Osteoporosis is the most common metabolic bone disease worldwide, estimated to affect more than 200 million people globally [1]. About 30% of women and 20% of men over 50 suffer from osteoporosis or osteoporotic fractures [1]. Osteoporotic fractures are not only associated with increased mortality in both sexes, but are also responsible for about 1% of the worldwide disability caused by prevalent noncommunicable diseases [1]. Current pharmacological interventions for osteoporosis focus on hormone replacement therapy and antiresorptive treatment with bisphosphonates [2]. Anabolic therapy with parathyroid hormone (PTH) peptides has also been used for severe osteoporosis [3]. In addition, prevention strategies such as physical exercise and dietary vitamin D were introduced to reduce lifetime risk of osteoporosis [4]. Despite these efforts, osteoporosis and osteoporotic fractures are still a serious public health issue, with low diagnosis and treatment rates. Deeper understanding of the pathophysiology of osteoporosis and predisposition to fracture is necessary [5].

Bone mineral density (BMD) is a widely used index for diagnosis of osteoporosis and fracture prediction [5]. Other traits, such as bone geometry, bone size, and fracture (the outcome of osteoporosis) have also been used in genetic studies of osteoporosis [6]. Osteoporosis involves an imbalance between the activity of osteoclasts and osteoblasts that determines bone remodeling [7]. Osteoclasts are derived from monocytes; they digest and remove mature bone tissue (a process known as resorption) [8]. Osteoblasts are derived from multipotent mesenchymal stem cells (MSCs); they synthesize bone matrix and form new bone that replaces previously resorbed tissue [8]. Bone remodeling is controlled by complex genetic and environmental factors that act together. Identification of genes that influence variations in bone-related traits will provide insight into the genetic architecture of osteoporosis [9].

In genetic studies of osteoporosis, candidate gene association analysis is a conventional approach for detecting genetic variants associated with disease susceptibility; however, a major limitation of the candidate gene approach is that the ‘right’ candidate genes are sometimes difficult to identify because of our limited knowledge about the pathophysiology of osteoporosis [10]. With recent advances in molecular genetic technologies, it has become feasible to perform whole-genome studies to search for osteoporosis risk genes [11]. This hypothesis-free approach has an important advantage in identification and assessment of susceptibility genes as it does not require any prior assumptions or knowledge about the genes [12]. Depending on experimental technologies and analytical approaches, genome-wide scans may include linkage, association, gene expression, proteomic, and epigenetic studies [13]. Here, we summarize recent findings of such genome-wide studies of osteoporosis and we discuss their implications for pathogenesis and for the development of targeted interventions.

Abstract
Osteoporosis, the most common type of bone disease worldwide, is clinically characterized by low bone mineral density (BMD) and increased susceptibility to fracture. Multiple genetic and environmental factors and gene-environment interactions have been implicated in its pathogenesis. Osteoporosis has strong genetic determination, with the heritability of BMD estimated to be as high as 60%. More than 80 genes or genetic variants have been implicated in risk of osteoporosis by hypothesis-free genome-wide studies. However, these genes or genetic variants can only explain a small portion of BMD variation, suggesting that many other genes or genetic variants underlying osteoporosis risk await discovery. Here, we review recent progress in genome-wide studies of osteoporosis and discuss their implications for medicine and the major challenges in the field.

Genome-wide approaches for identifying genetic risk factors for osteoporosis
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Osteoporosis and the search for risk factors
Osteoporosis is the most common metabolic bone disease and is estimated to affect more than 200 million people worldwide [1]. About 30% of women and 20% of men over 50 suffer from osteoporosis or osteoporotic fractures [1]. Osteoporotic fractures are not only associated with increased mortality in both sexes, but are also responsible for about 1% of the worldwide disability caused by prevalent noncommunicable diseases [1]. Current pharmacological interventions for osteoporosis focus on hormone replacement therapy and antiresorptive treatment with bisphosphonates [2]. Anabolic therapy with parathyroid hormone (PTH) peptides has also been used for severe osteoporosis [3]. In addition, prevention strategies such as physical exercise and
Genome-wide linkage studies

Genome-wide linkage studies (GWLSs) are conducted to identify genomic regions associated with inherited diseases or traits in generations of families comprising affected and unaffected individuals by examining whether molecular markers (usually microsatellite markers spaced throughout the entire human genome) co-segregate with the diseases or traits under study [14]. GWLSs are designed to identify genomic regions contributing to predisposition to complex human diseases, and they do not require any information on the potential functions of genomic regions [15]. Before the advent of genome-wide association studies (GWAS), GWLSs had been widely used for genetic mapping of human diseases, and have successfully mapped contributing genes for many Mendelian diseases.

Traditional GWLSs have focused on individual diseases or traits (such as BMD) using univariate analytical approaches. For example, Styrkarsdottir et al. [16] conducted a GWLS for hip and spine BMD in a large number of extended families with osteoporosis in Iceland and identified bone morphogenetic protein gene BMP2 on chromosome 20p12.3; they followed up this discovery with association analysis. Kammerer et al. [17] performed a GWLS using BMD data at the forearm and hip for 664 individuals from 29 Mexican-American families. They obtained evidence for quantitative trait loci (QTL) on chromosome 4p affecting forearm BMD overall, and on chromosomes 2p and 13q affecting hip BMD in men [17].

