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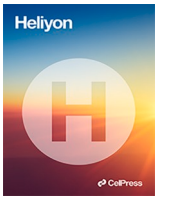

Research article

Potential drug targets for osteoporosis identified: A Mendelian randomization study

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A R T I C L E I N F O

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A B S T R A C T

Background: Osteoporosis is a prevalent global health condition, primarily affecting the aging population, and several therapies for osteoporosis have been widely used. However, available drugs for osteoporosis are far from satisfactory because they cannot alleviate disease progression. This study aimed to explore potential drug targets for osteoporosis through Mendelian randomization analysis.

Methods: Using cis-expression quantitative trait loci (cis-eQTL) data of druggable genes and two genome-wide association studies (GWAS) datasets related to osteoporosis (UK Biobank and FinnGen cohorts), we employed mendelian randomization (MR) analysis to identify the druggable genes with causal relationships with osteoporosis. Subsequently, a series of follow-up analyses were conducted, such as colocalization analysis, cell-type specificity analysis, and correlation analysis with risk factors. The association between potential drug targets and osteoporosis was validated by qRT-PCR.

Results: Six druggable genes with causal relationships with osteoporosis were identified and successfully replicated, including ACPP, DNASE1L3, IL32, PPOX, ST6GAL1, and TGM3. Cell-type specificity analysis revealed that PPOX and ST6GAL1 were expressed in all cell types in the bone samples, while IL32, ACPP, DNASE1L3, and TGM3 were expressed in specific cell types. The GWAS data showed there were seven risk factors for osteoporosis, including vitamin D deficiency, COPD, physical activity, BMI, MMP-9, ALP and PTH. Furthermore, ACPP was associated with vitamin D deficiency and COPD; DNASE1L3 was linked to physical activity; IL32 correlated with BMI and MMP-9; and ST6GAL1 was related to ALP, physical activity, and MMP-9. Among these risk factors, only MMP-9 had a high genetic correlation with osteoporosis. The results of qRT-PCR demonstrated that IL32 was upregulated while ST6GAL1 was downregulated in peripheral blood of osteoporosis patients.

Conclusion: Our findings suggested that those six druggable genes offer potential drug targets for osteoporosis and require further clinical investigation, especially IL32 and ST6GAL1.

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1. Introduction

Osteoporosis is a common skeletal disorder characterized by reduced bone mineral density, which makes bones more susceptible to fractures [1]. The global prevalence of osteoporosis is 18.3 % [2]. Considering the growing aging population, osteoporosis is steadily emerging as a prevalent global health condition. Bone homeostasis relies on the delicate balance between osteoclast-mediated bone resorption and mesenchymal lineage osteoblast-mediated bone formation, which involves a complex and tightly regulated processes [3]. Various drugs have been developed for clinical treatment of osteoporosis based on the complex mechanisms of bone homeostasis, including calcitonin, selective estrogen receptor modulators, bisphosphonates, and molecular-targeted drugs. However, many of these medications have serious side effects or are not suitable for long-term use [4]. Therefore, it is essential to identify more molecular drug targets for effective osteoporosis treatment.

Integrating genetics into medication development is a promising strategy for enhancing this process, as therapies supported by genetic evidence have a high likelihood of success in clinical trials [5,6]. Proteins encoded by druggable genes have been identified as valuable targets for drug development, including the development of small molecules or monoclonal antibodies [7,8]. Previous observational research has identified associations between protein levels and bone mineral density [9–11], but these findings could be influenced by confounding factors and reverse causation, obscuring the clarity of the causal relationship [12]. Mendelian randomization (MR) is a statistical approach that has been employed to investigate the causal link between exposure and outcome utilizing genetic instrumental variables (IVs) [13]. This approach can overcome issues such as reverse causation and the influence of unmeasured confounders that are commonly encountered in observational studies [14]. While randomized controlled trials (RCTs) are considered the gold standard for inferring causality, they are costly, resource-intensive, time-consuming, and may pose ethical limitations [15]. MR offers a way to explore drug targets and predict drug efficacy through mimicking RCTs [16,17]. In comparison to clinical trials, MR analysis provides benefits in terms of financial savings, efficient utilization of materials, and time efficiency. In drug-target MR analysis, this approach utilizes genetic variants (such as cis-expression quantitative trait loci (cis-eQTL) located in the genomic region of druggable genes) as proxies to assess causal associations between exposures and clinically relevant outcomes [18,19]. A recent study has identified putative causal proteins as druggable targets of osteoporosis by MR analysis [20]. However, genomic evidence for promising drug targets for osteoporosis has not been fully explored.

In this study, we aimed to explore the promising druggable genes with causal relationships to osteoporosis through MR analysis, followed by colocalization analysis, meta-analysis, gene-based association analysis, cell-type specificity analysis, and correlation analysis with risk factors. Moreover, we validated the association between potential drug targets and osteoporosis by qRT-PCR. The findings of our study will provide valuable insights into targeted treatment approaches for osteoporosis.

2. Data and methods

2.1. The cis-eQTL data associated with druggable genes

A total of 4302 druggable protein-coding genes located on the autosomal chromosomes were extracted from a previously published literature [7]. The cis-eQTL data [21] from a large-scale meta-analysis in 31,684 peripheral blood samples were downloaded from the eQTLGen Consortium. The cis-eQTL mapping was carried out in each cohort using a pipeline described previously [22]. Briefly, the pipeline involves selecting genes or expression probes within a 1 Mb window upstream and downstream of each SNP, centered on the gene or probe position. Associations between SNP-gene pairs are determined using Spearman correlation. Each cohort conducted ten permutations where genotype-expression mappings were shuffled before recalculating associations. Results from non-permuted and permuted datasets were meta-analyzed across all cohorts after each permutation round. For multiple-testing, meta-analyzed permutations were utilized to calculate the overall false discovery rate (FDR). These cis-eQTL data had complete statistical significance (FDR <0.05, ± 1 Mb per probe). The single nucleotide polymorphism (SNP) loci within ± 100 kb of the genome position of each gene were selected, and the eQTLs available for 2478 druggable genes located on the autosomal chromosomes were identified [7]. Furthermore, a series of quality control filtering on the loci within each gene were conducted, including removing weak IVs bias by EAF >1 %, P < 5e-8, and F-statistic >10, as well as removing linkage disequilibrium with an $r^2 < 0.1$ based on reference data from the European population in the 1000 Genomes Project [18]. The LDTrait tool (https://ldlink.nih.gov/?tab=ldtrait) was used to determine whether variants have previously been associated with a trait, thereby excluding confounding and publication bias.

2.2. Outcome data

Two genome-wide association studies (GWAS) datasets related to osteoporosis (ukb-b-17796 and finn-b-M13_OSTEOPOROSIS) were selected from the OpenGWAS database (https://gwas.mrcieu.ac.uk/). The ukb-b-17796 dataset was from UK Biobank and used as the discovery cohort. This dataset consisted of 1976 cases and 461,034 controls, with a total sample size of 463,010. The finn-b-M13_OSTEOPOROSIS dataset was obtained from the FinnGen database and utilized as the validation cohort. This dataset included 3203 cases and 209,575 controls, with a total sample size of 212,778. There is no sample overlap between the two osteoporosis datasets.

2.3. Other data

The PubMed database was searched for "(Osteoporosis) AND (risk factors)" and identified 15 potential risk factors related to

osteoporosis, including chronic obstructive pulmonary disease (COPD), body mass index (BMI), fat-free mass index (FFMI), sarcopenia, sex, age, frequent exacerbations of COPD, use of systemic corticosteroids, low levels of physical activity, systemic inflammation, vitamin D deficiency, increased bone turnover markers, matrix metalloproteinase-9 (MMP-9), parathyroid hormone (PTH), and alkaline phosphatase (ALP) [23]. Subsequently, the OpenGWAS database was searched, and GWAS summary data on seven of these risk factors were obtained, including COPD: ukb-b-13447; BMI: ukb-b-19953; physical activity: ieu-b-4860; vitamin D deficiency: finn-b-E4_VIT_D_DEF; MMP-9: prot-a-1921; PTH): prot-a-2431; and ALP: ebi-a-GCST005061.

