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Research article

Exploring the genetic association between immune cells and susceptibility to osteonecrosis using large-scale population data

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ABSTRACT

Objectives: Research shows a close association between aberrant immune reactions in osteonecrotic tissues and immune cell infiltration. However, due to limitations in sample size and dataset comprehensiveness, the causal relationship between them is not fully established. This study aims to determine whether there is a causal relationship using a larger and more diverse dataset.
Methods: We conducted a comprehensive Mendelian Randomization (MR) analysis to investigate the causal relationship between immune cell characteristics and osteonecrosis. Utilizing publicly available genetic data, we explored the causal relationships between 731 immune cell features and 604 cases from the FinnGen Finnish database, as well as 257 cases from the UK Biobank database with osteonecrosis data. The inverse-variance weighted (IVW) method was used for the primary analysis, and we employed sensitivity analyses to assess the robustness of the main results. In addition, considering data from the two databases used in this study, a meta-analysis was conducted on the significant immune cells associated with osteonecrosis (FDR <0.05).
Results: our findings suggested that specific immune cell signatures, such as $CD20^{-}$ % lymphocytes, CD62L-monocytes, and CD33br HLA DR^{+} $CD14^{-}$cells were associated with increased odds of osteonecrosis. In contrast, EM $CD4^{+}$ activated cells and DP ($CD4^{+}$ $CD8^{+}$) T cells were associated with decreased odds. Notably, osteonecrosis was associated with a potential decrease in CD45 on immature MDSC cell content.
Conclusion: From a genetic perspective, we demonstrated a close association between immune cells and osteonecrosis. These findings significantly enhance our understanding of the interplay between immune cell infiltration and the risk of osteonecrosis, contributing to the potential design of therapeutic strategies from an immunological standpoint.

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1. Introduction

Osteonecrosis is a pathological process in which bone cells die due to chronic inflammation, more common in middle-aged individuals, primarily occurring around joints, leading to mechanical involvement of joint cartilage, collapse of subchondral bone, and secondary osteoarthritis [1–3]. In the US, 10,000–20,000 osteonecrosis of the femoral head (ONFH) patients are newly found to be affected with the disease per year [4]. According to estimates, there are around 30 ONFH million patients worldwide [5]. If ONFH is not treated in a timely manner, the rate of collapse of the femoral head within 3 years is more than 80 %, with a high disability rate [6]. Causes of osteonecrosis include alcohol and glucocorticoid use, hypercholesterolemia, sickle cell anemia, and autoimmune diseases. It has been demonstrated that anti-resorptive medications (such as bisphosphonates and receptor activator of nuclear factor-κB ligand inhibitors like denosumab) or angiogenesis inhibitors can lead to medication-related osteonecrosis of the jaw (MRONJ) [7].

Osteonecrosis was traditionally attributed to osteoblast and osteocyte death, alongside abnormal osteoclast activation [8]. However, recent research has unveiled a close association between aberrant immune responses and immune cell infiltration in osteonecrotic tissues, highlighting signs of uncontrolled inflammation [9–11]. In necrotic bone tissue, inflammatory cytokines and chemokines perpetually recruit innate (macrophages, neutrophils, dendritic cells) and adaptive (T cells and B cells) immune cells. This cascade of events amplifies the inflammatory response, fostering bone resorption and inhibiting bone formation, thereby contributing to osteonecrosis [12,13]. However, observational studies have shown a notable deficiency of Vγ9Vδ2 T cells in osteoporotic patients undergoing n-BP therapy, experiencing bisphosphonate-associated osteonecrosis of the jaw (BAONJ), compared to untreated controls matched for age and gender [14]. This paradoxical finding challenges the conventional role of inflammatory cells, suggesting that immune cells may have context-specific effects. Given the critical role of immune cells in inflammation, understanding how innate and adaptive immune cells regulate inflammation related to osteonecrosis could provide valuable insights into its pathogenesis and treatment. To date, the vast majority of evidence regarding the association between immune cells and osteonecrosis comes from observational studies, which are often limited by confounding factors and reverse causation, thus preventing causal inference.

Mendelian Randomization (MR) is a data analysis technique used in epidemiological studies to establish causal relationships in genetics. It utilizes genetic variants strongly associated with exposure factors as instrumental variables to assess causal links between exposures and outcomes. In this study, we utilize recent large-scale Genome-Wide Association Study (GWAS) data summarizing statistics on immune traits, alongside osteonecrosis data from the FinnGen Finnish database and the UK Biobank database, and conducted a meta-analysis on the significant effects of immune cells on osteonecrosis. Our objective is to elucidate the pathophysiological role of immune cells in osteonecrosis by investigating the existence of a causal relationship between immune cells and the incidence of osteonecrosis. Understanding these risk factors associated with osteonecrosis progression will facilitate early diagnosis and treatment strategies.

