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Causal relationship between Interleukin-27 expression levels and osteoporosis: a bidirectional mendelian randomization study

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Abstract

Background This study aimed to evaluate the causal relationship between Interleukin-27 (IL-27) and osteoporosis by bidirectional Mendelian randomization (MR) analysis.

Methods Firstly, the genome-wide association study summary data of osteoporosis (finn-b-M13_OSTEOPOROSIS) and IL-27 levels (ebi-a-GCST90012017) were picked out from the Integrative Epidemiology Unit (IEU) OpenGWAS database. After filtrating instrumental variables (IVs), the bidirectional MR analysis between IL-27 levels and osteoporosis was performed by MR-Egger, Weighted median, Simple mode, Weighted mode, and Inverse variance weighted (IVW). Subsequently, the sensitivity analysis was adopted to evaluate the reliability of the MR results via the Heterogeneity, Horizontal pleiotropy test and Leave-One-Out (LOO) analysis. Finally, the enrichment analysis of genes corresponding to SNPs related to IL-27 levels derived from eQTLGen database was executed to explore in depth the biological function and regulatory mechanism of these genes on osteoporosis occurrence.

Results The bidirectional MR results based on IVW method revealed that IL-27 level as a risk factor was causally related to osteoporosis ($P=0.004$, odds ratio (OR) = 1.123, 95% confidence interval (CI) = 1.037–1.217), whereas osteoporosis was not in significant connection with IL-27 levels ($P>0.05$). In regard to the sensitivity analysis for forward MR results, there was no heterogeneity and horizontal pleiotropy, and no SNPs relevant to IL-27 levels existed severe bias, suggesting the reliability of forward MR analysis. Furthermore, a total of 74 genes corresponding to 26 SNPs of IL-27 levels were obtained and were mainly involved in immune and inflammatory pathways including MyD88-dependent toll-like receptor signaling pathway, Toll-like receptor signaling pathway, cytosolic DNA-sensing pathway and so forth.

Conclusions This study supported that IL-27 level as a risk factor was causally connected with osteoporosis and might regulate the disease occurrence and progression by means of immune and inflammatory mechanisms, which could provide important reference and evidence for further exploring the role of IL-27 in the development of osteoporosis.

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Keywords Osteoporosis, Interleukin-27, Mendelian randomization, Causality, Enrichment analysis

Background

Osteoporosis is a systemic bone disease accompanied with an increased risk of fracture and other complications [1, 2]. With the increasing aging of the global population, the incidence of osteoporosis is increasing year by year, and it has become one of the most common age-related diseases [3]. It has been confirmed that genetic factors, environmental factors and their interactions play an important role in the pathogenesis of osteoporosis [4–6]. Growing research on the process of bone remodeling suggests that immune inflammation plays an important role in the pathogenesis of osteoporosis [7, 8]. In the inflammatory state, the activation of inflammatory cells and inflammatory mediators leads to an increase in bone resorption process, thereby affecting bone remodeling balance. Abnormal activation or dysregulation of immune cells and immunomodulatory molecules may also negatively affect the skeletal system, leading to osteoporosis, fractures and other bone-related diseases [9–11]. Exploring bone remodeling, bone immunity and understanding the development of bone health and osteoporosis are important for further exploring the mechanisms of osteoporosis development and developing new treatment options in the future.

Interleukin (IL) levels have been proved to be associated with osteoporosis. However, most of these associations are based on observational studies. Therefore, their causal relationship remains unclear [12]. IL-27 is a heterodimeric cytokine composed of EBV-induced protein (EBI)3 and p28 subunits, a member of the IL-12 family, and shares homology with the p40 and p35 subunits of IL-12. IL-27 is mainly produced by activated macrophages and dendritic cells and plays an important role in the immune response [13]. In addition, IL-27 plays multiple roles in immune response, such as activation and regulation [14, 15]. IL-27 and its receptor subunits are expressed in bone cells. Specifically, IL-27 directly inhibits RANKL-induced osteoclastogenesis, a process critical for bone resorption. Furthermore, the levels of IL-27 are found to be reduced in the serum of female patients with osteoporosis, suggesting a potential role in the disease pathogenesis [16–20]. T cells play a regulatory role in bone homeostasis and osteoclast activation, and IL-27 can regulate the secretion of pro-inflammatory and anti-inflammatory cytokines by T cells [21]. Studies have shown that IL-27 can inhibit the secretion of IL-17 and down-regulate the expression of RANKL in CD4 T cells [22]. IL-27, as an important gene related to immune and inflammatory response, is also related to bone remodeling and bone immunity. Therefore, exploring the relationship between IL-27 and osteoporosis is of great significance for further exploring the pathogenesis and development of osteoporosis and developing new treatment schemes.