Univariate analysis generally ignores the relationship between study traits, and this may result in loss of statistical power in gene identification for osteoporosis [18]. The problem can partially be overcome by bivariate analysis, which can improve the power to detect QTLs, especially when the effects of QTLs are too small to be detected by univariate analysis. Between 2007 and 2009, bivariate GWLSs identified significant loci influencing BMD and other bone-related traits such as bone size, total body lean mass [19], body fat mass [20], and age at menarche [21].

In an effort to improve the power of GWLSs, Ioannidis et al. [22] conducted a meta-analysis of GWLSs of bone mass that included 11,842 individuals. This large-scale meta-analysis provided replication evidence for several QTLs identified in previous studies and also identified a new QTL on chromosome 18p11-q12.3 [22]. The limitations of GWLSs are related to limited statistical power, genetic heterogeneity, population stratification, sparse marker density, and phenotypic heterogeneity, which have resulted in difficulty in replicating study findings [18]. Nevertheless, GWLSs have provided valuable information for future fine-mapping studies and have also provided a basis for comparison and cross-validation of study results using different approaches (for example, gene expression and proteomic studies) [23]. So far, more than 60 QTLs for BMD have been reported on all chromosomes except chromosome Y [8], with a few replicated in multiple studies, such as 7p21-22, 11q12-13, 15q13, and Xq27 [8]. Table 1 summarizes the main findings of GWLSs for osteoporosis.

Genome-wide association studies

GWASs provide an unbiased approach by which a large number of participants are genotyped for dense genetic markers (normally single nucleotide polymorphisms, SNPs) covering the whole genome to identify susceptibility genes for human diseases or traits. Compared with GWLSs, GWASs can capitalize on all meiotic recombination events in a population, rather than only those in the families studied, and thus have higher statistical power and mapping resolution. Over the past decade, GWASs have become one of the most popular tools for gene mapping of complex human diseases or traits [24]. Various different strategies have been adopted in GWASs, including individual studies (for example, in single or multiple ethnicities) and meta-analyses.

GWASs for osteoporosis-related traits have primarily been performed for single ethnicities [25]. For example, Hsu et al. [26] integrated a GWAS of Caucasians with gene expression profiling of various human tissues and identified three novel genes associated with BMD - RAP1A (encoding a Ras oncogene family member), TBC1D8 (TBC1 domain family member 8), and OSBPL1A (oxysterol binding protein-like 1A) - and replicated the identification of OPG (osteoprotegerin) [26]. GWASs have also been conducted in populations of different ethnicities [27]. Koller et al. [28] found that SNPs (rs1298989 and rs1285635) in CATSPERB (encoding the catser channel auxiliary subunit B) were associated with femoral neck BMD in both premenopausal European-American women and African-American women [28]. Ichikawa et al. [29] found that one SNP in the C6orf97/ESRI gene (estrogen receptor 1) region was significantly associated with BMD in premenopausal white women and premenopausal black women.

Besides SNPs, other genetic variants have also been studied. Copy number variants (CNVs; that is, duplication or deletion of a relatively large segment of DNA) have been analyzed in GWASs [27]. For example, Liu et al. [30] reported a CNV (CNP267) on chromosome 2q12.2 that was significantly associated with hip bone size in both Chinese Han and Caucasian samples. A candidate gene, FHL2 (four-and-a-half-LIM gene), is located downstream of CNP267 [30].

A simple and efficient way to improve statistical power over individual GWASs is meta-analysis of multiple GWASs [31-33]. Recently, the Genetic Factors for Osteoporosis (GEFOS) consortium published a large
meta-analysis of GWASs of lumbar spine and femoral neck BMD [33]. Seventeen GWAS datasets were analyzed, comprising 32,961 individuals of European and East Asian ancestry [33]. A total of 56 loci (32 of which were new) were found to be associated with BMD, and several of these loci clustered within specific pathways, including those involved in mesenchymal stem cell differentiation, endochondral ossification, the RANKL/RANK/OPG pathway (involving receptor activator of nuclear factor (NF)-κB, its ligand, and osteoprotegerin) and the Wnt signaling pathway [33]. It should be noted that homogeneous phenotypes (for example, types of fractures or skeletal sites of BMD) are necessary for meta-analysis to provide dependable results.

In addition to univariate GWASs, multivariate GWASs have been conducted to identify pleiotropic genes underlying diseases with shared genetic susceptibility to reveal the interconnected pathophysiological networks for a spectrum of common human diseases [34]. A bivariate GWAS for femoral neck bone geometry and body lean mass - two major risk factors for musculoskeletal disease - was conducted in Chinese people and US Caucasians [35], and SNPs in four genes (the hexokinase gene HK2, the uromodulin gene UMOD, and microRNA genes MIR873 and MIR876) showed strong association with both traits [35].