2. Materials and methods

2.1. Study design

Using the TwoSampleMR R package (Version 0.5.7, https://mrcieu.github.io/TwoSampleMR/index.html), we conducted a Two-Sample MR analysis to assess the causal relationship between 731 immune cell features (grouped into 7 sets) and osteonecrosis. MR utilizes genetic variants as proxies for risk factors; therefore, the instrumental variables (IVs) in causal inference must satisfy three key assumptions [15]: (1) genetic variants are directly associated with the exposure; (2) genetic variants are unrelated to potential

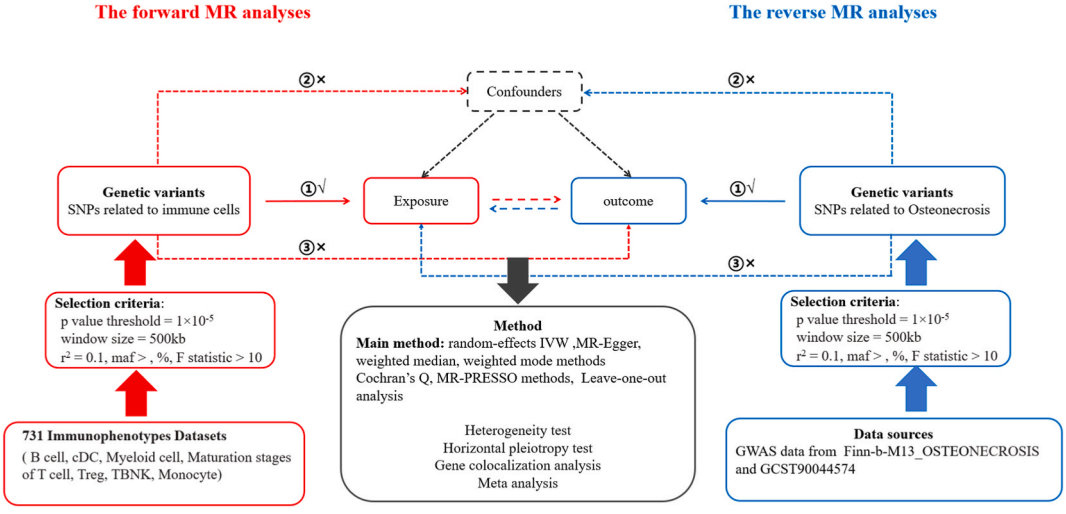

Fig. 1. Workflow of our research.

confounders between exposure and outcome; (3) genetic variants do not affect the outcome through pathways other than exposure. The specific flowchart is illustrated in Fig. 1.

2.2. Genome-Wide Association Study data on immune cells

This project sourced all immune cell data from the IEU OpenGWAS project platform (https://gwas.mrcieu.ac.uk/), developed by the MRC Integrative Epidemiology Unit at the University of Bristol. The platform integrates comprehensive open-access GWAS summary datasets, available for download as open files or through database queries. We downloaded data on 731 immune cells from the IEU OpenGWAS project platform. GWAS summary statistics for immune traits can be publicly accessed from the GWAS catalog (with IDs ranging from GCST0001391 to GCST0002121) [16]. A total of 731 immune phenotypes were included, comprising absolute cell (AC) counts (n = 118), median fluorescence intensity (MFI) reflecting surface antigen levels (n = 389), morphological parameters [17] (n = 32), and relative cell (RC) counts (n = 192). Specifically, MFI, AC, and RC phenotypes include B cells, cDC, mature stages of T cells, monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), and Treg panel. Meanwhile, the MP phenotype includes CDC and TBNK panel. The original GWAS for immune traits utilized data from 3757 European individuals with no overlapping cohorts.

2.3. Genome-Wide Association Study data on osteonecrosis

This study utilized two sets of osteonecrosis data. One set is from the FinnGen Finnish database (https://www.finngen.fi/en/access_results), with the code finn-b-M13_OSTEONECROSIS (ICD-10: M87). The FinnGen study, initiated in 2017, is a comprehensive nationwide cohort study integrating genetic data from Finnish biobanks with digital health record data from Finnish health registries. For finn-b-M13_OSTEONECROSIS, the data consists of 604 cases, 209,575 controls, and a total of 16,380,447 SNPs.The other set is from the UK Biobank database, with the code GCST90044574 (https://www.ebi.ac.uk/gwas/home). The UK Biobank study, a large multicenter cohort study conducted between 2006 and 2010, recruited over 500,000 European participants across the United Kingdom. For GCST90044574, the data includes 257 cases, 456,091 controls, and a total of 24,327,686 SNPs. Both datasets are derived from European populations, and there is no sample overlap between the UK Biobank and FinnGen datasets.