Generally speaking, more evidence is needed to assess whether there is a causal relationship between IL-27 and osteoporosis. Mendelian randomization (MR) is an epidemiological method that uses germline genetic markers as proxies or instrumental variables for presumptive risk factors [23]. To infer causal relationships between exposures (e.g., IL-27) and outcomes (e.g., osteoporosis), exposure susceptibility variants are used as tools. Because the gene sequences are randomly assigned, the association of the tool with exposure is not subject to any confounding factors, and it only correlates with the results through its effect on exposure. MR has the advantage of not being susceptible to confounding and reverse causal bias [24–26]. At present, the causal relationship between IL-27 and osteoporosis has not been reported, and more studies are needed to further clarify the nature and significance of this relationship.

In this study, a bidirectional MR analysis was performed based on the effective genetic variation information in the genome-wide association study (GWAS) data of IL-27 levels and osteoporosis to study their causal correlation The possible immune and inflammatory mechanisms involved in the causal relationship between IL-27 and osteoporosis were studied through enrichment analysis of IL-27 related genes based on GeneOntology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. This study could provide a new theoretical basis for further understanding of the occurrence and development of osteoporosis.

Methods

Study design

This study employed a bidirectional MR analysis to investigate the causal effect of IL-27 levels on osteoporosis with the help of GWAS summary data. The MR design abides by three key conceptions in respect of the genetic instruments: (1) its close association with IL-27 levels; (2) its association with osteoporosis solely through exposure factor, without alternative pathways; (3) its lack of association with any confounders in the exposure-outcome relationship [27]. The overview of the study design of the MR analysis was displayed in Fig. 1.

Data sources and summary

GWAS summary data of osteoporosis and expression level of IL-27 were derived from the Integrative Epidemiology Unit (IEU) OpenGWAS database (https://gwas.mrcieu.ac.uk/). The dataset of osteoporosis

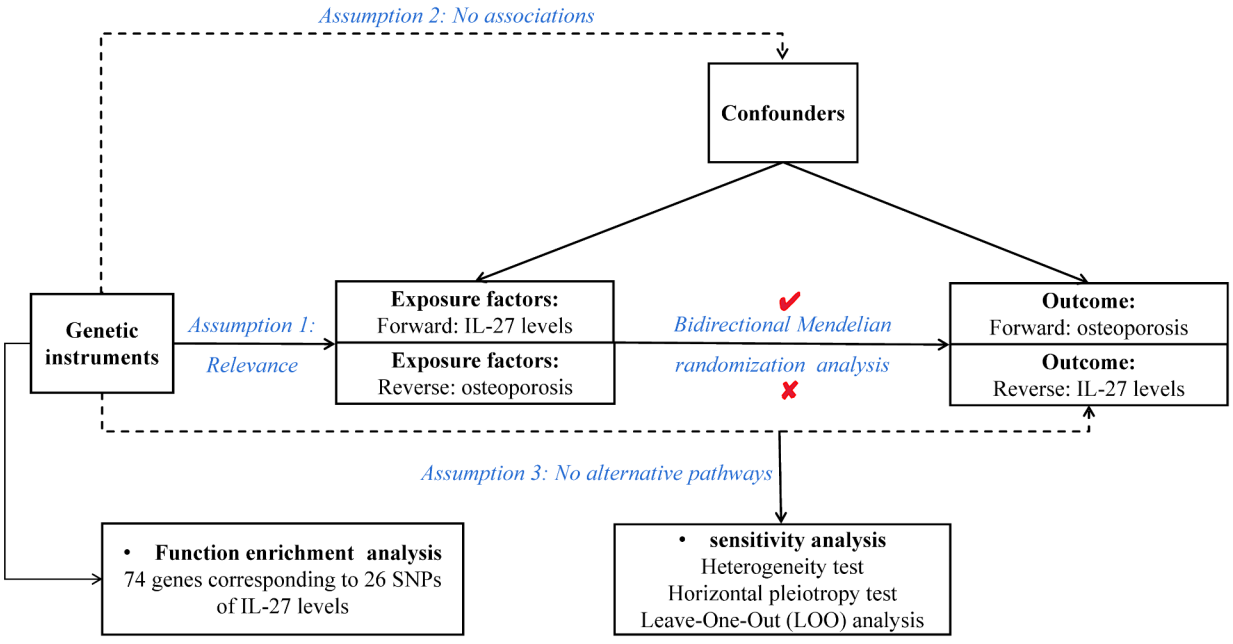

Fig. 1 The overview of the study design of the bidirectional Mendelian randomization (MR) analysis. MR estimates were calculated with five algorithms. The result of Inverse variance weighted (IVW) method was decisive for the judgment of causality between IL-27 levels and osteoporosis. The sensitivity analysis was adopted to evaluate the reliability of the MR results via the Heterogeneity, Horizontal pleiotropy test and Leave-One-Out (LOO) analysis