2.4. MR analysis for identifying genes with causal relationships with osteoporosis

MR analysis for eQTL data and osteoporosis were conducted using TwoSampleMR R package (version 0.5.7) [24]. The statistical methods for MR analysis were chosen as outlined in the previous literature [25]. In detail, after extracting the association estimates between the variants and either the exposures or outcomes, the direction of estimates was standardized based on effect alleles. The Wald ratio calculation method was utilized to estimate the causal effects when the number of IVs was 1. If more than one IV was available, the inverse variance weighted (fixed-effects) model was selected when the number of IVs was 2–3; and the inverse variance weighted method (multiplicative random-effects) was applied when the number of IVs was >3 [26]. A cut-off of 3 was selected for the random-effects model, as using more than 3 variants could introduce some heterogeneity among IVs. The multiplicative random-effects model permits heterogeneity among the causal estimates targeted by genetic variants through enabling over-dispersion within the regression model.

In the UK Biobank cohort, the genes with causal relationships with osteoporosis were identified with the threshold value of $p < 0.001275$ (false discovery rate (FDR) < 0.05). When these genes could be replicated in the FinnGen cohort, the significance threshold was set as $p < 0.005325$ (FDR <0.05).

2.5. Colocalization analysis

Based on MR-analysis results, the colocalization analysis was conducted to investigate whether SNPs associated with osteoporosis and eQTLs shared genetic variants. When GWAS signals and eQTLs co-localized, the genetic variants at the GWAS signals might have influenced the phenotype through altering the biological processes of gene expression. The probability of co-localization was calculated using the coloc (version 5.2.1) [27] in R package. The coloc package employed Bayesian algorithms and allows estimation of the probability of shared causal genetic variants using summary data.

In this analysis, in the context of SNP causality within a region of Q variants (typically SNPs), each trait could be represented by a binary vector of length Q with values (0, 1), where 1 indicated a causal association with the trait and at most one entry was non-zero. Each possible pair of vectors (for traits 1 and 2, termed "configuration") could be assigned to one of five hypotheses: H0 (no association with either trait), H1 (association with trait 1, not with trait 2), H2 (association with trait 2, not with trait 1), H3 (association with trait 1 and trait 2, two independent SNPs), and H4 (association with trait 1 and trait 2, one shared SNP). The colocalization problem can be re-formulated as evaluating the support for all configurations in hypothesis H4. The method was Bayesian, integrating over all possible configurations, with prior probabilities defined at the SNP level. Posterior probabilities can then be computed for each hypothesis by summing the probabilities of all configurations and combining them with the prior. This procedure yielded five posterior probabilities (PP.H0, PP.H1, PP.H2, PP.H3, and PP.H4). A large PP.H3.abf indicated strong evidence for two independent causal SNPs associated with each trait. Conversely, a large PP.H4.abf supported a single variant affecting both traits.

2.6. Meta-analysis of two osteoporosis GWAS datasets

Using the GWAS summary data of UK Biobank and FinnGen cohorts, a meta-analysis was performed using Linux Metal [28] to combine the data from the two cohorts. The sample size estimation for meta-analysis was conducted using METAL [29]. The results were visualized using Manhattan and QQ plots that were drawn using the CMplot package (version 4.3.1) [30]. The SNP with a p-value $< 1^{-5}$ was considered as suggestive loci, and the nearest genes associated with these loci were visualized in the Manhattan plot [31].

2.7. Analysis of osteoporosis-associated genes and functional enrichment analysis

Based on data from meta-analysis, the genes associated with osteoporosis were analyzed utilizing MAGMA (version 1.10) [32] with the threshold value of $p < 0.05$. The osteoporosis-associated genes obtained from MAGMA and the causal genes identified from MR analysis were then subjected to functional enrichment analysis using WebGestalt to examine the enriched pathways and functions related to osteoporosis.

2.8. Cell-type specificity analysis

To determine whether druggable genes were cell-type specific in human bone samples, the single-cell expression of druggable genes was visualized using the Seurat package (version 4.1.1) based on the single-cell RNA sequencing (scRNA-seq) data of human embryonic bone (GSE143753 dataset) [33]. This dataset was downloaded from the Gene Expression Omnibus (GEO) database and included 16 subclusters from limb bud and long bone samples, comprising a total of 35,570 cells. We adopted the filtering method, preserving 35,570 cells of annotated cell types. Additionally, we employed uniform manifold approximation and projection (UMAP)

for dimension reduction to map the cell types and gene expression levels based on the original literature published by He et al. [33].

2.9. Correlation analysis with risk factors

The GWAS summary data of risk factors for osteoporosis were downloaded from OpenGWAS database. Then, their genetic correlation with data from UK Biobank cohort, FinnGen cohort, and meta-analysis was analyzed using the LDSC software (version 1.0.1) [34].

2.10. Sample Preparation

From October 2023 to December 2023, peripheral whole blood samples from 9 osteoporosis patients (7 females and 2 males; average age 68 ± 9.7) and 10 healthy adults (9 males and 1 females; average age: 30 ± 7.0) in the Honghui Hospital, Xi'an Jiaotong University were obtained. The study was reviewed and approved by the Ethics Committee of Honghui Hospital, Xi'an Jiaotong University. Each participant provided written and signed informed consent. Then, we use human peripheral monocyte-negative isolation kit (IPHASE) separated blood monocyte from whole blood following the manufacturer's recommendation. Real-time quantitative PCR was performed according to our previous study (25). The primers for genes are displayed in Table 1. All experiments were performed at least three times independently.

2.11. Statistical analysis

The gene expression data were presented as mean ± standard deviation (SD). The differences between osteoporosis patients and healthy controls were analyzed with *t*-test. Statistical analyses were carried out with SPSS version 22.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Overall analysis plan

The overall scheme of the analyses is depicted in Fig. 1. We first extracted 4602 druggable genes from a previous study published by Finan et al. [7] and selected 2478 druggable genes based on cis-eQTL data in 31,684 peripheral blood samples. Next, using the MR approach, we identified druggable genes associated with osteoporosis based on the discovery cohort (UK Biobank cohort) and replicated using the validation cohort (FinnGen cohort). For significant MR results, a series of follow-up analyses were conducted, including colocalization analysis, meta-analysis of two GWAS datasets, gene-based association analysis, cell-type specificity analysis, and correlation analysis with risk factors.

3.2. Identification of druggable genes with causal relationships with osteoporosis

Using cis-eQTL data from the eQTLGen Consortium, 2478 druggable genes were identified as exposure variables. Using the discovery cohort (UK Biobank cohort) as outcome variables, MR analysis was performed and 54 druggable genes associated with osteoporosis were identified with FDR <0.05. Further using these 54 druggable genes as exposure variables, six druggable genes with causal relationships with osteoporosis were successfully replicated using the validation cohort (FinnGen cohort) as outcome variables, including acid phosphatase, prostate (ACPP), deoxyribonuclease 1-like 3 (DNASE1L3), interleukin 32 (IL32), protoporphyrinogen oxidase (PPOX), ST6 beta-galactosamide alpha-2,6-sialyltranferase 1 (ST6GAL1), and transglutaminase 3 (TGM3). In addition, we performed pleiotropy and heterogeneity analyses on the results associated with these six genes. We found that apart from DNASE1L3, which might have potential heterogeneity, there was no pleiotropy or heterogeneity in the other results. The results of MR analysis for two cohorts are shown in Table 2.

Table 1
The information of the primers' sequencing.