2.4. Selection of instrumental variables (IVs)

According to recent studies, the significance level for each immune trait's Instrumental Variable (IV) is set at 1×10^{-5}. For the osteonecrosis phenotype, we adjusted the significance level to 5×10^{-6} [16,18]. Using PLINK software [19](version v1.90) and the clump function, we performed linkage disequilibrium (LD) pruning on these SNPs (LD r^2 threshold set at 0.1, window distance <500 kb) [20], where LD r^2 was calculated based on 1000 genomes as the reference panel. We computed the Phenotypic Variance Explained (PVE) and F-statistic for each IV to assess IV strength and avoid bias from weak instrumental variables. Subsequently, IVs with low F-statistics ($F < 10$) were removed, as well as instrumental variables with a minimum allele frequency (MAF) less than 1 %, retaining the remaining instrumental variables for further analysis.

2.5. Statistical analysis

First, the inverse variance weighted (IVW) approach evaluate the relationships among immune cells and osteonecrosis as the primary approach. After extracting the association estimates linking the instruments and outcomes, and harmonize the directional orientation of these estimates as the effect alleles, we applied the Wald estimator in our computation of MR estimates for each instrument, which allowed us to derive estimates of the causal effect. The IVW method's findings can be considered reliable if every SNP fulfills the MR assumptions which should be without horizontal pleiotropy. Cochran's Q test was utilized to assess heterogeneity among estimates derived from individual SNP. In cases without heterogeneity, a fixed-effects model was applied, while a random-effects model was employed in the presence of heterogeneity to yield more robust and reliable estimations. If there was not significant for heterogeneity ($P < 0.05$), a fixed-effects model was applied, otherwise, the random-effects model was used to provide more reliable estimate.

Further sensitivity analyses were employed to substantiate the robustness of results, including the weighted-median method, MR-Egger regression, the MR-PRESSO test and leave-one-out analyses [21]. The Weighted Median estimators generate reliable causal effects as long as fewer than half of the contributing information is sourced from invalid IV. In MR-Egger regression, an intercept term *P*-value less than 0.05 is deemed statistically significant and serves as an indicator of directional pleiotropy. The MR-PRESSO test is utilized to assess pleiotropic biases, and remove outliers to correct the pleiotropic effect. Leave-one-out analyses were performed to identify potentially influential SNPs. The variation in results before and after the exclusion of a SNP serves as an indicator of the association's stability. The stability of the association can be ascertained by examining the variation in results before and after the exclusion of a SNP. The scatter plot illustrates that the results are not influenced by outliers, and the funnel plot demonstrates the robustness of the correlation without heterogeneity.

Considering the use of data from two databases in this study, we performed meta-analysis (meta_6.5–0) on the results of significant immune cells associated with osteonecrosis (FDR <0.05). The model used in the meta-analysis was determined based on the heterogeneity (I^2) of the meta results from the two datasets. If heterogeneity is >50 %, a random-effects model was chosen; if heterogeneity is ≤ 50 %, a fixed-effects model was selected. The significance threshold for meta results was set at $P < 0.05$. Finally, we also performed the reverse MR analysis to explore the reverse causal relationships between osteonecrosis and immune cells.