(finn-b-M13_OSTEOPOROSIS) was comprised of 3,203 cases and 209,575 controls, with a total of 16,380,452 single nucleotide polymorphisms (SNPs). The dataset ebi-a-GCST90012017 associated with IL-27 levels contained 21,758 samples and 13,102,608 SNPs.

Data pre-processing

The "extract_instruments" function in R package "TwoSampleMR" (v.0.5.6) [28] was in the employ of reading exposure factors and screening instrumental variables (IVs), so as to ascertain the SNPs dramatically connected with exposure factors as IVs (Forward: $P<5\times10^{-8}$; Reverse: $P<5\times10^{-7}$), followed by the elimination of SNPs with linkage disequilibrium (clump=TRUE; r2=0.001; Forward / Reverse kb=100 / 10000). Whereafter, the "harmonise_data" function in "TwoSampleMR" was hired to harmonize the effect equipotential with effect size after removing the SNPs significantly associated with the outcome. Incidentally, the exposure factor was IL-27 levels and outcome was osteoporosis in forward MR analysis, which were swapped as outcome and exposure in reverse MR analysis.

Bidirectional MR analysis

The "mr" function in "TwoSampleMR" with five algorithms was employed to proceed bidirectional MR analysis, including MR Egger [29], Simple mode, Weighted mode [30], Weighted median [31], and Inverse variance weighted (IVW) [32]. Noteworthily, the result of IVW method was decisive for the judgment of causality between IL-27 levels and osteoporosis, referring to the following result of Cochran's Q test in Heterogeneity analysis at the same time. The scatter plot, forest plot and funnel plot were created to exhibit MR results. Subsequently, the sensitivity analysis was executed to decide the dependability of above MR results by dint of the Heterogeneity, Pleiotropy and Leave-One-Out (LOO) test. The F-score is a more direct and quantitative assessment metric that accurately reflects the strength of the association between the instrumental variable and the target exposure variable. The R package 'TwoSampleMR' (version 0.6.4) was used to calculate the F-score in this study.

Functional enrichment of genes related to SNPs

In order to identify the SNPs playing an important regulatory role in the expression of specific genes to deeper understand the gene function and regulatory networks, the cis-expression quantitative trait loci (cis-eQTL) genes associated with IL-27 levels were retrieved on the strength of eQTLGen database, followed by the enrichment analysis based on GO and KEGG databases via R package "clusterProfiler" [33]. The results of GO and KEGG enrichment were visualized by bar diagram and bubble diagram, respectively.

Statistical analysis

All MR statistical analyses were conducted using TwoSampleMR (v.0.5.6) in R software.

Results

IL-27 levels was causally associated with an increased risk of osteoporosis

The features of genetic variants associated with exposure factor irrelevant to outcome were presented in Supplementary Tables 1–2. After screening, a total of 30 SNPs related to IL-27 levels and 4 SNPs correlated with osteoporosis were identified as IVs for forward and reverse MR analysis, respectively. As presented in Supplementary Table 3, there was a strong causal relationship between IL-27 levels and osteoporosis based on IVW method ($P=0.004$) in forward MR analysis, and IL-27 levels were a risk factor for osteoporosis (odds ratio (OR) = 1.123, 95% confidence interval (CI) = 1.037–1.217). The causal effects evaluated through Weighted median and Weighted mode methods demonstrated the similar results. In addition, there was no significant relation between osteoporosis and IL-27 levels ($P>0.05$) in reverse MR analysis (Supplementary Table 4). The scatter plot of causal effect of IL-27 levels on osteoporosis presented a positive correlation (slope > 0) in accordance with the previous MR results (Fig. 2A). The forest plot was utilized to evaluate the diagnostic efficiency of each SNP for osteoporosis, indicating that the overall effect of IVW models was prominent (Fig. 2B). The valid SNPs in forward MR analysis were distributed symmetrically in the funnel plots, which was consistent with Mendel's second law random grouping (Fig. 2C). The F-score calculation results showed that the F-scores of SNPs were all greater than 10, indicating a good correlation between each SNP and osteoporosis (Supplementary Table 5).

