family members rely on ATP for the efflux of pharmaceutical agents (175). Their expression is influenced by lncRNAs, which consequently affect the resistance of malignant cells (176, 177).

In samples from human osteosarcomas and in the cell lines MG63 and KH-OS, which exhibit DXR resistance, there is excessive expression of long non-coding RNA Forkhead box protein C2 antisense 1 (FOXC2-AS1) (169). In human tissue samples from osteosarcomas, this finding is associated with an adverse clinical prognosis, and *in vitro*, it is linked with the enhancement of resistance to DXR in the same cell lines. Conversely, FOXC2-AS1 knockdown promotes the susceptibility of cells from osteosarcomas to DXR and reduces osteosarcoma cell resistance to DXR in cell lines, including MG63/DXR and KH-OS/DXR, through FOXC2 inhibition. ABCB1 expression is also promoted by FOXC2, which adds to the drug resistance seen in osteosarcomas (169). Thus, the amplification of ABCB1 expression is a common pathway to DXR resistance in cells from osteosarcomas caused by FOXC2-AS1 and FOXC2. FOXC2-AS1 appears to have two potential roles. Firstly, it may be a useful marker in patients with osteosarcoma to highlight potential DXR resistance, and secondly, it represents a possible treatment target for increasing tumor susceptibility to DXR.

Cell lines from osteosarcomas that display resistance to methotrexate, an extremely potent chemotherapeutic agent used in this type of neoplasia, have been demonstrated to contain amplified lung cancer-associated transcript 1 (LUCAT1) expression (178). In addition, LUCAT1 can interact with ABCB1 through miR-200c, which combines with the 3'UTR of ABCB1 and is regulated by LUCAT1. Accordingly, if elevated levels of LUCAT1 were detected in human osteosarcomas, it can be used as a predictive biomarker in individuals with osteosarcomas.

Patients with osteosarcomas and tumor dissemination to the lungs and a poor response to chemotherapy demonstrated

heightened osteosarcoma doxorubicin-resistance related to up-regulated lncRNA (ODRUL) expression. Notably, the lncRNA ODRUL may diminish DXR susceptibility in osteosarcoma cells owing to its ability to amplify ABCB1 expression (179). Furthermore, several lncRNAs, such as ENST00000563280 and NR-036444, were recognized from a lncRNA-mRNA co-expression network. These engage with genes including ABCB1, HIF1A and FOXC2, which may contribute to elevated levels of osteosarcoma resistance to DXR (180).

Sustained cell survival necessitates prompt and apposite reparation of any injury to DNA. Many miRNAs might control the responsiveness of osteosarcoma cells to treatment with radiation by influencing these mechanisms. Apoptosis brought about by cisplatin is significantly influenced by lncRNA, long intergenic non-coding RNA 161 (LINC00161). Additionally, this lncRNA ameliorates the resistance of osteosarcoma cells by targeting the signaling axis comprising the miR-645-interferon-induced with tetratricopeptide repeats 2 (IFIT2) (181).

DXR resistance in osteosarcoma samples has been linked to amplified LINC00922 expression, the silence of which inhibits osteosarcoma growth. One of LINC00922's functionalities is to sponge miR-424-5p, which decreases transcription factor TFAP2C expression. Consequently, it has been proposed that a feedback loop, including TFAP2C, LINC00922, and miR-424-5p, is important in DXR resistance (182).

CTA levels have been demonstrated to be diminished in cells that are resistant to DXR (130). Significantly, enhancing CTA levels led to a notable rise in two recognized miR-2010 targets, including caspase-8-associated protein 2 and apoptosis-inducing factor, mitochondrion-associated 3 (183). Correspondingly, the reparation of CTA may therefore potentially diminish cell survival through competitive bonding with miR-201, enhancing apoptosis and suppressing autophagy. Moreover, increased SNHG12 transcript levels have been noted in cells from osteosarcomas that demonstrated DXR resistance, as opposed to those that are susceptible to this drug at cytotoxic levels. The mechanism of action of SNHG12 in cells from osteosarcomas arises through its capacity to sponge miR-320a, which has an inhibitory influence on the expression of MCL1 (101).

Anomalies in drug breakdown form a second key pathway that brings about resistance to pharmaceutical reagent. Important enzymes within these metabolic pathways can be affected by several lncRNAs. Enzyme systems that are key to the activation and inactivation of many drugs include the cytochrome P450 (CYP) system, and the glutathione-S-transferase (GST) and the uridine diphosphoglucuronosyltransferase (UGT) superfamilies (184). Numerous pharmaceutical reagents used in the treatment of malignancy have to be activated by the body's metabolism, which could lead to drug resistance if this process is impaired by neoplastic cells (167). Elevated CYP3A4/5 levels have been associated with adverse clinical outcomes in individuals with osteosarcomas (185). Furthermore, after the administration of DXR or cisplatin, glutathione-S-transferase P1 (GSTP1) levels were found to be elevated, and amplified GSTP1 expression in SAOS-2 cell lines from osteosarcomas was linked with heightened vulnerability to resistance to these two pharmaceutical reagents (186).

7 Summary and future prospects

Osteosarcoma, an extremely aggressive malignancy, is typically characterized by invasion at the site of origin, remote dissemination, and recurrence. There is an exigent requirement to comprehend the disease processes underlying this cancer type to facilitate the innovation of *de novo* clinical treatments. The above review has detailed the abundance of evidence that substantiates the possibility that lncRNAs are key actors in the onset, growth, invasion, systemic dissemination, and resistance to pharmaceutical agents exhibited by osteosarcomas. The expression amplification or downregulation of these lncRNAs means that they can display either oncogenic or tumor-suppressive properties, respectively. Examples include HOTAIR, MALA T1, H19, TUG1, MEG3, and TUSC7.

A range of pathways have been identified concerning lncRNAs, such as host gene targeting, signal pathway involvement, and ceRNA activity. Several lncRNAs have been determined to function as autonomous indicators of prognosis, including the likelihood of the presence of drug resistance to modern treatments such as DXR and cisplatin. Such data pertaining to lncRNAs are encouraging for the future innovation of clinically applicable biological indicators for the diagnosis and prediction of survival, as well as potential targets for treatment. LncRNA can be targeted by using small interfering RNA, antisense oligonucleotide, ribozyme, aptamer, and miRNA. These methods have long been evaluated for targeting key cancer-related genes, and they are at different stages of clinical trials. For example, flavonoids are negatively correlated with the risk of colorectal cancer. These bioactive compounds can target the lncRNA/Wnt pathway to reduce the side effects of anticancer drugs (187). Simvastatin, a potential therapeutic drug for colorectal cancer immunotherapy, promotes anti-tumor immunity by inhibiting lncRNA SNHG29-mediated YAP activation and inhibiting PD-L1 expression (188). In addition, due to the ability of oligonucleotides to enter cells and specifically target RNA that cannot be accessed by antibodies, oligonucleotide drugs show stronger target specificity than small molecule drugs and reduce potential side effects. Therefore, lncRNA-based oligonucleotide drugs are being studied and are expected to make further progress. Nevertheless, research investigating the role played by lncRNAs in osteosarcoma is still in its infancy and predominantly in the pre-clinical phase. Despite all this, recent experimental data are extremely promising regarding the utility of lncRNAs as future diagnostic and prognostic clinical indicators, and as treatment targets in patients with osteosarcoma.

Author contributions

SH: Writing – original draft, Writing – review & editing. XH: Writing – original draft, Writing – review & editing. GL: Supervision, Writing – review & editing. SW: 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

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Microbiota-bone axis in ageing-related bone diseases

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Bone homeostasis in physiology depends on the balance between bone formation and resorption, and in pathology, this homeostasis is susceptible to disruption by different influences, especially under ageing condition. Gut microbiota has been recognized as a crucial factor in regulating host health. Numerous studies have demonstrated a significant association between gut microbiota and bone metabolism through host-microbiota crosstalk, and gut microbiota is even an important factor in the pathogenesis of bone metabolism-related diseases that cannot be ignored. This review explores the interplay between gut microbiota and bone metabolism, focusing on the roles of gut microbiota in bone ageing and aging-related bone diseases, including osteoporosis, fragility fracture repair, osteoarthritis, and spinal degeneration from different perspectives. The impact of gut microbiota on bone metabolism during aging through modification of endocrinology system, immune system and gut microbiota metabolites are summarized, facilitating a better grasp of the pathogenesis of aging-related bone metabolic diseases. This review offers innovative insights into targeting the gut microbiota for the treatment of bone ageing-related diseases as a clinical therapeutic strategy.

KEYWORDS

aging, skeletal degenerative diseases, bone metabolism, gut microbiota, bone

1 Introduction

Bone is an organ that experiences continuous remodeling by osteoblasts and osteoclasts. Osteoblasts are responsible for the production of type I collagen, osteocalcin, and alkaline phosphatase, which serve as scaffolds for the deposition of calcium and phosphorus, facilitating the formation of new bone (1). On the contrary, osteoclasts, responsible for bone resorption, attach to the bone surface and secrete hydrogen ions, leading to the dissolution of the bone matrix and the release of mineral deposits from the bone. During aging, debilitating skeletal diseases like osteoporosis occur due to the inability to maintain bone homeostasis. This phenomenon primarily arises from the overstimulation of osteoclasts or the suppression of osteoblasts, leading to a higher rate of bone resorption compared to bone formation.

Somatic cells have a limited lifespan. During the 1960s, Leonard Hayflick first described the limited replicative potential of normal cultured human fibroblasts and termed this phenotype "cellular senescence" (2). These cells are causally implicated in aging and in an ever-expanding list of diseases (3). Accumulating evidence has indicated that aging is often accompanied by degradation of the bone microenvironment, leading to decreased differentiation and compromised bone repair capacity (4). As the human body undergoes the ageing process, senescent cells occur and release senescence-associated secretory phenotype (SASP) factors, including IL-6 and GCA, into the bone microenvironment (5). The escalating burden of senescent cells with age disrupts tissue structure and function, emerging as a significant factor in elevating disease susceptibility and mortality among the elderly. Osteoporosis, sarcopenia, degenerative disc disease, and osteoarthritis are musculoskeletal disorders that are closely related to the ageing process. Focus on more detailed aspects. The aging behavior of stem cells, macrophages and osteocytes plays an important role in the aging of bone tissue (6). It has been demonstrated that senescence of skeletal stem cells in mice leads to greater release of pro-inflammatory and pro-resorptive factors into the bone senescence microenvironment leading to bone senescence (7). Senescent macrophages have also been shown to influence bone senescence by promoting inflammation and the release of granular calprotectin (GCA) (8). Also, chronic inflammation accelerates the aging of immune cells, leading to weakened immune function and an inability to remove senescent cells and inflammatory factors, resulting in a vicious cycle of inflammation and aging. As for the osteocytes, previous studies have suggested that possible characteristics of aged osteocytes include, but are not limited to, impaired mechanosensitivity, accumulated cellular senescence, dysfunctional perilacunar/canalicular remodeling (PLR), and degenerated lacuna-canalicular network (LCN). The aging osteocytes can exhibit mechanical- and endocrine-responsive properties to the aging microenvironment (9). Targeted elimination of senescent cells from ageing tissues has the potential to postpone the onset and progression of age-related diseases. Additionally, counteracting oxidative and inflammatory stressors may decelerate the formation of senescent cells, thereby offering insights into potential therapeutic interventions (10).

The interactions between gut metabolism and bone health have been investigated, encompassing various mechanisms such as gut metabolites, the immune system, intestinal epithelial barrier function, nutrient absorption metabolism, endocrinology, hormone function, and modulation of the gut-brain-skeleton axis to impact bone metabolism (Figure 1). It is well known that gut microbes occupy a central position in gut metabolism. Intestinal microorganisms encompass a diverse array of commensal and pathogenic microorganisms, such as bacteria, archaea, viruses, and fungi, that colonize the intestinal mucosa. Comprising 1014 species of bacteria and possessing 150 times more genes than the human body, this entity has been referred to as the "forgotten organ of the human body" (11). *Firmicutes* and *Bacteroidetes* constitute the main components of the gut microbiota in both mice and humans (12). The ratio of the two is of great importance for bone metabolism and the improving of the ratio is advantageous to suppress age-induced chronic inflammation and oxidative damage in skeletal tissue (13).

The gut microbiota represents a crucial element of the gut barrier (14), the integrity of the gut barrier is closely associated with gut permeability. Dysbiosis of the gut microbiota may contribute to the development of systemic low-grade inflammation and the activation of the immune system by causing intestinal barrier dysfunction, increased intestinal permeability and leakage of toxic bacterial metabolites into the circulation (15). And numerous studies have indicated that this progress are linked to a multitude of processes that govern bone mass, mechanical function, marrow production, development, metabolism, osteoporosis (16), inflammation, fracture risk, and cancer in bone.

Throughout the process of co-evolution with the host, the composition of the gut microbiota undergoes continual modifications as individuals age, transitioning from infancy to old age. It is inevitable that changes in the gut microbiota occur concomitantly with organismal ageing. The gut microbiota composition in older adults is distinguished by diminished diversity and stability, decreased expression of genes responsible for producing short-chain fatty acids (SCFA), reduced capacity to break down glycoconjugates, heightened proteolytic activity, and, at the genus level, an increase in Proteobacteria enrichment. The enrichment of Proteobacteria, along with an elevated proportion of the Bacteroidetes and Clostridium genera, was observed (17). Various metabolites present in the gut microbiota exhibit potent anti-inflammatory and antioxidant properties, potentially playing a role in mitigating the development of inflammatory and tumorigenic conditions linked to the ageing process. Dysbiosis of the gut can lead to chronic inflammatory stress in the body, potentially contributing to cellular senescence and worsening degenerative bone diseases (18) (Figure 2).

2 Mechanism of gut microbiota metabolites affecting bone metabolism

2.1 Metabolites of gut microbiota

The gut microbiota significantly impacts bone metabolism through its modulation of short-chain fatty acids (SCFAs). SCFAs are primarily generated through the fermentation of carbohydrates like dietary fiber, resistant starch, and oligosaccharides by anaerobic bacteria in the colon. SCFAs can inhibit LPS-induced inflammatory responses, and the uptake of sufficient SCAFs may be associated with the inhibition of senescence. It has been demonstrated that SCFAs can bind to GPR41 (FFAR3)/GPR43 (FFAR2) receptors on cell membranes or passively diffuse into cells to activate histone deacetylase (HDAC) to block the NF-κB signaling pathway and inhibit the inflammatory response (19). SCFAs also directly exhibit a dual regulatory effect on osteoclasts. SCFAs have the capacity to promote the differentiation of regulatory T cells (Treg) through the inhibition of histone deacetylase, thus SCFAs can attenuate osteoclast differentiation and formation. Conversely, SCFAs can also prompt naive T cells to differentiate into T helper 17 (Th17) cells, which secrete the receptor activator of nuclear factor-κB

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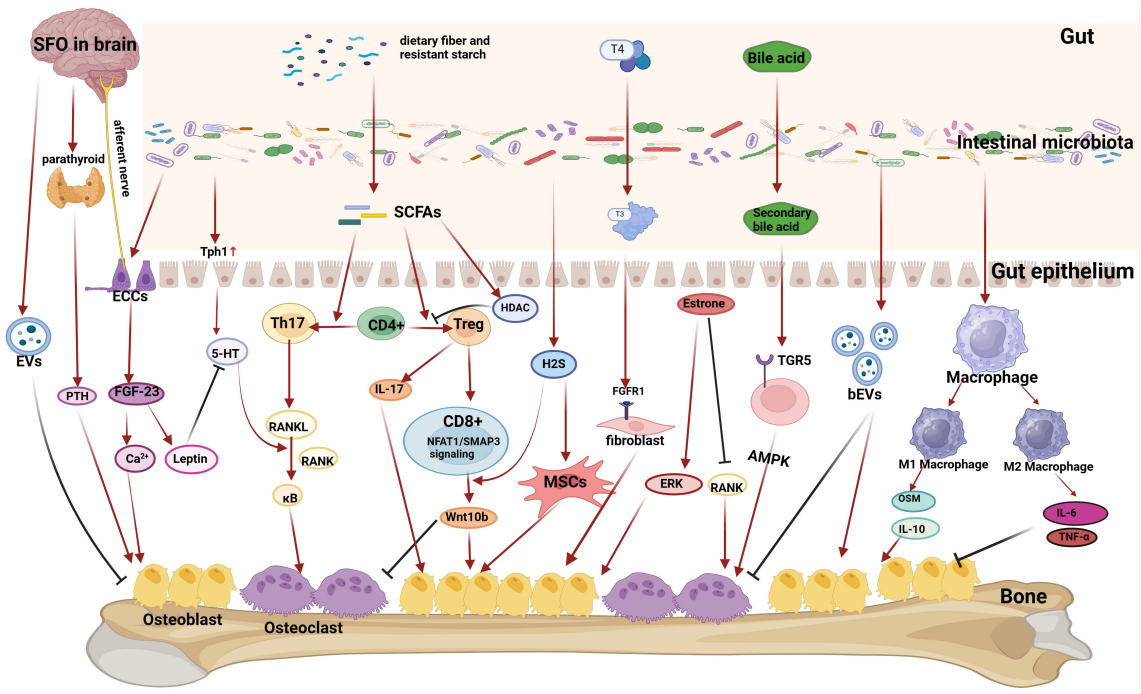

FIGURE 1
Mechanisms by which gut microbiota affect bone metabolism. (1) Gut microbiota can act on intestinal secretory cells (ECCs), which can affect the subpial region of the brain (SFO) through the gut-brain axis, and then affect the release of parathyroid hormone from the parathyroid glands, which ultimately affects the absorption of calcium and bone formation, and also affect the serum calcium level and thus affect the formation of bone by influencing the FDF-23 content, and also affects the regulation of the leptin content and the role of 5-HT. The brain can also release extracellular vesicles (EVs) to inhibit osteoblasts after stimulation of the gut-brain axis. (2) Gut microbiota can affect 5-HT absorption by upregulating Tph1 protein expression in intestinal epithelial cells, and 5-HT can amplify the role of the RANKL pathway in promoting osteoclast differentiation. (3) Gut microbiota can break down dietary fiber and other substances to produce short-chain fatty acids (SCFAs), which regulate the Th17-Treg balance, and then play a dual role in regulating osteoblasts and osteoclasts differentiation through the RANKL pathway or the Wnt10b pathway. Gut microbiota can break down food to produce H2S, which acts on mesenchymal stem cells (MSCs) to promote osteoblast differentiation. (4) The gut microbiota can influence material transformation to regulate bone metabolism. Gut microbiota plays a crucial role in the conversion of thyroid hormone T4 to a more active form of T3, which can act on the FGFR1 receptor of fibroblasts to promote osteoblast differentiation. Gut microbiota plays an important role in the conversion of bile acids into secondary bile acids, which can act on the TGR5 receptor of cells to activate the AMPK pathway to promote osteoblast differentiation. (5) Gut microbiota can influence the amount of estrogen. Increasing estrogen levels in postmenopausal women by influencing gut microbiota can promote osteogenesis and improve osteoporosis. Gut microbiota can also release bacterial-derived extracellular vesicles (bEVs) that act on bone to influence bone metabolism. (6) Gut microbiota can affect macrophage differentiation. M2-type macrophages can secrete factors such as OSM and IL-10 to promote osteogenesis, while M1-type macrophages can secrete pro-inflammatory factors such as TNF-α and IL-6 to inhibit osteogenesis and promote osteoblastogenesis.

ligand (RANKL). This, in turn, triggers the activation of nuclear factor-κB upon binding to RANK. The nuclear factor-κB promotes the expression of osteoclasts. SCFAs have the capacity to influence osteoblasts by prompting naïve CD4+ cells to transform into Treg cells within the bone marrow. They also facilitate the interaction between NFAT and SMAD, leading to the activation of Wnt10b and subsequently triggering Wnt signaling in osteoblasts. This process ultimately enhances bone formation (20). Additionally, SCAFs can enhance bone formation through the regulation of IGF-1 production. SCAFs can also activate osteoblasts by inducing the expression of glucagon-like peptide-1 (GLP-1), which plays a crucial role in osteoblast function. The secretion of glucagon-like peptide-1 (GLP-1) aims to improve bone strength (21).

The gut microbiota can also act on bone via 5-HT. Intestinal-derived 5-HT, which accounts for 95% of 5-HT *in vivo*, is mainly synthesized by enterochromaffin cells via the rate-limiting enzyme [tryptophan hydroxylase 1 (Tph1)], and it has been reported that bacteria of the genus Clostridium stimulate the production of 5-HT by enteroendocrine cells by inducing the expression of Tph1 in enterochromaffin cells (22). Cui et al. reported that inhibition of Tph1 expression affected osteoblast proliferation and differentiation causing high bone mass syndrome (23). Stone et al. reported that intestinal-derived 5-HT amplified the effects of RANKL on osteoclastogenesis in mice (24). Thus, The gut microbiota plays a crucial role in modulating the interaction between osteoblasts and osteoclasts through its impact on the synthesis of the pivotal rate-limiting enzyme, Tph1, thereby influencing the levels of enteric 5-HT.