Although the number of GWASs has grown dramatically during the last five years [36,37], GWASs have several limitations, such as small sample size, dependence on minor allele frequency and genetic effects, stringent statistical significance, and the difficulty of replication across studies [38]. Using empirical data, we recently performed theoretical analyses to address the usefulness and limitations of GWASs and meta-analyses (our unpublished observations). The results suggested that discordant findings in GWASs and meta-analyses are not unexpected, even for true susceptible genes. We concluded that although meta-analyses can detect many more true and novel loci for complex diseases than individual GWASs, they should not be used as a gold standard to evaluate the results of individual GWASs. In particular, individual GWASs in homogeneous populations can detect true disease genes that meta-analyses might have low power to replicate.

So far, over 30 GWASs have been published on osteoporosis and related traits [25,26,28-57], and these have identified over 50 genes or genomic loci; examples are shown in Table 2. About 20 genes have also been detected in multiple GWASs (Table 2). Some of the identified genes are involved in well-established pathways, such as the RANKL/RANK/OPG pathway and the Wnt signaling pathway, that are important for bone metabolism [44].

### Table 1. Examples of genome-wide linkage studies of osteoporosis and related traits*

| Study participants | No. of markers | Phenotype | Results | Refs |
|--------------------|---------------|-----------|---------|------|
| 3,730 men and 4,374 women from the Framingham Osteoporosis Study | 209,546 SNPs. Genotypic call rates ≥97%; HWE P ≥ 0.01; MAF ≥0.2 | Hip and spine BMD heel ultrasound, geometric indices of the hip | For BMD, 9p and 11p, LOD ≥3.0; for ultrasound, 4p, LOD = 3.9, 16p, LOD = 3.8, 22p, LOD = 4.0; for femoral neck width, 7p, LOD ≥5.0 | [23] |
| 4,498 individuals from 451 pedigrees | 410 | TBLM and spine BMD in women | 15q13, LOD = 4.86 | [19] |
| 1,323 individuals from 207 extended Icelandic families | 1,100 | Hip BMD | 16q, LOD = 1.99 | [16] |
| 664 individuals from 29 Mexican-American families | 416 | Forearm BMD | 4p, LOD = 4.33; 12q, LOD = 2.35 | [17] |
| 11,842 individuals from 9 groups and 1,008 | Varied between 270 and 1,008 | Lumbar spine BMD only in women; lumbar spine BMD in both women and men; femoral neck BMD | LOD >1.6 for 1p13.3-q23.3, 12q24.31-qter, 3p25.3-p12.1, 11p12-q13.3, 19q32-q42.3, 18p11-q12.3, 9q31.1-q33.3, 17p12-q21.33, 14q13.1-q24.1, 9q21.1-q23.1, and 5q14.3-q23.2 | [22] |

*HWE P, Hardy-Weinberg Equilibrium P-value; LOD, logarithm of odds; MAF, minor allele frequency; TBLM, total body lean mass.
Table 2. Examples of genome-wide-association studies of osteoporosis and related traits

| Genotyping                        | Total markers | Significant markers | Discovery participants | Replication participants | Phenotype | P-value        | Candidate gene/related pathway | Refs   |
|-----------------------------------|---------------|---------------------|------------------------|--------------------------|-----------|----------------|---------------------------------|--------|
| Affymetrix 500K array set         | 342,854 SNPs  | rs9630182, rs2036417, rs7125774, rs8057551, rs8061992, rs7199138 | 495 females and 488 males, unrelated | 2,557 phenotyped white individuals from 750 families | Femoral neck BMD | 3.98×10^{-5} to 6.74×10^{-3} | IL21R and PTH/PTH pathway | [52]   |
| Affymetrix 500K array set         | 281,533 SNPs  | rs13182402          | 700 elderly Chinese Han | 906 Chinese, 4,054 US Midwest Caucasians, 2,953 US Framingham samples | BMD; low-trauma osteoporotic fractures | 2.08×10^{-8} to 6.39×10^{-6} | ALDH7A1 | [40]   |
| Infinium assay                    | 564,214 SNPs  | rs2273061           | 800 unrelated Hong Kong Chinese females | 720 Hong Kong and 17,378 of European or Asian descent | BMD | 5.27×10^{-4} to 3.47×10^{-5} | JAG1; Wnt and Notch pathways | [46]   |
| High-density oligonucleotide arrays | 224,507 SNPs  | rs7605378           | 1,747 (190 cases and 1,557 controls) in Japan | 5,206 (2,092 cases, 3,114 controls) in Japanese | 1.51×10^{-4} | FON2 |                      | [45]   |
| Affymetrix genome-wide human SNP array 6.0 | 689,368 SNPs | rs17743190, rs3857454, rs3907327, rs17799762, rs17799805, rs1385530, rs17799462, rs3857453, rs3857449, rs10484943, rs199670, rs16890720 | 1,627 Han adults | 1,728 from Midwestern US (Kansas City, MO and Omaha, NE) | Spine bone size | 6.2×10^{-5} to 1.8×10^{-6} | HMGN3 | [50]   |
| Affymetrix GeneChip human mapping SNP 6.0 array | 194 CNVs | CNP267               | 1,627 Chinese Han | 2,286 unrelated US Caucasians | Hip bone size | 4.73×10^{-3} and 5.66×10^{-3} | FHL2 | [30]   |
| Meta-analysis                     | 1,200 SNPs    | 467 SNPs            | 19,195 Northern Europeans | Femoral neck and lumbar spine BMD | <5×10^{-8} | SPTBN1, CTNMB1, MEPE, STARD3NL, FLA22B0, ARHGAP1, DCCDS, SOX6, FOXL1, HDACS ESR1, SP7, and others; Wnt and MAPK pathways | [31]   |
| Meta-analysis                     | 96 SNPs       | 32,061 of European and East Asian ancestry | Replication in 50,153 individuals | Lumbar spine and femoral neck BMD | <5×10^{-8} | FAM210A, SLC25A13, LRPS, MEPE, SPTBN1, DIX1; RANK-RANKL-OPG and Wnt pathways | [33]   |