Gene	Primers	
	Forward	Reverse
ACPP	CAAGACTGGTCCACGGAGTGTA	AGCAGAGTCCACGGCGAATGTG
DNASE1L3	TGGTTGAGGTCTACACGGACGT	GTCAGTCCTCAAGCGGATGTTC
IL32	TCAAAGAGGGCTACCTGGAGAC	TCTGTTGCCTCGGCACCGTAAT
PPOX	AGCCACTGCTTGGTCCATCTAC	CTGTGAGCAGTCAGGAATTGCC
ST6GAL1	CTGAATGGGAGGGTTATCTGCC	ACCTCAGGACTGCGTCATGATC
TGM3	ATGGCAGGTGTTGGATGCTACC	CCGCGAAGATAAAGGGCATGTC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

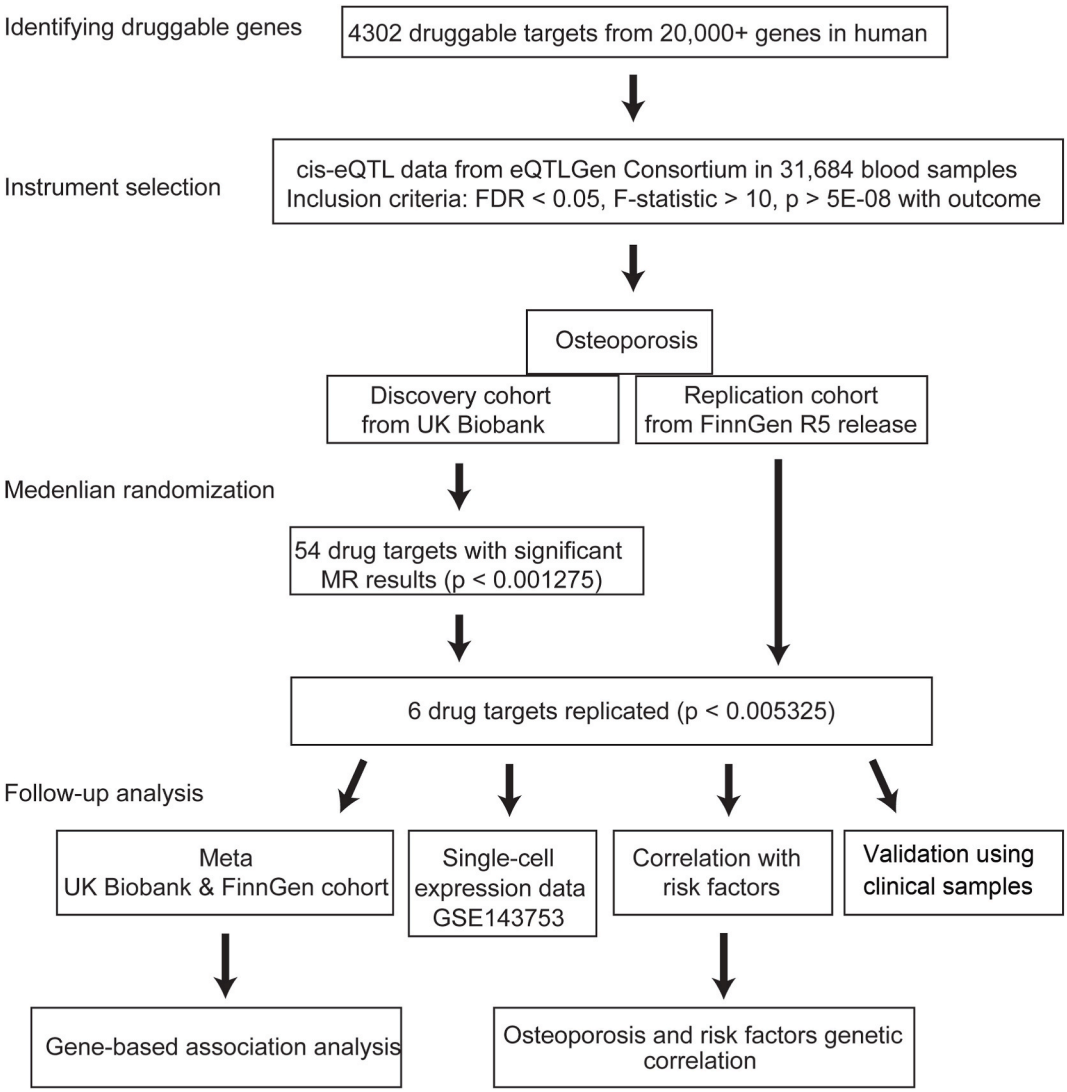

Fig. 1. Overview of the study design.

3.3. Colocalization analysis for eQTLs and osteoporosis GWAS summary data

To determine the probability that SNPs associated with osteoporosis and eQTLs shared causal genetic variants, we conducted a colocalization analysis. The results showed that the PP.H4.abf of the 12 pairs (six druggable genes with two GWAS cohorts) were all lower than 0.8 (Table 3), suggesting that there was no shared causal variant between the druggable genes from MR analysis and osteoporosis.

3.4. Meta-analysis results of GWAS summary data from UK Biobank and FinnGen cohorts

To analyze the genetic loci associated with osteoporosis, we conducted a meta-analysis of GWAS summary data from UK Biobank and FinnGen cohorts. We identified a total of 29 significant genetic loci with the significance threshold set at p-value $< 1^{-5}$. The nearest genes of these suggestive loci were visualized in the Manhattan plot (Fig. 2A). Additionally, we calculated the genomic inflation factor of meta-analysis datasets. The QQ plot indicated no population stratification in the results (Fig. 2B).

3.5. Analysis of osteoporosis-associated genes and functional enrichment analysis

To explore the osteoporosis-associated genes, we performed a gene-based association analysis based on the aforementioned meta-analysis results. We analyzed whether SNPs were located within a 20-kb window upstream or downstream of the gene, with linkage disequilibrium calculated based on the data from the European population of the 1000 Genomes Project Phase 3. With the threshold

Table 2
Mendelian randomization results.

Genes	UK Biobank cohort					FINNGEN cohort				
	SNPs	OR (95 % CI)	P-value	Heterogeneity-Qpval	Pleiotropy-pval	SNPs	OR (95 % CI)	P-value	Heterogeneity-Qpval	Pleiotropy-pval
ACPP	4	1.000 (0.999,1.000)	1.79E-04	0.966	0.876	17	1.090 (1.034,1.149)	1.45E-03	0.985	0.910
DNASE1L3	3	0.998 (0.997,0.999)	1.23E-03	0.047	0.444	12	1.269 (1.089,1.479)	2.23E-03	0.321	0.357
IL32	7	0.999 (0.998,0.999)	7.08E-05	0.549	0.959	22	1.167 (1.076,1.265)	1.86E-04	0.279	0.869
PPOX	5	1.001 (1.001,1.001)	1.19E-19	0.997	0.925	11	1.372 (1.118,1.685)	2.50E-03	0.218	0.058
ST6GAL1	11	0.999 (0.999,1.000)	1.23E-04	0.947	0.841	23	0.841 (0.770,0.919)	1.31E-04	0.903	0.468
TGM3	4	0.998 (0.998,0.999)	3.22E-09	0.939	0.936	9	0.861 (0.775,0.957)	5.32E-03	0.903	0.658

Table 3
The results of colocalization analysis.

Trait1	Trait2	nsnps	PP.H0.abf	PP.H1.abf	PP.H2.abf	PP.H3.abf	PP.H4.abf
ACPP	finn	471	0	0.91097	0	0.015903	0.073127
ACPP	ukb	268	0	0.999894	0	2.58E-05	8.07E-05
DNASE1L3	finn	325	######	0.95922	######	0.025857	0.014923
DNASE1L3	ukb	177	######	0.998539	######	0.000641	0.00082
IL32	finn	324	0	0.543129	0	0.022964	0.433907
IL32	ukb	157	0	0.999332	0	0.000403	0.000264
PPOX	finn	188	######	0.932844	######	0.016015	0.051141
PPOX	ukb	57	######	0.999737	######	1.77E-05	0.000245
ST6GAL1	finn	413	######	0.924004	######	0.016935	0.059062
ST6GAL1	ukb	154	######	0.99979	######	5.26E-05	0.000158
TGM3	finn	364	8.92e-318	0.948564	1.36e-319	0.014447	0.036989
TGM3	ukb	141	9.28e-318	0.999871	2.67e-322	2.84E-05	0.000101