Additionally, the gut microbiota can modulate bone metabolism through bile acids. Bile acids are substances that facilitate digestion. Upon entering the intestine, primary bile acids undergo conversion into secondary bile acids through the influence of the gut microbiota. The membrane-bound G protein-coupled bile acid receptor (TGR5) serves as the receptor for secondary bile acids. The interaction between secondary bile acids and TGR5 has the potential to stimulate osteoclast differentiation via the AMPK

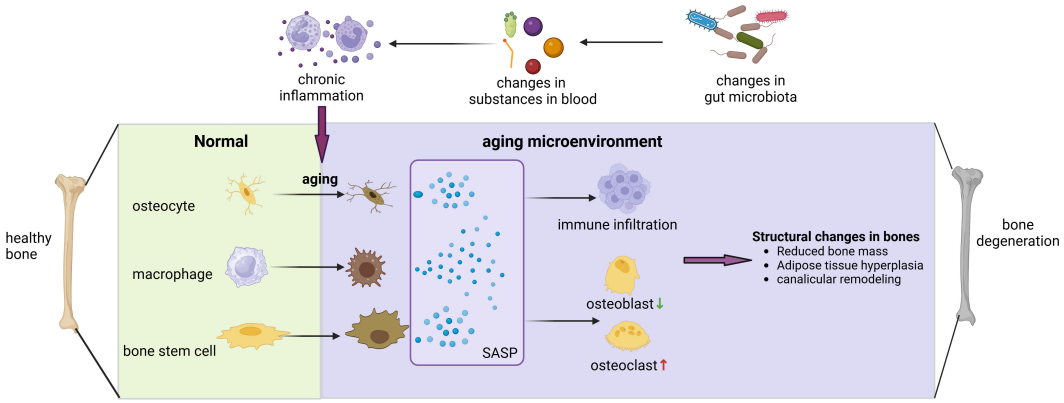

FIGURE 2
Gut microbiota can contribute to bone degenerative diseases by affecting the bone aging microenvironment. Alterations in the composition of gut microbiota can affect LPS entry, bile acid metabolism, short-chain fatty acid absorption, and other processes that induce widespread chronic inflammation in the body, and chronic inflammation has been shown to have a close association with cellular senescence. When osteoblasts, macrophages, and skeletal stem cells become senescent, they release SASP, which exacerbates the inflammatory infiltration of the bone microenvironment, promotes osteoblasts and inhibits osteogenesis, leading to various changes in bone structure and ultimately triggering the development of degenerative bone diseases.

signaling pathway. The differentiation of osteoclasts can be influenced by the gut microbiota, thereby regulating bone metabolism through the modulation of bile acid conversion (25). The relationship between bile acids and aging has also been demonstrated. Studies have shown that when bile acid metabolism is disturbed after adverse changes in the structure of the gut microbiota there is a significant increase in the body's inflammation levels and an increase in the incidence of aging-related diseases, including Alzheimer's disease and cardiovascular disease (8), but the relationship between bile acids and bone aging diseases remains to be demonstrated.

2.2 Affecting substance absorption

The gut microbiota can impact bone metabolism through its influence on nutrient absorption and modulation of nutrient levels within the body. On the one hand, The production of short-chain fatty acids (SCFAs) leads to a direct decrease in the pH of the intestinal lumen (26), enhancing mineral solubilization which leads to the conversion of more calcium into a soluble form. This soluble calcium then permeates intestinal cells via passive exchange, thereby augmenting the body's calcium absorption. Consequently, this process influences bone formation. On the other hand, A fraction of the substance undergoes degradation within the gastrointestinal tract, specifically in the intestines, leading to the generation of bioactive peptides that exhibit various biological activities. Small peptide molecules have the ability to adsorb, bind, and transport calcium and other essential elements within the human body, thereby enhancing their absorption and utilization rates. For instance, casein phosphopeptide, which acts as a carrier for Ca2+, has been documented to exhibit preventive effects against osteoporosis in ovariectomized mice (27). Wang et al. discovered that the gut microbiota has the ability to reprogram lipid absorption and metabolism in the mouse intestine through the inhibition of long-chain non-coding RNA (lncRNA) Snhg9 expression in small intestinal epithelial cells (28).

2.3 Modulating the immune system

As described in the previous section on the senescence microenvironment, when senescent cells are present in the bone metabolic environment and secrete SASP or when the level of inflammation is increased due to the regulation of other factors it can induce bone degenerative diseases by promoting immune responses, such as elevating the level of inflammatory factors such as IL-6, recruiting neutrophils, and inducing the conversion of macrophages to the M1 type to disrupt the equilibrium between osteoclasts and osteoblasts (5). Gut microbiota can play an important role in the inflammatory level of the bone metabolic environment by, for example, releasing LPS into the bloodstream to induce an immune response, and thus the gut microbiota-immune system-bone senescence microenvironment is an important pathway for the disruption of bone homeostasis and the development of disease.

Previous studies have suggested the function of T cells in affecting bone metabolism. TGF-β and IL-2 stimulate T cells to differentiate into Treg cells. Additionally, TGF collaborates with certain pro-inflammatory factors like IL-6 and IL-21 to enhance the differentiation of naïve T cells into Th17 cells (29). On one hand, regulatory T (Treg) cells possess the capability to impede osteoclast maturation and differentiation via a pathway mediated by cytotoxic T lymphocyte-associated protein 4 (CTLA-4) (30). On the other hand, Treg cells can regulate the production of Wnt10b in CD8+ T cells (31), which in turn can trigger the activation of the Wnt/β-catenin signaling pathway. The reduction of beta-catenin levels is linked to diminished bone formation. This dynamic is evident in the

Th17-Treg homeostasis, where a higher differentiation of CD4+ T cells into Treg cells leads to the inhibition of osteoclasts and a facilitation of bone formation.

Macrophages also play an important regulatory role in bone metabolism and bone aging. F4/80 macrophages located in proximity to the periosteal and endosteal surfaces, commonly referred to as "osteomacs," have been demonstrated to oversee the mineralization process of osteoblasts in laboratory settings and uphold the functionality of mature osteoblasts in living organisms (*in vivo*) (32). Furthermore, oncostatin M (OSM) and interleukin-10 (IL-10) secreted by macrophages have been shown to promote the differentiation and mineralization of osteoblasts (5). The inhibition of osteogenesis is caused by TNF-α and IL-6, which are produced by activated M1-like macrophages (33). In the context of immune-mediated bone metabolism, the RANKL (receptor activator of NF-kappa B ligand)-RANK-OPG axis and the immunoreceptor tyrosine-based activation motif (ITAM) pathway are pivotal in regulating both normal bone remodeling and pathological bone conditions (34).

The gut microbiota has the capacity to regulate bone metabolism through its impact on the immune system. In terms of affecting the T cells, the *probiotic bacterium Lactobacillus acidophilus* has been shown to inhibit bone loss in mice that have undergone ovariectomy (OVX) by modulating the balance between regulatory T cells (Treg) and T helper 17 cells (Th17) (35). The colonization of gnotobiotic mice with Indigenous *Clostridium species* led to a heightened accumulation of colonic Tregs, consequently fostering bone formation (36). T regulatory (Treg) cells have the capacity to secrete IL-17, which is crucial for bone formation *in vivo* and has been shown to alleviate osteoporosis in mice post-ovariectomy (OVX). Additionally, filamentous bacteria have been found to enhance the production of IL-17, suggesting a potential contributory role in bone development (37). In terms of affecting Macrophage, it is reported that the gut microbiota can promote Bone Marrow-Derived Macrophages to secret pro-inflammatory factors like IL-6, IL-12p40 (38). And IL-6, IL-12/p40 have been reported to be closely associated with bone ageing diseases such as osteoarthritis and osteoporosis (39).

2.4 Affecting the internal secretion

From a local perspective of gastrointestinal hormones, the gastrointestinal (GI) tract is recognized as one of the largest endocrine organs in the human body. Enteroendocrine cells (ECCs) in GI play a crucial role in secreting a diverse array of gastrointestinal hormones that exert a significant regulatory influence on bone metabolism. For instance, leptin is synthesized by primary and P cells in the gastric fundus region. It has been documented to potentially stimulate fibroblast growth factor 23 (FGF-23) (40), influencing osteocalcin, 5-HT, serotonin levels, and other pathways that modulate bone growth (41). Glucagon-like peptide-1 (GLP-1) has been documented to stimulate bone formation and suppress bone resorption (42). Meanwhile, the use of a GLP-1 receptor agonist activated the PKA/CREB pathway to exhibit anti-inflammatory activity in a rat model of osteoarthritis, suggesting that GLP-1 may be associated with the formation of the skeletal senescence microenvironment and influence the onset of degenerative bone diseases (43). The gut microbiota has the capacity to impact the secretion of gastrointestinal hormones by intestinal secretory cells, thereby potentially influencing bone metabolism (44).

From a systemic perspective of the endocrine system, the gut microbiota can concurrently impact the activity of various hormones, including thyroid hormones, sex hormones, growth hormones, and other hormones, thereby exerting an indirect influence on bone metabolism. The gut microbiota plays a significant role in the conversion of thyroid hormones. Approximately 20% of thyroxine (T4) is transformed into the active form of triiodothyronine (T3) within the intestine. T3, in turn, stimulates the proliferation and differentiation of osteoblasts by activating Fibroblast Growth Factor Receptor 1 (FGFR1) (45). The presence of excessive pathogens or inflammation in the intestinal tract can reduce the conversion of thyroid hormones in the gut. Consequently, bone reconstruction is impacted, potentially resulting in osteoporosis. Estrogen deficiency has been associated with bone loss (46). In a study where estrogen-deficient mice were administered Lactobacillus acidophilus, a reduction in bone resorption markers and an improvement in bone formation were observed (47). The colonization of germ-free mice with gut microorganisms has been shown to markedly elevate serum insulin-like growth factor-1 levels, thereby stimulating bone growth (48).

2.5 Through gut-brain-bone axis

The gut-brain axis refers to a complex network of bidirectional connections that integrate neural, hormonal, and immune signals between the gastrointestinal tract and the brain. On one hand, signals originating from the brain impact gastrointestinal homeostasis processes through the autonomic nervous system and the hypothalamic-pituitary-adrenal axis. On the contrary, the gut microbiota can also be detected through numerous metabolites by specialized cells in the gut, such as enteroendocrine cells (EEC), enterochromaffin cells (ECC), and primary or secondary afferent nerve endings. These cells are responsible for perceiving bacterial metabolites and subsequently transmitting neural signals to the brain. The gut microbiota has the capacity to engage with immune cells within the gut, resulting in both local and systemic immune activation. This interaction leads to the generation of circulating metabolites that can traverse the blood-brain barrier, reaching the central nervous system and exerting a direct influence on neural activity. Consequently, this process impacts bone metabolism through the intricate mechanism known as the brain-bone axis.

With regard to the brain-bone axis, Yang et al. found that activation of GABAergic neurons and Glutinergic neurons in specific subfornical organ (SFO) regions of the brain using chemical genetics resulted in decreased or increased PTH hormones, respectively, which in turn resulted in decreased or increased bone density (49). Shen et al. found that brain-derived extracellular vesicles(EVs) in Alzheimer's disease(AD) patients

significantly inhibited osteogenic differentiation of BMSCs and promoted their lipogenic differentiation, inducing bone loss and fat accumulation in the bone marrow cavity (50). Given that gut microbiota has many effects on the brain, and that the brain also plays an important role in the regulation of bone metabolism, the gut-brain-bone axis will be a very promising area of study.

2.6 Via extracellular vesicles

Bacterial extracellular vesicles (bEVs) are functional nanovesicles secreted by a variety of gut microbiota bacteria. These vesicles contain a diverse array of biomolecules derived from the parent bacteria and play a role in numerous signaling pathways within the host organism. BEVs are linked to systemic diseases, such as bone disorders. Xie et al. introduced a new model for regulating bone metabolism through Enterobacteriaceae functional extracellular vehicles (EVs). Their research demonstrated that Enterobacteriaceae and the probiotic *Akkermansia muciniphila* (Akk) are significant in enhancing bone formation and suppressing bone metabolism within bone tissues. This is achieved through the release of extracellular vehicles (EVs) into bone tissues, which mitigate bone loss in postmenopausal mice with osteoporosis (51). A recent investigation demonstrated that extracellular vehicles (EVs) released by *Lactobacillus animalis* have the potential to influence the femoral head, mitigating glucocorticoid-induced necrosis through the enhancement of bone formation, vascularization, and anti-apoptotic mechanisms (52).

3 The impact of gut microbiota on ageing-related skeletal degenerative diseases

3.1 Gut microbiota and osteoporosis

Osteoporosis is a systemic skeletal disorder distinguished by reduced bone density and deterioration of the microarchitecture of bone tissue, resulting in heightened bone fragility and vulnerability to fractures. Osteoporosis is a prevalent disease, and global statistics indicate that the population of individuals with osteoporosis has surpassed 200 million (53). The epidemiological survey conducted on osteoporosis in China revealed that the prevalence of osteoporosis among individuals aged over 50 is 19.2%. Among them, 32.1% are women and 6.9% are men (54). Postmenopausal osteoporosis constitutes the predominant demographic among women affected by osteoporosis.

The dysregulated gut microbiota impacts bone remodeling equilibrium through the mechanisms, resulting in a higher rate of bone resorption compared to bone formation, ultimately causing a reduction in bone mass (54). Numerous inflammatory factors can induce local inflammation and alter the mechanical characteristics of bone, ultimately resulting in osteoporosis. Sjögren et al. discovered that mice with gut microbiota deficiency exhibit approximately 40% higher bone mineral density in the distal femur compared to normal mice. This phenomenon may be attributed to the lower presence of osteoclasts in the gut-deficient mice in comparison to normal mice, while the quantity of osteoblasts does not show significant variance. Consequently, this leads to decreased bone resorption and subsequent bone loss (55). Furthermore, the levels of osteolytic cytokines, such as IL-6 and TNF-α, were notably diminished in the bones of germ-free mice. This suggests that the decrease in osteoclasts in germ-free mice could potentially be attributed to immune system regulation and inflammatory responses in the aging microenvironment. In postmenopausal women with osteoporosis, a decrease in gut microbiota diversity may result in lower circulating estrogen levels. This is because a healthy gut microbiota plays a role in estrogen regulation through the secretion of β-glucuronidase, an enzyme that converts estrogen into its active form. Research has indicated a correlation between estrogen deficiency and osteoporosis (56). Targeting the gut microbiota may exert a therapeutic impact on osteoporosis. Chevalier et al. documented that *Mucinophilic acromyces* have the potential to enhance trabecular volume, junction density, and thickness. This enhancement contributes to the improvement of bone biomechanical strength in adult female and young male mice. Consequently, it aids in the prevention of ovariectomy-induced bone loss and serves as a treatment for osteoporosis (57).

3.2 Gut microbiota and fragility fracture repair

As previously stated, the elderly demographic is susceptible to osteoporosis, rendering them more vulnerable to fractures that are harder to mend. An osteoporotic fracture, also known as a fragility fracture, is a type of fracture that occurs due to minor trauma, such as a fall from a standing height or lower (58). It is considered a severe complication of osteoporosis. The most prevalent osteoporotic fracture involves the vertebrae, while the most severe osteoporotic fracture is a hip fracture (59). Osteoporotic fractures pose significant harm and represent a primary contributor to disability and mortality among elderly individuals. Within one year following a hip fracture, approximately 20% of patients may succumb to various complications. Moreover, around 50% of patients may experience disability, leading to a significant decline in their quality of life (60). Additionally, treating fragility fractures in the elderly population is often more challenging. Hence, mending fragility fractures is a research topic of social significance.

The gut microbiota contributes to the process of repairing fragility fractures. Roberts et al. demonstrated that the administration of *Bifidobacterium longum* to elderly mice expedited the formation of bone callus post-fracture, augmented bone healing, and enhanced the biomechanical characteristics of the fractured limb. During the recuperation period following a fracture in mice, the administration of *Bifidobacterium longum* helped preserve the integrity of the intestinal barrier, preventing the fracture-induced decline in gut function and systemic inflammatory responses (61). Liu et al. demonstrated the potential of *probiotic Akkermansia muciniphila* in promoting fracture healing in mice by reducing gut permeability and

inflammation (62). As a result, the senescent microenvironment in the new bone is inhibited, contributing to healing at the fracture site.

3.3 Gut microbiota and osteoarthritis

Osteoarthritis (OA) is a chronic degenerative disease affecting articular cartilage, commonly observed in middle-aged and elderly individuals. It is characterized by the progressive deterioration of articular cartilage, sclerotic hyperplasia of subchondral bone, the development of periarticular bony outgrowths, and synovial lesions. OA can result in joint pain, dysfunction, and may lead to joint deformity or disability (63).

Lei et al. conducted a fecal sequencing study involving 1,388 subjects, of whom approximately 5% had symptomatic hand osteoarthritis (SHOA). The study revealed notable alterations in pathways associated with amino acid, carbohydrate, and lipid metabolism in the fecal gut of individuals with symptomatic hand osteoarthritis. Furthermore, they discovered a higher relative abundance of Cholera species. *Vibrio desulfuricans species* are present. A decreased relative abundance of *Rousselotiella spp* was observed in the fecal gut (64). In a study, 440 fecal macrogenomes (221 inflammatory arthritis patients and 219 controls) were analyzed. The results suggest an interaction between host genetics, the immune system and gut microbiota in the onset, progression and severity of arthritis (65). Schott et al. discovered a significant reduction in intestinal bifidobacteria in obese rats. This alteration in the gut microbiota composition resulted in the initiation of systemic inflammatory responses, leading to the aggregation of macrophages in the synovial membrane (66). Consequently, this process accelerated the advancement of osteoarthritis. The restoration of gut microbiota through the supplementation of oligofructose resulted in decreased levels of systemic inflammation, potentially mitigating symptoms of arthritis. An elevated population of lactic acid bacteria plays a crucial role in preserving the integrity of the intestinal mucosal barrier, thereby impeding the translocation of bacterial endotoxin or LPS and hindering the progression of osteoarthritis (64). Furthermore, a randomized, double-blind, placebo-controlled clinical study conducted on individuals with knee osteoarthritis demonstrated that the probiotics *Lactobacillus casei Shirota* and *Streptococcus thermophilus* (TCI633) exhibit beneficial effects in ameliorating knee osteoarthritis (67).

3.4 Gut microbiota and spinal degeneration disease

Spinal degenerative diseases (SDD) encompass a broad term that includes degenerative conditions affecting spinal structures such as osteoporosis, facet osteoarthritis, intervertebral disk degeneration, lumbar spinal canal stenosis, and spinal sarcopenia (68). Increased inflammatory stress linked to intestinal dysbiosis fosters the senescence of spinal muscle and skeletal cells, leading to the accumulation of diverse senescent cells in spinal structures (18). This accumulation triggers local and systemic inflammation through the senescence-associated secretory phenotype (SASP), thereby playing a role in the progression of SDD.

For a long time it was thought that the intervertebral disc (IVD) was a sterile environment, but now more and more experiments have shown that the IVD also has its own special microbiota and that the microbiota plays an important role in the maintenance of homeostasis in the IVD and in the development of intervertebral disc degeneration (IDD). The gut microbiota has been shown to influence the development of IDD through migration and promotion of inflammation. There are three potential mechanisms widely accepted by which the gut microbiota can induce IDD are: (1) translocation of the bacteria across the gut epithelial barrier and into the intervertebral disc (IVD), (2) regulation of the mucosal and systemic immune system, and (3) regulation of nutrient absorption and metabolites formation at the gut epithelium and its diffusion into the IVD (69). Rajasekaran et al. highlighted that there exists a distinction in the microbiome composition between healthy discs and degenerative or herniated discs. They observed that bacteria residing on discs have the ability to attract additional inflammatory cells through the secretion of inflammatory mediators like IL-6 and TNFα (70), leading to chronic inflammation and thus promoting disc degeneration and the ageing process (71).

Kawaguchi et al. found a correlation between ossification of the posterior longitudinal ligament (OPLL) and leptin as well as chronic inflammation. They also suggested that dysbiosis in gut microbiota influences bone metabolism in connection with chronic inflammation-related diseases and the neurotransmitter leptin. This indicates that gut microbiota dysbiosis may play a role as a pathological factor in OPLL (72). In lumbar spinal stenosis (LSS) patients, inflammatory responses were detected in the typical thickened ligamentum flavum. The factors of lumbar spinal stenosis (LSS), including intervertebral disc disease (IVDD) and facet osteoarthritis (OA), are known to have a relationship with gluteal muscles (GM). Moreover, the correlation between lumbar spinal stenosis (LSS) and chronic inflammation-related disorders like diabetes and metabolic syndrome, along with the inflammation detected in ligament thickening in individuals with LSS, implies a plausible link between LSS and gut microbiota. This suggests the potential existence of a gut-ligament axis in LSS (72).

In vivo or *in vitro* experimental studies in humans or animals on the mechanisms and relationships between gut microbiota and the development of degenerative bone diseases are summarized as Table 1.

4 Treatment and prevention of skeletal diseases by means of gut microbiota

Given the increasing recognition of the involvement of the gut microbiota in bone health, various investigations have explored the potential interventions in the gut microbiota for the treatment or prevention of bone diseases by inhibiting the inflammatory

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TABLE 1 Summary of studies investigating the impact of gut microbiota on ageing-related skeletal degenerative diseases.