*About 20 genes have been detected in multiple GWASs, including ARHGAP1 [31,32], C6orf57 [29,32], CLCN7 [25,43], CTNMB1 [31,53], DCCDS [31,53], ESR7 [29,31,32,48,53,57], FLA22B0 [31,53,55], FOXL1 [31,53], GPR177 [31,53,54], HDACS [31,53], LRPA4 [31,32,56,57], LRPS [31,33,53], MEPE [31,33,53], PTH [43,52], STARD3NL [31,53], SOST [53,56], SOX6 [31,41,51,53], SPTBN1 [31,33,57], TNFSF11 [55,56], TNFRSF11A [31,37,55,56], TNFSF11B [31,53,56,57], and ZBTB40 [31,53].

Overlap between results obtained from GWLSs and GWASs has been limited. COL1A1 (encoding Collagen type I α1) is among the very few genes detected by both GWLS and GWAS [18,43]. The limited overlap may be due to false positive or false negative results, or because the linkage approach is more likely to find functional rare variants that are enriched in large pedigrees, whereas GWAS mainly aims to identify common variants [46,47].

Gene expression studies
Unlike GWASs and GWLSs, which search for genetic variants at the DNA level, gene expression analysis can
simultaneously detect gene expression (at the RNA level) for tens of thousands of genes. It is a powerful scanning tool to investigate biochemical processes and intermediates of pathways that are linked with pathophysiology of osteoporosis [58]. Gene expression studies of osteoporosis have been conducted both in vivo and in vitro.

Because of the difficulty in obtaining sufficient amounts of fresh osteoblasts, osteoclasts, and osteocytes, most gene expression studies have been conducted using cell cultures. In vivo studies using blood monocytes have also been reported. Monocytes are important sources of cytokines and chemokines, are involved in immune system functions and have been related to bone metabolism. Peripheral blood monocytes (PBMs) are the precursors of osteoclasts [59], and thus represent an important sample for studying the molecular mechanisms of osteoporosis in vivo. Chen et al. [60] found that STAT1 (encoding signal transducer and activator of transcription 1) was significantly upregulated in the low versus the high BMD groups in both Chinese and Caucasian people, suggesting its importance in the etiology of osteoporosis. However, this was a pilot study conducted with a relatively small sample size and the findings need to be confirmed in other populations.

In vitro studies of osteoporosis using cell cultures have provided a wealth of information on the pathophysiology of osteoporosis [61]. Trost et al. [62] conducted a study on cultures of osteoblasts isolated from osteoporotic and non-osteoporotic human bone tissue samples. They found higher levels of protein synthesis and lower levels of cell proliferation in osteoblasts from osteoporotic tissue than in those from non-osteoporotic tissue [62]. MSCs, the precursors of osteoblasts, have also been investigated in gene expression studies of osteoporosis [63]. Kulterer et al. [64] identified the expression of ID4 (encoding inhibitor of DNA binding domain dominant negative helix-loop-helix protein), CRYAB (alpha-crystallin B chain), and SORT1 (sortilin) in osteogenic differentiation of MSCs. Furthermore, Tanabe et al. [65] identified four genes - EPHA5 (ephrin type-A receptor 5 gene), NOV (nephroblastoma overexpressed), NDN (necdin), and RUNX2 (runt-related transcription factor 2) - as stage-specific markers of osteogenic differentiation of MSCs.

Gene expression studies have also provided useful information about the molecular mechanisms of bone healing [66-68]. Oleanolic acid was reported to have an osteoprotective effect in rats with ovariectomy-induced osteoporosis; its ability to stimulate osteoblastic differentiation might be related to the Notch signaling pathway [69]. Li et al. [70] explored the anabolic and catabolic effects of intermittent and continuous treatments with three different PTH peptides in bone metabolism. A large number of genes, including SLP1 (encoding secretory leukocyte peptidase inhibitor), TFPI2 (tissue factor pathway inhibitor), SOCS3 (cytokine signaling suppressor) and GRO1 (melanoma growth stimulating activity α), were verified to be functional in the regulation of bone remodeling using PTH treatment [70]. These investigations might thus provide insights into mechanisms underlying PTH treatment of osteoporosis.

High-throughput microarrays have been informative in genetic studies of osteoporosis, but studies using PBMs and MSCs are not expected to be as useful as studies of osteoclasts, osteoblasts, and osteocytes. Because of the relatively small sample size and relatively poor signal/noise ratio for genes with low expression levels, most microarray technology using human blood or monocyte samples has produced results that have not been replicated well. Also, novel transcripts, gene fusion, and alternative splicing may not be detected because of the dependence on commercial chips. To address these issues, transcriptome sequencing (RNA-seq) could be an attractive alternative [71].