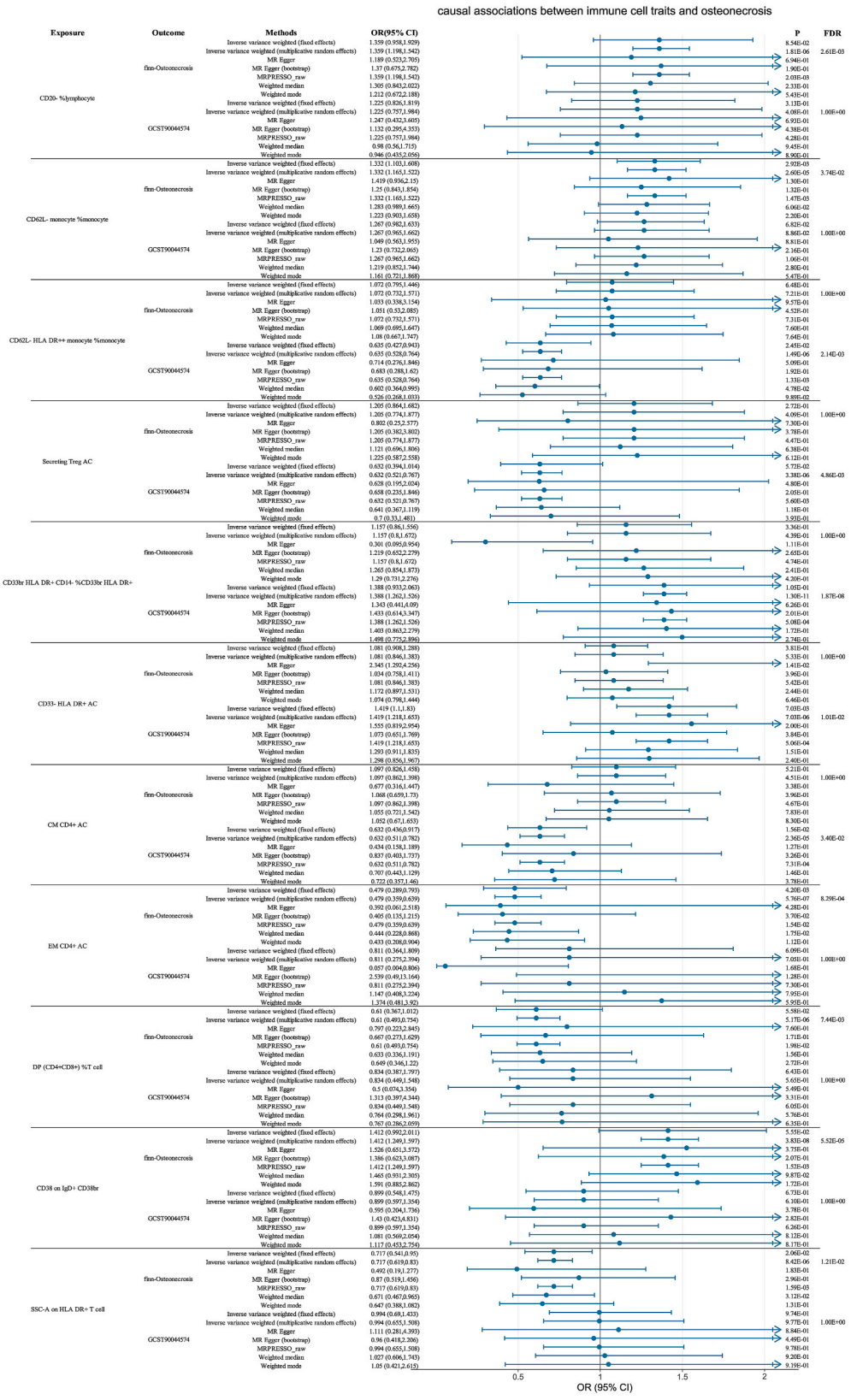

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Fig. 2. Forest plots showed the causal associations between osteonecrosis and immune cell traits. IVW: inverse variance weighting; CI: confidence interval.

3. Results

3.1. Exploration of the causal impact of immunophenotypes on osteonecrosis

According to the method described, we used a significance threshold of 1×10^{-5} to filter out the relevant loci from the 731 immunophenotype data as exposure IV loci for the osteonecrosis outcome. A two-sample Mendelian randomization was conducted with osteonecrosis data as the outcome variable, and the *P*-values for the respective models were calculated, as shown in Supplementary Table 1. The inverse variance-weighted method was chosen as the primary method for significance determination. When the number of instrumental variables exceeded 3, we opted for the inverse variance-weighted random-effects model. For cases with 2–3 instrumental variables, the inverse variance-weighted fixed-effects model was selected. Bonferroni correction was applied to the results. Overall, 11 immunophenotypes showed significant associations with osteonecrosis after Bonferroni correction (Fig. 2). Among these, 2 were from B cell phenotypes, 2 from cDC phenotypes, 2 from Maturation stages of T cell phenotypes, 2 from Myeloid cell phenotypes, 2 from TBNK phenotypes, and 1 from Treg phenotype. We conducted sensitivity analyses, heterogeneity testing, and leave-one-out validation on the obtained results. After systematically excluding SNPs, the MR results showed minimal fluctuation,