Author	species	Dysbiosis or treatment of microbiota	mechanisms	outcomes
Osteoporosis				
Chevalier et al. (57)	Mice	Mucinophilic acromyces	elevating the polyamine levels	preventing the ovariectomy-induced bone loss
Sjögren et al. (55)	Mice	Deficiency of gut Microbiota	increasing osteoclasts and osteolytic cytokines	promoting bone mineral density in the distal femur
Wang et al. (54)	Human	Blautia	chronic inflammation	inducing bone loss
		Parabacteroides	chronic inflammation	inducing bone loss
		Lachnoclostridium	chronic inflammation	inducing bone loss
		Klebsiella	chronic inflammation	inducing bone loss
		Ruminococcaceae	/	maintaining bone mass
		Prevotella	/	maintaining bone mass
Fragility fracture repair				
Roberts et al. (61)	Mice	Bifidobacterium longum	preserving the integrity of the intestinal barrier	Promoting bone healing
Liu et al. (62)	mice	Akkermansia muciniphila	decreasing inflammation	Promoting bone healing
osteoarthritis				
Lei et al (67)	Human	Cholera species	Increasing inflammation	Promoting OA
		Rousselotiella spp	Decreasing inflammation	Demoting OA
Schott et al. (66)	Rat	bifidobacteria	Decreasing inflammation	Demoting OA
Weng et al. (64)	Mice	lactic acid bacteria	preserve the integrity of the intestinal barrier	Demoting OA
Lei et al (67)	Human	probioticsLactobacillus casei Shirota	/	ameliorating OA
		Streptococcus thermophilus	/	ameliorating OA
Morimoto T (72)	Rat	Clostridium butyricum	Decreasing inflammation	demoting OA
Degeneration disease				
Rajasekaran et al. (70)	Human	Firmicutes	immuno-protection	demoting IDD
		Actinobacteria	immune-protection	demoting IDD
		Proteobacteria	immune-protection	demoting IDD
		Bdellovibrio	preying gram-negative bacteria	demoting IDD
		gram-negative bacteria	releasing LPS and inducing inflammation	promoting IDD
		Prevotella	increasing inflammation	promoting IDD

response in the senescent microenvironment or directly promoting the osteogenic process.

Probiotic therapy represents a feasible approach. Supplementation with *Lactobacillus animalis* has been documented to offer benefits in averting osteonecrosis of the femoral head via an extracellular vesicular mechanism (52). *Lactobacillus helveticus* HY7801 has demonstrated prophylactic and therapeutic properties in a murine arthritis model by increasing IL-10 expression in CD4+ T cells (73). Moreover, the administration of *Bifidobacterium longum* was found to suppress post-fracture weight reduction and lumbar spine bone density loss in a model of fractures in elderly female mice (61). The oral administration of *Clostridium butyricum* to a rat model with knee osteoarthritis resulted in a notable decrease in serum inflammation and bone metabolism markers, including COX-2 and IL-6 molecules. Additionally, this treatment demonstrated efficacy in maintaining knee cartilage and synovium integrity, as well as reducing fibrous tissue formation (72).

Fecal microbiota transplantation (FMT) involves transferring functional gut amicrobiota from the feces of a healthy individual into the gastrointestinal tract of a patient to restore normal intestinal function. This approach has demonstrated effectiveness in manageing systemic conditions like multiple sclerosis and cancer (74). While there is currently no research on the use of FMT for degenerative bone diseases, this paper highlights the substantial evidence linking intestinal dysbiosis to these conditions. Therefore, FMT has the potential to restore a healthy gut microbiota and may be a promising strategy for treating degenerative bone diseases.

Hormone therapy involving estrogen supplementation, blunting the adverse effects of GCs. Chronic glucocorticoid treatment triggers the senescence of bone-marrow adipocyte (BMAd) lineage cells and induces bone deterioration (75). A study found that delivering recombinant human ANG (rhANG) in vascular endothelial cells to antagonize cellular senescence can blunt the adverse effects of GCs on the growing skeleton (74). Creating a warm environment is also a good strategy. In *in vivo* experiments with mice, a more uniform distribution of gut microbiota abundance was noted in mice exposed to a temperature of 34°C. This led to a decrease in alterations in the transcriptional pathway related to bone remodeling caused by estrogen deficiency, ultimately leading to a notable amelioration of osteoporosis (57). Photobiomodulation (PBM) refers to a light therapy technique that employs non-ionizing light sources such as lasers, light-emitting diodes (LEDs), and broadband light within the visible and infrared spectrum to elicit local or systemic effects. PBM has the potential to modulate the gut microbiota, which plays a crucial role in disease management (76). In a study by Bicknell et al., the impact of infrared laser treatment (904 nm; 700 Hz pulse frequency, 861.3 total joules) administered to the abdomen of breast cancer patients was assessed. The treatment was conducted

three times a week for 11 weeks. The study revealed an augmentation in the population of beneficial gut bacteria such as *Akkermansia, Faecalibacterium, and Roseburia*, along with a reduction in pathogenic microorganisms (77).

5 Conclusion

This paper examined the fundamental role of the gut microbiota in bone metabolism and the ageing process. It explores the diverse mechanisms through which the gut microbiota influences bone metabolism, consolidates relevant research on the impact of the gut microbiota on conditions such as osteoporosis, fracture healing post-fragility fracture, osteoarthritis, and spinal degenerative diseases. Furthermore, it outlines the connections and current therapeutic strategies for addressing gut microbiota involvement in age-related degenerative skeletal diseases. This paper aims to enhance comprehension of the intestinal-bone axis, improve insight into the pathogenesis of ageing-related bone metabolic disorders, and offer insights for the advancement of therapeutic strategies targeting the gut microbiota. In the realm of therapeutic strategies, the existing approaches predominantly focus on metabolic pathways, exemplified by the use of estrogen supplementation in postmenopausal osteoporosis. Further investigation is warranted to directly target the gut microbiota, potentially influencing the management of bone degenerative conditions. Hormonal therapies have accumulated significant experience; however, further research is needed to address the side effects of estrogenic drugs more effectively. This may involve the exploration of developing estrogen receptor analogs. Warm environment therapy and photobiomodulation offer innovative approaches for addressing degenerative bone diseases. The trend of personalized medicine is increasingly prevalent in healthcare. Sequencing and targeting the individual gut microbiota is a promising approach to fulfill the requirements for personalized medicine. The advancement of additional probiotic medications holds the potential to expand treatment options for degenerative bone conditions. Additionally, fecal transplants represent a promising avenue for personalized therapeutic interventions. The efficacy of Fecal Microbiota Transplantation (FMT) in degenerative bone diseases necessitates further validation through additional experiments. Moreover, careful consideration should be given to the efficacy and safety of FMT therapies. Establishing precise treatment criteria and implementing rigorous screening protocols for donor feces are essential components in this regard.

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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

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Poor bone health in Duchenne muscular dystrophy: a multifactorial problem beyond corticosteroids and loss of ambulation

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Duchenne muscular dystrophy (DMD) is a progressive, fatal muscle wasting disease caused by X-linked mutations in the dystrophin gene. Alongside the characteristic muscle weakness, patients face a myriad of skeletal complications, including osteoporosis/osteopenia, high susceptibility to vertebral and long bone fractures, fat embolism post-fracture, scoliosis, and growth retardation. Those skeletal abnormalities significantly compromise quality of life and are sometimes life-threatening. These issues were traditionally attributed to loss of ambulation and chronic corticosteroid use, but recent investigations have unveiled a more intricate etiology. Factors such as vitamin D deficiency, hormonal imbalances, systemic inflammation, myokine release from dystrophic muscle, and vascular dysfunction are emerging as significant contributors as well. This expanded understanding illuminates the multifaceted pathogenesis underlying skeletal issues in DMD. Present therapeutic options are limited and lack specificity. Advancements in understanding the pathophysiology of bone complications in DMD will offer promising avenues for novel treatment modalities. In this review, we summarize the current understanding of factors contributing to bone problems in DMD and delineate contemporary and prospective multidisciplinary therapeutic approaches.

KEYWORDS

DMD, skeletal abnormality, osteoporosis, myokines, muscle and bone crosstalk

1 Introduction

Duchenne Muscular Dystrophy (DMD) is a debilitating disease characterized by progressive muscle wasting due to X-linked recessive mutations in the dystrophin (*DMD*) gene (1). While the decline in motor function is well-documented, DMD patients face a myriad of other challenges, including intellectual disabilities, depression,

delayed puberty, gastrointestinal complications, and skeletal abnormalities (2). Among these, skeletal issues such as osteoporosis, fragility fractures, scoliosis, short stature, and fracture-associated fat embolism (3) significantly impact patients' quality of life (1, 2).

Poor skeletal health is one of the most overlooked aspects of this disease despite causing considerable morbidity. Low bone mineral density (BMD) (osteoporosis/osteopenia) is prevalent in DMD boys, especially those on glucocorticoid treatment, which poses a significant risk of pathological fractures (2, 4). Up to 60% of patients experience low-trauma extremity fractures (usually the distal femur, tibia, or fibula) by age 15 (5), and vertebral fractures occur in 30% to 60% patients (6). If left untreated, vertebral fractures can lead to chronic back pain and spine deformity, while leg fractures can cause premature, permanent loss of ambulation (2, 7, 8). Death due to fat embolism syndrome after long-bone fractures has also been reported in DMD boys (1, 3, 9). Moreover, reduced growth and short stature are also common (10, 11), further adding to the burden of skeletal issues in DMD.

The cause of poor bone health in DMD is complicated. It was previously thought that corticosteroid use and decreased mechanical stimulus from loss of ambulation (12), both of which are well-known to independently affect bone health (13), are the primary drivers of bone loss in DMD. These factors both substantially contribute to osteoporosis and increased fracture risk but do not completely explain the pathogenesis. Patients still develop osteoporosis and experience fractures without corticosteroid treatment and before loss of ambulation (12, 14). Thus, poor bone health cannot be explained solely by corticosteroid use or loss of ambulation, indicating that there are other mechanisms contributing to these aspects of disease. Indeed, recent evidence suggests that the pathogenesis of poor bone health is far beyond corticosteroid usage and muscle weakness, implicating nutritional deficiencies, hormonal imbalances, systemic inflammation, myokine release from dystrophic muscle, and vascular dysfunction in contributing to the deterioration of bone health to varying degrees, as summarized in Figure 1 (15–18). These factors, to varying degrees, disturb bone homeostasis by preventing bone deposition by osteoblasts and promoting bone resorption by osteoclasts, thus disrupting BMD and bone microarchitecture in DMD patients (18–21). Further understanding of these mechanisms will help us better understand DMD's impact on bone health, identify novel therapeutic targets, develop therapeutic approaches, and better manage this debilitating issue.

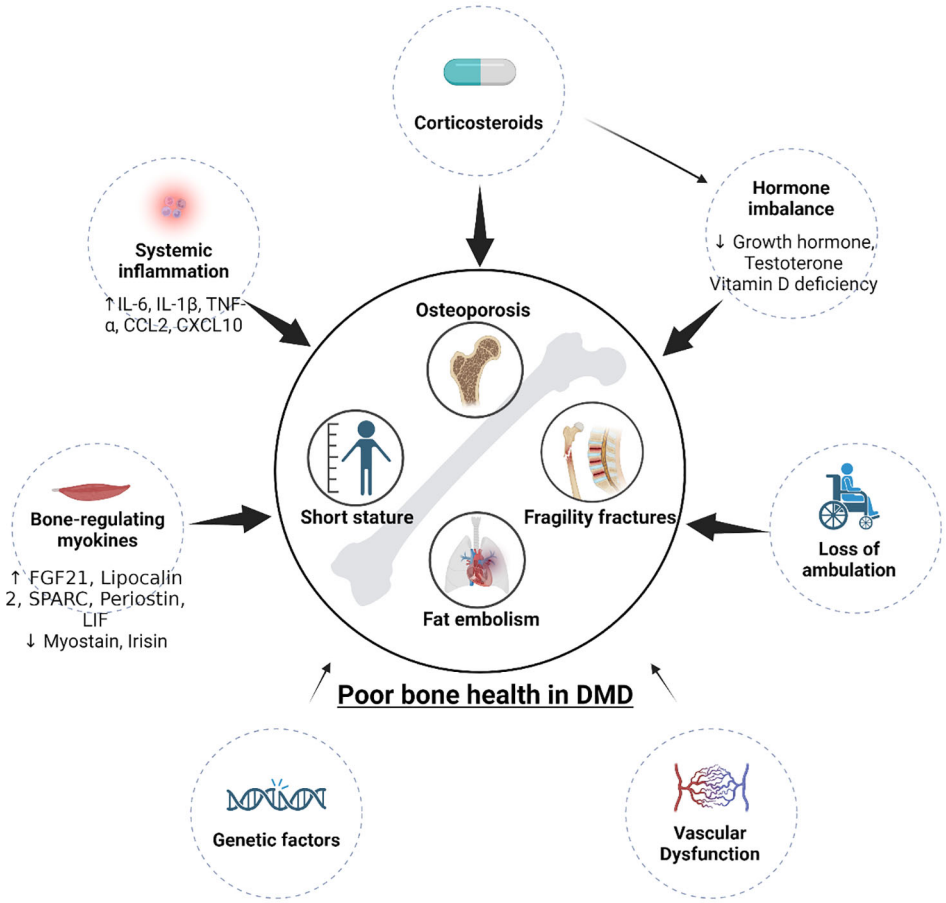

FIGURE 1
Pathogenesis (contributors) of poor bone health in DMD. Multiple factors contribute to short stature and osteoporosis. Corticosteroids, loss of ambulation, vascular dysfunction, hormone imbalance (GH and testosterone deficiencies), systemic inflammation, and dystrophic myokines all contribute to the poor bone health in DMD. Genetic factors that may contribute to poor bone health has not been explored.

We currently lack comprehensive guidelines for managing bone problems in DMD. The DMD Care Considerations Working Group (DCCWG) recommends lateral spine radiographs to detect vertebral fractures every 2-3 years in corticosteroid-naïve patients and every 1-2 years for those treated with corticosteroids (22). However, monitoring of bone mineral density (BMD) via dual energy X-ray absorptiometry (DEXA) scanning is not recommended as routine management since there is no established correlation between BMD and fracture risk for this population (2, 23). Therapeutic intervention in the form of intravenous bisphosphonates is primarily recommended after detection of severe pathological fractures (2). Excitingly, emerging strategies, including physical therapy (PT), innovative bone-sparing corticosteroids, anti-resorptive agents, hormone replacement therapy, and targeted antibodies offer promise for improving bone health in DMD.

Despite the significant impact on patient's lives, skeletal health in DMD remains an understudied area. In this review, we aim to critically analyze existing evidence, providing an up-to-date assessment of risk factors, pathogenic mechanisms, and emerging therapeutic avenues for addressing skeletal issues in DMD.

2 Risk factors and mechanisms of poor bone health in DMD

2.1 Immobilization and reduced mechanical loading

As DMD progresses, patients experience a gradual loss of mobility, often leading to wheelchair use by the second decade of life. Bone health is closely linked to mechanical stimulus (13), with increased loading promoting bone deposition while chronic unloading favors bone resorption (24). As DMD advances, there is diminished force on the skeleton. This is especially pronounced in the transition from the ambulatory to non-ambulatory phase, after which there is a marked decrease in BMD (12). This reduction in mechanical loading has long been considered a primary driver of impaired bone health in DMD patients.

2.2 Corticosteroid side effects

Corticosteroids, namely prednisone and deflazacort, are mainstay therapies in DMD, albeit non-curative. Their use has revolutionized DMD treatment and significantly improved survival rates by delaying the need for mechanical ventilation and preserving cardiac function (6, 25–27). They prevent muscle deterioration, which is thought to be by improving sarcolemma healing and reducing inflammation (27–29). However, their long-term use is associated with significant adverse effects, including osteoporosis, pan-hormonal suppression, mood disturbances, and metabolic dysfunction (6, 30–33). Numerous studies have demonstrated increased fracture risk and growth retardation in corticosteroid-treated patients (5, 25, 30, 32, 34–37), with earlier initiation and longer treatment durations correlating with higher risk (36, 38, 39).

Long-term corticosteroid use is known to cause osteoporosis through both direct and indirect mechanisms. Corticosteroids directly inhibit bone formation and increase bone resorption by acting on osteoblasts and osteoclasts (40, 41), leading to impaired bone microarchitecture and low BMD (19). On average, 6-12% of BMD is lost in the first year of corticosteroid treatment and 3% is lost every year after that (41). Beyond their direct effects on osteoblasts and osteoclasts, corticosteroids impair intestinal calcium absorption (42) and contribute to vitamin deficiency (4, 43), which indirectly affect bone homeostasis.

Corticosteroids also significantly contribute to growth retardation in DMD patients. Although growth retardation occurs in corticosteroid-naïve DMD patients through mechanisms which are not fully understood (10, 44), corticosteroid usage significantly worsens this manifestation of the disease (39, 45–47). Corticosteroids exert direct toxicity to the growth plate by inducing chondrocyte apoptosis (48, 49). They further affect growth plate physiology by interfering with the growth hormone (GH) – insulin-like growth factor-1 (IGF-1) axis. Corticosteroids inhibit endogenous GH production (42, 50) and antagonize the peripheral action of GH and IGF-1 (51). Furthermore, GH and testosterone act synergistically to promote bone growth during puberty (52). Corticosteroid-induced hypogonadotropic hypogonadism and consequent testosterone deficiency further impair the partnership of the GH-IGF-1 axis and testosterone, which substantially contributes to corticosteroid-induced short stature if used before puberty (47, 52).

2.3 Vitamin D deficiency

Studies have shown that up to 84% of DMD patients have a documented vitamin D deficiency at some point, even with supplementation (5, 38). Multiple aspects of disease may contribute to this phenomenon. Loss of ambulation is linked to lower vitamin D levels, possibly due to reduced sunlight exposure (14). Corticosteroids may be a major contributor to vitamin D deficiency and resistance to supplementation by increasing breakdown (4, 43). DMD patients also have elevated fat mass, which increases vitamin D distribution into fat and reduces its availability (53–55). Additionally, lower serum vitamin D binding protein levels were observed in DMD patients, which reduces vitamin D stores, further explaining resistance to supplementation (56, 57). Despite a high prevalence of vitamin D deficiency in DMD patients, the relative impact of vitamin D supplementation on bone health in this patient population is still elusive. Retrospective and cohort studies have not consistently correlated supplementation with improved BMD or reduced fracture risk (5, 16), questioning the importance of vitamin D deficiency as a contributor to poor bone health in DMD. However, resistance to supplementation demonstrates a need for more effective strategies to reach adequate vitamin D levels in this population.

2.4 Systemic inflammation

Muscle degeneration and systemic inflammation are characteristic features of DMD (58, 59), leading to increased circulating levels of pro-inflammatory cytokines, which in turn negatively impact bone metabolism (60). Elevated levels of inflammatory cytokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNF-α), chemokine ligand 2 (CCL2), and CXC motif chemokine ligand 10 (CXCL10) have been reported in DMD animal models and patients, which may contribute to bone loss in DMD (summarized in Table 1) (59, 61, 62). IL-1β, IL-6, and TNF-α all have been shown to increase bone resorption by stimulating osteoclast activity and differentiation (63). Likewise, CCL2 and CXCL10 also affect osteoclastogenesis and increase bone resorption (64–66). So far, the effects of these cytokines on bone metabolism have been mostly studied in inflammatory, autoimmune and cancer conditions. Only IL-6 has been directly studied in DMD, showing increased osteoclast formation in *ex vivo* murine calvaria bone cultures treated with mdx mouse serum, which was reduced following treatment with IL-6 neutralization antibody (7).

TABLE 1 Cytokines and myokines involved in bone loss in DMD.

Cytokine/ myokine	Effects on bone	Effects on muscle	Levels in DMD	Benefit of inhibition in DMD
IL-6	Increases osteoclast differentiation and osteoblast differentiation, but with a net resorptive effect leading to osteoporosis (63, 67, 102)	Muscle degeneration, inflammation, exhaustion of stem cells, fibrosis, adipogenesis (103)	Increase (7, 59, 62, 104, 105)	Mdx mice treated with anti-IL-6 had increased muscle diameter, decreased fibrosis, lower serum CK. No effects on diaphragm or heart (104). Calvaria explants treated with DMD patient serum had attenuated bone resorption when treated with anti-IL-6 (7)
TNF-α	Suppresses bone formation, induces resorption (102)	Frequently induces atrophy, but is involved in muscle regeneration in certain contexts (106)	Increase (59, 62, 105)	Inhibition reduces muscle damage and degeneration (107, 108) Reduces fibrosis but decreases ejection fraction and left ventricle thickness (109)
IL-1β	Suppresses bone formation, induces resorption (102)	Induces skeletal muscle catabolism (110)	Increase (59, 62, 105)	IL-1Ra inhibition in mdx mice improved forelimb grip strength but did not affect EDL function (111)
CCL2, CXCL10	Induces resorption (64–66)	CCL2 stimulates regeneration at low concentrations, but inhibits regeneration at too high of concentrations (112, 113) CXCL10 increases myogenic differentiation *in vitro* but has no effect *in vivo* (114)	Increase (61, 62, 105)	CCL2 inhibition reduced diaphragm inflammation (115) CXCL10 has not been studied.
RANKL	Elevated RANKL induces bone resorption (63, 116)	Implicated in pathogenesis of hypertrophic cardiomyopathy, inhibition improves muscle function in a variety of disease states (117)	Decrease (73, 118)	Improves cardiac function (119) Improves skeletal muscle function (72) Treats osteoporosis (72, 120, 121) in dystrophic mice and DMD patients
FGF21	Likely promotes resorption (76, 79, 122, 123)	Promotes muscle atrophy (75)	Increase (62, 77)	Inhibition improves osteoporosis (77)
Myostatin	Induces osteoporosis (67, 87, 89, 124)	Negatively regulates muscle fiber number and size (84)	Decrease (62)	Improved muscle strength, BMD in mdx mice (88, 125–128) Trend improvement in muscle and bone in humans, trial prematurely ended due to adverse effects (94, 129)
Irisin	Increases bone growth, BMD (96)	Promotes hypertrophy, prevents atrophy (97)	Decrease (62)	Supplementation improved muscle strength, mass in mdx mice (130)
Lipocalin 2	Inhibits linear growth, inhibits bone deposition, and induces bone resorption (101)	Inhibits myogenesis (131)	Increase (62, 100)	Increased trabecular volume, improved grip strength, reduced diaphragm fibrosis (100)
SPARC, periostin	Thought to be pro-osteogenic (132–134), but effect as a myokine has not been evaluated	SPARC: unclear, may be pro- or anti-regenerative, may be dependent on context, alters metabolism (135–137) Periostin may inhibit myogenesis, promote fibrosis (138, 139)	Increase (62)	Has not been studied
Other elevated myokines – LIF, OSM	Overexpression leads to osteopetrosis, knockout leads to smaller bones (140)	Promotes regeneration after damage (141)	Increase (62)	May improve dystrophic phenotype by improving repair, preventing fibrosis (142–144)

Description of what is known about effect on bone, muscle, and effect of supplementation or inhibition on DMD, if studied. All studies are preclinical animal studies unless otherwise specified.