Reliability of the forward MR results was illustrated by sensitivity analysis

With respect to the sensitivity analysis, there was no heterogeneity (Q = 22.153; $P=0.814$) and horizontal pleiotropy (Intercept = 0.000182; $P=0.985$) for the MR analysis between IL-27 levels and osteoporosis based on IVW approach with the help of Cochran's Q test (Supplementary Table 6) and MR-Egger test (Supplementary Table 7). Meanwhile, there was no exaggerated influence on the model effect by LOO method by eliminating SNPs one by one (Fig. 3). In conclusion, IL-27 as a risk factor was causally influential on osteoporosis occurrence.

Seventy-four cis-eQTL genes corresponding to SNPs of IL-27 levels were mainly enriched in immune and inflammatory pathways

In total, 74 cis-eQTL genes corresponding to 26 SNPs of IL-27 levels were obtained for enrichment analysis based on eQTLGen database (Supplementary Table 8). The co-expression network of these genes and IL-27 was structured on the basis of GeneMANIA database, which principally participated in co-expression (52.82%), physical interactions (15.70%), genetic interactions (13.50%), pathway (10.93%) and so forth (Fig. 4A). Furthermore, these 74 genes were enriched in 265 GO terms, including 199 in biological process (BP), 36 cellular components (CC) and 30 molecular functions (MF), such as positive regulation of cytokine production, toll-like receptor signaling pathway and MyD88-dependent toll-like receptor signaling pathway in BP; phagocytic vesicle membrane, phagocytic vesicle and endocytic vesicle membrane in CC; hydrolase activity acting on glycosyl bonds, NAD + nucleosidase activity and NAD + nucleotidase cyclic ADP-ribose generating in MF (Fig. 4B, Supplementary Table 9). A total of 80 KEGG pathways were involved in these genes, including Toll-like receptor signaling pathway, cytosolic DNA-sensing pathway, Th17 cell differentiation and so on (Fig. 4C, Supplementary Table 10).

Discussion

In the current large-scale MR and genetic association study, using currently available comprehensive genetic data on bone mineral density and osteoporosis, we observed a causal effect of IL-27 and osteoporosis. In this study, we used bidirectional MR analysis to explore the causal relationship between levels of the inflammatory cytokine IL-27 and osteoporosis. We found a strong causal effect of IL-27 levels on osteoporosis, on the other hand, osteoporosis was not significantly associated with IL-27 levels via reverse MR analysis. Moreover, bioinformatics approaches suggested that IL-27 levels might regulate the onset and progression of osteoporosis through immune and inflammatory mechanisms.

he imbalance of bone remodeling is the pathophysiological process of osteoporosis. And T cells and their products are key regulators of bone remodeling [16, 34]. In the absence of estrogen, Th17 cell differentiation is enhanced, leading to increased osteoclast production and bone loss. IL-27 plays a major role in T cell regulation. IL-27 reduces rheumatoid arthritis by inhibiting Th17 cells and increasing T-regulatory cells. In this MR study, we found a possible positively causal relationship between IL-27 levels and osteoporosis, suggesting that IL-27 levels may play an important role in bone metabolism. It has been found that IL-27 negatively regulates the process of osteoclastogenesis [35]. In addition, IL-27 receptor subunits were also expressed in primary osteoblasts. However, IL-27 had no significant effect on the expression of osteogenesis-related proteins or osteoblast proliferation [35, 36]. Although IL-27 negatively regulates osteoclast formation and bone resorption, its role in osteoporosis and its causal relationship with osteoporosis