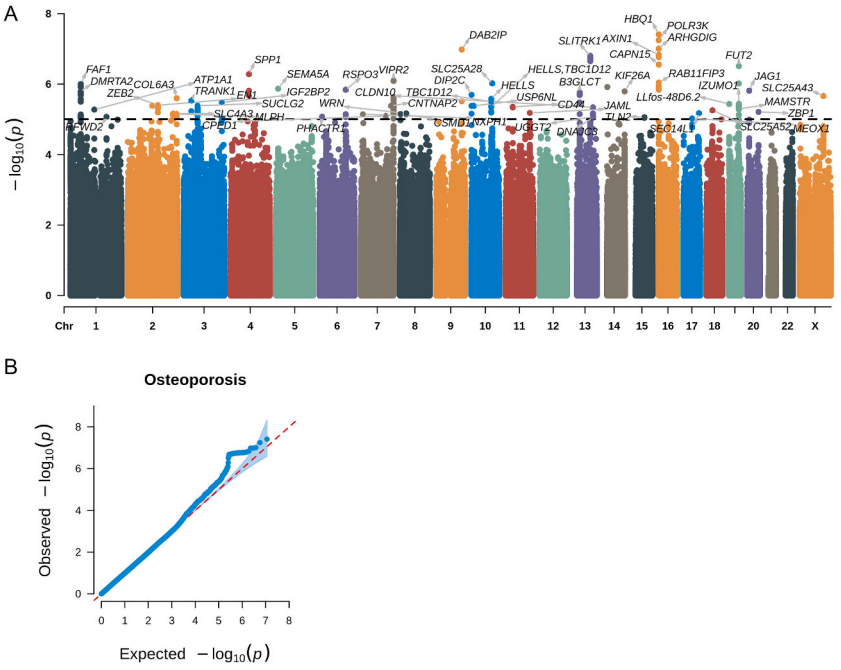

Fig. 2. Meta-analysis results of GWAS summary data from UK Biobank and FinnGen cohorts. A: Manhattan plot showed the nearest genes of these suggestive loci. B: QQ plot showed no population stratification in the meta-analysis results. GWAS: genome-wide association studies.

value of $p < 0.05$, 936 trait-associated genes were found, among which DNASE1L3 and PPOX were also identified by MR analysis. Subsequently, these osteoporosis-associated genes obtained from MAGMA and the causal genes identified from MR analysis were then subjected to functional enrichment analysis. The results showed these genes were remarkably enriched in KEGG pathways such as ribosome and allograft rejection; GO BP terms like vesicle localization and cell junction organization; GO CC terms such as Golgi stack and mitochondrial membrane part; and GO MF terms like amide binding and transcription coactivator activity. The top 10 KEGG pathways and GO terms are displayed in Fig. 3A and B, respectively, according to the normalized enrichment score (NES).

3.6. Cell-type specificity analysis in the bones

Based on GSE143753 dataset, we analyzed the expression levels of druggable genes in human bone cells. Following the filtering steps previously described by He et al. [33], 35,570 cells and corresponding 16 subclusters were obtained in the limb bud and long bone samples. The UMAP plot of 16 subclusters is shown in Fig. 4A. We then used the Seurat package to map the expression of six druggable genes (ACPP, DNASE1L3, IL32, PPOX, ST6GAL1, and TGM3) onto the UMAP plot. The results showed that PPOX and ST6GAL1 were evenly expressed in all cell types. IL32, ACPP, DNASE1L3, and TGM3 were expressed in specific cell types. For instance, IL32 and DNASE1L3 were mainly expressed in epithelium; ACPP was primarily expressed in macrophage and chondroblast; and TGM3 was expressed in limb bud mesenchyme and myoprogenitor (Fig. 4B).

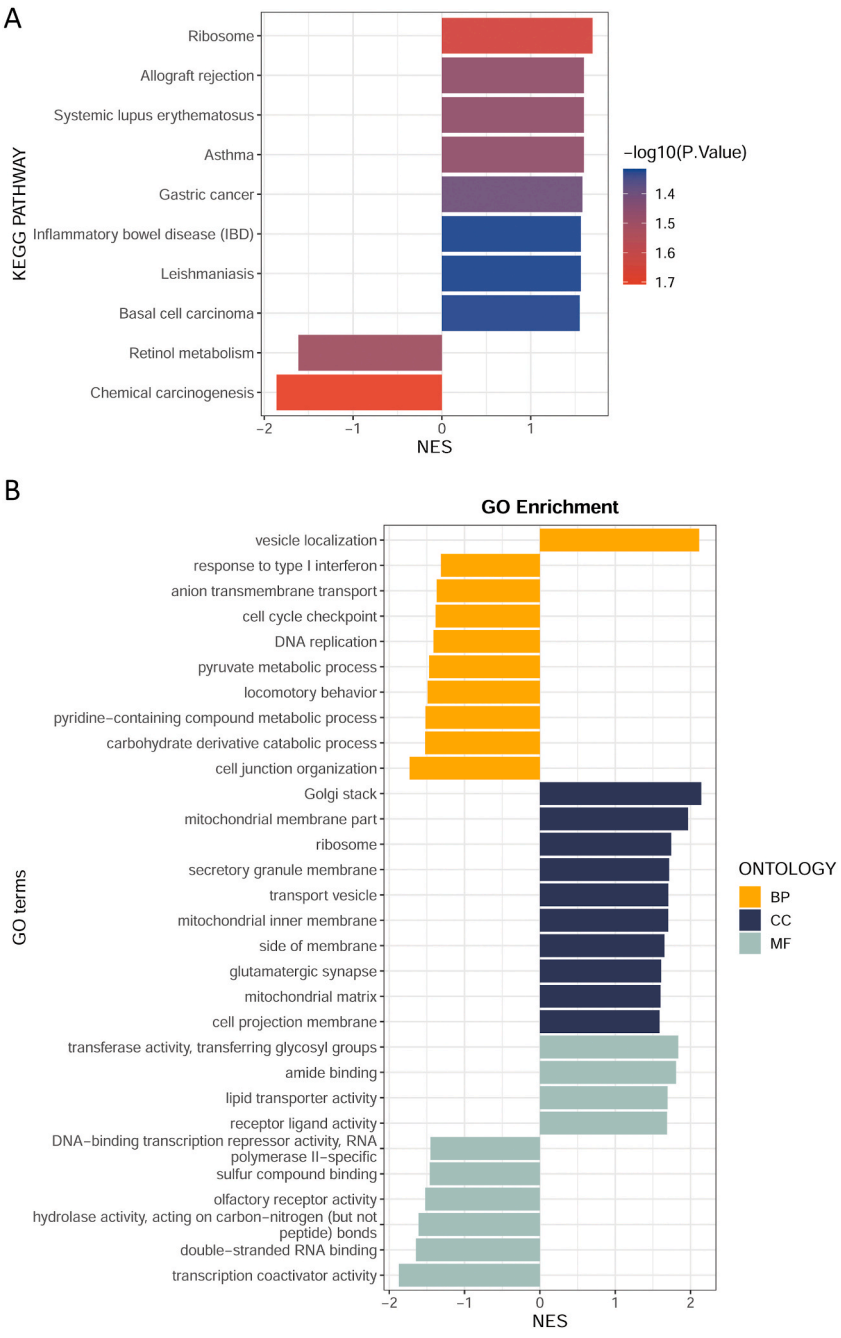

Fig. 3. Functional enrichment results for osteoporosis-associated genes obtained from MAGMA and the causal genes identified from MR analysis. A: The top 10 KEGG pathways. B: The top 10 GO terms. MR: mendelian randomization; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Ontology; BP: biological process; CC: Cellular component; MF: molecular function; NES: normalized enrichment score.

3.7. Correlation between druggable genes and risk factors

The GWAS summary data of seven risk factors for osteoporosis were downloaded, including vitamin D deficiency, COPD, physical activity, BMI, MMP-9, ALP and PTH. We then explored the associations between six druggable genes and osteoporosis risk factors. Using six druggable genes as exposure variables and seven risk factors as outcome variables, MR analysis was performed. As results, ACPP was associated with vitamin D deficiency and COPD; DNASE1L3 was associated with physical activity; IL32 was associated with BMI and MMP-9; and ST6GAL1 was associated with ALP, physical activity, and MMP-9 (Fig. 5A). We further explored the genetic correlations between osteoporosis and risk factors based on data from UK Biobank cohort, FinnGen cohort, and their meta-analysis