Taken together, recent gene expression studies have shown that osteoporosis involves numerous genes and pathways (some of which were also identified in GWLs or GWASs and some of which were novel) with complex regulatory mechanisms that are controlled by hormones, cytokines, or various receptors [72-74]. Examples of these studies are summarized in Table 3.

**Proteomic studies**

Proteomics is the large-scale study of proteins, allowing analyses of the entire complement of proteins in a cell or sample simultaneously, and made possible today by technological advances in computing and data processing. These approaches have been used to characterize biochemical interactions and protein signaling in bone remodeling [75,76]. Like gene expression studies, recent proteomic studies of osteoporosis have focused on osteoblasts and osteoclasts and their precursors [77-80].

Saad and Hofstaetter [81] identified 16 proteins that may have a role in osteoblast matrix mineralization. Choi et al. [82] investigated differentiation of MSCs and osteoblastogenesis and identified SMOC1 (SPARC-related modular calcium-binding protein) as an important extracellular matrix protein in osteoblast differentiation. By using both gene and protein expression analyses in aged bone, MSC-derived adipocytes were also shown to have potential roles in regulating osteoblast differentiation through transforming growth factor β (TGF-β)-mediated canonical Wnt signaling [83]. A proteomic study conducted using PBMs identified a novel annexin protein, ANXA2, that was upregulated twofold in Caucasians with extremely low BMD compared with those with extremely high BMD [39]. ANXA2 protein significantly promoted monocyte migration across an endothelial barrier in vitro; this suggested that elevated ANXA2
protein expression levels in subjects with low BMD may be involved in increased PBM migration to bone resorption surfaces in vivo, where higher numbers of osteoclasts might resorb bone at higher rates resulting in decreased BMD [39].

A proteomic approach was used to show that specific antibodies could suppress bone turnover [84]. Kostenuik et al. [85] also reported that the human monoclonal antibody denosumab bound to human RANKL but not to murine RANKL, human TRAIL, or other human TNF family members in direct binding assays. Knock-in technology was applied to create ‘huRANKL’ mice by replacement with a human RANKL fragment encoded primarily by the fifth exon of the RANKL gene [85]. In young huRANKL mice, denosumab and OPG-Fc (an osteoprotegerin-immunoglobulin Fc segment complex) each reduced the osteoclast surfaces of trabecular bone (spongy bone or cancellous bone) by 95% and also increased bone density and volume [85]. In adult huRANKL mice, denosumab reduced bone resorption, increased the bone mass of both cortical bone (a dense type of bone tissue) and cancellous bone (the spongy inner layer of bone), and improved trabecular microarchitecture [84]. Subsequently, Kendler et al. [84] separated 504 postmenopausal women over 55 with low BMD who had been receiving alendronate therapy (which is used to slow down bone loss and increase bone density) for at least 6 months into two groups of continued weekly alendronate therapy or subcutaneous denosumab therapy. Transition to denosumab produced greater increases in BMD at all measured skeletal sites and a greater reduction in bone turnover than did continued alendronate, with a similar safety profile in both groups [84].

Like gene expression studies, proteomic studies have also been conducted using relatively small sample sizes, and there have been difficulties in replicating results. Although proteomic approaches are still at an early stage in the bone research field, they represent one of the most promising methods for generating insights for human osteoporosis and have shown promise for the identification of novel proteins and genes [86-90]. Examples of recent proteomic studies of osteoporosis are summarized in Table 4.

**Epigenome-wide studies**

Epigenetic regulation is important for sustaining normal growth and development of animals [91]. Dysregulation of these mechanisms is involved in many human diseases, such as cancer, intellectual disability, and immunodeficiency, and in aging [91-94]. DNA methylation, histone modifications, and RNA-mediated mechanisms are all known to have key roles in epigenetic regulation, and they are being investigated in bone disease [95].

DNA methylation involves the covalent transfer of a methyl group to the fifth carbon of cytosine in CpG dinucleotides in the genome [96]. The influence of CpG methylation in human bone has been investigated. Hsiao et al. [97] found that suppression of the TRIP10
promoter by DNA methylation resulted in acceleration of MSC-to-osteocyte differentiation [97]. Delgado-Calle et al. reported [98] that the SOST gene (encoding sclerostin, an inhibitor of bone formation) was dramatically upregulated by demethylating agent AzadC, and that DNA methylation strongly hindered RANKL and OPG expression [99].

Histone modifications can regulate gene expression by influencing interactions between DNA and histones, for example by acetylation and methylation of conserved lysine residues in the amino-terminal tail domains [100]. Histone modification has been studied in a variety of cancers, such as prostate, breast, lymphoma, and ovarian cancer [54]. However, studies of osteoporosis are scarce, with the limited information suggesting a role in developmental processes that may be related to bone metabolism [101].

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RNA interference (RNAi) and noncoding RNAs (such as microRNAs) have drawn much attention in the field [95]. You et al. [102] reported that the zinc finger protein Zfp467 stimulated osteoblast differentiation of cultured adipose-derived stem cells, and that Zfp467-targeted RNAi could restore bone function and structure in an ovariectomy-induced osteoporotic mouse model.