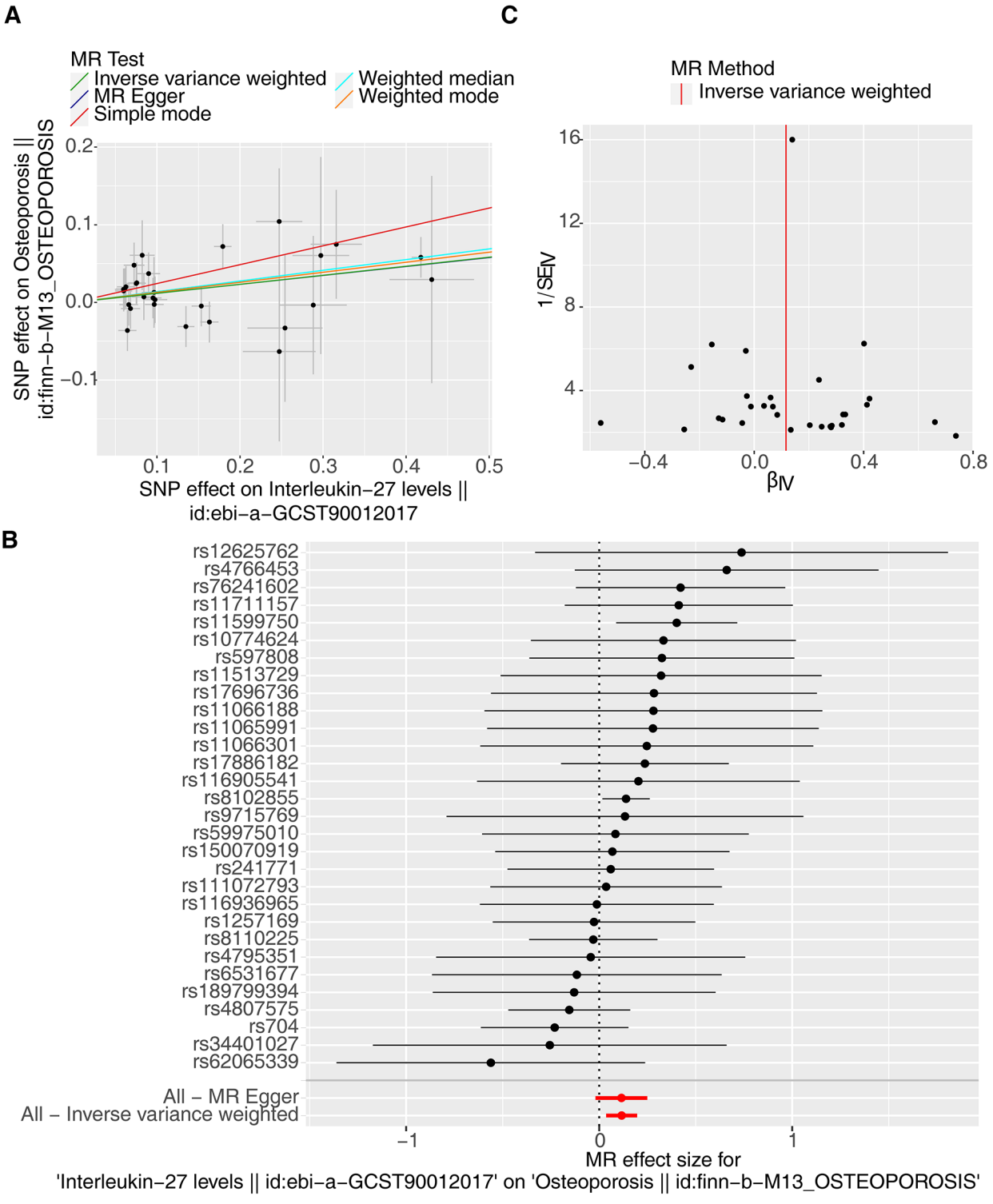

Fig. 2 Presentation of forward MR analysis for causal effect of IL-27 levels on osteoporosis. **(A)** Scatter plot for single nucleotide polymorphisms (SNPs) effect of IL-27 levels on osteoporosis. **(B)** Forest plot of MR analysis for causal effect of IL-27 levels on osteoporosis. **(C)** Funnel plot exhibited that SNPs distribution in forward MR analysis was consistent with Mendel's second law random grouping