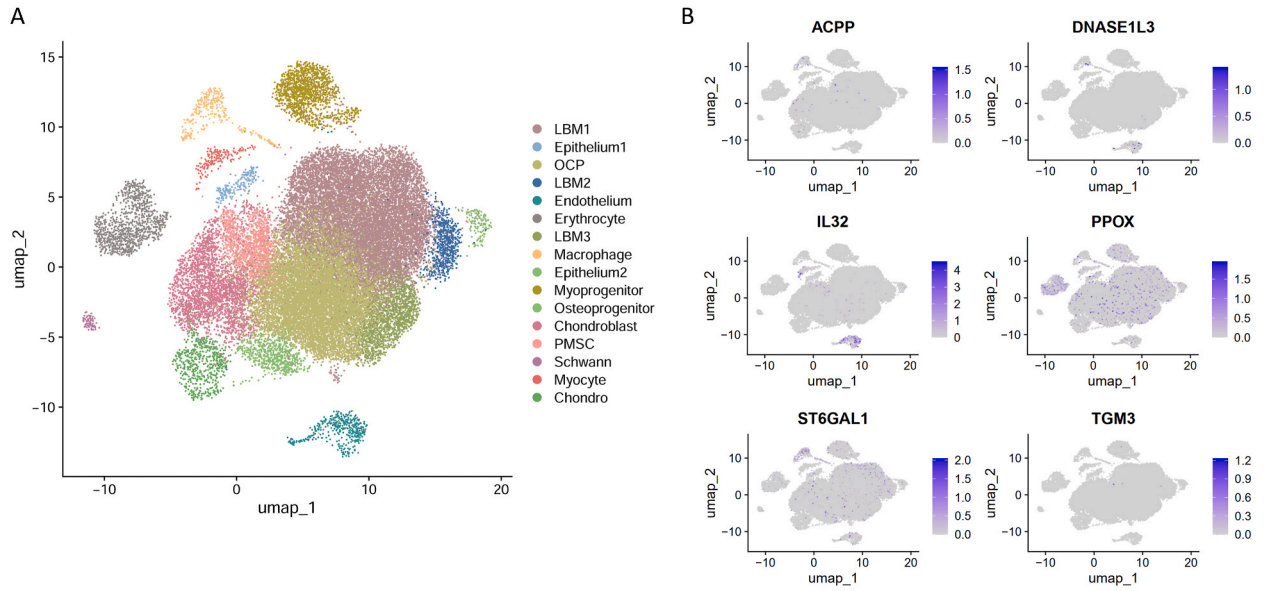

Fig. 4. Cell-type specificity analysis in the bones. A: UMAP plot of cell types from limb buds and long bones that has been previously published by He et al. [33]. B: UMAP plot of gene expression in different cell types of bone tissues. UMAP: uniform manifold approximation and projection.

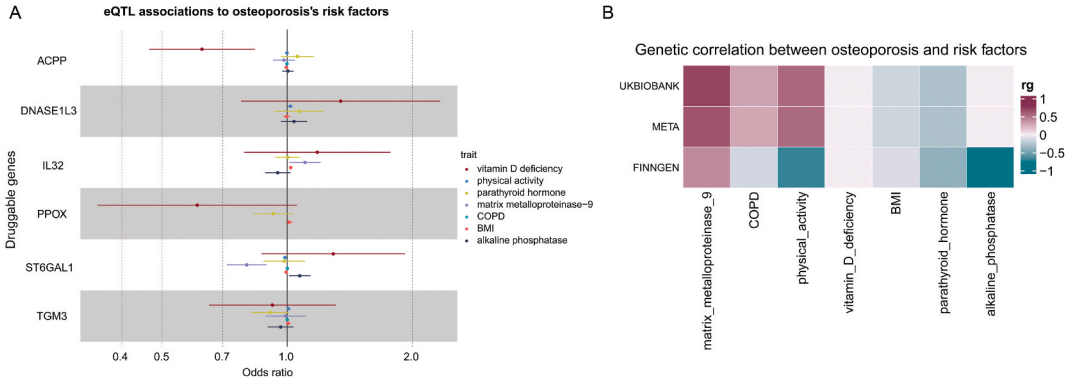

Fig. 5. Correlation between druggable genes and risk factors. A: MR results of druggable genes and risk factors for osteoporosis. B: Heatmap of genetic correlation between osteoporosis and risk factors.

data. The results showed that only MMP-9 had a high genetic correlation with osteoporosis (Fig. 5B).

3.8. External validation of druggable genes for osteoporosis

To validate the association between the druggable genes, including ACPP, DNASE1L3, IL32, PPOX, ST6GAL1, and TGM3, and osteoporosis, we performed validation experiments in human peripheral blood. The results of qRT-PCR demonstrated that IL32 was upregulated while ST6GAL1 was downregulated in peripheral blood in the osteoporosis patients compared with those in the healthy adults (Fig. 6). There was no differential expression in ACPP, DNASE1L3, PPOX, and TGM3 between two groups (Fig. 6).

4. Discussion

To identify putative druggable genes that could potentially provide protection against osteoporosis, we performed MR analysis by integrating the cis-eQTL data of druggable genes and two osteoporosis GWAS datasets. As results, six druggable genes with causal relationships with osteoporosis were identified and successfully replicated, including ACPP, DNASE1L3, IL32, PPOX, ST6GAL1, and TGM3. Cell-type specificity analysis revealed that PPOX and ST6GAL1 were expressed in all cell types in the bone samples, while IL32, ACPP, DNASE1L3, and TGM3 were only expressed in specific cell types. The GWAS data showed there were seven risk factors for osteoporosis, including vitamin D deficiency, COPD, physical activity, BMI, MMP-9, ALP and PTH. Furthermore, we analyzed the relationships between these genes and risk factors. The results showed that ACPP was associated with vitamin D deficiency and COPD;

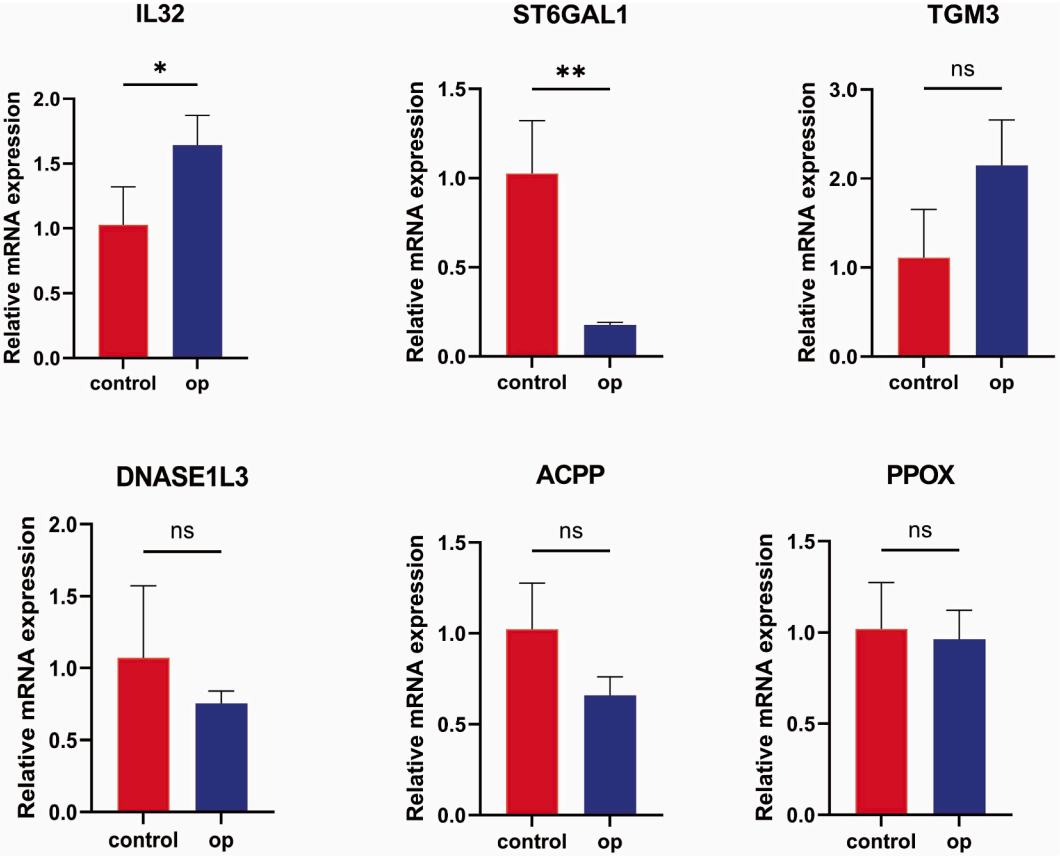

Fig. 6. Validation of druggable genes for osteoporosis. Relative expression levels of the 6 druggable genes. Differences between groups were compared with a *t*-test. *, $P < 0.05$; **, $P < 0.01$.