2.5 Myokines

Recent studies have highlighted that myokines secreted from skeletal muscle exert regulatory effects on bone metabolism (67, 68). While the precise composition of myokines from dystrophic muscle remains to be fully elucidated, several myokines have been implicated in modulating bone turnover in DMD, including receptor activator of nuclear factor κ B ligand (RANKL), fibroblast growth factor (FGF) 21, myostatin, lipocalin 2, and irisin (62). Understanding the roles of these myokines in DMD-associated osteoporosis is essential for exploring them as potential therapeutic targets. The current understanding of the roles of those myokines is summarized in Table 1.

2.5.1 RANKL

RANKL, in conjunction with its membrane receptor RANK and the soluble decoy receptor osteoprotegerin (OPG), plays a pivotal role in osteoclast differentiation and bone remodeling (69). Notably, the expression of RANK, RANKL, and OPG is not confined to bone; all three are also expressed in muscle (70, 71). Skeletal muscle in dystrophic mouse models has been shown to exhibit elevated RANKL expression (62). While the precise function of this axis in skeletal muscle remains elusive, systemic neutralization of RANKL in dystrophic mice improved both skeletal muscle function (70) and bone strength in mdx mice (21, 72), which suggests dual function of RANKL on skeletal muscle and bone. Despite one study finding paradoxically lower levels of RANKL, OPG, and RANKL/OPG ratio in patients compared to healthy controls (73), the concept that the RANKL/RANK/OPG system may interconnect bone and muscle in DMD warrants further investigation.

2.5.2 FGF21

FGF21 is a member of the endocrine FGF19 subfamily, an atypical group of FGFs secreted into systemic circulation, acting as endocrine hormones (74, 75). While FGF21 is primarily expressed by the liver and adipose tissue, it can also be secreted in large quantities from muscle tissue under pathological conditions, including mitochondrial dysfunction and muscular dystrophy (62, 76–78). In dystrophic mice, considerable amounts of FGF21 are expressed in muscle tissue and contribute to the elevated circulating levels of FGF21 (62). Blockade of FGF21 via neutralizing antibodies has shown promise in improving bone phenotype in a severe DMD mouse model (77). The impact of FGF21 on musculoskeletal system is largely unknown, with emerging evidence implicating that it may play important roles in homeostasis of both skeletal muscle and bone [as reviewed recently in (75)]. Although not fully understood, it is likely that FGF21 regulates bone homeostasis via multiple direct and indirect mechanisms. Recent studies have demonstrated that bone is a direct target of FGF21 since osteoclasts, bone marrow adipocytes, and mesenchymal stem cells express the obligate FGF21 co-receptor β-klotho (77, 79). Furthermore, FGF21 can interact with the GH/IGF-1 signaling pathway (80), which plays a crucial role in protein synthesis and bone homeostasis (81). FGF21 inhibits the action of GH/IFG-1 on proliferation and differentiation of chondrocytes directly at the growth plate (82, 83), potentially implicating its pathological role in growth retardation as well.

2.5.3 Myostatin

Myostatin, also known as growth differentiation factor 8 (GDF-8), is a member of the transforming growth factor-beta (TGF-β) superfamily and is known for its inhibitory effects on both muscle and bone growth (84–86). Myostatin acts as a catabolic stimulus, resulting in muscle fiber atrophy (17). In addition to its anti-myogenic effects, it negatively regulates bone homeostasis by inducing bone resorption (85, 87) by promoting osteoclastogenesis and inhibiting osteogenesis (86, 88). Notably, the effect of myostatin on bone loss are potentiated in the context of decreased mechanical loading (87, 89, 90). Despite downregulation of myostatin in mdx mice (62) and in DMD patients (91–93), myostatin inhibition has long been considered a promising intervention to reverse pathology in muscle-wasting diseases, including DMD (94). However, there has been a failure to translate positive results of myostatin inhibition in animal models to humans (94), which will be discussed in the treatment section (Section 3).

2.5.4 Irisin

Irisin, an exercise-induced myokine, is a cleaved product of secreted fibronectin type II domain containing 5 (FNDC5) (95). Unlike myostatin, irisin serves as an anabolic regulator, influencing both muscle growth and bone health (96, 97). However, the expression levels of irisin in dystrophic muscle, particularly in DMD, remains unclear. While one study reported mild decreases in serum irisin levels in Becker muscular dystrophy (98), others have found increased levels in exercised dystrophic mice (99). Another study noted reduced *Fndc5* mRNA levels in skeletal muscle of dystrophic mice, but increased protein levels, indicating intricate posttranslational regulation of FNDC5/irisin in DMD (62). The function of irisin in regulating muscle and bone homeostasis in DMD is still unknown.

2.5.5 Lipocalin 2

Originally identified as an adipokine, elevated levels of lipocalin 2 have been reported in the muscles of mdx mice (100). Transgenic mice overexpressing lipocalin exhibit decreased BMD and short stature due to growth plate abnormalities (101), suggesting a potential role for lipocalin 2 in the development of osteoporosis and short stature in DMD. Inhibition of lipocalin in mdx mice via either *Lcn2* knockout or the administration of lipocalin monoclonal antibody has shown increased trabecular volume, enhanced grip strength, and decreased diaphragm fibrosis (100), indicating that it may play an important role in both skeletal muscle and bone pathologies in DMD.

2.5.6 Other myokines

A plethora of other bone-regulating myokines have been identified as upregulated in dystrophic muscle. In addition to the previously mentioned myokines, leukemia inhibiting factor (LIF), secreted protein acidic and rich in cysteine (SPARC), and periostin have been found to be elevated at both the transcript and protein levels (62). However, their roles in regulating bone homeostasis in DMD or other conditions have not been studied.

Together, these findings collectively suggest that altered levels of multiple potent myokines contribute to the pathogenesis of DMD-associated bone loss. Notably, increased/decreased expression of both pro- and anti-osteogenic factors indicate a complex interplay within the skeletal muscle and bone microenvironment. Despite the complexity, the net effect of these factors is bone loss in DMD. Further research is warranted to elucidate the exact effects, underlying mechanisms, and relative contributions of these myokines, as well as to validate their clinical relevance. Understanding the interplay between these myokines and their impact on muscle function and bone health is crucial for developing targeted therapeutic interventions for DMD.

2.6 Vascular dysfunction

Bone is intricately connected to vascular function throughout its development, growth, maintenance, and healing processes (145). Vasculature is essential for adequate delivery of oxygen and nutrients, thus shaping the metabolic environment. Additionally, significant cross-talk exists between vascular endothelial cells and adjacent bone cells, with mutual influence on osteogenesis, bone resorption, and angiogenesis (145). Notably, impaired angiogenesis has been associated with osteoporosis (145).

Vascular dysfunction in skeletal muscle is well-documented in the context of DMD. Dystrophin, known for its structural role in cytoskeletal stabilization, binds to neuronal nitric oxide synthase (nNOS) in skeletal muscle fibers, facilitating nitric oxide (NO) synthesis for vasorelaxation (146). In DMD, the absence of dystrophin results in the loss of nNOS from the sarcolemma and its significant downregulation in the cytoplasm, leading to functional ischemia contributing to muscle damage and impaired regeneration (147). Dystrophic muscles exhibit not only vascular dysfunction, but also low vessel density and impaired angiogenesis (148–152). In addition to skeletal muscle fibers, smooth muscle cells (152–154) and endothelial cells (148, 150) also express full-length dystrophin, suggesting its crucial role in regulating vasodilation, vasocontraction, and angiogenesis [refer to recent review (155)]. However, little is known regarding the functional dysregulation of dystrophin expression out of skeletal muscle fibers in DMD. Furthermore, no studies have directly evaluated vasculature in dystrophic bone, even though it is reasonable to hypothesize that dystrophin mutations may affect bone vasculature via NOS. One study evaluating fracture healing in mdx mice observed decreased vessel density in early repair stages, correlating with delayed healing (18). The study also noted abnormally high inflammation after fracture (18), which brings to question whether impaired fracture healing was due to angiogenic dysfunction, excessive inflammation, or both. The question of whether bone exhibits vascular anomalies in DMD remains unanswered. A deeper understanding of the role of angiogenesis, vascular function, and $NO^{\bullet}$ signaling in DMD pathogenesis in both muscle and bone may unveil potential targets for enhancing muscular, cardiovascular, and bone health.

3 Treatments: current and future

Current DMD treatment is negligent to bone health. While vitamin D is often used to prevent osteoporosis, most patients still experience osteoporosis and pathological fractures (5). Bisphosphonate use is typically reserved for use following pathological fractures. Disappointing statistics regarding rates of fracture, especially those which lead to loss of ambulation, indicate that current management of bone health is inadequate in DMD patients. Furthermore, patient age is important to consider in managing bone health in DMD. In pediatric patients, growth retardation is of great concern, while in adult patients, osteoporosis and pathological fracture are of most concern. Optimal timing of therapeutic intervention requires more research.

The intricate relationship between muscle and bone along with the impact of long-term corticosteroid usage necessitate an integrated, multi-factorial approach to the management of DMD bone issues. Combining strategies to address multiple aspects of disease while minimizing off-target effects of current treatment hold the most promise. As the field gains more understanding of disease pathogenesis, more effective and targeted therapeutic avenues can be explored. Below, we will discuss potential therapeutics at varying states of investigation, which are also summarized in Table 2. As research continues to advance, a more comprehensive toolkit of interventions is expected to emerge.

3.1 Monitoring bone health and early detection of fractures

Due to the high incidence of bone issues in DMD patients, frequent monitoring of bone health in this patient population is important for early detection and intervention. Vertebral fracture affects 30-60% of patients, many of which are asymptomatic (2). Current guidelines emphasize early fracture detection, and thus recommend routine lateral spine radiographs at an interval of every 1-2 years in corticosteroid-treated patients and every 2-3 years in those who are corticosteroid-naïve (2). DEXA scans may be used as an adjacent method of monitoring BMD but are not of particular emphasis since there is no established cutoff for what may warrant intervention (1, 8). Additionally, BMD Z-score may be underestimated in DMD patients since they often have a younger bone age than chronological age, which could alter estimation of fracture risk (23). Early detection of fracture, however, does not prevent fracture. Further research should be done to correlate BMD with fracture risk to establish guidelines for when to start prophylactic treatment.

3.2 Physical therapy

Current guidelines published by the DCCWG recommend PT as an integral part of DMD management (22). However, there is no consensus on protocols, leading to inconsistent recommendations

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TABLE 2 Treatments for bone comorbidities in DMD.

Conservative Treatments			
Treatment	**Evidence for**	**Limitations**	**References**
PT: strength training	Only exercise validated for muscle growth in other populations	Concern for exercise-induced muscle damage, ischemia, poor regeneration, negative cardiovascular effects	(13, 161–166)
PT: Aerobic exercise	Well-tolerated by DMD patients, may prolong walking	Unclear if there are any positive effects on bone	(99, 231, 232)
PT: Low-intensity vibration	Attenuates muscle and bone loss	None known	(170–172)
PT: Cyclic electrical muscle stimulation	Promising results in other immobilization conditions	Potentiated bone loss in a dystrophic mouse model	(168, 173)
PT: Assisted standing		Lacked efficacy in a small trial	(160)
Vitamin D & calcium Supplementation	Improves BMD	Unclear if vitamin D reduces fracture risk	(5, 16, 202)
Pharmacological Treatments			
Corticosteroids: Intermittent versus daily dosing	Believed to prevent adverse effects	Not as effective as daily dosing at preventing disease progression, unclear if it prevents bone loss	(31, 178–180)
*Vamorolone	No growth suppression, equal efficacy to prednisone, no effect on bone turnover markers	No direct evaluation of BMD or fracture risk in clinical trials	(182–186, 188, 233)
Bisphosphonates	Increases BMD	No strong evidence demonstrating increased fracture risk, concern for adverse effects (e.g. acute phase reaction, hypocalcemia, hypophosphatemia, adrenal insufficiency, decreased bone turnover)	(15, 34, 191, 193–197)
Denosumab	Increases BMD in patients, increases muscle strength and cardiovascular function in mice	Difficult to stop without rebound bone loss	(21, 72, 119–121, 199, 201)
GH/IGF-1 supplementation	Increases growth velocity, may be more effective in conjunction with testosterone and/or bisphosphonates	No improvement of muscle function, no effect on BMD	(203–207)
Testosterone	Preserves muscle mass, increases growth velocity, prevents bone loss, induces puberty	Benefits do not persist past termination of treatment	(212–216)
*Teriparatide/PTH	More effective than bisphosphonates at improving BMD, anabolic agent, decreases fracture risk	Not recommended before puberty	(218–220, 222)
Preclinical therapies			
Tocilizumab (anti-IL-6)	Prevented bone loss *in vitro*, prevents muscle degeneration in mice	Bone-protective effects have yet to be studied *in vivo*	(7, 63, 104)
Anakinra (IL-1R antagonist)	Attenuates bone loss in autoimmune conditions, improves forelimb strength in mice	Bone-protective effects have yet to be studied in DMD	(63, 111, 226)
Adalimumab (anti-TNF-α)	Attenuates bone loss in autoimmune conditions, decreases muscle degeneration in mice	May be detrimental to cardiovascular health, effect on bone has yet to be studied in DMD	(63, 107, 109, 226)
Anti-FGF21	Improves BMD in mice	Unknown effect on muscle, unknown adverse effects	(77)
Anti-Lipocalin 2	Improves bone, increases strength, decreases diaphragm degeneration	Unknown adverse effects	(100, 101)
Irisin supplementation	Increases muscle mass and strength, decreases degeneration in DMD models, increases bone growth and BMD in other mouse models	Unknown effect on bone in DMD	(96, 130)
LIF supplementation	Increases muscle repair, decreases fibrosis in DMD models, overexpression mice have increased bone deposition	Unknown adverse effects, unknown effect on bone in DMD	(142–144)
*Myostatin inhibition	Increases muscle mass, strength, and decreases fibrosis, increases bone mass and strength in mice	Not effective in clinical trials	(88, 94, 125–128, 229, 230)

Conservative treatments, pharmacological treatments, and treatments under investigation at the preclinical or clinical stage and their outcomes and limitations. *Indicates under investigation in clinical trials, italics indicates under investigation at the pre-clinical stage.

between physicians and physical therapists based solely on provider experience and anecdotal evidence (156). Moreover, the current focus of PT is to improve or maintain muscle function and is not focused on improving bone health. Currently, only low-intensity and aerobic exercise are recommended (156–158). Investigation into modalities such as low-intensity vibration and assisted standing have been explored with some benefit but are not yet standard of care (159, 160). All of them show some benefit to maintain muscle function in clinical studies [A more detailed summary of these studies can be found in a recent review by Spaulding and Selsby (157)]. Unfortunately, no studies have evaluated any type of exercise for improving bone health in DMD patients (156).

When discussing PT to improve bone health in healthy populations, high-load resistance training or repetitive high-impact movements are optimal to increase muscle mass and increase BMD (13). However, for DMD patients, there are several concerns, including exercise-induced muscle damage, ischemia, regenerative ability of muscle, and effects on cardiovascular function. It is recommended to provide sub-maximal loading to reduce the risk of muscle damage (156). However, the scientific basis of this recommendation is not definitive. Numerous studies have demonstrated that dystrophic muscle is more prone to damage following exercise than healthy muscle (161–163), and satellite cells in dystrophic muscle have impaired regenerative capacity (164–166). However, some studies also indicate that dystrophic muscle has a high functional regenerative capacity and recovers function well after contractile injury (162, 167), indicating that higher intense exercise may not lead to a functional deficiency as we thought. There is certainly more to investigate regarding exercise regimens and regenerative capacity of dystrophic muscle and how that may affect bone.

In addition to exercise, some modalities, such as low-intensity vibration, assisted standing, repetitive electrical muscle stimulation, and high-dose compressive loading, have shown benefits in other muscle-wasting and immobility related bone conditions (168, 169). Of these, electrical muscle stimulation and low-intensity vibration can also attenuate muscle loss (170–172), thus potentially addressing both muscle and bone problems in DMD. So far, electrical muscle stimulation has been evaluated in mice, while low-intensity vibration and assisted standing have been evaluated in humans. One study found that electrical muscle stimulation, which provides cyclic loading, worsened bone loss in mdx mice, potentially by increasing release of harmful myokines (173). This has not been evaluated in humans with DMD, so it is unclear if this result would hold. In patients, assisted standing, meant to return body-weight stimulus to bone, did not prevent bone loss in four wheelchair-bound DMD patients (160). Low-intensity vibration is FDA-approved to treat osteoporosis in adults and is thought to increase BMD by stimulating osteocytes and mesenchymal stem cells, although the exact mechanism is unclear (170, 171). It has also shown some efficacy in increasing BMD in DMD patients and in other pediatric populations and is generally well-tolerated (13, 159, 174), warranting further exploration of this modality.

The optimal PT protocols to promote maintenance or gain of muscle function and bone still require much more mechanistic and clinical exploration. The complexities of muscle damage and regeneration, its crosstalk with bone, differences in the response of muscle and bone to different types of exercise, and potential confounders of vascular dysfunction, leave many unanswered questions. The optimal protocol may not be intuitive, and likely should include additional modalities targeting bone.

3.3 Optimal corticosteroid treatment and alternatives

Corticosteroids play a pivotal role in preserving muscle function by mitigating inflammation; however, their usage is accompanied by a range of adverse effects, such as excessive weight gain, adrenal insufficiency, behavioral changes, Cushing's syndrome, stunted growth, and osteoporosis. The optimization of corticosteroid administration including the choice of agent, dosing, timing, and frequency remains a dynamic area of research in the field, as recently reviewed by Biggar et al. (175).

Intermittent dosing of corticosteroids has been explored as an alternative to daily administration with hopes of mitigating side effects while maintaining efficacy. However, results have been inconsistent. Studies have found that intermittent dosing is at least better than placebo (176, 177), but it is not as effective at slowing disease progression as daily dosing (31, 178–180). One RCT of weekend versus daily dosing found a statistically significant increase in growth and BMD in the weekend group compared to the daily group, but patients' heights and BMD were not statistically significantly different at the end of the study (179). Despite this being a theoretically appealing option, evidence suggests that intermittent dosing is inferior for disease progression and does not substantially reduce the risk of bone loss.

Ongoing efforts in treatment development focus on generating more selective corticosteroids to balance efficacy and side effects. Vamorolone is a synthetic corticosteroid which is more selective for the glucocorticoid receptor while antagonizing the mineralocorticoid receptor, which should theoretically reduce side effects, especially bone-related side effects (181). In preclinical studies, vamorolone improved skeletal muscle repair with minimal impact on hormonal regulation, growth, and immune suppression (181–183). Phase III clinical trials have shown comparable or superior efficacy to prednisone in retaining muscle function while preventing growth suppression (184–186). Vamorolone's potential bone-sparing properties are also noteworthy, as evidenced by its neglectable effects on bone growth and trabecular thickness in mice (181, 187) and decreased markers of bone turnover in patients (186, 188). However, further clinical investigations are required to ascertain whether it is truly less detrimental to bone than prednisone.

3.4 Anti-resorptive therapy

Anti-resorptive therapies, such as bisphosphonates and denosumab, are widely used for osteoporosis treatment (189, 190). There is currently no consensus on the use of antiresorptive therapies to manage osteoporosis in DMD, but bisphosphonates are commonly used following severe fracture (2, 191). Bisphosphonates inhibit osteoclast attachment to the bone surface and induce cell apoptosis

(190), while denosumab, a monoclonal antibody targeting RANKL, prevents osteoclast formation (192).

DCCWG recommends intravenous bisphosphonate treatment following a pathological fracture (vertebral Genant grade 2 or 3 or low-trauma long bone fracture) (2). However, it is still unclear whether bisphosphonates are beneficial as a prophylactic treatment. Landfeldt et al. recently published a systematic review on bisphosphonate use in corticosteroid-treated DMD patients (191). Notably, there is good quality evidence that bisphosphonate use increases BMD in DMD patients, but it is unclear if this translates to a lower fracture risk (15, 34, 191, 193, 194). Unfortunately, the rate of adverse effects in DMD patients is high, with acute phase reaction, hypocalcemia and hypophosphatemia, and precipitation of adrenal insufficiency of particular concern (191, 195). Of further concern, histomorphometric analyses indicate that bisphosphonates globally decrease bone turnover (1, 196, 197), which is problematic during bone development (198). Currently, bisphosphonates are one of the few options for treating osteoporosis but may not be the best option for young DMD patients.

Denosumab has also been used in pediatric populations (189) and has shown promising results in DMD. Case reports demonstrate improved BMD without notable side effects, indicating its relative effectiveness and tolerability in children (120, 121, 189). Notably, denosumab and other anti-RANKL therapies may also improve muscle strength and cardiac function, as reported in mouse models of DMD (21, 72, 119, 199). One consideration when starting denosumab treatment is that it cannot be stopped abruptly; its effects on BMD decline rapidly following termination of treatment (72, 200, 201). This is easily remedied by a short course of bisphosphonates (72, 200, 201). Given the favorable outcomes observed in both muscle and bone in DMD patients and animal models, further investigation into the use of denosumab for this population is warranted.