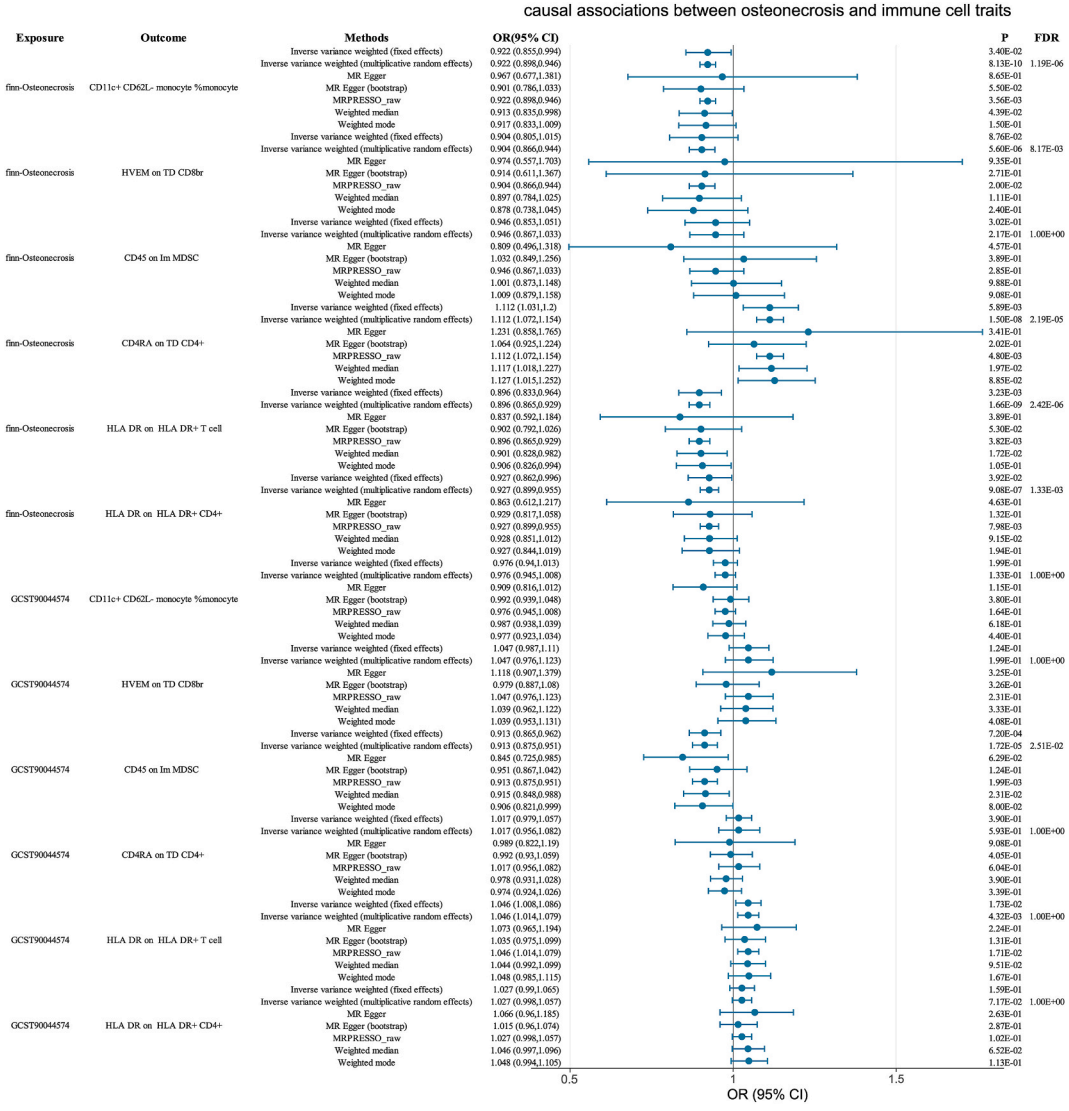

Fig. 3. Forest plots showed the causal associations between immune cell traits and osteonecrosis by using different methods. IVW: inverse variance weighting; CI: confidence interval.

indicating the stability of the results. As described in the methods, we employed the MR-Egger method to assess model pleiotropy and heterogeneity and used MR-PRESSO to evaluate pleiotropy. The test results for all immunophenotypes showing a significant relationship with osteonecrosis are provided in Supplementary Table 2. The results indicate the absence of heterogeneity and horizontal pleiotropy (Q_pval >0.05, intercept_pval >0.05).

Following the described methods, we conducted a meta-analysis of the 11 significant results from positive Mendelian randomization. If meta OR = 1, it indicates no association between the exposure factor and the disease; if meta OR > 1, it suggests a positive correlation between the exposure factor and the disease; if meta OR < 1, it indicates a negative correlation (protective factor). Among these, five results showed meta-significance (P < 0.05) (Supplementary Fig. 1): $CD20^-$ %lymphocyte, CD62L-monocyte %monocyte, CD33br HLA DR^+ $CD14^-$ %CD33br HLA DR^+, EM $CD4^+$ AC, DP ($CD4^+$ $CD8^+$) %T cell. $CD20^-$ %lymphocyte exhibited no heterogeneity (I2 = 0 %) for FinnGen and UK Biobank, so a fixed-effects model was chosen. The OR (95 % CI) result was 1.35 (1.20–1.53), with a *P*-value <0.01, suggesting a potential increase in the likelihood of osteonecrosis. CD62L-monocyte %monocyte showed no heterogeneity (I2 = 0 %) for FinnGen and UK Biobank, so a fixed-effects model was chosen. The OR (95 % CI) result was 1.32 (1.17–1.49), with a *P*-value <0.01, indicating a potential increase in the likelihood of osteonecrosis. CD33br HLA DR^+ $CD14^-$ % CD33br HLA DR^+ exhibited no heterogeneity (I2 = 0 %) for FinnGen and UK Biobank, so a fixed-effects model was chosen. The OR (95 % CI) result was 1.37 (1.25–1.50), with a *P*-value <0.01, suggesting a potential increase in the likelihood of osteonecrosis. EM $CD4^+$ AC showed no heterogeneity (I2 = 0 %) for FinnGen and UK Biobank, so a fixed-effects model was chosen. The OR (95 % CI) result was 0.50 (0.38–0.66), with a *P*-value <0.01, indicating a potential decrease in the likelihood of osteonecrosis. DP ($CD4^+$ $CD8^+$) %T cell exhibited no heterogeneity (I2 = 0 %) for FinnGen and UK Biobank, so a fixed-effects model was chosen. The OR (95 % CI) result was 0.63 (0.52–0.77), with a *P*-value <0.01, suggesting a potential decrease in the likelihood of osteonecrosis. Scatter plots, funnel plots, and leave-one-out validation plots for the five immune cells are depicted in Supplementary Figs. 2–11.