DNASE1L3 was linked to physical activity; IL32 was correlated with BMI and MMP-9; and ST6GAL1 was related to ALP, physical activity, and MMP-9. Among these risk factors, only MMP-9 had a high genetic correlation with osteoporosis. The results of qRT-PCR demonstrated that IL32 was upregulated while ST6GAL1 was downregulated in peripheral blood of osteoporosis patients. These data provide evidence for candidate drug targets for osteoporosis.

Analyzing druggable genomics can provide important information for the development of drugs that prevent osteoporosis. Recently, MR analysis of drug targets has become an essential approach in drug development [8]. Herein, using the MR method, six druggable genes (ACPP, DNASE1L3, IL32, PPOX, ST6GAL1, and TGM3) were identified and successfully replicated using two osteoporosis cohort (UK Biobank and FinnGen cohorts). DNASE1L3 is a Ca2+/Mg2+-dependent endonuclease belonging to the DNase superfamily. DNASE1L3 contributes to cytokine secretion after inflammasome activation [35]. NLRP3 inflammasome and cytokine secretion (IL-1β and IL-18) play crucial roles in the pathogenesis of osteoporosis by modulating the differentiation of osteoblasts and osteoclasts [36]. IL-32 is an inflammatory cytokine that can induce production of cytokines such as IL-6, TNFα, and IL-18 from macrophages and dendritic cells [37,38]. Lee et al. reported that IL-32 gamma played a crucial role in promoting bone formation in osteoblastic cells and preventing osteoporosis [39]. ST6GAL1 is a sialyltransferase that adds α2–6 sialic acids to N-glycosylated proteins. It is reported that ST6GAL1 can promote immunoglobulin G (IgG) production in B cells and enhance blood IgG titers [40]. Furthermore, IgG can suppress glucocorticoid-induced osteoporosis via FcgRI [41]. Transglutaminases are enzymes that play a role in bone remodeling by regulating the differentiation, migration, and fusion of osteoclasts, which are specialized cells responsible for breaking down bone tissue [42]. One of transglutaminases, TGM3, has been shown to attenuate skin inflammation via inhibiting NF-κB activation [43], suggesting a potential relationship between TGM3 and the NF-κB pathway. Activation of the NF-κB pathway is a key mechanism against osteoporosis [44]. In our study, we found that apart from DNASE1L3, there was no pleiotropy or heterogeneity in the MR results on these druggable genes, confirming the credibility of MR results. Additionally, qRT-PCR revealed that IL32 was upregulated while ST6GAL1 was downregulated in peripheral blood in the patients with osteoporosis. These findings suggest that upregulation of IL32 may promote osteoporosis via involving in inflammatory processes, while downregulation of ST6GAL1 may contribute disease development via affecting immune responses. Taken together, we conclude that these genes, especially IL32 and ST6GAL1 may be promising drug targets for osteoporosis prevention.

Bone is a complex tissue that consists of various cell types to maintain bone homeostasis [45,46]. In the process of osteoporosis, multiple cell types can secrete various proinflammatory chemokines and cytokines [47,48], which can, in turn, recruit osteoclast

precursors and mainly act on osteoblasts and marrow stromal cells, affecting bone homeostasis. Given the heterogeneity between single cells, scRNA-seq analysis can help to reveal the possible cell type-specific gene targets and pathways that probably play an important role in osteoporosis [49]. Moreover, advances in scRNA-seq technology and GWAS have advanced the biological and therapeutic understanding of trait-relevant cell types or states [50]. To better understand the osteoporosis-relevant cell type-specific druggable targets, we further analyzed the expression of our identified six druggable genes in human bone samples using cell-type specific gene expression data. Our results showed that PPOX and ST6GAL1 were expressed in all cell types of the limb bud and long bone samples. The widespread expression across different cell types indicate that these genes may be involved in fundamental processes that are essential for bone development, maintenance, or remodeling, and targeting these genes may have a potential impact on multiple aspects of bone physiology during osteoporosis development. In addition, we found that IL32, ACPP, DNASE1L3, and TGM3 were expressed in specific cell types. For instance, IL32 and DNASE1L3 were mainly expressed in the epithelium, while ACPP was primarily expressed in macrophages and chondroblasts. These data provide important insights into the development of novel cell-based therapeutic targets for osteoporosis.

Since the progression and prognosis of osteoporosis are significantly affected by risk factors [51], a better understanding of the correlation between drug target genes and risk factors of osteoporosis can provide a reference for individual treatment. Herein, we obtained the GWAS summary data on seven risk factors of osteoporosis (COPD, BMI, physical activity, vitamin D deficiency, MMP-9, parathyroid hormone, and ALP) and conducted MR analysis to explore the causal relationships between our identified druggable genes and these risk factors. COPD and osteoporosis are closely linked [52], and patients with COPD have a high incidence of osteoporosis (37.62 %) [53]. BMI is found to be associated with bone mineral density and lower BMI predicts an increased risk of osteoporosis in Chinese people [54]. Physical activity is believed to promote bone growth and maintain bone density, thus playing a role in osteoporosis prevention and management [55,56]. Vitamin D regulates the absorption of calcium (Ca^{2+}) and is essential for bone homeostasis. Deficiency of vitamin D can cause or exacerbate osteoporosis; thus, both calcium and vitamin D intake are recommended to prevent osteoporosis [57]. MMP-9 is expressed in bone tissue and functions as a key player in bone loss during the process of osteoporosis [58]. MMP-9 plays an essential role in initiating osteoclastic resorption in osteoporosis through removing the collagenous layer from the bone surface, which is a prerequisite for demineralization to occur [59]. Alkaline phosphatase is found to be elevated in cases of osteoporosis and osteopenia, indicating a correlation between ALP and osteoporosis [60]. Our MR analysis revealed that ACPP was associated with vitamin D deficiency and COPD; DNASE1L3 was linked to physical activity; IL32 was correlated with BMI and MMP-9; and ST6GAL1 was related to ALP, physical activity, and MMP-9. These data imply that the expression of these genes may be influenced by risk factors. More importantly, among all risk factors, only MMP-9 showed a high genetic correlation with osteoporosis, indicating the potential significance of MMP-9 and its associated genes as drug targets for osteoporosis. Considering that risk factors and drug target genes both play important roles in disease prevention and treatment, it is recommended to identify both the drug target genes and assess the risk factor status of individuals during drug development. Designing personalized treatment plans based on their specific conditions can contribute to enhancing treatment efficacy and minimizing the risk of adverse drug reactions.

This study has some limitations. Firstly, the druggable genes were identified based on publicly available data and the generalizability of this study is restricted due to the samples in the datasets only comprising individuals of European descent. Therefore, the results may be affected by regional and racial disparities and extrapolating these findings to populations of diverse genetic ancestries necessitates additional research to ensure broader applicability. Secondly, MR could not fully reproduce clinical trials (as the MR results did not yet directly reflect the practical effect size) and perfectly predict a drug effect. The clinical application of these druggable genes should be evaluated by more clinical trials. Thirdly, no positive colocalization results were observed, indicating the absence of a shared causal variant between the druggable genes and osteoporosis, which down-toned our argument and questioned the notion of an association driven by specific target genes within disease [61]. While a positive colocalization finding often indicates a non-zero MR estimate, numerous scenarios may produce a non-zero MR estimate without evidence for colocalization. The reasons for the discrepancy between MR and colocalization results were as follows: 1) the exposure and outcome exhibit distinct yet correlated causal variants, violating the MR assumptions; 2) there are a paucity of robust associations with the outcome; and 3) the complexity of the genetic region, such as allelic heterogeneity or multiple biological mechanisms affecting the same genetic region can introduce challenges in interpreting colocalization analyses [62]. The absence of colocalization in findings does not inherently invalidate the target, but it does necessitate further exploration into the factors contributing to the lack of colocalization. This might include a more thorough evaluation of data sources and the appropriateness of the exposure trait selected for analysis. Overall, MR studies can only make preliminary conclusions about causal relationships and further investigation is needed to understand the potential implications of these druggable genes in therapeutic interventions. Lastly, qRT-PCR showed that there was no differential expression in ACPP, DNASE1L3, PPOX, and TGM3 between osteoporosis patients and healthy controls, which will impact the broader applicability of the final conclusions. This phenomenon may be due to the limited validation samples and the complexity of osteoporosis, which likely results from multiple genetic variations and environmental factors. Further research is needed to investigate the roles of these genes in the disease and explore other potential influencing factors to better understand the relationship between these genes and osteoporosis.