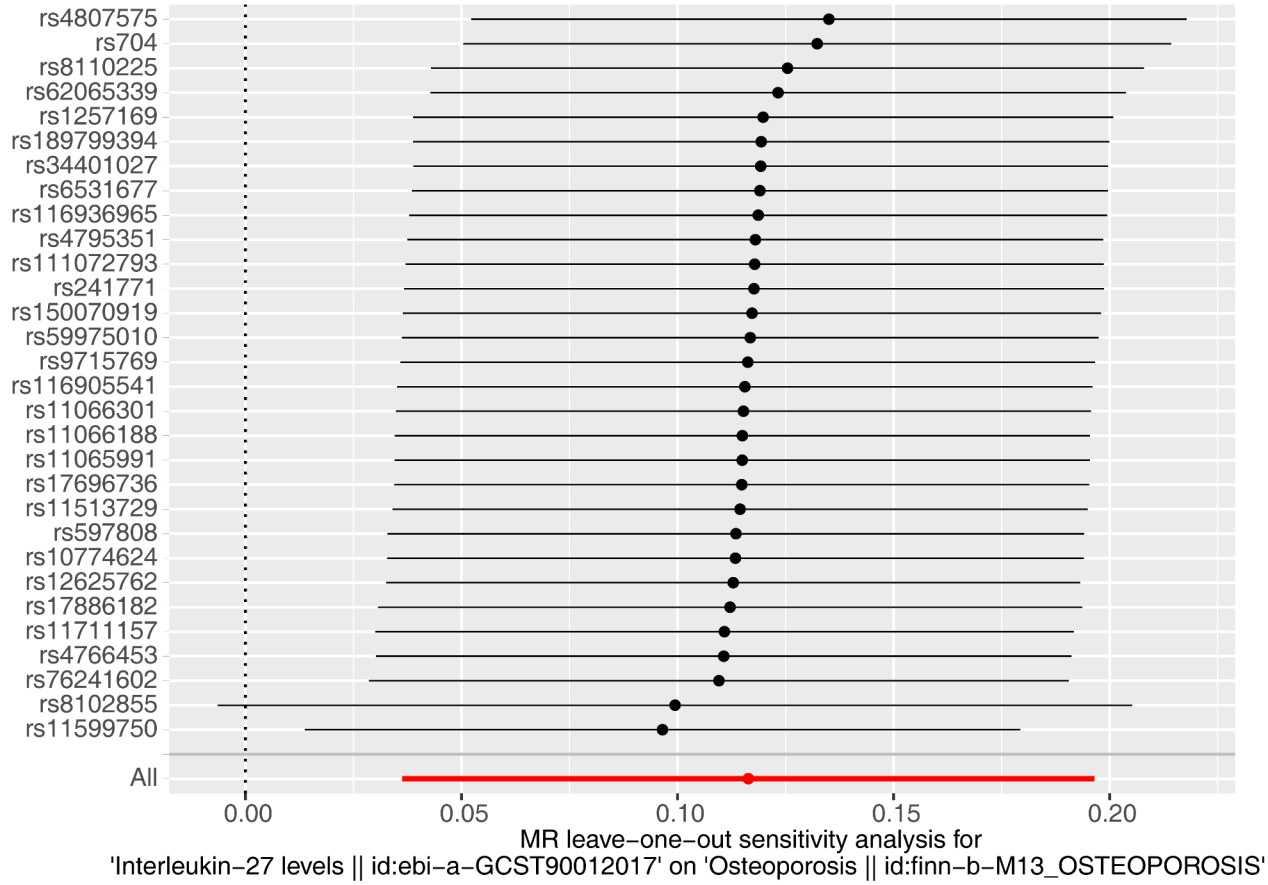

Fig. 3 The forest plot of the Leave-One-Out (LOO) sensitivity analysis by calculating the MR results of the remaining SNPs after removing the SNPs one by one. The smooth black dot line reflects the robustness of MR results

are unclear. Other studies have reported that IL-27 is produced in circulation and negatively regulates inflammation, suggesting that IL-27 plays an anti-inflammatory role in joint pathology [37]. However, it has also been reported that IL-17 induces the expression of IL-27 while aggravating rheumatoid arthritis, questioning the role of IL-27 in the process of bone resorption and bone destruction.

One MR Study has reported that many types of immune cells have a negative or positive causal relationship with osteoporosis, which contribute to the study of immune mechanisms in osteoporosis [38]. In our study, IL-27 expression levels had a correlation strength with osteoporosis, and IL-27 levels were able to increase the probability value of osteoporosis by 1.6% (OR=1.016). Tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-23, IL-27, and all pro-inflammatory mediators may exacerbate the progression of osteoporosis by destroying cartilage and bone due to increased osteoclast activity [39]. IL-27 inhibits T and B cell proliferation to suppress osteoclastogenesis [17, 22]. In this work, the results of enrichment analysis were involved in Th17 cell differentiation and Toll-like receptor signaling pathway, suggesting that the mechanism of IL-27 in osteoporosis is closely linked to immune inflammatory pathways.

In physiological and pathophysiological environments, osteoblast generation is regulated by an intricate cytokine network. In this network, positive regulatory factors such as IL-18, IFN-γ, CT-1 and OSM promote osteoblast generation, while negative regulatory factors such as TNF-α, TNF-β, IL-1α, IL-4, IL-23, IFN-α, IFN-β and CNTF inhibit osteoblast generation. Both positive and negative regulatory factors maintain bone homeostasis and health [40]. IL-6 family cytokines play an important role in determining bone structure by controlling longitudinal and radial bone growth, craniofacial development, and normal bone remodeling processes that maintain bone structure. IL-6 is an early recognized factor that stimulates osteoclast formation. The mechanism of IL-6 is not directly acting on hematopoietic osteoclast precursors, but stimulating the release of "bone transmitters" to promote bone formation on the periosteum through cortical osteocyte network [41, 42]. As an immune regulatory factor, IL-27 not only inhibits the differentiation of Th17