3.2. Exploration of the causal effect of osteonecrosis onset on immunophenotypes

According to the described methodology, the significance level for osteonecrosis was set at 5×10^{-6}. We filtered for minimum allele frequency (MAF) > 1 %, F-value >10, and after screening, six instrumental variables remained for FinnGen database Osteonecrosis. Additionally, eleven instrumental variables were retained after screening for UK Biobank database Osteonecrosis. The threshold of 5×10^{-6} was utilized as the significance threshold for the selected osteonecrosis-associated loci as instrumental variable (IV) sites. A total of 731 immune phenotype datasets were employed as outcome variables for a two-sample Mendelian randomization analysis. *P*-values for the respective models were computed, and the results are presented in Supplementary Table 3. We selected the inverse variance-weighted method as the primary method for determining significance. When the number of instrumental variables exceeds 3, we opted for the random-effects model using the inverse variance-weighted method. When the number of instrumental variables ranged from 2 to 3, we chose the fixed-effects model with the inverse variance-weighted method and performed Bonferroni correction on the results. The results indicated that six immune cell-related phenotypes exhibited post-correction significance (see Fig. 3). Among these, one was associated with the cDC phenotype, two with Maturation stages of T cell phenotype, two with TBNK phenotype, and one with Myeloid cell phenotype. To assess the sensitivity of reverse MR, conduct heterogeneity tests, and validate using leave-one-out method, we observed that the MR results did not exhibit significant fluctuations after systematically removing SNPs. This suggests the stability of the results. For causally significant phenotypes, we employed the MR-Egger method to assess model pleiotropy and heterogeneity, and MR_PRESSO to evaluate pleiotropy. All immune traits with significant relationships were tested, and the results, as shown in Supplementary Table 4, indicated no evidence of heterogeneity or horizontal pleiotropy in the significant relationships (Q_pval >0.05, intercept_pval >0.05). A meta-analysis was conducted on the six post-correction significant results (FDR <0.05) from reverse Mendelian randomization. Among them, the CD45 on Im MDSC cell type exhibited post-meta significance (Supplementary Fig. 12). Both FinnGen and UK Biobank datasets showed 0 % heterogeneity (I^2) for CD45 on Im MDSC, so a fixed-effects model was chosen. The odds ratio (OR) with a 95 % confidence interval (CI) was 0.92 (0.88–0.85), and the *p*-value was <0.01. As the meta OR was <1, and the meta *p*-value was <0.01, it indicates a significant negative association between osteonecrosis and CD45 on Im MDSC, suggesting that osteonecrosis is associated with a decrease in CD45 on Im MDSC content.

4. Discussion

Based on extensive publicly available genetic data, this study explores the causal relationship between 731 immune cell phenotypes and osteonecrosis. Using bidirectional Mendelian randomization, it reveals that 11 cell types show significant associations (FDR <0.05) with increased or decreased risk of osteonecrosis. The occurrence and development of osteonecrosis are associated with significant causal relationships (FDR <0.05) with an increase or decrease in six immune cell types. However, the effect sizes of the bidirectional causal relationships between certain immune cells and osteonecrosis varied across the two osteonecrosis datasets. We conducted a meta-analysis of the two distinct datasets. The final analysis revealed that $CD20^-$ % lymphocyte cell type, CD62L-monocyte % monocyte cell type, CD33br HLA DR + $CD14^-$ % CD33br HLA DR + cell type, and EM $CD4^+$ AC cell type increase the likelihood of osteonecrosis. Conversely, DP ($CD4^+$ $CD8^+$) % T cell cell type decreases the likelihood of osteonecrosis, and osteonecrosis is potentially associated with a decrease in CD45 on Im MDSC cell type. Upon exploring causal relationships, immune cell types linked to osteonecrosis do not exhibit a reverse relationship.