3.4 Vitamin D and calcium supplementation

Calcium and vitamin D supplementation are strongly recommended for DMD patients (22). DMD patients are particularly susceptible to vitamin D deficiency because of corticosteroid use, obesity, and low levels of vitamin D binding protein, meaning supplementation needs to be aggressive (2000 IU/day) to reach adequate levels in most patients (53). Cohort studies do not find an association between vitamin D supplementation and fracture risk, but an RCT found that adequate supplementation improved BMD (5, 16, 202). Although vitamin D supplementation alone does not fully prevent osteoporosis in DMD (5), due to its simplicity, low risk, and possible benefit, vitamin D supplementation should be a pillar of maintaining bone health in DMD patients.

3.5 Hormone therapy

Endocrine management is an important aspect of DMD treatment given the hormonal imbalances induced by corticosteroids. GH and testosterone therapy have been investigated to restore hormonal levels to normal ranges and promote linear growth. Additionally, to improve bone health, PTH therapy via teriparatide, a PTH analogue, has been explored as a strategy specifically to improve bone quality and prevent osteoporosis.

The role of GH in bone problems in DMD has been extensively studied, particularly due to growth retardation observed in DMD patients. Although there is no clear evidence of GH dysregulation in DMD patients who are not treated with corticosteroids (44), corticosteroid use is well-known to impair the GH axis and GH secretion (42, 51). Both GH supplementation and inhibition have been tested in DMD patients, but yielded controversial and inconclusive results. Supplementation, either via recombinant human GH (rhGH) or IGF-1, improved in growth velocity but failed to demonstrate motor function improvement (203–205). Moreover, there has been no documented improvement in BMD with GH or IGF-1 treatment in mdx mice or patients, possibly due to the short duration of these trials. GH secretagogues have been explored in mice, including ghrelin and the synthetic secretagogues EP80317 and JMV2894. Both studies demonstrated decreased muscle fibrosis and inflammation, and increased muscle strength (206, 207), but neither study evaluated the bone phenotype in these mice. Conversely, the idea that GH inhibition might be beneficial stemmed from the observation that a patient with DMD and GH deficiency had no clinical evidence of muscular weakness before initiation of GH replacement therapy; and treatment with human GH resulted in appearance of symptoms of easy fatigability and proximal muscle weakness, which suggest that increased growth may worsen muscle function in DMD (208). However, clinical trials investigating mazindol, a postulated inhibitor of growth hormone release, have shown inconsistent effects on growth suppression and no benefits on muscle strength (209, 210). In light of limited data and ongoing controversy, routine use of rhGH or secretagogues in DMD population is not recommended. Any decision to use rhGH should be made on a case-by-case assessment with biochemical evidence of growth hormone deficiency, weighing the risks and benefits.

Delayed or absent pubertal development in DMD patients on glucocorticoids is common due to hypogonadotropic hypogonadism (211). Testosterone therapy is recommended for pubertal induction in these individuals, typically initiated by age 14 (22, 212–214). Clinical trials have shown that testosterone therapy not only induces puberty but also preserves muscle mass, improves growth velocity, and prevents bone deterioration (212–216). However, these benefits are observed only during treatment. A recent follow up study on patients that were treated with testosterone for 2 years demonstrated persistent lower testosterone levels and testicular volumes than adult reference values; muscle functional volume increased during the intervention but declined in the years after cessation of supplementation (214). Given the known additional advantages of testosterone on bone health, muscle, and well-being (217), the benefits of restoring testosterone to normal physiologic levels are deemed to outweigh potential risks. Nevertheless, further research is warranted to elucidate the optimal timing, duration, and regimen

for testosterone supplementation in DMD patients and its potential impact on bone health.

PTH administration in DMD mouse models improved or maintained BMD in several studies (218–220). Research on corticosteroid-induced bone loss suggests that PTH may be more effective than bisphosphonates (221). Although its safety and efficacy in improving bone health in DMD still need to be validated in clinical trials, a small cohort treated with teriparatide, a PTH analogue, demonstrated decreased fracture risk (218, 222).

As previously mentioned, multiple hormonal pathways are affected in DMD. This complexity prompted an investigation to evaluate the efficacy of hGH, testosterone, and zolendronic acid (ZA, a bisphosphonate) in various combinations after vertebral fractures (15). The study revealed that hGH and testosterone, either alone or in conjunction with ZA, significantly delayed the occurrence of subsequent vertebral fractures compared to ZA treatment alone. Moreover, multivariate analysis demonstrated that hGH was the greatest contributor in enhancing this protective effect (15). This study highlights the intricate hormonal imbalances contributing to osteoporosis in DMD patients, which point out the need for a holistic management strategy that targets these hormonal disruptions.

3.6 Novel anti-inflammatory and immunomodulatory agents

In addition to corticosteroids, monoclonal antibodies targeting specific immune modulators are increasingly used to treat inflammatory conditions. Tocilizumab, an anti-IL-6 receptor monoclonal antibody, is used to treat certain autoimmune conditions and may attenuate bone loss (223–225). *Ex vivo* and *in vitro* experiments found that tocilizumab rescued the anti-osteogenic/pro-osteoclastogenic effect induced by DMD patient serum (7). In mdx mice, tocilizumab treatment improved skeletal muscle phenotype by improving muscle diameter, reducing fibrosis, and decreasing serum CK, suggesting it has promise for altering disease progression (104). Although no studies have evaluated its effect on bone *in vivo*, this may be a promising avenue to treat muscle and bone degeneration. Anti-IL-1 receptor antagonist anakinra and anti-TNF-α monoclonal antibody adalimumab are also used to treat autoimmune diseases and may also attenuate bone loss (63, 226). Neither has been tested for effects on bone in DMD models, but they have been studied for their effects on muscle. Anti-TNF-α treatment decreased muscle damage, degeneration, and fibrosis in mdx mice, but appeared detrimental to cardiac function (107, 109). Anti-IL-1R treatment in mdx mice improved forelimb strength (111). The effects of these treatments on disease progression and osteoporosis in DMD requires more investigation, but thus far tocilizumab appears to be most promising.

3.7 Novel therapies targeting myokines

Myokines secreted from dystrophic muscle significantly contribute to the development of bone abnormalities in DMD. Targeting these myokines holds considerable promise for mitigating dystrophic bone loss. Of the myokines discussed, RANKL, FGF21, myostatin, irisin, and lipocalin 2 have been studied in mouse models. Only myostatin inhibition has been translated to the clinic.

In preclinical studies, inhibition of FGF21 or lipocalin 2, and supplementation of irisin or LIF have been studied with some promising results. Inhibition of FGF21 via neutralizing antibody increased BMD in a severe DMD mouse model by decreasing osteoclastogenesis (77). The effect of FGF21 inhibition on dystrophic muscle has yet to be determined. Lipocalin 2 has been studied in dystrophic mice by neutralizing antibody and global knockout of *lcn2*, which resulted in increased trabecular volume, grip strength, and reduced diaphragm fibrosis (100). A study by *Reza* et al. (130) revealed that recombinant irisin treatment increased muscle mass and strength in addition to reducing muscle fibrosis and necrosis, although its effects on bone are unknown. Several studies have also evaluated LIF supplementation, which improved dystrophic phenotype by improving repair and preventing fibrosis (142–144), but given its role in promoting solid tumor progression, further research should be cautious of potential oncogenic effects (227). With the popularity of monoclonal antibody treatments, inhibition of FGF21 and lipocalin 2 could be promising therapeutic targets. Irisin could be supplemented by recombinant protein or mimetics (97, 228). While the effects of FGF21 on muscle and irisin and LIF on bone are unstudied, their known roles in muscle and bone pathology warrant further investigation in pre-clinical models.

In preclinical studies involving DMD animal models, myostatin inhibition led to increased muscle mass and strength, and decreased fibrosis (125–128, 229), as well as improved bone mass and strength (88). However, these positive results have not translated to positive outcomes in clinical trials (230). Three distinct myostatin inhibitors have been developed and all have failed in clinical trials [as recently reviewed in (94, 230)]. The discrepancy of efficacy between animal models and humans is likely due to the differing expression levels of myostatin between mdx mice and human patients. Myostatin levels in mice are approximately 5 to 9-fold higher than in humans (124). Thus, further exploring direct inhibition of myostatin is unlikely to be fruitful. However, increased understanding of myostatin action, such as downstream signaling pathways, may provide alternative druggable targets.

4 Conclusions

The pathogenesis of bone abnormalities in DMD is multifactorial, extending beyond corticosteroid usage and loss of ambulation. While factors such as mechanical loading, corticosteroid usage, nutritional deficiencies, hormonal imbalances, systemic inflammation, and myokine dysregulation contribute to bone health deterioration in DMD patients, the precise interplay among these factors requires further elucidation. Moreover, the management of bone health in DMD necessitates a multidisciplinary approach that includes PT, nutritional supplementation, hormonal therapy, anti-inflammatory interventions, and potential myokine-targeted therapies. It is evident that a more comprehensive clinical management plan is imperative to address the complex pathophysiology of bone

abnormalities in DMD and must take into account patient age. Future research efforts should focus on unraveling the underlying mechanisms driving bone pathology in DMD and developing tailored therapeutic strategies to mitigate bone loss and improve skeletal health outcomes.

Author contributions

AH-N: Writing – review & editing, Writing – original draft. DC: Writing – review & editing, Writing – original draft. KM: Writing – review & editing, Writing – original draft. LW: Writing – review & editing, Writing – original draft. HL: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, 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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The association between primary frozen shoulder and serum lipids may be overestimated: evidence based on retrospective observational studies and Mendelian randomization

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Background: Previous studies have shown that dyslipidemia is significantly associated with primary frozen shoulder and may be a risk factor for the development of primary frozen shoulder. However, these findings may be biased by a number of confounding factors. We investigated the association between serum lipids and primary frozen shoulder by retrospective analysis and two-sample Mendelian randomization (MR) methods.

Methods: This retrospective observational study included 284 patients with primary frozen shoulder diagnosed from October 2020 to October 2023 at four centers as the experimental group. Patients with diabetes and thyroid dysfunction were excluded. The control group consisted of age- and sex-matched people who underwent a health checkup. We compared total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) between the two groups. Genetic variants for the serum lipids and frozen shoulder were then extracted from large-scale genome-wide association studies. Causal effects were assessed using Inverse variance weighting (IVW), Weighted median, MR-Egger, simple and weighted models in MR analysis.

Results: The analysis showed that after excluding patients with diabetes and thyroid dysfunction, the serum lipids (TC, TG, HDL, and LDL) in the primary frozen shoulder group were no different from those of normal individuals. None of the MR methods found significant causal evidence between them.

Conclusions: Dyslipidemia in patients with primary frozen shoulder may be influenced by confounding factors such as diabetes and thyroid dysfunction. These findings deepen our understanding of primary frozen shoulder risk factors.

KEYWORDS

primary frozen shoulder, serum lipid, Mendelian randomization, correlation, causality

1 Introduction

For Primary frozen shoulder, also known as "adhesive capsulitis of the shoulder", is a common shoulder disorder characterized by spontaneous pain and limited shoulder motion (1). The major risk factors for primary frozen shoulder that have been reported include age, female gender, diabetes mellitus, and thyroid diseases (2–4). Because the mechanisms of primary frozen shoulder development are unknown, identifying possible risk factors is particularly important for the diagnosis and clinical management of primary frozen shoulder.

Bunker's study (5) provided the first indication that dyslipidemia may be a risk factor for the pathogenesis of frozen shoulder. This was based on the finding that the pathologic manifestations of frozen shoulder are very similar to Dupuytren's disease, which is known to be associated with hyperlipidemia. Subsequently Bunker et al. (6) further found that fasting serum triglyceride (TG) and cholesterol levels were significantly higher in patients with frozen shoulder than in healthy controls. However, the significance of this study is limited by the fact that the effects of confounding factors (such as diabetes mellitus and thyroid dysfunction) were not considered. Dyslipidemia is frequent in patients with diabetes mellitus and thyroid disease, and it is not possible to identify dyslipidemia as an independent risk factor for primary frozen shoulder (7, 8). With this in mind, Sung et al. (9) conducted a case-control study after excluding patients with diabetes mellitus and thyroid dysfunction. Although the results had significance in continuous and categorical values, the actual differences between the two study groups of these serum lipid levels were small. The relationship between primary frozen shoulder and dyslipidemia remains ambiguous, therefore strong evidence of a correlation is needed. And to the best of our knowledge, there has not been a study exploring the causal relationship between primary frozen shoulder and dyslipidemia.

In this study, we performed a multicenter retrospective analysis to assess the correlation between total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), TG, and primary frozen shoulder. The patients with already diagnosed diabetes mellitus and thyroid dysfunction were excluded. We then used two-sample Mendelian randomization (MR) to assess the causal relationship between primary frozen shoulder and dyslipidemia. This method avoids interference from reverse causal associations and potential confounders encountered in conventional randomized controlled trials by using the random distribution of genetic variation and simulating the randomization process of a randomized controlled experiment (10).

2 Materials and methods

2.1 Clinical study design

This retrospective observational study included patients with primary frozen shoulder diagnosed from October 2020 to October 2023 at four centers as the experimental group. The control group consisted of age- and sex-matched people who underwent a health checkup. The inclusion criteria were as follows:(a) Patients in the experimental group met the diagnostic criteria for primary frozen shoulder (11), including painful freezing phase, adhesive phase, and resolution phase; (b) The control population showed normal shoulder motion and no shoulder symptoms. The exclusion criteria were as follows: (a) Patients diagnosed with type 1 diabetes (12) and type 2 diabetes (13); (b) Patients diagnosed with hyperthyroidism (14) and hypothyroidism (15); (c) Patients with previous shoulder surgery or trauma.

The clinical data of all enrolled patients were collected, including gender, age, TC, TG, HDL, and LDL. The study was conducted under the Declaration of Helsinki and was approved by the ethics committees of each participating center. As the study was retrospective, the requirement for informed consent was waived.

2.2 Mendelian randomization study design

In this study, causal association analysis with outcome variables was performed by selecting Single nucleotide polymorphisms (SNPs) loci significantly associated with exposure factors as instrumental variables (IVs) and using the two-sample MR method. MR analysis needs to satisfy the following three core hypotheses: ① There was a strong correlation between IVs and exposure factors; ② IVs and any confounders associated with exposure-outcome were not relevant; ③ IVs affect outcomes only through exposure factors (16).

2.3 Sources of GWAS summary statistics

Summary-level GWAS data for hypothyroidism (17), hyperthyroidism (17), type 1 diabetes (18), type 2 diabetes (17), lipid traits (HDL, LDL, and TG) (19), and primary frozen shoulder (20) were sourced from published genome-wide association studies. All GWAS datasets utilized in this study were obtained from FinnGen and the UK Biobank and are publicly accessible via the IEU Open GWAS database summary website (https://gwas.mrcieu.ac.uk/). Summary information on the datasets used in this study is presented in Table 1. In addition, sex-stratified summary statistics for lipid traits and primary frozen shoulder were obtained from the UK Biobank (https://www.nealelab.is/uk-biobank) to analyze sex-specific genetic effects.

2.4 Selection of instrumental variables

SNPs in exposure factors were screened by setting a genome-wide significance threshold ($P < 5.0 \times 10^{-8}$). Second, independent SNPs without linkage disequilibrium (LD) were used as IVs according to the PLINK clumping algorithm (LD cut-off of $r^2 < 0.001$ within a 10 000-kilobase clumping window). The F statistic ($F = beta^2/se^2$) was used to assess the strength of genetic tools for all SNPs. We selected strong IVs with F-statistics above 10 for subsequent analysis. However, due to the large number of SNPs associated with lipid traits, we raised the F-statistic threshold to 100 to further optimize the quality of the instrumental variables and ensure the precision and robustness of the causal effect estimates. Additionally, SNPs associated with confounding factors (diabetes mellitus and thyroid dysfunction) were excluded when analyzing the causal associations between lipid traits and primary frozen shoulder.

2.5 Mendelian randomization analysis

In this study, inverse variance weighting (IVW) (21) was used as the primary method of analysis, with MR Egger (22), weighted median (23), simple mode, and weighted mode (24) as secondary methods of analysis.

Individual differences between IVs were tested using IVW and MR-Egger methods, and Cochran's Q statistic and p-value were used to determine the presence of heterogeneity. To determine the possibility of horizontal pleiotropy, the MR-Egger intercept method calculates the intercept term from linear regression analysis. The study's overall pleiotropy and horizontal pleiotropic outliers were evaluated using the MR pleiotropy residual sum and outlier (MR-PRESSO) test (25).

2.6 Statistical analysis

Statistical analysis was performed using SPSS 26.0 software. Continuous variables that satisfy normal distribution are expressed as mean ± standard deviation, and comparisons between groups are made using the independent samples t-test. Categorical variables were expressed as percentages, and comparisons between groups were made using the Chi-square test. The "TwoSampleMR" and "MR-PRESSO" packages in R (version 4.3.0) were used for MR analysis between exposures and results. The R script used in this analysis is provided in the Supplementary Table 1. The results are expressed as odds ratio (OR) and 95% confidence interval (CI) for each standard deviation. $P < 0.05$ was considered as statistical significance.

3 Results

3.1 No difference in serum lipids between primary frozen shoulder patients and normal subjects

The analysis showed that after excluding patients with diabetes mellitus and thyroid dysfunction, the serum lipids (TC, TG, HDL, and LDL) in the primary frozen shoulder group were no different from those of normal individuals (Table 2).

TABLE 1 Details of GWAS datasets used in the study.

Variable	ID	Sample size	SNPs	Population	Year
Hypothyroidism	ebi-a-GCST90018862	410,141	24,138,872	European	2021
Hyperthyroidism	ebi-a-GCST90018860	460,499	24,289,279	European	2021
Type 1 diabetes	ebi-a-GCST90014023	520,580	59,999,551	European	2021
Type 2 diabetes	ebi-a-GCST90018926	490,089	24,167,560	European	2021
HDL	ieu-b-109	403,943	12,321,875	European	2020
LDL	ieu-b-110	440,546	12,321,875	European	2020
TG	ieu-b-111	441,016	12,321,875	European	2020
Frozen shoulder	ebi-a-GCST90000512	451,099	15,184,371	European	2021

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

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TABLE 2 Serum lipids levels in the study and control groups.

Characteristic	Frozen shoulder group (n = 284)	Control group (n = 284)	t/χ2	*P*
Male sex	125 (44%)	133(47%)	0.455	0.500
Age (years)	55.11 ± 5.94	55.25 ± 6.04	0.287	0.774
TC (mmol/L)	5.07 ± 0.72	4.99 ± 0.88	-1.321	0.187
TG (mmol/L)	1.19 ± 0.31	1.17 ± 0.32	-0.662	0.508
HDL (mmol/L)	1.26 ± 0.37	1.28 ± 0.41	0.627	0.531
LDL (mmol/L)	2.63 ± 0.70	2.54 ± 0.75	-1.509	0.132

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

3.2 Effect of diabetes mellitus and thyroid dysfunction on primary frozen shoulder

We first evaluated the causal relationships between hypothyroidism, hyperthyroidism, type 1 diabetes mellitus, and type 2 diabetes mellitus with primary frozen shoulder by MR analysis. For subsequent analysis, 63 SNPs associated with hypothyroidism, 9 SNPs associated with hyperthyroidism, 76 SNPs associated with type 1 diabetes mellitus, and 170 SNPs associated with type 2 diabetes mellitus were identified (Supplementary Tables 2-5). The results revealed that the development of primary frozen shoulder was positively associated with hypothyroidism (OR = 1.055, 95% CI, 1.011–1.101, $P = 0.015$), hyperthyroidism (OR = 1.083, 95% CI, 1.030–1.138, $P = 0.002$), type 1 diabetes mellitus (OR = 1.028, 95% CI, 1.012–1.044, $P < 0.001$), and type 2 diabetes mellitus (OR = 1.042, 95% CI, 1.002–1.085, $P = 0.042$) (Figure 1). These findings provide additional evidence that hypothyroidism, hyperthyroidism, type 1 diabetes mellitus, and type 2 diabetes mellitus are independent risk factors for the development of primary frozen shoulder.

3.3 Effect of serum lipids on primary frozen shoulder

We first evaluated whether serum lipids have a causal effect on the onset of frozen shoulder without excluding potential confounders. The results indicated a positive association between LDL and the onset of primary frozen shoulder, while HDL and TG were negatively associated with its development (Supplementary Figure 1). However, significant heterogeneity and pleiotropy were observed in this analysis, which violates the core assumptions of MR analysis and indicates the influence of confounding factors (Supplementary Figure 1). In subsequent MR analyses of serum lipids and primary frozen shoulder, SNPs associated with these confounding factors will be excluded.