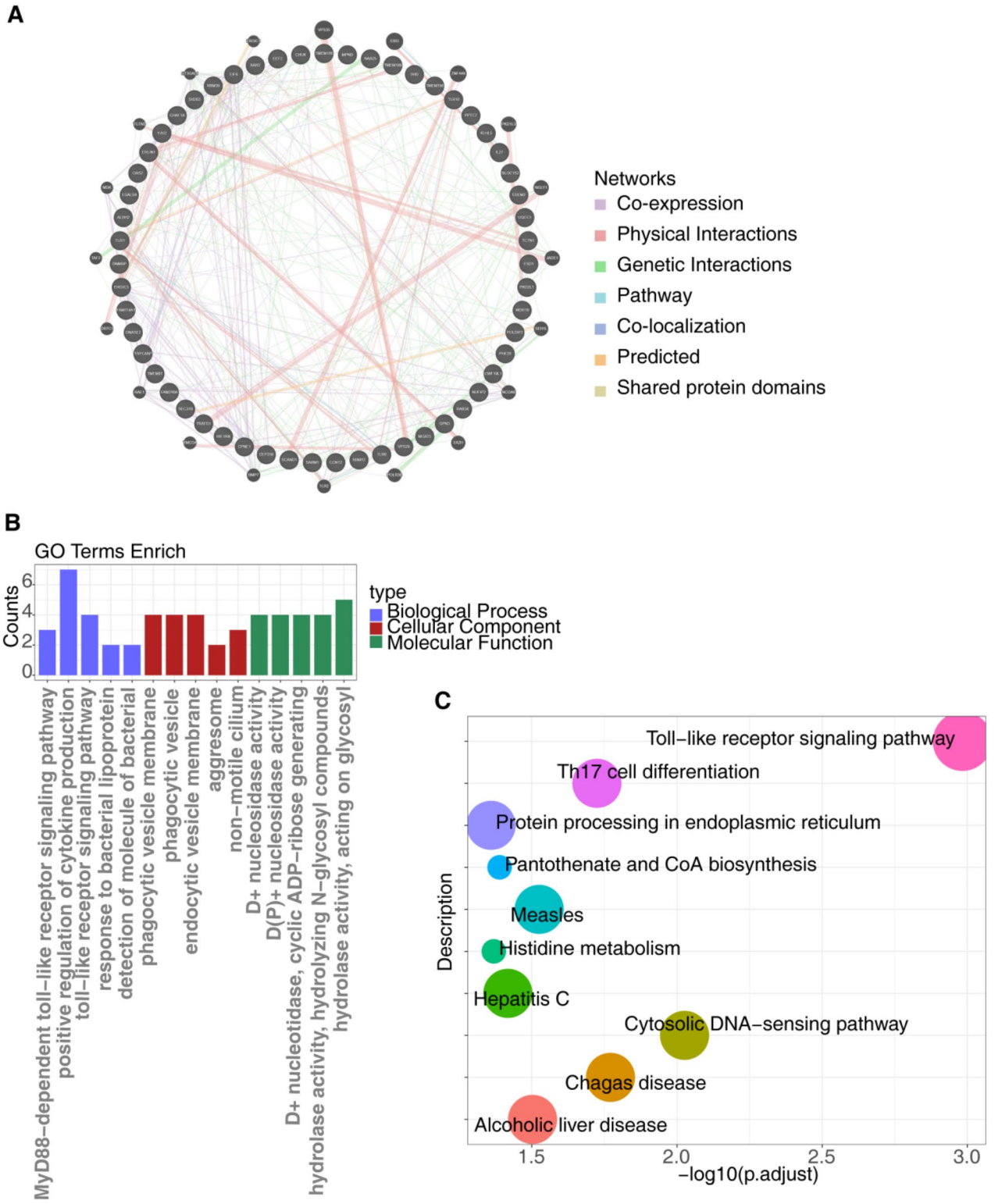

Fig. 4 Functionality exploration of 74 cis-eQTL genes corresponding to 26 SNPs of IL-27 levels on osteoporosis. **(A)** The co-expression network and function hypothesis analysis of cis-eQTL genes based on GeneMANIA website. **(B)** Bar chart of Gene Ontology (GO) results of the cis-eQTL genes. **(C)** Bubble chart of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the cis-eQTL genes

cells, but also directly acts on osteoclast precursor cells to inhibit c-Fos through STAT1-dependent pathway, thereby blocking the osteoclast generation process mediated by RANKL and effectively alleviating the disease of inflammatory bone destructive diseases, such as rheumatoid arthritis [43].