Two types of T cells, EM $CD4^+$ AC and DP ($CD4^+$ $CD8^+$) %T cell, have been shown to decrease the likelihood of osteonecrosis. T cells are vital components of cell-mediated adaptive immunity, possessing antigen-specific receptors on their surface that can recognize antigens presented by antigen-presenting cells in the MHC complex [22]. T cells can be divided into helper T cells (Th),

cytotoxic T lymphocytes (CTL), and regulatory T cells (Treg). These T cell subtypes collectively maintain the balance between bone formation and resorption by secreting osteoprotegerin (OPG) and RANKL or regulating the local inflammatory microenvironment, thus influencing bone metabolism [23]. Interestingly, Th and CTL participate in the progression of osteonecrosis, while Treg alleviates its progression. Studies have found that the imbalance of T cell subtypes may be related to the pathophysiological processes of ONFH, with a decrease in CD4/FoxP3 T regulatory cells possibly linked to an increase in RANKL T cells in the bone marrow of GC-ONFH femoral heads, potentially promoting bone absorption and femoral head collapse [24]. CD4/CD8 DP T cells, as a relatively obscure subset of lymphocytes with regulatory properties, have controversial roles [25]. Some studies have reported potential cytotoxicity in diseases such as lymphocytic choriomeningitis virus (LCMV), HIV, and cancer, while others suggest inhibitory phenotypes in cancer, systemic sclerosis, inflammatory bowel disease, and skin immune responses [26]. EM $CD4^+$ AC, considered a mature stage of T cells and typically identified as effector memory (EM) $CD4^+$ T cells, serve as a critical signaling node that initiates pro-inflammatory programs within the innate immune system. By binding to CD40 and TNFR on myeloid cells, these effector memory $CD4^+$ T cells induce destructive innate inflammation and autoimmune pathology [27]. This suggests that EM $CD4^+$ T cells may play a significant role in the inflammatory processes of osteonecrosis. Our MR Provides a new direction for investigating the immune pathogenesis of EM $CD4^+$ AC and DP ($CD4^+$ $CD8^+$) %T cells in osteonecrosis.

$CD20^-$ %lymphocyte, as a B cell, has been proven to increase the likelihood of osteonecrosis. B cells or B lymphocytes secrete antibody molecules to initiate adaptive humoral immune responses and present antigens to activate specific T cell immunity [28]. B cells contribute to maintaining a normal bone microenvironment, and abnormal quantities of certain B cell subtypes may be associated with osteonecrosis. Previous studies have found that the percentage of activated B cells ($CD86^+$ $CD19^+$) in the circulation of ONFH patients is correlated with disease severity, and serum TNF-α levels are positively correlated with the collapse index of femoral heads in alcohol-induced ONFH patients [29]. Alcohol abuse may induce systemic inflammation through the activation of TLR4-related pathways. The activation of the TLR4 pathway stimulates the production of TNF-α, which is a death factor. Members of the TNF-α family can regulate the development of B cells in the bone marrow and the activity of peripheral B cells, thereby promoting the development of ONFH [30–32]. Additionally, the OPG/RANKL system is another pathway through which B cells influence bone metabolism. Pre-B cells, immature B cells, and antibody-secreting B cells (plasma cells) block the RANK/RANKL system by producing large amounts of OPG, inhibiting osteoclast differentiation. Conversely, activated B cells secrete RANKL in a pro-inflammatory state, activating osteoclast formation [17,33,34]. In summary, different subtypes of B cells regulate bone metabolism by exerting different regulatory effects on osteoblasts and osteoclasts, thereby influencing the progression of osteonecrosis. Reducing harmful B cell subtypes may be a therapeutic approach for osteonecrosis, and future research should explore this avenue.

Additionally, we found that the CD33br HLA DR^+ $CD14^-$ %CD33br HLA DR^+ cell type and CD62L-monocyte %monocyte cell type increase the risk of osteonecrosis. HLA-DR is a surface receptor of MHC class II cells encoded by the leukocyte antigen complex on human chromosome 6p21. In a large case-control study of cancer patients receiving intravenous bisphosphonate therapy, half of whom were diagnosed with ARONJ, the frequencies of DRB1*15, DQB1*06:02, DRB1*01, and DQB1*05:01 were significantly increased in the ARONJ group compared to unaffected cancer patients. These results suggest that MHC class II polymorphism represents a genetic risk factor for the development of ARONJ and indicate that inflammation and infection may play a significant role in the pathogenesis of ARONJ [35]. CD62L-monocyte %monocyte cell type is a type of dendritic cell, and dendritic cells have two roles in bone immunity: (1) under the stimulation of receptor activator of nuclear factor κ b ligand (RANKL) released by T cells, dendritic cells can differentiate into osteoclasts, and the newly formed osteoclasts participate in local bone remodeling; (2) dendritic cells can present processed antigens through major histocompatibility complex (MHC) class I and II molecules or secrete pro-inflammatory or anti-inflammatory cytokines such as IL-12 p70, IL-4, and IL-17, severely affecting the type of T cell response [36–38] CD62L (L-selectin) is a type I transmembrane glycoprotein and cell adhesion molecule that facilitates the adhesion of leukocytes to endothelial cells, enabling their transmigration across vascular walls to participate in immune responses [39]. This indicates that CD62L is critical in the migration and adhesion of dendritic cells, allowing them to move to and interact within inflamed tissues, thereby modulating immune responses and either exacerbating or mitigating bone damage.