A total of 151 SNPs (HDL 58, LDL 43, and TG 50) were identified for use in MR analysis by screening the acquired SNPs according to our study design criteria (Supplementary Tables 6-8). The main MR analysis results were as follows: HDL (OR = 1.007, 95% CI, 0.931–1.090, $P = 0.857$); LDL (OR = 1.000, 95% CI, 0.911–1.098, $P = 0.998$); TG (OR = 0.963, 95% CI, 0.876–1.057, $P =$

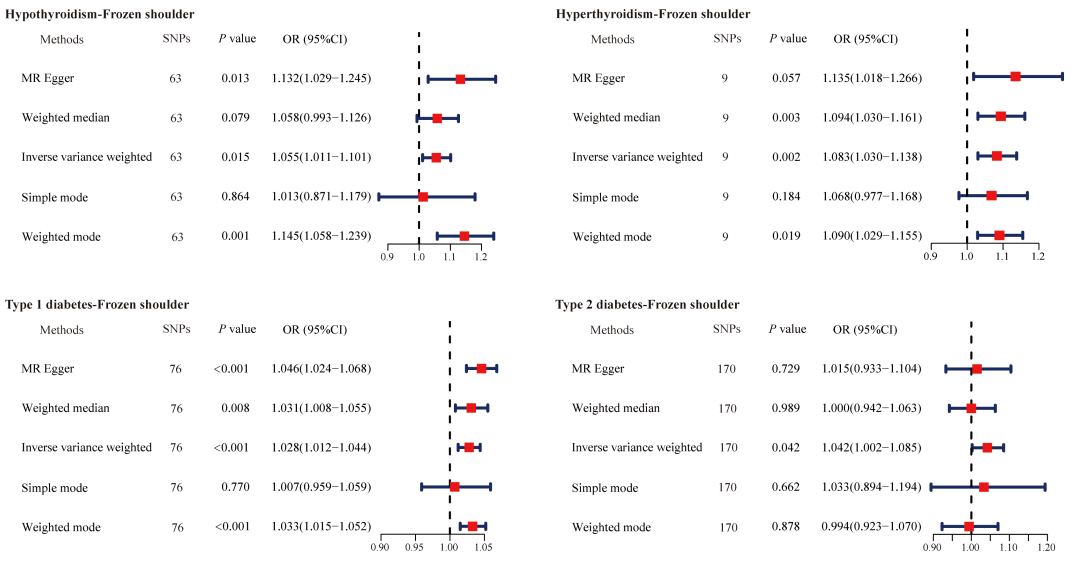

FIGURE 1
The causal effects of diabetes mellitus and thyroid dysfunction on primary frozen shoulder using two-sample Mendelian randomization methods. SNPs, single nucleotide polymorphisms; OR, odds ratios; CI, confidence interval.

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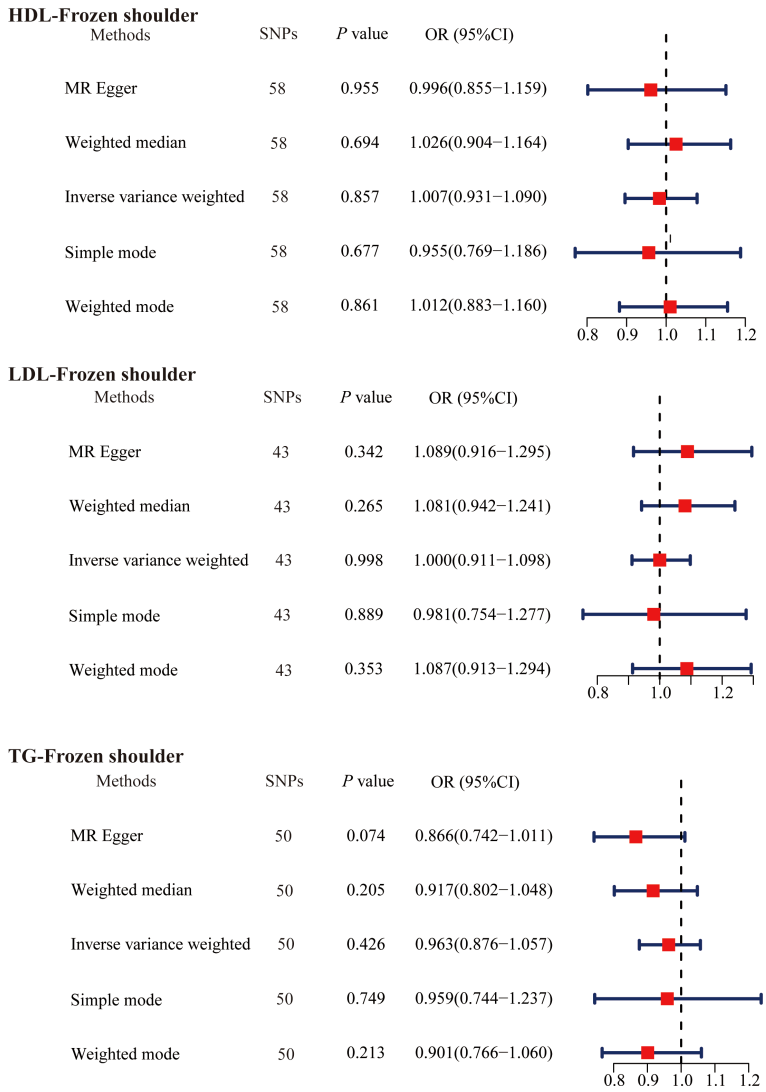

FIGURE 2
The causal effects of serum lipids on primary frozen shoulder using two-sample Mendelian randomization methods. SNPs, single nucleotide polymorphisms; OR, odds ratios; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

0.426) (Figure 2). No correlation was found between serum lipids and the risk of developing primary frozen shoulder by any of the MR analysis methods in this study.

Given that gender is an independent risk factor for primary frozen shoulder, we also evaluated the causal relationship between serum lipids and the gender-stratified onset of primary frozen shoulder. Specifically, we identified 60 SNPs (HDL-primary frozen shoulder in females), 60 SNPs (HDL-primary frozen shoulder in males), 42 SNPs (LDL-primary frozen shoulder in females), 43 SNPs (LDL-primary frozen shoulder in males), 51 SNPs (TG-primary frozen shoulder in females), and 51 SNPs (TG-primary frozen shoulder in males) as IVs (Supplementary Tables 9-14). However, none of the MR analysis methods revealed a causal association between serum lipids and the gender-specific onset of primary frozen shoulder (Figure 3).

3.4 Effect of primary frozen shoulder on serum lipids

We then explored whether the primary frozen shoulder contributes to abnormal serum lipid levels. We adjusted the threshold to $P < 5.0 \times 10^{-6}$ screening to obtain primary frozen shoulder -associated SNPs. Based on the design criteria, 21 SNPs (primary frozen shoulder-HDL), 23 SNPs (primary frozen shoulder-LDL), and 21 SNPs (primary frozen shoulder-TG) were finally obtained as IVs for MR analysis of the effect of primary frozen shoulder on serum lipids (Supplementary Tables 15-17). The results of the IVW method of primary frozen shoulder on serum lipids were analyzed as follows: primary frozen shoulder on HDL (OR = 0.997, 95% CI, 0.983–1.012, $P = 0.711$); primary frozen shoulder on LDL (OR = 0.991, 95% CI, 0.978–1.003, $P = 0.134$);

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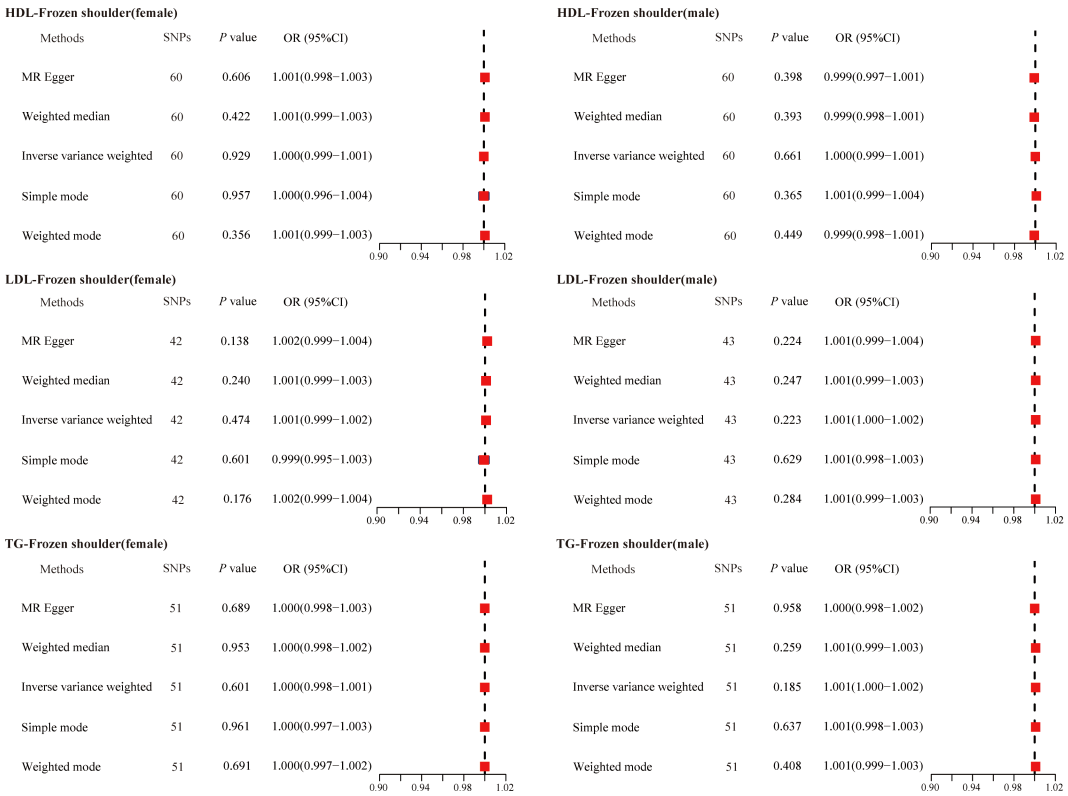

FIGURE 3
The causal effects of serum lipids on gender-stratified primary frozen shoulder using two-sample Mendelian randomization methods. SNPs, single nucleotide polymorphisms; OR, odds ratios; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

primary frozen shoulder on TG (OR = 0.999, 95% CI, 0.986–1.013, P = 0.942) (Figure 4). All results did not find any effect of the primary frozen shoulder on serum lipids.

The causal association between primary frozen shoulder and sex-stratified abnormal serum lipid levels was then analyzed in a similar manner. The IVs used for MR analysis consisted of 25 SNPs (primary frozen shoulder-HDL in females), 25 SNPs (primary frozen shoulder-HDL in males), 27 SNPs (primary frozen shoulder-LDL in females), 27 SNPs (primary frozen shoulder-LDL in males), 25 SNPs (primary frozen shoulder-TG in females), and 25 SNPs (primary frozen shoulder-TG in males) (Supplementary Tables 18-23). Consistent with these findings, no evidence was found to support a causal association between primary frozen shoulder and sex-specific changes in serum lipid levels (Figure 5).

3.5 Assessment of IVs validity and robustness in MR analysis

The Cochran Q test showed no intergenic heterogeneity among the IVs used for MR analysis in this study (Table 3). Moreover, neither the MR-Egger intercept test nor the MR-PRESSO global test showed signs of multidimensionality (Table 4). The funnel plot shows that the dispersion of causal association effects is largely symmetric and the results are not potentially biased (Supplementary Figure 2). In addition, "leave-one-out" sensitivity analyses showed that after eliminating each SNP in turn, the results of IVW analyses for the remaining SNPs were similar to the analyses that included all SNPs (Supplementary Figure 3).

4 Discussion

Previous clinical studies have shown that dyslipidemia and primary frozen shoulder are closely related, and may be a risk factor for the development of primary frozen shoulder (9). In the present study, we obtained different results for the first time. After excluding patients with diabetes mellitus and thyroid dysfunction, there was no difference in serum lipid levels between the primary frozen shoulder group and the normal control group. In addition, the results of MR analyses also indicated that there was no causal relationship between dyslipidemia and primary frozen shoulder.

An analysis of data based on the National Health Insurance Research Database of Taiwan found that patients with hyperlipidemia had a 1.5-fold higher risk of adhesive capsulitis than did healthy controls (26). Several potential mechanisms may explain how blood lipids could influence primary frozen shoulder. In individuals with dyslipidemia, inflammatory factors such as interleukins and tumor necrosis factor-alpha are often elevated, which may promote the development of primary frozen shoulder through inflammatory pathways (27, 28). Additionally, alterations

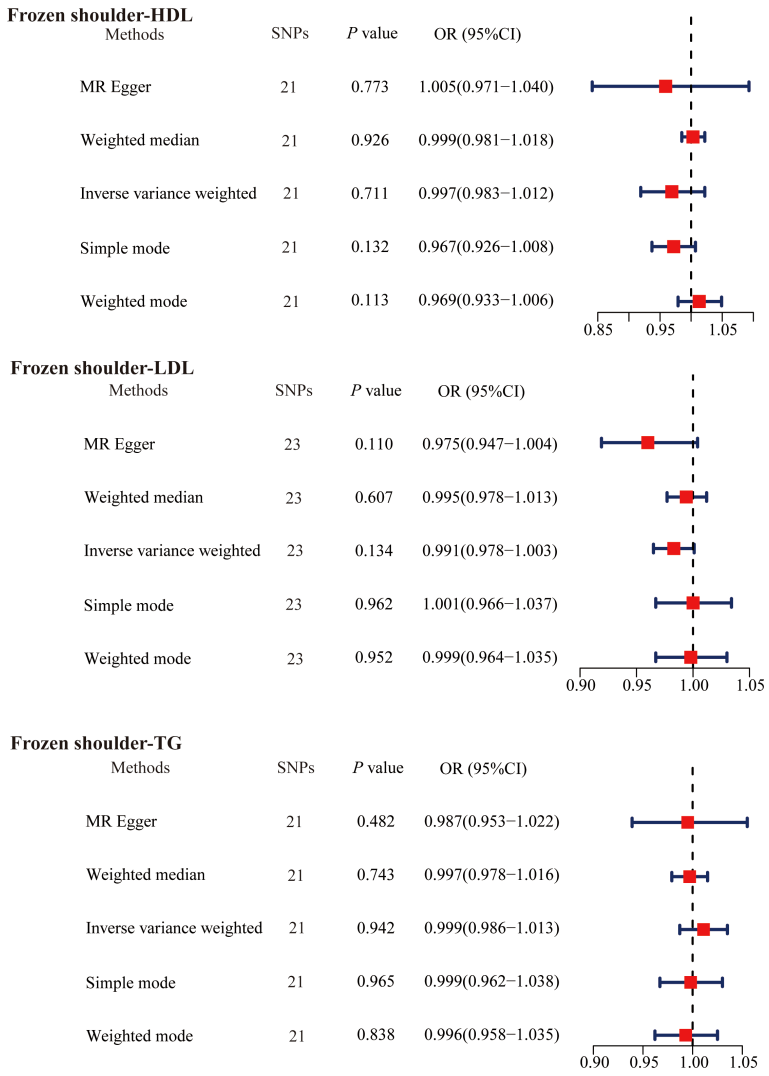

FIGURE 4
The causal effects of primary frozen shoulder on serum lipids using two-sample Mendelian randomization methods. SNPs, single nucleotide polymorphisms; OR, odds ratios; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

in lipid metabolism could affect the function of synovial tissue in the shoulder joint. Lipids influence the integrity of cell membranes and signaling pathways, including those associated with peroxisome proliferator-activated receptors and the NF-kB pathway, both of which play crucial roles in inflammation and fibrosis (29). Of note, the study also found that the use of statins did not prevent frozen shoulder in patients with hyperlipidemia (26). This result is thought-provoking. Diabetes mellitus and thyroid dysfunction are independent risk factors for the development of primary frozen shoulder (4). And dyslipidemia is an important component of diabetic metabolic syndrome and thyroid dysfunction (7, 8). We therefore hypothesized that serum lipid levels in patients with primary frozen shoulder may be affected by diabetes mellitus and thyroid dysfunction. This was confirmed in the multicenter retrospective analysis conducted in this study.

In addition, although some single-center, small-sample studies have demonstrated a relationship between dyslipidemia and frozen shoulder. However, no study has been able to confirm whether dyslipidemia is a cause, an associated cofactor, or a consequence of primary frozen shoulder. In this study, the causal genetic link between frozen shoulder and serum lipids was examined using a two-sample MR approach based on a sizable GWAS database. To the best of our knowledge, this is the first study to use MR methods to assess the causal relationship between serum lipids and frozen shoulder. Unfortunately, the results of the MR analysis did not reveal a direct link between frozen shoulder and serum lipids.

The above evidence indicates that diabetes mellitus and thyroid dysfunction interfere with the relationship between dyslipidemia and the risk of developing primary frozen shoulder to the extent that the association is overestimated. This further explains why the use of statins does not protect hyperlipidemic patients from primary frozen shoulder. Although serum lipids improve with statins, diabetes mellitus and thyroid dysfunction may persist. This observation emphasizes the need for a more nuanced understanding of risk factors for primary frozen shoulder in clinical practice. For instance, clinicians should focus on

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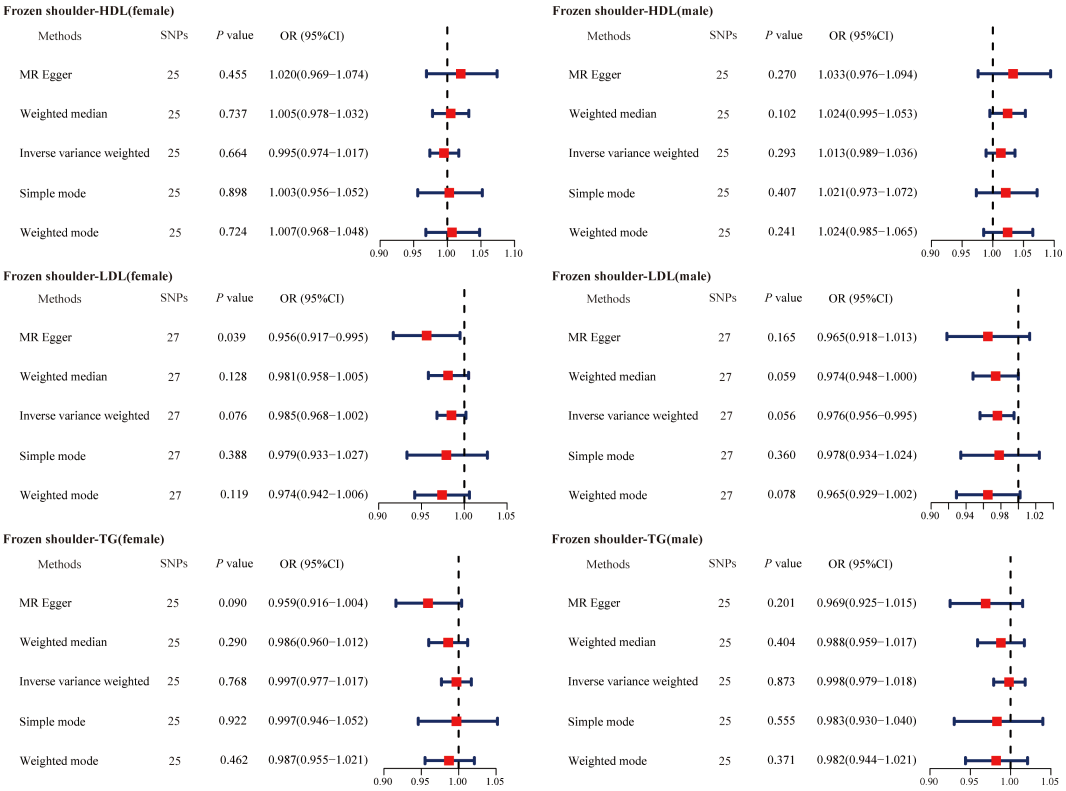

FIGURE 5
The causal effects of primary frozen shoulder on gender-stratified serum lipids using two-sample Mendelian randomization methods. SNPs, single nucleotide polymorphisms; OR, odds ratios; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

TABLE 3 Heterogeneity test.

Exposure	Outcome	Heterogeneity test (MR-Egger)			Heterogeneity test (IVW)		
		Cochran's Q	Q_df	*P*	Cochran's Q	Q_df	*P*
Hypothyroidism	FS	77.067	61	0.080	80.361	62	0.058
Hyperthyroidism	FS	8.893	7	0.260	10.043	8	0.262
Type 1 diabetes	FS	81.404	74	0.260	87.440	75	0.154
Type 2 diabetes	FS	199.712	168	0.058	200.303	169	0.051
HDL	FS	56.262	56	0.465	56.293	57	0.502
HDL	FS-females	73.666	58	0.081	74.222	59	0.087
HDL	FS-males	64.084	58	0.272	64.67491	59	0.285
LDL	FS	38.404	41	0.587	39.709	42	0.572
LDL	FS-females	32.598	40	0.791	32.598	40	0.791
LDL	FS-males	34.403	41	0.757	34.403	41	0.757
TG	FS	52.311	48	0.310	55.373	49	0.247
TG	FS-females	46.677	49	0.568	47.474	50	0.575
TG	FS-males	58.081	49	0.176	59.406	50	0.170
FS	HDL	29.611	19	0.057	30.001	20	0.070
FS	HDL-females	30.741	23	0.129	32.165	24	0.123
FS	HDL-males	32.387	23	0.0925	33.221	24	0.100

(Continued)

TABLE 3 Continued

Exposure	Outcome	Heterogeneity test (MR-Egger)			Heterogeneity test (IVW)		
		Cochran's Q	Q_df	*P*	Cochran's Q	Q_df	*P*
FS	LDL	19.120	21	0.577	20.430	22	0.556
FS	LDL-females	25.418	25	0.439	27.921	26	0.362
FS	LDL-males	31.225	25	0.182	31.538	26	0.209
FS	TG	24.185	19	0.189	24.903	20	0.205
FS	TG-females	28.475	23	0.198	32.500	24	0.115
FS	TG-males	23.732	23	0.419	25.668	24	0.370

FS, frozen shoulder; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

TABLE 4 Pleiotropy test.