In summary, some novel factors and mechanisms have been identified in a limited number of epigenetic studies of bone pathogenesis. However, further epigenome-wide studies will be needed to investigate the role of these epigenetic mechanisms in osteoporosis.

**Implications for pathogenesis**

Most of the genetic variants or genomic regions that have so far been identified by GWASs of osteoporosis-related traits have been intronic or intergenic [71]. These variants or regions could be transcription factor binding sites that regulate or affect gene expression [71], but precisely how they might influence bone mass awaits further investigation. Ultimately, GWAS is an indirect genetic mapping approach that relies on linkage disequilibrium, so further studies are needed to pinpoint functional variants by deep sequencing and functional molecular studies.

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**Table 4. Examples of proteomic studies of osteoporosis**

| Participants or cells/treatment | Identified protein expression | Related functions/pathways | Refs |
|--------------------------------|-----------------------------|---------------------------|------|
| Human bone MSCs                | 64 secreted proteins upregulated, especially SMOC1 | Osteoblast mineralization | [82] |
| Human PBMs                     | ANXA2                       | Osteoclast differentiation | [39] |
| Human osteocytes               | LRP4                        | A sclerostin interaction partner | [87] |
| Chinese with low BMD           | SOD 2 upregulated           | Located in circulating monocytes (potential osteoclast precursors) | [89] |
| Osteoblast differentiation in mouse osteoprogenitor MC3T3-E1 cells | Upregulation of IQGAP1, gelsolin, moesin, radixin, and CFL1 | Cytoskeleton regulation | [79] |
|                               | Upregulation of FLNA, LAMA1, LAMA5, COL1A1, COL3A1, COL4A6, COL5A2; downregulation of COL4A1, COL4A2, and COL4A4 | Focal adhesion signaling | [79] |
| Osteoblast differentiation in MC3T3-E1 preosteoblast cells/leukocyte common antigen-related tyrosine phosphatase | ALP, BSP, DLX5, OCN, and RUNX2 | Erk activation | [80] |
| Osteoblasts and osteoclasts induced from RAW 264.7 macrophage cell line (from murine blood)/Er-Xian Decoction treatment | In osteoblasts: 8 proteins upregulated | Hmgb1, acidic ribosomal phosphoprotein P0, histone H2, carbonyl reductase 1, ATP synthase, aldolase A, and GDIα | [78] |
|                               | In osteoblasts: 3 proteins downregulated | Carbonic anhydrase 3, prohibitin, hemiferrin, far upstream element-binding protein | [78] |
|                               | In osteoclasts: 3 proteins upregulated | Vimentin, protein disulfide isomerase associated 3 and α-fetoprotein | [78] |
|                               | In osteoclasts: 1 protein downregulated | Calnexin | [78] |
| Rat primary calvarial osteoblast/Kaempferol | 9 proteins upregulated, 9 downregulated | Including cytoskeletal proteins, intracellular signaling protein, chaperone, extracellular matrix protein, and proteins involved in glycolysis and cell-matrix interactions | [77] |
| Ovariectomized rats            | SOD1, ATP synthase, and COMT | Protection against bone loss | [90] |

*a*For gene abbreviations and explanations of pathways, see Table 5.
Table 5. Key pathways associated with osteoporosis

| Genes                        | Full names                                                                 | Refs                |
|------------------------------|-----------------------------------------------------------------------------|---------------------|
| **RANK/RANKL/OPG pathway**    |                                                                            |                     |
| TNFSF11                      | TNF (ligand) superfamily, member 11 (RANKL)                                 | [55,56]             |
| TNFRSF11A                    | TNF receptor 11a, NF-κB activator (RANK)                                    | [26,37,55]          |
| TNFRSF11B                    | TNF receptor 11b (OPG)                                                     | [26,53,56]          |
| **TNF-R1/TRAIL signaling pathway** |                                                                           |                     |
| CFLAR                        | CASP8 and FADD-like apoptosis regulator                                      | [37]                |
| NFKB1                        | Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1         | [37]                |
| TNFSF10                      | TNF (ligand) superfamily, member 10                                         | [37]                |
| TNFRSF11B, TNFRSF10B         | TNF receptors                                                              | [37]                |
| TRAF3                        | TNF receptor-associated factor 3                                            | [37]                |
| **Wnt signaling pathway**     |                                                                            |                     |
| CTNNB1                       | B-catenin                                                                  | [26,53]             |
| DKK1                         | Dickkopf-related protein 1                                                  | [33,74]             |
| LRP4, LRP5                   | Lipoprotein receptor related peptides                                       | [32,33,53,56,57]    |
| RSPO3                        | R-spondin 3                                                                | [25]                |
| SOX6                         | Sclerostin                                                                 | [56,57]             |
| WNT4, WNT15                  | Wnt proteins                                                               | [61]                |
| **Autophagy regulation pathway** |                                                                           |                     |
| ATG5, ATG7, ATG12             | Autophagy-related proteins                                                 | [36]                |
| IFNA4, IFNA5, IFNA7, IFNA8, IFNA12, IFNA14, IFNA16, IFNA17, IFNA21 | Interferon α proteins                                                      | [36]                |
| PIK3C3                       | Phosphatidylinositol 3-kinase catalytic subunit type 3                      | [36]                |
| **Other pathways**            |                                                                            |                     |
| ALDH7A1                      | Aldehyde dehydrogenase 7 family, member A1                                 | [33]                |
| ARHGAP1                      | Rho GTPase activating protein 1                                             | [26,32]             |
| COL1A1                       | Collagen, type I, α1                                                       | [43,66,79]          |
| DCCDS                        | Doublecortin domain containing 5                                           | [26,53]             |
| ESR1                         | Estrogen receptor 1                                                        | [29,32,48]          |
| FLJ42280                     | Putative uncharacterized protein FLJ42280                                   | [26,53,55]          |
| FDL1                         | Forkhead box L1                                                            | [26,53]             |
| GALNT2                       | UDP-N-acetyl-d-galactosamine: polypeptide N-acetylgalactosamyltransferase 3 | [25]                |
| HDAC5                        | Histone deacetylase 5                                                      | [26,53]             |
| JAG1                         | Jagged 1 (Notch ligand)                                                    | [46]                |
| MEF2C                        | Myocyte enhancer factor 2C                                                 | [26]                |
| MEPE                         | Matrix extracellular phosphoglycoprotein                                    | [26,33,53]          |
| STARD3NL                     | STARD3 amino-terminal like protein                                         | [26,53]             |
| SOX6                         | Sex-determining region Y-box 6                                             | [26,41,51]          |
| SP7                          | Sp7 transcription factor                                                   | [26]                |
| SPTBN1                       | Spectrin, β, non-erythrocytic 1                                            | [26,33,58]          |
| TGFBR1                       | TGF, β receptor 1                                                          | [66]                |