We observed a potential decrease in the CD45 on Im MDSC cell type in osteonecrosis. Myeloid-derived suppressor cells (MDSCs) were first proposed in 2007 to describe non-uniform immature myeloid cells found in pathological environments [40]. Mononuclear phagocytes (MNPs) (including monocytes, macrophages, and dendritic cellsx') and granulocytes (neutrophils, mast cells, basophils, and eosinophils) constitute the main population of innate immune cells, playing unique and specific roles in pathogen defense and maintaining the integrity of body tissues. In various conditions, especially in late-stage cancer, MNPs and granulocytes have been well-documented as immunosuppressive and/or regulatory mediators [41–43]. The role of MDSCs in autoimmune diseases is more complex. Preliminary studies on the collagen-induced arthritis (CIA) model in mice suggest that inhibitory MDSCs accumulate in the spleen, and the transfer of MDSCs can reduce the severity of rheumatoid arthritis (RA) by blocking the pro-inflammatory immune response of $CD4^+$ T cells [44]. M-MDSCs isolated from CIA mice BM can also inhibit B cell proliferation and function, improving the prognosis of CIA [45]. Recently, it has been reported that MDSCs may have opposite effects, with MDSC infiltration in the joints of mice with RA arthritis being positively correlated with disease activity, while the frequency of MDSCs in peripheral blood is negatively correlated with the number of TH17 cells [46]. This study conducted a two-sample MR analysis based on the published results of large GWAS cohorts, with a sample size of approximately 650,000 people, providing robust genetic evidence for the reported associations.

This study conducted a two-sample MR analysis based on the published results of large GWAS cohorts, with a sample size of approximately 650,000 people, providing robust genetic evidence for the reported associations. The conclusions of this study are based on genetic instrumental variables, and causal inference is made using a variety of MR analysis methods. The results are robust and were not confounded by horizontal pleiotropy and other factors. However, there were certain limitations in our study that should be addressed when interpreting the results. First, even when multiple sensitivity analyses are performed, horizontal pleiotropy cannot be

fully assessed. Second, due to the lack of individual information, we cannot conduct further stratified analysis of the population. Third, the study was based on a European database, so the conclusion cannot be extended to other ethnic groups, which would limit the generalizability of our results. Finally, even though we adhered to the STROBE-MR statement, not all of its recommendations could be met for the restricted availability of information (e.g., we were unable to determine whether overlapping individuals were enrolled between the exposure and outcome).

5. Conclusion

We have demonstrated the causal associations between immune cells and Osteonecrosis through a comprehensive bidirectional MR analysis, highlighting the complex pattern of interactions between the immune system and Osteonecrosis, contributing to the potential design of therapeutic strategies from an immunological standpoint.

Data availability statement

Immune cell data are available at https://gwas.mrcieu.ac.uk/, with IDs ranging from GCST0001391 to GCST0002121. The first set of osteonecrosis data is available at https://www.finngen.fi/en/access_results with the identifier finn-b-M13_OSTEONECROSIS (ICD-10: M87). The second set of osteonecrosis data is available at https://www.ebi.ac.uk/gwas/with the identifier GCST90044574.

List of abbreviations

Not applicable.

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Animal ethics

This study does not involve animal or human trials.

CRediT authorship contribution statement

Chen Meng: Writing – original draft, Conceptualization. **Baochuang Qi:** Data curation. **Huan Luo:** Data curation. **Zhifang Tang:** Data curation. **Junxiao Ren:** Formal analysis. **Hongxin Shi:** Data curation. **Chuan Li:** Data curation. **Yongqing Xu:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yongqing Xu reports financial support was provided by Yunnan Orthopedic Trauma Clinical Medical Center (No. ZX20191001). Yongqing Xu reports financial support was provided by Yunnan Orthopedics and Sports Rehabilitation Clinical Medicine Research Center (No. 202102AA310068). Yongqing XU reports financial support was provided by Clinical Key Subject Construction Project of PLA. Yongqing Xu reports financial support was provided by medical key subject of Joint Logistic Support Force of PLA. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34547.

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