Exposure	Outcome	Horizontal pleiotropy test (MR-Egger)		MR-PRESSO
		Intercept	*P*	Global test *P*-value
Hypothyroidism	FS	-0.0081	0.112	0.051
Hyperthyroidism	FS	-0.0124	0.373	0.361
Type 1 diabetes	FS	-0.0066	0.122	0.155
Type 2 diabetes	FS	0.0022	0.482	0.050
HDL	FS	0.0007	0.862	0.500
HDL	FS-females	<-0.0001	0.511	0.107
HDL	FS-males	<-0.0001	0.467	0.315
LDL	FS	-0.0058	0.260	0.576
LDL	FS-females	<-0.0001	0.187	0.757
LDL	FS-males	<-0.0001	0.500	0.600
TG	FS	0.0075	0.100	0.267
TG	FS-females	<-0.0001	0.376	0.580
TG	FS-males	<-0.0001	0.296	0.209
FS	HDL	-0.0008	0.622	0.068
FS	HDL-females	-0.0027	0.313	0.148
FS	HDL-males	-0.0022	0.450	0.093
FS	LDL	0.0017	0.265	0.561
FS	LDL-females	0.0032	0.129	0.350
FS	LDL-males	0.0012	0.621	0.217
FS	TG	0.0013	0.462	0.215
FS	TG-females	0.0041	0.084	0.117
FS	TG-males	0.0032	0.184	0.389

FS, frozen shoulder; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

managing systemic conditions like diabetes and thyroid dysfunction, which may indirectly influence the development of frozen shoulder. This shift in focus could lead to more targeted and effective preventive strategies, reducing unnecessary interventions aimed solely at lipid management in such patients. At the same time, we believe that there may be other potential factors contributing to the relationship between lipids and primary frozen shoulder that we have not yet focused on or observed.

Larger genetic and intervention studies are needed to further investigate the pathophysiology of serum lipids and primary frozen shoulder.

The greatest strength of our study was the combination of retrospective and MR analyses, which reduced the effects of reverse causality and confounding factors. Secondly, we performed a gender-stratified MR analysis to identify gender-specific causal associations. Finally, we used multiple MR analysis methods, which increased the credibility and authenticity of our findings. In clinical settings, similar MR analyses could be applied to identify and validate causal risk factors for other musculoskeletal or metabolic disorders. This evidence-based approach could guide the development of precision medicine strategies, ensuring that interventions are directed at modifiable risk factors with proven causal links to disease outcomes.

Inevitably, the current study has some limitations. First, we may not be able to exclude all confounders and eliminate biased estimates of causal inference. Confounding factors such as subclinical disease states, different stages of disease progression, diet, and lifestyle may influence the relationship between serum lipids and primary frozen shoulder. Second, the GWAS dataset we use is primarily from European-origin populations. The population included in previous clinical studies and our retrospective analysis consisted of Asian individuals. The emergence of specific traits varies across racial and ethnic groups due to their different living environments and genetic backgrounds. This therefore limits the application of our findings to other populations of different races. Finally, the sample size included in this study was too small because serum lipids are not a routine examination for patients with primary frozen shoulder.

5 Conclusion

In conclusion, retrospective observational studies and MR analyses did not reveal correlations and causal genetic relationships between serum lipids and primary frozen shoulder. These findings deepen our understanding of risk factors for primary frozen shoulder. Further studies on the pathophysiologic relationship between serum lipids and primary frozen shoulder are warranted.

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 author.

Ethics statement

The studies involving humans were approved by Zhengzhou Central Hospital Affiliated to Zhengzhou University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin due to the retrospective nature of the study.

Author contributions

YZ: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. WF: Data curation, Writing – original draft. YW: Data curation, Writing – original draft. TD: Data curation, Writing – original draft. DL: Conceptualization, Methodology, Supervision, Writing – review & editing. YS: Conceptualization, Data curation, Methodology, Supervision, Visualization, Writing – original draft, 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/fendo.2024.1363018/full#supplementary-material

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Causal associations between osteoporosis and HBV infection across Asian and European populations: evidence from Mendelian randomization and colocalization analysis

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Introduction: Clinical studies have demonstrated a potential association between chronic hepatitis caused by hepatitis B virus (HBV) infection and osteoporosis. However, the causal relationship between HBV infection and osteoporosis remains to be determined.

Methods: We investigated whether HBV infection is causally associated with osteoporosis using Mendelian randomization (MR) in East Asian and European populations, respectively. The data we utilized were obtained from the genome-wide association studies (GWAS) database. Various MR methods, including inverse variance weighted (IVW), MR Egger, weighted median, simple median and simple mode were employed to estimate the association between HBV infection and osteoporosis. Heterogeneity analysis and sensitivity tests were performed to ensure the robustness of the results. Bayesian co-localization (coloc) analysis was also applied to calculate the posterior probability of causal variants and to identify common genetic variants between HBV infection and osteoporosis.

Results: MR analysis indicated that HBV infection increased the risk of osteoporosis onset in two East Asian cohort (IVW, OR = 1.058, 95% CI = 1.021 to 1.097, P = 0.002 and OR = 1.067, 95% CI = 1.029 to 1.106, P < 0.001). However, a clear effect of genetic susceptibility to HBV on the enhanced risk of osteoporosis was not observed in two European cohort (IVW, OR = 1.000, 95% CI = 0.999 to 1.001, P = 0.171 and OR = 1.003, 95% CI = 0.981 to 1.025, P = 0.780). Additional MR methods and sensitivity analyses further validated the reliability and robustness of our results. Bayesian co-localization analysis revealed co-localization of HBV infection and osteoporosis on STAT4 at rs11889341based on East Asian GWAS data.

Conclusions: Our study identified a causal relationship between HBV infection and osteoporosis in East Asian and European populations. These results provided strong evidence that HBV infection augmented the risk of developing osteoporosis in East Asian populations and provided novel therapeutic targets.

KEYWORDS

osteoporosis, HBV, STAT4, Mendelian randomization, Colocalization analysis

Introduction

Osteoporosis is the most prevalent bone metabolic disease characterized by decreased bone mineral density (BMD), structural deterioration of bone tissue as well as increased risk of fracture (1). It is estimated that osteoporosis affects more than 200 million people worldwide and occurs primarily in the elderly, especially in menopausal women (2). In addition, the results of recent research showed that among the subjects they investigated, 95% of patients with osteoporosis had at least one coexisting condition, and they were also at higher risk of developing joint disease, arthritis, chronic low back pain, depression and chronic heart failure (3). Notably, fragility fractures are a major complication of osteoporosis, with a mortality rate of up to 30% within 1 year of fragility fracture occurrence (4). With the tendency of an aging population, osteoporosis will cause a wider impact and more alarming healthcare costs, which will dramatically affect global health and economic issues. Therefore, we are supposed to emphasize the search for potential risk factors of osteoporosis and the exploration of new therapeutic targets.

Hepatitis B virus (HBV) infection is globally widespread and more than 2 billion people are influenced by HBV infection (5). Owing to the hepatophilic nature of HBV, HBV infection may lead to a broad spectrum of liver diseases, including hepatitis, cirrhosis, hepatocellular carcinoma and so on (6). Non-hepatic complications in patients with chronic liver diseases, particularly cirrhosis, include hepatic osteodystrophy. Notably, osteoporosis is the most common form of hepatic osteodystrophy (7). Recent works have explored the potential association between liver diseases arising from chronic hepatitis B and bone health indicators such as BMD and the incidence of osteoporosis. Zhang et al. (8) found that lumbar spine and hip BMD were significantly lower and the occurrence of osteoporosis was much higher in the cirrhotic group of hepatitis B as compared to the control group. Not only that, Schiefke et al. (9) also identified a meaningful reduction in BMD in non-cirrhotic patients with chronic hepatitis B infection. However, Mora et al. (10) measured BMD in untreated adolescents vertically infected with HBV and discovered that neither lumbar spine BMD nor whole-body BMD in HBV-infected patients differed significantly from that of controls. These results were not entirely consistent. In addition, most of the previous studies were observational studies focusing on the correlation between chronic liver disease, especially cirrhosis, and osteoporosis, and mainly used BMD as an observational index. Given the confounding factors and other reasons, they may not be robust enough to establish causality. Therefore, we need an approach to determine whether HBV infection is directly linked to an increased risk of osteoporosis and to explore the biomolecules that are critical in this process.

Currently, Mendelian randomization (MR) is widely recognized as a promising tool for addressing questions in epidemiology and human biology, based on the wide availability of genetic data and the tremendous growth of genome-wide association studies (GWAS) (11). MR employs genetic variants as instrumental variables (IVs) to investigate causal relationships between exposures and outcomes (12). Compared with traditional observational studies, MR is less susceptible to confounding factors and also minimizes the potential risk of reverse causality bias. Thus, MR may present stronger evidence for reflecting causal effects. In this study, we conducted MR analysis with available publicly GWAS datasets of HBV infection and osteoporosis in two populations including East Asia and Europe to explore the causal relationship between HBV infection and osteoporosis. In addition, to verify the MR results and to detect pivotal molecules linking the connection between HBV infection and osteoporosis, we performed Bayesian co-localization (coloc) analysis to calculate the posterior probability of causal variants and to identify shared genes for HBV infection and osteoporosis.

Abbreviations: BMD, bone mineral density; HBV, hepatitis B virus; MR, Mendelian randomization; GWAS, genome-wide association studies; IVs, instrumental variables; COLOC, co-localization; SNPs, single nucleotide polymorphisms; LD, linkage disequilibrium; IVW, inverse variance weighting; WM, weighted median; OR, odds ratio; CI, confidence intervals; eQTL, expression quantitative trait loci; PPH4, posterior probability of hypothesis 4; CTLs, cytotoxic T-lymphocytes; Ub, ubiquitin; HBcAg, hepatitis B core antigen; CTP, cytoplasmic transduction peptide; JAK 2, janus kinase 2; Tyk 2, tyrosine kinase 2; HLA-A2, human leukocyte antigen-A2; IL-2, interleukin-2; DCs, dendritic cells; CCL12, chemokine (C-C motif) ligand; RANKL, receptor activator of nuclear factor kappa-B ligand; ALI, acute lung injury; CCR 2, chemokine (C-C motif) receptor 2.

Methods

Study design

The study design is described in Figure 1. We conducted MR in each of the East Asian and European populations to explore the possible causal relationship between HBV infection and osteoporosis. Based on previous reports in the literature and the GWAS database, we screened single nucleotide polymorphisms (SNPs) closely associated with HBV infection as IVs, including data from both East Asian and European populations. Notably, valid IVs in MR studies are required to fulfill three core assumptions: 1) the association assumption, where IVs need to be directly related to exposure factors, 2) the independence assumption, in which IVs are not related to confounders of exposure and outcome, and 3) the exclusivity assumption, where IVs should only be able to influence outcomes through exposure factors. The GWAS summary dataset cited in this study was approved by the review committee, as provided in the original article. Therefore, this study did not require relevant ethical permission.

This Mendelian randomization study strictly follows the guidelines outlined in the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) statement for reporting (Supplementary Table S5) (13, 14).

GWAS data sources and genetic instrument variables

All summary statistics used in this study were obtained from the IEU open gwas project, Finn R11 database and GWAS catalog database (Table 1). The GWAS data for Chronic hepatitis B in East Asian populations were mainly derived from the Japan BioBank project, including 8,885,805 SNPs and 212,453 East Asian-origin individuals, among which 1,394 were cases and 211,059 were controls (Chronic hepatitis B dataset: bbj-a-99). Exposure summary data for the European population were based on the study by Sakaue et al. (15), containing 19,079,722 SNPs, and 351,885 European individuals, among which 145 were cases and 351,740 were controls (Chronic hepatitis B infection: ebi-a-GCST90018804). Furthermore, to minimize potential bias due to population heterogeneity, the population characteristics of the MR studies we carried out were corresponding with two different outcome datasets in both populations. Specifically, the pooled data for osteoporosis were also taken from East Asian (Osteoporosis Dataset: bbj-a-13 and ebi-eas-GCST90018667) and European (Non-cancer illness code, self-reported: osteoporosis Dataset: ukb-b-12141 and finngen_R11_M13_OSTEOPOROSIS) populations.

Instrumental variables

We eliminated IVs that exhibited a substantial correlation with exposure based on the following standards. First, SNPs were associated with exposure at a genome-wide significant level ($P < 5 \times 10^{-6}$). Moreover, applying the Thousand Genomes Project's East Asian and European reference panels, we executed an SNP aggregation procedure using the PLINK algorithm, which retained SNPs with linkage disequilibrium (LD) $r^2 < 0.001$ in a 10,000-kb window for IVs. Finally, the potency of IVs was evaluated employing the F-statistic: $F= (N-k-1)/k \times R^2/(1-R^2)$, where N denotes the total number of samples in the GWAS study, k is the number of IVs utilized in the analysis, and R2 is used to measure regression model fit. It is worth noting that F<10 is usually considered a weak IV and needs to be removed from the research to prevent weak instrumental bias (16).

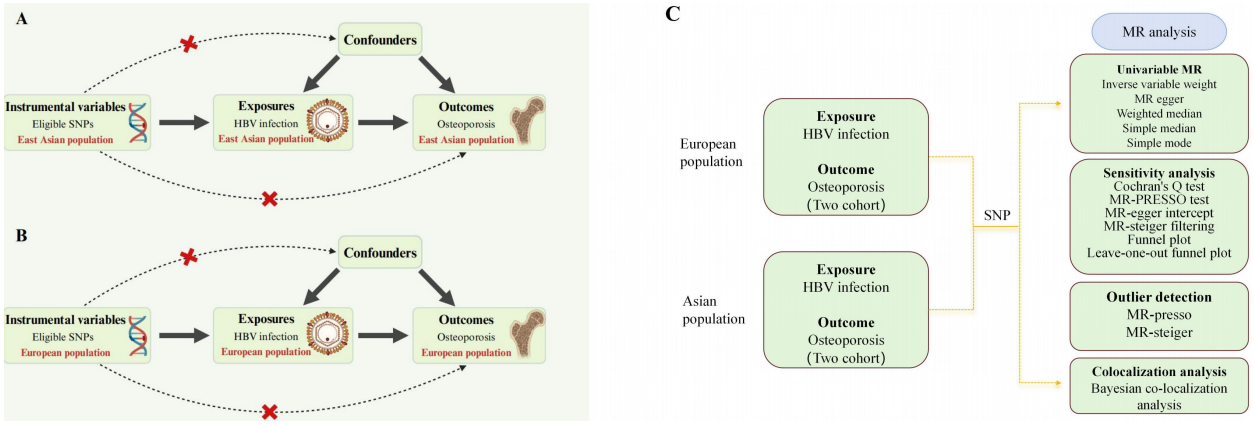

FIGURE 1
Overall study design based on Mendelian randomization analysis. SNPs, single nucleotide polymorphisms; **(A)** presents the three main Mendelian randomization hypotheses for the Asian population in this study, while **(B)** displays the corresponding hypotheses for the European population. In both figures, the selected instrumental variables are assumed to influence the outcome solely through the exposure and not through any direct association with confounding factors or the outcome itself. **(C)** illustrates the overall workflow of the study. First, we obtained GWAS data and applied five different Mendelian randomization methods to perform two-sample Mendelian randomization in the two populations. This was followed by sensitivity and heterogeneity analyses to assess the stability of the study conclusions, and finally, a colocalization analysis was conducted. HBV, hepatitis B virus.

TABLE 1 The characteristics of GWAS studies on this Mendelian randomization.

IEU-ID	Phenotype	SNPs	Cases/ controls	Ethnicity	Data link
bbj-a-99	Chronic hepatitis B infection	8,885,805	1,394/211,059	East Asian	https://gwas.mrcieu.ac.uk/datasets/bbj-a-99/
ebi-a-GCST90018804	Chronic hepatitis B infection	19,079,722	145/351,740	European	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018804/
bbj-a-137	Osteoporosis	8,885,805	7,788/204,665	East Asian	https://gwas.mrcieu.ac.uk/datasets/bbj-a-137/
ukb-b-12141	Osteoporosis	9,851,867	7,547/455,386	European	https://gwas.mrcieu.ac.uk/datasets/ukb-b-12141/
ebi-eas-GCST90018667	Osteoporosis	13,309,275	9,794/168,932	East Asian	https://www.ebi.ac.uk/gwas/studies/GCST90018667
finngen_R11_M13_OSTEOPOROSIS	Osteoporosis	20,093,941	9,046/429,826	European	https://r11.risteys.finregistry.fi/endpoints/M13_OSTEOPOROSIS

SNPs, single nucleotide polymorphisms.

Bayesian co-localization analysis

Bayesian coloc analysis is often utilized to identify whether two phenotypes are motivated by the same causal variant in a region (17). We integrated aggregated data from both exposure and outcome GWAS datasets, then used the "coloc" package with default parameters (https://github.com/chr1swallace/coloc) to calculate the posterior probability of HBV infection and osteoporosis, which is crucial to determine whether they share causal variables. In this study, we focused on the posterior probability of hypothesis 4 (PPH4) in the Bayesian co-localization analysis, which is the posterior probability that HBV infection and osteoporosis are significantly associated with a SNP locus in a certain genomic region and are driven by the same causal variant locus. Typically, PPH4 ≥ 75% is considered evidence of co-localization, suggesting that genetic variants are shared between HBV and osteoporosis and may be involved in regulating their association (18).

Statistical analysis

In our study, we employed five different approaches to estimate the causal relationship between exposure (hepatitis B) and outcome (osteoporosis). Among them, standard inverse variance weighting (IVW) served as the predominant method of assessment. IVW is based on valid and mutually independent IVs that weight random variable measures by the inverse of the outcome's variance (se^2) (19). Additionally, IVW is characterized by regressions that do not account for the presence of an intercept term. Although IVW is regarded as an effective method and is widely used in MR studies, errors may arise when heterogeneity and horizontal pleiotropy are present. Therefore, we performed complementary analyses including MR Egger, weighted median (WM), simple median and simple mode in order to obtain more accurate results. MR-Egger regression takes the presence of an intercept term into account and is capable of assessing horizontal pleiotropy of genetic variation, where a significantly non-zero intercept indicates that the IVs we used may have horizontal pleiotropy (20). WM is the median of the distribution function obtained by ranking all SNP effect values according to their weights, and it yields robust estimates when at least 50% of the information comes from valid IVs (21). The results assessed through the above techniques were deemed statistically significant when the p-value was <0.05. Besides, the effect of the results was estimated by the odds ratio (OR) and the corresponding 95% confidence intervals (CI).

To ensure that the results were stable and reliable, we also tested for heterogeneity, horizontal pleiotropy and sensitivity. We used IVW and MR-Egger to detect heterogeneity between each SNP. Heterogeneity was quantified by calculating the Cochran Q-statistic, and a P-value > 0.05 indicated the absence of heterogeneity and more reliable results (22). Additionally, we visualized outliers using scatterplots and funnel plots. To test for horizontal pleiotropy, we resorted to intercept p-values gained from MR Egger regression as well as MR-Presso global test, and reassessed the causal effect estimates after removing outlier SNPs (23, 24). Moreover, we also conducted leave-one-out analyses to assess the influence of individual SNPs on the causal effects of exposure and outcome. The leave-one-out method indicates reliable results if all error lines are simultaneously on the 0 side after the removal of an SNP (25).

All analyses were performed in the statistical software R (version 4.2.1) utilizing the "TwoSampleMR (version 0.5.7)", "MRPRESSO (version 1.0)" and "coloc" packages.

Results

Instrumental variables for Mendelian randomization

Supplementary Tables S1, S2 provide detailed information on the SNPs used in this study for the East Asian and European populations, respectively. The East Asian population included a total of 27 SNPs, while the European population included 23 SNPs. All of these SNPs had F values greater than 10, indicating the absence of weak instrumentation and effectively demonstrating the potency of the exposure. Moreover, there was no direct correlation between the IVs and osteoporosis, ensuring the validity of the instrumental variables in this study.

Causal effects of HBV infection on the development of osteoporosis

Mendelian randomization analysis revealed a significant causal relationship between HBV infection and osteoporosis in the East Asian population, with consistent results across two different datasets. In the bbj-a-137 dataset, where osteoporosis was the outcome, the results were as follows: IVW (OR = 1.058, 95% CI = 1.021 to 1.097, P = 0.002), MR Egger (OR = 1.113, 95% CI = 1.024 to 1.210, P = 0.026), Weighted median (OR = 1.055, 95% CI = 1.006 to 1.107, P = 0.027), Simple median (OR = 1.040, 95% CI = 0.989 to 1.094, P = 0.128), and Simple mode (OR = 1.045, 95% CI = 0.953 to 1.145, P = 0.369). In the GCST90018667 dataset, where osteoporosis was also the outcome, the results were as follows: IVW (OR = 1.067, 95% CI = 1.029 to 1.106, P < 0.001), MR Egger (OR = 1.107, 95% CI = 1.019 to 1.202, P = 0.038), Weighted median (OR = 1.071, 95% CI = 1.015 to 1.130, P = 0.012), Simple median (OR = 1.070, 95% CI = 1.012 to 1.131, P = 0.017), and Simple mode (OR = 1.072, 95% CI = 0.979 to 1.173, P = 0.160). The main results for the East Asian population are shown in Figure 2A. However, this relationship did not show statistical significance in the European population. In the Finnish osteoporosis dataset, the IVW result was (OR = 1.003, 95% CI = 0.981 to 1.025, P = 0.780), and in the UKB osteoporosis dataset, the IVW result was (OR = 1.000, 95% CI = 0.999 to 1.001, P = 0.171). The remaining results are shown in Figure 2B. The scatter plots were all presented in Figure 3.