*Gene abbreviations: AGER, Advanced glycosylation end product-specific receptor; ALP1, Actinin-associated LIM protein; ANXA2, Annexin A2; BMP2, Bone morphogenetic protein; BSP, Binder of sperm; C6orf97, Chromosome 6 open reading frame 97; CBFA1, Core binding factor A1; CF11, Coflin 1; CLCN7, Chloride channel 7; COL1, Collagen; COMT, Catechol-O-methyltransferase; CRYPB, α-crystallin B chain; CT5K, Cathepsin K; CXC12, Chemokine ligand 2; CXC4, Chemokine receptor 4; DLX5, Distal-less homeobox 5; EPHAS5, Ephrin A receptor 5; ERK, Extracellular signal-regulated kinase; FAM210A, Family with sequence similarity 210A; FLG, Filaggrin; G0S2, G0S2 kinase-like protein; GPR177, G protein-coupled receptor 177; GPRC5C, G protein-coupled receptor; GRO1, Melanoma growth stimulating activity α; Hmgb1, High mobility group protein; HMGN3, High mobility group nucleosomal binding domain 3; IBSP, Integrin-binding sialoprotein; ID4, Inhibitor of DNA binding dominant negative helix-loop-helix protein; IGFR-1, Insulin-like growth factor; IL, Interleukin; IL21R, Interleukin 21 receptor; IGGAP1, I2 motif containing GTPase activating protein; LAMA, Laminin α; MMP9, Matrix metallopeptidase 9; NAM, Necdin; NDV, Nephroblastoma overexpressed; OCN, Osteocalcin; P3, P3 oncogene; PHEX, Phosphate regulating endopeptidase homolog; PTH, Parathyroid hormone; PTHrP, PTH-related protein; RUNX2, Run-related transcription factor 2; SLC25A13, Aspartate/glutamate carrier; SLP6, Secretory leukocyte peptidase inhibitor; SOCS3, Cytokine signaling suppressor 3; SOD1, Superoxide dismutase 1, soluble; SOD2, Superoxide dismutase 2, mitochondrial; SORT1, Sortilin 1; STARD3NL, STARD3 amino-terminal like gene; STAT1, Signal transducer and activator of transcription 1; TFPI2, Tissue factor pathway inhibitor; ZBTB40, Zinc finger and BTB domain containing 40. This pathway also includes TNFSF11, TNFRSF11A, and TNFRSF11B."
Several genes or proteins have shown remarkable association with osteoporosis-related traits in genome-wide studies, and these have become interesting candidates for further studies. For example, both GWASs and gene expression studies have shown that DKK1 (encoding a dickkoph-related protein) [31,74], SOST [53], MEPE (encoding matrix extracellular phosphoglycoprotein) [33], SOX4 (sex-determining region Y-box 4 gene) [25] and the spectrin gene SPTBN1 [31,67] were strongly associated with BMD. COL1A1 [18,43,66,79] is a candidate gene whose association with BMD has been confirmed by four types of genome-wide approaches (GWLS, GWAS, gene expression, and proteomic studies). Also, results from GWAS, gene expression, and proteomic studies have confirmed that RANK, RANKL, OPG, and LRP4 (encoding lipoprotein receptor related peptide 4) have important roles in regulating BMD.