The IFN family plays an important role in bone remodeling. Studies have shown that the ability of hematopoietic stem cells (HSCs) to form new cell colonies in the bone marrow is hindered by IFN-γ, and at the same time, it induces the differentiation and apoptosis process of human CD34+bone marrow cells. Differentiation of CD34+cells is a natural and necessary step in the hematopoietic process, but IFNγ -induced over differentiation may accelerate the depletion of the HSCs pool. This depletion phenomenon in turn poses a threat to the immune system as it reduces the number of HSCs available for regeneration, potentially leading to an overall decline in immune system function [44]. TNF-α, as a key pro-inflammatory cytokine, plays a crucial role in immune response and bone metabolism. TNF-α mainly enhances the immune response by enhancing macrophage activation and antigen presentation, inhibits osteoblast activity at specific differentiation stages, and stimulates the proliferation and differentiation of osteoclasts, thereby regulating bone reconstruction [45].

Toll-like receptors (TLR) stimulation can induce osteocytes to secrete IL-27 cytokines, and the expression of autocrine IL-27 seems to be mediated by TLR signaling pathway and accessory cytokine driving pathway [46]. In our study, we utilized functional enrichment analysis to study the mechanism of action of IL-27 levels on osteoporosis. GO enrichment and KEGG enrichment results showed that some immune and inflammation-related pathways were enriched, such as MyD88-dependent Toll-like receptor signaling pathway, regulation of cytokine production, Toll-like receptor signaling pathway. Immunity system regulation and imbalances in cellular activity may affect the normal physiological state of skeletal tissue, which in turn may be associated with problems such as osteoporosis [47, 48].

This study has the following advantages. Most previous studies on the relationship between IL-27 and bone-related diseases have relied on cellular experiments and animal models, limiting the ability to establish a causal relationship between IL-27 and osteoporosis. We analyzed the association between IL-27 and osteoporosis using MR analysis, thereby minimizing the influence of confounding factors and ensuring valid conclusions of causality. Our study utilized pooled data from GWAS meta-analyses of IL-27 levels and osteoporosis, ensuring the reliability of our MR analysis. In our selection of datasets, we took into account a number of factors, including but not limited to sample size, SNP coverage, data quality, and availability, as the abundance of SNPS is critical for genetic association studies. The abundance of SNPS is critical for genetic association studies because it increases the likelihood that we will detect potential genetic variants, thereby improving the sensitivity and accuracy of the study. It was with this in mind that we ultimately chose the current dataset with the richest number of SNPS to maximize the effectiveness of our study. Despite these strengths, there are some limitations to our findings. For example, this study focused on European populations, which naturally raises an important question: Do our findings apply equally to individuals of non-European? This is an area that needs to be explored in the future. In addition, the absence of gender statistics in the original study and the failure to analyze gender as an independent variable in this study may have affected the comprehensiveness and depth of the results to some extent. In future research, future studies could consider combining multiple data sets for analysis to more fully explore the genetic basis of osteoporosis.

Conclusions

Through the comprehensive analysis of MR method, we obtained a causal relationship between IL-27 expression level and osteoporosis, and bioinformatics analysis revealed that the causal relationship between IL-27 and osteoporosis may involve immune and inflammatory mechanisms. Further study of the causal relationship between immune inflammation and osteoporosis as well as disease-related mechanisms is necessary for future development.

Abbreviations

IL-27	Interleukin-27
MR	Mendelian randomization
IVW	Inverse variance weighted
LOO	Leave-One-Out
OR	Odds ratio
CI	Confidence interval
EBI	EBV-induced protein
GWAS	Genome-wide association study
GO	GeneOntology
KEGG	Kyoto Encyclopedia of Genes and Genomes
IEU	Integrative Epidemiology Unit
SNPs	Single nucleotide polymorphisms
cis-eQTL	Cis-expression quantitative trait loci
BP	Biological process
CC	Cellular components
MF	Molecular functions
TLR	Toll-like receptors

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12891-024-07765-8.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

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Not applicable.

Author contributions
YX wrote the manuscript and prepared data. YZ and CL prepared the figures and tables. JZ and FL performed the statistical analysis. RS modified the manuscript. All authors analyzed the results, wrote the main manuscript, and reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The authors declare that all investigations were conducted in conformity with ethical standards.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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