In conclusion, this MR analysis revealed six druggable genes with causal relationships to osteoporosis. Assessing the expression patterns of these druggable genes across different cell types of bone tissues and studying their association with risk factors could offer crucial information to guide the development of effective therapeutic strategies for osteoporosis.

Ethics approval and consent to participate

The study was reviewed and approved by the Ethics Committee of Honghui Hospital, Xi'an Jiaotong University. Each participant provided written and signed informed consent.

Not applicable.

Consent for publication

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Guolong Zhao: Formal analysis, Data curation. **Qian Wang:** Investigation, Conceptualization. **Ning Duan:** Formal analysis, Data curation. **Kun Zhang:** Writing – review & editing. **Zhong Li:** Writing – review & editing. **Liang Sun:** Conceptualization. **Yao Lu:** Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] B. Liang, G. Burley, S. Lin, Y.-C. Shi, Osteoporosis pathogenesis and treatment: existing and emerging avenues, Cell. Mol. Biol. Lett. 27 (1) (2022) 72.
[2] N. Salari, H. Ghasemi, L. Mohammadi, E. Rabieenia, S. Shohaimi, M. Mohammadi, The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis, J. Orthop. Surg. Res. 16 (1) (2021) 1–20.
[3] L.G. Raisz, Pathogenesis of osteoporosis: concepts, conflicts, and prospects, J. Clin. Invest. 115 (12) (2005) 3318–3325.
[4] S. Song, Y. Guo, Y. Yang, D. Fu, Advances in pathogenesis and therapeutic strategies for osteoporosis, Pharmacol. Therapeut. 237 (2022) 108168.
[5] A.D. Hingorani, V. Kuan, C. Finan, F.A. Kruger, A. Gaulton, S. Chopade, et al., Improving the odds of drug development success through human genomics: modelling study, Sci. Rep. 9 (1) (2019) 18911.
[6] E.A. King, J.W. Davis, J.F. Degner, Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval, PLoS Genet. 15 (12) (2019) e1008489.
[7] C. Finan, A. Gaulton, F.A. Kruger, R.T. Lumbers, T. Shah, J. Engmann, et al., The druggable genome and support for target identification and validation in drug development, Sci. Transl. Med. 9 (383) (2017) eaag1166.
[8] A.F. Schmidt, C. Finan, M. Gordillo-Marañón, F.W. Asselbergs, D.F. Freitag, R.S. Patel, et al., Genetic drug target validation using Mendelian randomisation, Nat. Commun. 11 (1) (2020) 3255.
[9] M.M. Martínez-Aguilar, D.I. Aparicio-Bautista, E.G. Ramírez-Salazar, J.P. Reyes-Grajeda, A.H. De la Cruz-Montoya, B. Antuna-Puente, et al., Serum proteomic analysis reveals vitamin D-binding protein (VDBP) as a potential biomarker for low bone mineral density in Mexican postmenopausal women, Nutrients 11 (12) (2019).
[10] M.M. Al-Ansari, S.M. Aleidi, A. Masood, E.A. Alnehmi, M. Abdel Jabar, M. Almogren, et al., Proteomics profiling of osteoporosis and osteopenia patients and associated network analysis, Int. J. Mol. Sci. 23 (17) (2022).
[11] D. Huang, Y. Wang, J. Lv, Y. Yan, Y. Hu, C. Liu, et al., Proteomic profiling analysis of postmenopausal osteoporosis and osteopenia identifies potential proteins associated with low bone mineral density, PeerJ 8 (2020) e9009.
[12] K. Trajanoska, F. Rivadeneira, Using mendelian randomization to decipher mechanisms of bone disease, Curr. Osteoporos. Rep. 16 (5) (2018) 531–540.
[13] X. Liu, L. Nie, Y. Zhang, Y. Yan, C. Wang, M. Colic, et al., Actin cytoskeleton vulnerability to disulfide stress mediates disulfidptosis, Nat. Cell Biol. 25 (3) (2023) 404–414.
[14] D.A. Lawlor, R.M. Harbord, J.A. Sterne, N. Timpson, G. Davey Smith, Mendelian randomization: using genes as instruments for making causal inferences in epidemiology, Stat. Med. 27 (8) (2008) 1133–1163.
[15] J. Zheng, M. Frysz, J.P. Kemp, D.M. Evans, G. Davey Smith, J.H. Tobias, Use of mendelian randomization to examine causal inference in osteoporosis, Front. Endocrinol. 10 (2019) 807.
[16] A. Hingorani, S. Humphries, Nature's randomised trials, Lancet 366 (9501) (2005) 1906–1908.
[17] M.V. Holmes, M. Ala-Korpela, G.D. Smith, Mendelian randomization in cardiometabolic disease: challenges in evaluating causality, Nat. Rev. Cardiol. 14 (10) (2017) 577–590.
[18] Y. Chen, X. Xu, L. Wang, K. Li, Y. Sun, L. Xiao, et al., Genetic insights into therapeutic targets for aortic aneurysms: a Mendelian randomization study, EBioMedicine 83 (2022).
[19] Y. Cao, Y. Yang, Q. Hu, G. Wei, Identification of potential drug targets for rheumatoid arthritis from genetic insights: a Mendelian randomization study, J. Transl. Med. 21 (1) (2023) 1–17.
[20] Z. Wu, K.G. Yang, T.-P. Lam, J.C.Y. Cheng, Z. Zhu, W.Y.-W. Lee, Genetic insight into the putative causal proteins and druggable targets of osteoporosis: a large-scale proteome-wide mendelian randomization study, Front. Genet. 14 (2023).