These significant risk factors can be grouped into different pathways, which provide insights into the pathogenesis of osteoporosis. For example, LRP-4, -5, and -6 can bind Wnt ligands to form a receptor complex and initiate the Wnt signaling pathway, which is involved in differentiation and growth of cell types such as osteoblasts or osteoclasts [103]. However, these proteins are also essential oncogenic receptors that may increase the risk of cancers by interacting with components of the Wnt signaling pathway [104]. RANK, RANKL, and OPG are known to be involved in different biological pathways that are important for bone mass regulation, such as the RANKL pathway, the TNF-R1 signaling pathway and the TRAIL pathway [103]. Table 5 summarizes the key genes, loci, and pathways associated with osteoporosis and related traits.

From genome-wide approaches to targeted interventions

Although a large number of genome-wide linkage and association studies on osteoporosis have been published over the past decade, there have been many unreplicated results [27]. As mentioned earlier, factors that may significantly contribute to inconsistencies in genetic studies of osteoporosis include inadequate statistical power, population stratification, genetic heterogeneity, experimental errors, and limited coverage of genomic regions [27]. The occurrence of gene-gene interactions and gene-environment interactions might also contribute to these challenges.

Significant gene-gene interaction effects have been found to influence osteoporosis risk in different studies [103]. For example, a role for the CD40/CD40L system was revealed in bone metabolism regulation [105]. Pineda et al. [105] conducted an association study of BMD with SNPs in CD40 and CD40L genes. The study indicated a strong interaction between polymorphisms in these genes that might have a synergistic role in BMD regulation [105]. Also, the role of environmental factors and their potential interactions with key genes or loci requires further study. Environmental factors such as dietary intake and medication can trigger gene responses and gene-gene interactions. For example, Sonoda et al. [106] found that the SNPs rs2077647 and rs2234693 in the estrogen receptor α gene were significantly associated with osteoporosis risk, and that the osteoporotic haplotype CC at these SNPs was also significantly associated with alcohol drinking [106].

The confounding factors and complicated nature of osteoporosis indicate that appropriate study design and interpretation of results are crucial to ensure reproducibility. Therefore, it is also important to use multidisciplinary approaches, such as gene expression profiling, proteomics, and epigenetics, which may complement each other and provide information for cross-validation. Combinations of different genome-wide analyses have recently been used in bone research. Lei et al. [59] performed gene expression profiling using monocytes from 26 Chinese and 20 Caucasian premenopausal women with extremely high or low BMD, and identified a list of differentially expressed genes, some of which were further confirmed by GWAS. By combining information from gene expression and genome-wide association studies, Farber et al. [107] identified the ASXL2 gene as a novel regulator of BMD and osteoclastogenesis. Although integrating data from multidisciplinary studies could be challenging, such approaches may help to identify some of the most interesting susceptibility genes and factors for osteoporosis, and could provide leads for the development of new targeted preventive interventions and treatments.

The estrogen receptor gene ESR1 has been reported to be involved in bone metabolism in numerous studies [29,31,32,48,53,57]. In current osteoporosis treatments, estrogen or estrogen-like medications known as estrogen receptor modulators have been clinically applied to provide protection against postmenopausal bone loss [106]. Such medications can exclusively target the estrogen receptor and produce estrogen-like effects in the bone, in addition to their effects in decreasing the occurrence of breast cancer [106].

Similarly, the role of the RANK/RANKL/OPG pathway in osteoporosis has been confirmed in many studies and involves several susceptibility genes and factors that influence bone modeling [103]. Denosumab, which is a specific antibody that targets RANKL, may reduce reproduction of osteoclasts and may be useful as an approach for restoring bone metabolism balance. Administration of denosumab has been reported to improve BMD and to reduce fractures in postmenopausal women who suffer from osteoporosis, although some side effects have been reported [108].
In gene expression studies, PTH has shown an ability to stimulate both bone resorption and new bone formation [70]. PTH has been used for the treatment of severe osteoporosis [108]. The effects of intermittent or continuous PTH treatment on bone metabolism are still under investigation to understand the underlying mechanisms and optimize its application to osteoporosis [109].

**Concluding remarks and future perspectives**

Despite extensive efforts, currently there is not sufficient information to allow effective assessment of osteoporosis risk. The majority of the recently identified risk factors are still pending further investigation, and it is therefore too early to define novel biological factors as preventive or treatment targets for osteoporosis. This does not imply that current genome-wide approaches are futile, but rather indicates that appropriate implementation of these studies might help to reduce potential bias and confounding factors.

Genome-wide approaches individually have specific limitations. Gene expression is a complex process that is regulated simultaneously and interactively at DNA, RNA, protein, epigenomic, and environmental levels. Therefore, a genomic convergence or systems biology approach that integrates the information from studies such as GWLs, GWASs, DNA sequencing, gene expression, proteomics (including studies of post-translational modifications), epigenomics, and gene-environment studies may help facilitate the identification of key pathways that are globally involved in the pathogenesis of osteoporosis and osteoporotic fractures. Ultimately, the functional relevance of the identified variants then needs to be confirmed by in vivo and/or in vitro molecular biology studies.

**Abbreviations**

BMD: bone mineral density; CNV, copy number variation; GWAS, genome-wide association study; GWLS, genome-wide linkage study; MSC, mesenchymal stem cell; PBM, peripheral blood monocyte; PTH, parathyroid hormone; QTL, quantitative trait loci; SNP, single nucleotide polymorphism. For gene abbreviations see Table 5.

**Competing interests**

The authors declare that they have no competing interests.

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