Sensitivity analysis results

The sensitivity analysis of this study included tests for heterogeneity and horizontal pleiotropy (Table 2). In the East Asian population, both IVW and MR Egger methods were employed to assess heterogeneity among the IV estimates. No significant heterogeneity was observed, indicating the robustness of the results (bbj-a-137: IVW Q = 17.414, p = 0.235; MR Egger Q = 15.390, p = 0.284; ebi-eas-GCST90018667: IVW Q = 10.690, p = 0.470; MR Egger Q = 9.759, p = 0.462). Additionally, pleiotropy testing for the effect of HBV infection on osteoporosis showed no significant horizontal pleiotropic bias, as indicated by both MR Egger regression intercept analysis and the MR-Presso global test (bbj-a-137: Egger intercept = -0.021, MR Egger p = 0.214, RSS = 20.555, Global test p = 0.251; ebi-eas-GCST90018667: Egger intercept = -0.014, MR Egger p = 0.357, RSS = 3.223, Global test P = 0.458). Funnel plots for both IVW and MR Egger also suggested no horizontal pleiotropy (Supplementary Figure S2). Furthermore, a leave-one-out sensitivity analysis revealed that removal of any

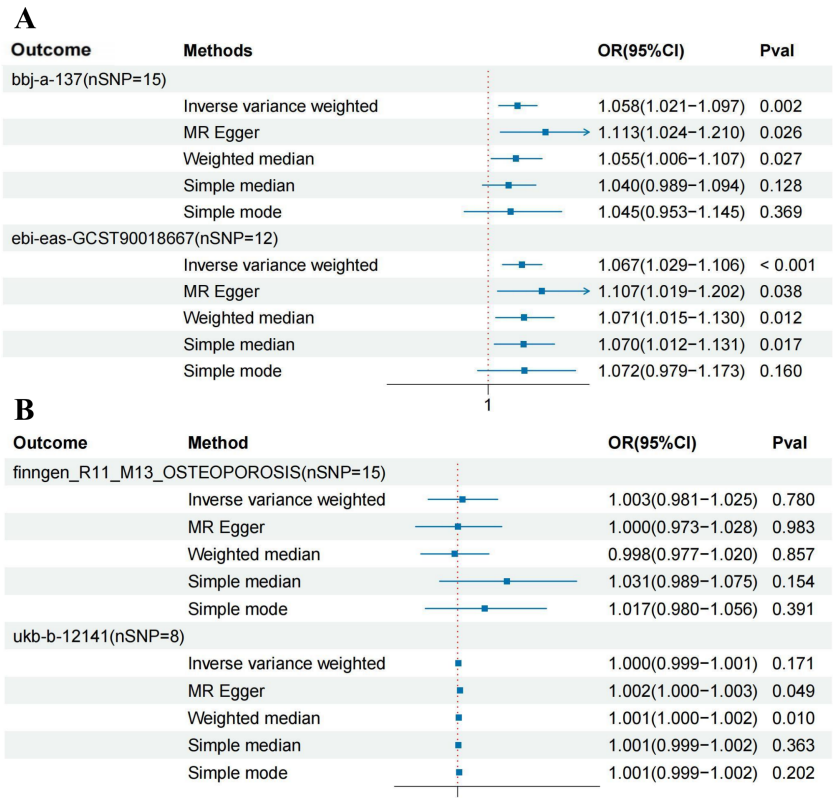

FIGURE 2
Forest plot showing the causal relationship between hepatitis B virus infection and osteoporosis. **(A)** East Asian population ('bbj-a-99' on 'bbj-a-137' AND 'bbj-a-99' on 'ebi-eas-GCST90018667'). **(B)** European population ('ebi-a-GCST006355' on 'finngen_R11_M13_OSTEOPOROSIS' AND 'ebi-a-GCST006355' on 'ukb-b-12141'). HBV, hepatitis B virus; MR, Mendelian randomization; IVW, inverse variance weighted; OR, odds ratio; CI, confidence intervals.

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TABLE 2 Sensitivity analysis of the causal association between hepatitis B virus infection and the risk of osteoporosis.

Exposures	Outcomes	Heterogeneity						Pleiotropy				
		IVW			MR Egger			MR Egger			MR-Presso	
		Q	Q_df	Q_pval	Q	Q_df	Q_pval	egger-intercept	se	pval	RSS	Global test Pval
bbj-a-99	bbj-a-137	17.414	14	0.235	15.390	13	0.284	-0.021	0.016	0.214	20.555	0.251
ebi-a-GCST006355	ukb-b-12141	21.436	16	0.162	21.209	15	0.130	0.00017	0.00042	0.694	24.165	0.156
bbj-a-99	GCST90018667	10.690	11	0.470	9.759	10	0.462	-0.014	0.015	0.357	3.223	0.458
ebi-a-GCST006355	finngen_R11_M13_OSTEOPOROSIS	26.571	14	0.022	26.302	13	0.015	0.005	0.012	0.721	36.612	0.049

IVW, inverse variance weighted; RSS, residual sum of squares.

single SNP did not significantly alter the results, with all lines remaining on the same side of zero (Supplementary Figure S1). The same sensitivity analysis was also conducted in the European population; while potential heterogeneity and pleiotropy were observed, these results were not statistically significant, and therefore no further analysis was conducted.

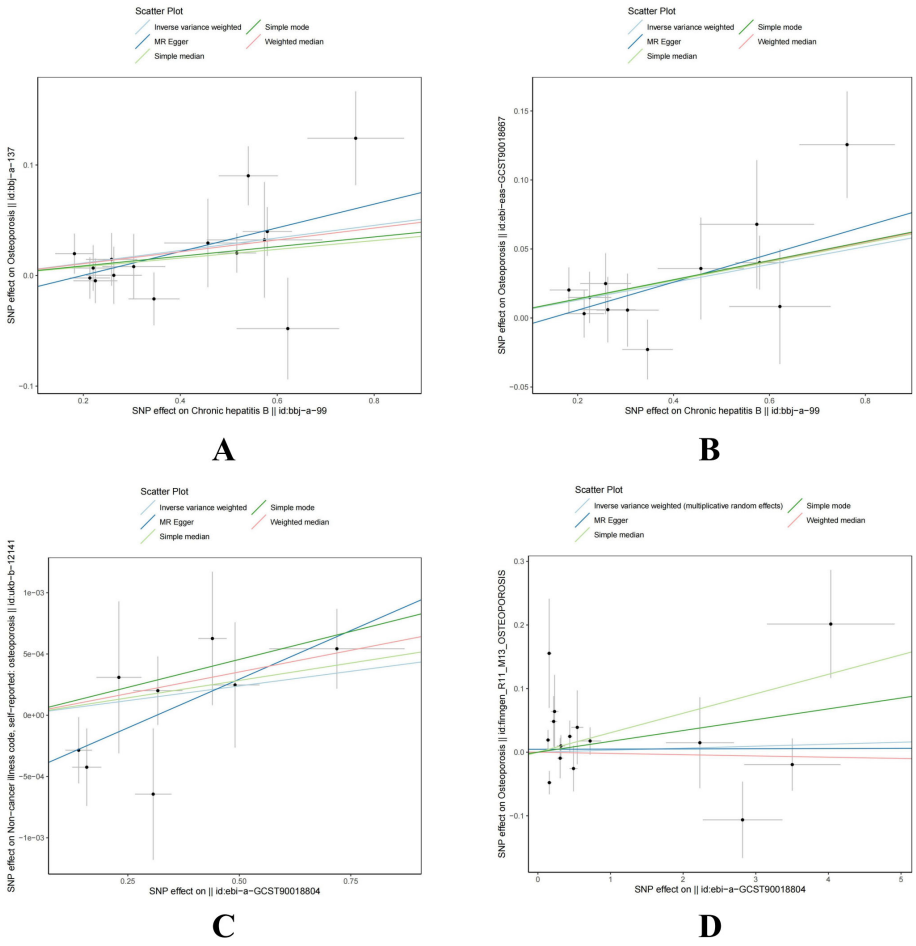

FIGURE 3
Scatter plot of genetic causality between hepatitis B virus infection and osteoporosis using different MR methods. **(A)** East Asian population ('bbj-a-99' on 'bbj-a-137'). **(B)** East Asian population ('bbj-a-99' on 'ebi-eas-GCST90018667'). **(C)** European population ('ebi-a-GCST006355' on 'ukb-b-12141'). **(D)** European population ('ebi-a-GCST006355' on 'finngen_R11_M13_OSTEOPOROSIS'). MR, Mendelian randomization; IVW, inverse variance weighted; SNP, single nucleotide polymorphism; HBV, hepatitis B virus.

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Bayesian co-localization analysis

Since the results in the East Asian population showed a positive causal relationship between HBV infection and osteoporosis, we conducted a GWAS-GWAS colocalization analysis for both exposure and outcome in this population. Figure 4 illustrates the distribution of SNPs across the three datasets used in the analysis. The colocalization results indicated that the rs11889341 locus may be a key site for the association between HBV infection and osteoporosis in the East Asian population. Specifically, the colocalization analysis between bbj-a-99 and bbj-a-37 at rs11889341 yielded a PPH4 value of 0.793, with results for other loci presented in Supplementary Table S3. Additionally, the colocalization analysis between bbj-a-99 and ebi-eas-GCST90018667 at rs11889341 yielded a PPH4 value of 0.815, with further results shown in Supplementary Table S4. Notably, rs11889341 is located in the gene region of the signal transducer and activator of transcription 4 (STAT4) on chromosome 2. Therefore, we speculate that STAT4 may be a key molecule mediating the causal relationship between HBV infection and osteoporosis.

Discussion

To our knowledge, this is the first MR study to investigate the potential causal relationship between HBV infection and osteoporosis. In the presented study, we conducted MR analyses in East Asian and European populations, respectively, intending to comprehensively explore the causal relationship between HBV infection and osteoporosis. We found that genetically predicted HBV infection was strongly associated with an elevated prevalence of osteoporosis in East Asian individuals. However, no effect of HBV infection on osteoporosis was observed in European populations. In addition, the evidence provided by the sensitivity analysis further supported the reliability and stability of our findings. Interestingly, we searched for the shared genetic variant rs11889341 (located in the gene region of STAT4) between HBV infection and osteoporosis in a given genomic region by performing Bayesian co-localization analysis, confirming the potential of a shared genetic basis between HBV infection and osteoporosis.

STAT4, a key mediator of inflammation and tumor development, is involved in various signaling pathways, and after phosphorylation, it dimerizes and translocates to the nucleus to act as a transcription factor (26). Recent studies have shown that STAT4 expression is positively correlated with HBV, particularly the rs7574865 genotype, which may increase the incidence of chronic HBV infection (27). Notably, Lu et al. (28) found that rs11889341 in STAT4 was associated with HBV infection, aligning with our co-localization results. STAT4 also plays a crucial role in the activation of HBV-specific CTLs, which are essential for controlling HBV infection (29, 30). Additionally, Chen et al. (31) showed that stimulating the JAK2/STAT4 pathway increased CTL activity and inhibited HBV replication. Interestingly, STAT4 has also been linked to osteoporosis. Studies using RT-qPCR, Elisa, and Mendelian randomization demonstrated elevated STAT4 expression in osteoporosis and its positive correlation with IL-2 (32), a known contributor to osteoporosis. Furthermore, single-cell analysis showed a negative correlation between STAT4 and activated dendritic cells (DCs), which differentiate into osteoclasts and promote bone resorption, further linking STAT4 to osteoporosis (33). These findings suggest that STAT4 may enhance osteoporosis risk by

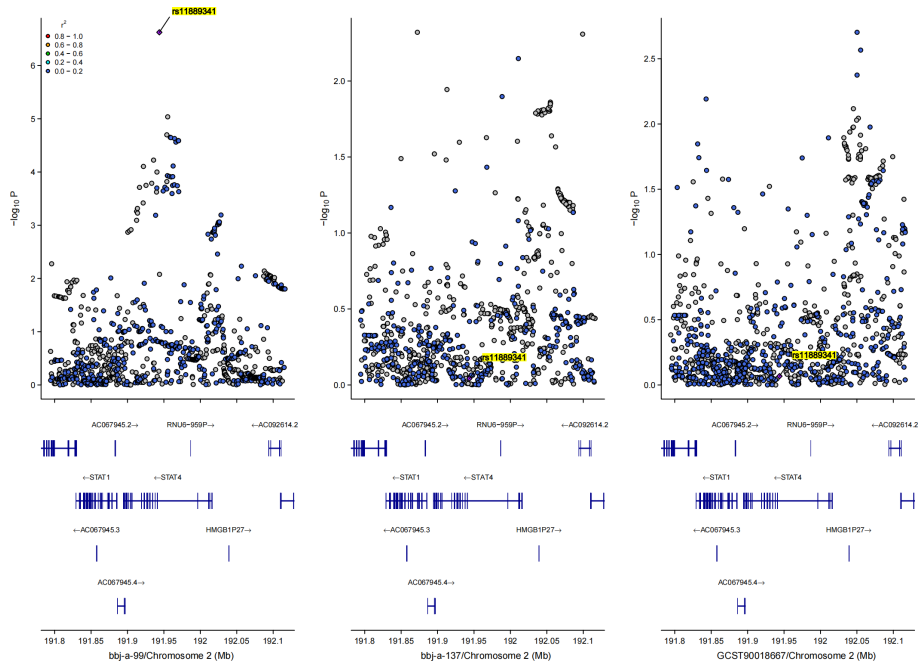

FIGURE 4
Bayesian co-localization analysis results for the causal relationship between hepatitis B virus and osteoporosis in East Asian population, rs11889341 was highlighted.

facilitating IL-2 production and promoting osteoclast activity. In summary, although not many studies have been conducted on the effect of STAT4 in HBV and osteoporosis, especially the relationship between STAT4 and osteoporosis. However, the current study suggested that STAT4 may be a novel biomarker for the prevention and treatment of HBV and osteoporosis, which is in agreement with our findings. Importantly, our results combined with previous studies hinted that STAT4 may be a pivotal molecule in the process of increasing the prevalence of osteoporosis under HBV infection (Supplementary Figure S3). However, this is still our speculation and additional researches are required to ascertain the molecular mechanism by which STAT4 regulated the process that HBV infection contributed to the development of osteoporosis.

Another interesting finding from our study was that the causal association between HBV infection and osteoporosis was observed exclusively in the Asian population. One reason for this is that the prevalence of HBV infection in the Asian population is likely much higher than in the European population, and the epidemiological burden in Asia results in a larger number of individuals who are positive for the virus, which leads to a higher overall proportion of individuals with osteoporosis (34). In this context, the pressure for antiviral treatment in the Asian population has also increased significantly, and antiviral therapy may have detrimental effects on bone metabolism, contributing to decreased bone mineral density (35). Additionally, differences in lifestyle and diet may also contribute to the increased susceptibility to osteoporosis following HBV infection. For example, diet and lifestyle habits can affect vitamin D levels, which in turn influence bone mineral density. While this study primarily focused on genetic variations, it is important to note that lifestyle and dietary factors are environmental influences. Compared to Europeans, Asians generally have lower levels of vitamin D, which may unintentionally increase the risk of osteoporosis in HBV-infected individuals (36).

Our study has several distinct strengths. We utilized the most up-to-date and comprehensive GWAS data available on HBV infection and osteoporosis, and explored the potential causal relationship between HBV infection and osteoporosis in two populations, East Asia and Europe, respectively. Moreover, corresponding homogeneous populations were used in both population studies, which minimized potential population heterogeneity and increased the applicability of the results. In addition, our study focused on whether common mechanisms underlie the causal relationship between HBV infection and osteoporosis, which will assist in the design of targeted measures to combat HBV infection and osteoporosis. However, there are limitations to this study. First, relying on existing data resources, we only used data from GWAS to investigate the causal relationship between HBV infection and osteoporosis, this data source greatly limits the scope of our analysis, restricting it to the exploration of the broad causal relationship between the two diseases. Given that our data heavily relies on raw GWAS data, we were unable to conduct further subgroup analyses, such as by age, gender, and other factors. These variables are crucial for understanding osteoporosis, and therefore, further investigations into these relationships should be explored using more specific clinical data. Future studies could consider supplying more favorable evidence by using other data sources for validating and establishing a causal relationship. Besides, although we conducted MR analyses in two populations, East Asia and Europe, these results may not be extended to the whole population due to regional and ethnic differences.

Conclusion

We established a causal relationship that HBV infection may increase the risk of osteoporosis development and searched for common genetic variants between HBV infection and osteoporosis with this MR study. Our findings may have significant implications for the prevention and clinical management of these diseases.

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

We used summary-level data from publicly available GWAS studies which have received ethical approval from their respective institutional review boards and informed consent from all participants. No administrative permissions were required to access the data.

Author contributions

ZL: Writing – original draft, Formal analysis, Project administration, Supervision, Writing – review & editing. JC: Writing – original draft, Methodology. KL: Writing – original draft, Resources. YW: Writing – original draft, Supervision. ZL: Supervision, Writing – original draft. RC: Writing – original draft, Resources. ZC: Validation, Writing – review & editing. ZT: Writing – review & editing, Visualization. YH: Writing – review & editing, Formal analysis, Project administration. YL: Formal analysis, Writing – review & editing. BW: Writing – review & editing, Software, Supervision. SC: Writing – review & editing, 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1419303/full#supplementary-material

SUPPLEMENTARY FIGURE 1
"Leave-one-out" plots for the causal association between hepatitis B virus infection and osteoporosis. **(A)** East Asian population ('bbj-a-99' on 'bbj-a-137'). **(B)** East Asian population ('bbj-a-99' on 'ebi-eas-GCST90018667'). **(C)** European population ('ebi-a-GCST006355' on 'ukb-b-12141'). **(D)** European population ('ebi-a-GCST006355' on 'finngen_R11_M13_OSTEOPOROSIS').

SUPPLEMENTARY FIGURE 2
Funnel plots for the causal association between hepatitis B virus infection and osteoporosis. **(A)** East Asian population ('bbj-a-99' on 'bbj-a-137'). **(B)** East Asian population ('bbj-a-99' on 'ebi-eas-GCST90018667'). **(C)** European population ('ebi-a-GCST006355' on 'ukb-b-12141'). **(D)** European population ('ebi-a-GCST006355' on 'finngen_R11_M13_OSTEOPOROSIS').

SUPPLEMENTARY FIGURE 3
Molecular mechanism of STAT4 as a key molecule in HBV infection and osteoporosis. Fusion proteins Ub-HBcAg-CTP and CTP-HBcAg-Tapasin could up-regulate the expression levels of JAK2, Tyk2, STAT1, and STAT4 in T-lymphocytes, which in turn activated the JAK2/STAT4 signaling pathway. The activation of the JAK2/STAT4 signaling pathway enhanced the percentage of CTLs, inducing HBV-specific CTLs immune response and ultimately combating HBV infection. In addition, STAT4 may add to the risk of osteoporosis through two pathways: inducing DCs to be derived into osteoclasts and activated, promoting IL-2 production. Notably, Palbociclib may suppress osteoporosis by mediating STAT4 mRNA degradation through its action on miR-141-3p. Furthermore, CCL12 could stimulate RANKL production in bone marrow stromal cells of acute lung injury mice via the CCR 2/JAK 2/STAT 4 axis, which consequently promoted trabecular bone loss and elevated bone resorption *in vivo*, and manifested as osteoporosis. HBV, hepatitis B virus; Ub, ubiquitin; HBcAg, hepatitis B core antigen; CTP, cytoplasmic transduction peptide; JAK2, janus kinase 2; STAT4, signal transducer and activator of transcription 4; CCL12, chemokine (C-C motif) ligand 12; CCR2, chemokine (C-C motif) receptor 2; RANKL, receptor activator of nuclear factor kappa-B ligand; RANK, receptor activator of nuclear factor kappa-B; CTLs, cytotoxic T-lymphocytes; IL-2, interleukin-2.

SUPPLEMENTARY TABLE 1
All SNP data used for the analysis of the Asian population.

SUPPLEMENTARY TABLE 2
All SNP data used for the analysis of the European population.

SUPPLEMENTARY TABLE 3
Results of the GWAS-GWAS colocalization analysis for the Asian population ('bbj-a-99' on 'bbj-a-137').

SUPPLEMENTARY TABLE 4
Results of the GWAS-GWAS colocalization analysis for the Asian population ('bbj-a-99' on 'ebi-eas-GCST90018667').

SUPPLEMENTARY TABLE 5
STROBE-MR-checklist.

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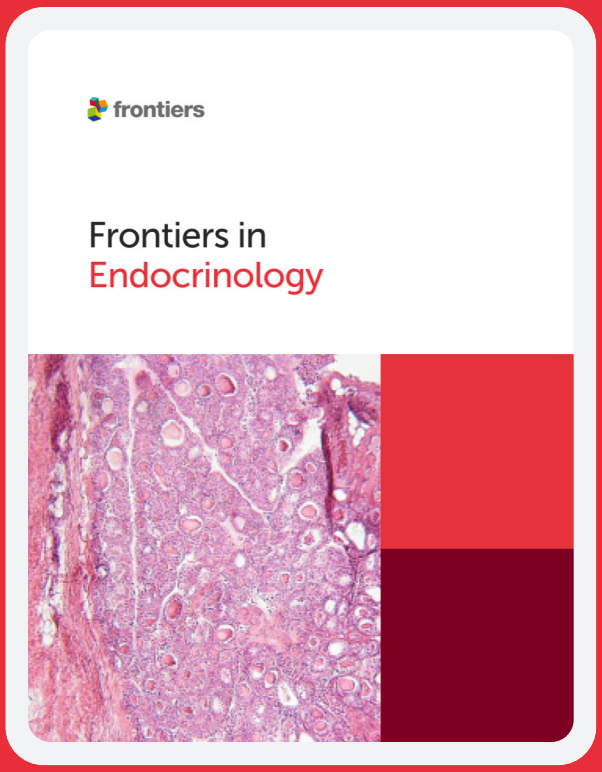

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