[21] U. Võsa, A. Claringbould, H.-J. Westra, M.J. Bonder, P. Deelen, B. Zeng, et al., Large-scale cis-and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression, Nat. Genet. 53 (9) (2021) 1300–1310.
[22] H.J. Westra, M.J. Peters, T. Esko, H. Yaghootkar, C. Schurmann, J. Kettunen, et al., Systematic identification of trans eQTLs as putative drivers of known disease associations, Nat. Genet. 45 (10) (2013) 1238–1243.
[23] Y.-W. Chen, A.H. Ramsook, H.O. Coxson, J. Bon, W.D. Reid, Prevalence and risk factors for osteoporosis in individuals with COPD: a systematic review and meta-analysis, Chest 156 (6) (2019) 1092–1110.
[24] G. Hemani, J. Zheng, B. Elsworth, K.H. Wade, V. Haberland, D. Baird, et al., The MR-Base platform supports systematic causal inference across the human phenome, Elife 7 (2018) e34408.
[25] L. Chen, J.E. Peters, B. Prins, E. Persyn, M. Traylor, P. Surendran, et al., Systematic Mendelian randomization using the human plasma proteome to discover potential therapeutic targets for stroke, Nat. Commun. 13 (1) (2022) 6143.
[26] L. Chen, J.E. Peters, B. Prins, E. Persyn, M. Traylor, P. Surendran, et al., Systematic Mendelian randomization using the human plasma proteome to discover potential therapeutic targets for stroke, Nat. Commun. 13 (1) (2022) 6143.
[27] C. Giambartolomei, D. Vukcevic, E.E. Schadt, L. Franke, A.D. Hingorani, C. Wallace, et al., Bayesian test for colocalisation between pairs of genetic association studies using summary statistics, PLoS Genet. 10 (5) (2014) e1004383.
[28] C.J. Willer, Y. Li, G.R. Abecasis, METAL: fast and efficient meta-analysis of genomewide association scans, Bioinformatics 26 (17) (2010) 2190–2191.
[29] J.B. Gebhart, Route of hysterectomy for benign indications, J. Gynecol. Surg. 37 (2) (2021) 98–100.
[30] L. Yin, H. Zhang, Z. Tang, J. Xu, D. Yin, Z. Zhang, et al., rMVP: a memory-efficient, visualization-enhanced, and parallel-accelerated tool for genome-wide association study, Dev. Reprod. Biol. 19 (4) (2021) 619–628.
[31] Y. Li, K. Cao, G. Zhu, W. Fang, C. Chen, X. Wang, et al., Genomic analyses of an extensive collection of wild and cultivated accessions provide new insights into peach breeding history, Genome Biol. 20 (2019) 1–18.
[32] C.A. de Leeuw, J.M. Mooij, T. Heskes, D. Posthuma, MAGMA: generalized gene-set analysis of GWAS data, PLoS Comput. Biol. 11 (4) (2015) e1004219.
[33] J. He, J. Yan, J. Wang, L. Zhao, Q. Xin, Y. Zeng, et al., Dissecting human embryonic skeletal stem cell ontogeny by single-cell transcriptomic and functional analyses, Cell Res. 31 (7) (2021) 742–757.
[34] B. Bulik-Sullivan, H.K. Finucane, V. Anttila, A. Gusev, F.R. Day, P.-R. Loh, et al., An atlas of genetic correlations across human diseases and traits, Nat. Genet. 47 (11) (2015) 1236–1241.
[35] G. Shi, K.N. Abbott, W. Wu, R.D. Salter, P.A. Keyel, Dnase1L3 regulates inflammasome-dependent cytokine secretion, Front. Immunol. 8 (2017) 522.
[36] N. Jiang, J. An, K. Yang, J. Liu, C. Guan, C. Ma, et al., NLRP3 inflammasome: a new target for prevention and control of osteoporosis? Front. Endocrinol. 12 (2021) 752546.
[37] S.-H. Kim, S.-Y. Han, T. Azam, D.-Y. Yoon, C.A. Dinarello, Interleukin-32: a cytokine and inducer of TNFα, Immunity 22 (1) (2005) 131–142.
[38] F. Ribeiro-Dias, R. Saar Gomes, L.L. de Lima Silva, J.C. Dos Santos, L.A. Joosten, Interleukin 32: a novel player in the control of infectious diseases, Journal of Leucocyte Biology 101 (1) (2017) 39–52.
[39] E.-J. Lee, S.-M. Kim, B. Choi, E.-Y. Kim, Y.-H. Chung, E.-J. Lee, et al., Interleukin-32 gamma stimulates bone formation by increasing miR-29a in osteoblastic cells and prevents the development of osteoporosis, Sci. Rep. 7 (1) (2017) 40240.
[40] E.E. Irons, P.R. Punch, J.T. Lau, Blood-borne ST6GAL1 regulates immunoglobulin production in B cells, Front. Immunol. 11 (2020) 617.
[41] L. Jiang, W. Qiu, X. Wang, X. Duan, X. Han, T. Yu, et al., Immunoglobulin G inhibits glucocorticoid-induced osteoporosis through occupation of FcγRI, iScience 26 (10) (2023).
[42] H. Sun, M.T. Kaartinen, Transglutaminase activity regulates differentiation, migration and fusion of osteoclasts via affecting actin dynamics, J. Cell. Physiol. 233 (9) (2018) 7497–7513.
[43] S. Ling, B. Xu, Y. Luo, X. Fang, X. Liu, A. Wang, et al., Transglutaminase 3 attenuates skin inflammation in psoriasis by inhibiting NF-κB activation through phosphorylated STAT3–TET3 signaling, J. Invest. Dermatol. 142 (11) (2022) 2968–2977. e10.
[44] W. Wang, J. Bai, W. Zhang, G. Ge, Q. Wang, X. Liang, et al., Protective effects of punicalagin on osteoporosis by inhibiting osteoclastogenesis and inflammation via the NF-κB and MAPK pathways, Front. Pharmacol. 11 (2020) 696.
[45] N.A. Sims, N.C. Walsh, Intercellular cross-talk among bone cells: new factors and pathways, Curr. Osteoporos. Rep. 10 (2012) 109–117.
[46] R.C. Chai, Single-cell RNA sequencing: unravelling the bone one cell at a time, Curr. Osteoporos. Rep. 20 (5) (2022) 356–362.
[47] D. Grčević, A. Sanjay, J. Lorenzo, Interactions of B-lymphocytes and bone cells in health and disease, Bone 168 (2023) 116296.
[48] K. Kaur, M.-W. Ko, N. Ohanian, J. Cook, A. Jewett, Osteoclast-expanded super-charged NK-cells preferentially select and expand CD8+ T cells, Sci. Rep. 10 (1) (2020) 20363.
[49] Y. Wang, Q. Wang, Q. Xu, J. Li, F. Zhao, Single-cell RNA sequencing analysis dissected the osteo-immunology microenvironment and revealed key regulators in osteoporosis, Int. Immunopharm. 113 (2022) 109302.
[50] Y. Ma, Y. Zhou, D. Jiang, W. Dai, J. Li, C. Deng, et al., Integration of human organoids single-cell transcriptomic profiles and human genetics repurposes critical cell type-specific drug targets for severe COVID-19, Cell Prolif. 57 (3) (2024) e13558.
[51] W. Tański, J. Kosiorowska, A. Szymańska-Chabowska, Osteoporosis-risk factors, pharmaceutical and non-pharmaceutical treatment, Eur. Rev. Med. Pharmacol. Sci. 25 (9) (2021).
[52] A. de Sire, L. Lippi, V. Aprile, D. Calafiore, A. Folli, F. D'Abrosca, et al., Pharmacological, nutritional, and rehabilitative interventions to improve the complex management of osteoporosis in patients with chronic obstructive pulmonary disease: a narrative review, J. Personalized Med. 12 (10) (2022) 1626.
[53] A.N. Bitar, S.A.S. Sulaiman, I.A.H. Ali, I. Khan, A.H. Khan, Osteoporosis among patients with chronic obstructive pulmonary disease: systematic review and meta-analysis of prevalence, severity, and therapeutic outcomes, J. Pharm. BioAllied Sci. 11 (4) (2019) 310.
[54] R. Cui, L. Zhou, Z. Li, Q. Li, Z. Qi, J. Zhang, Assessment risk of osteoporosis in Chinese people: relationship among body mass index, serum lipid profiles, blood glucose, and bone mineral density, Clin. Interv. Aging (2016) 887–895.
[55] M.B. Pinheiro, J. Oliveira, A. Bauman, N. Fairhall, W. Kwok, C. Sherrington, Evidence on physical activity and osteoporosis prevention for people aged 65+ years: a systematic review to inform the WHO guidelines on physical activity and sedentary behaviour, Int. J. Behav. Nutr. Phys. Activ. 17 (1) (2020) 1–53.
[56] Prescribing physical activity for the prevention and treatment of osteoporosis in older adults, in: L.B. McMillan, A. Zengin, P.R. Ebeling, D. Scott (Eds.), Healthcare, MDPI, 2017.
[57] M. De Martinis, A. Allegra, M.M. Sirufo, A. Tonacci, G. Pioggia, M. Raggiunti, et al., Vitamin D deficiency, osteoporosis and effect on autoimmune diseases and hematopoiesis: a review, Int. J. Mol. Sci. 22 (16) (2021) 8855.
[58] A. Azevedo, A.F. Prado, S. Feldman, F.A. de Figueiredo, M.C.G. Dos Santos, J.P.M. Issa, MMPs are involved in osteoporosis and are correlated with cardiovascular diseases, Curr. Pharmaceut. Des. 24 (16) (2018) 1801–1810.
[59] B. Bragdon, O. Moseychuk, S. Saldanha, D. King, J. Julian, A. Nohe, Bone morphogenetic proteins: a critical review, Cell. Signal. 23 (4) (2011) 609–620.
[60] M.K. Saha, P. Agrawal, S.G. Saha, V. Vishwanathan, V. Pathak, S.V. Saiprasad, et al., Evaluation of correlation between salivary calcium, alkaline phosphatase and osteoporosis-a prospective, comparative and observational study, J. Clin. Diagn. Res.: J. Clin. Diagn. Res. 11 (3) (2017) ZC63.
[61] X. Lv, Y. Shang, Y. Ning, W. Yu, J. Wang, Pharmacological targets of SGLT2 inhibitors on IgA nephropathy and membranous nephropathy: a mendelian randomization study, Front. Pharmacol. 15 (2024) 1399881.
[62] V. Zuber, N.F. Grinberg, D. Gill, I. Manipur, E.A.W. Slob, A. Patel, et al., Combining evidence from Mendelian randomization and colocalization: review and comparison of approaches, Am. J. Hum. Genet. 109 (5) (2022) 767–782.