Osteoporosis and Sarcopenia 10 (2024) 151–156

Contents lists available at ScienceDirect

Osteoporosis and Sarcopenia

journal homepage: www.elsevier.com/locate/afos

Original article

Effects of bone metabolism on hematopoiesis: A Mendelian randomization study

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ARTICLE INFO

Keywords:
Mendelian randomization
bone mineral density
Hematopoiesis
Osteoimmunology

ABSTRACT

Objectives: Osteoblast is known to regulate hematopoiesis according to preclinical studies but the causal relationship in human remains uncertain. We aimed to evaluate causal relationships of bone mineral density (BMD) with blood cell traits using genetic data.

Methods: Summary statistics from the largest available genome-wide association study were retrieved for total body BMD (TBBMD), lumbar spine BMD (LSBMD), femoral neck BMD (FNBMD) and 29 blood cell traits including red blood cell, white blood cell and platelet-related traits. Using two-sample Mendelian randomization (MR) approach, inverse-variance weighted method was adopted as main univariable MR analysis. Multivariable MR (MVMR) analysis was conducted to evaluate whether the casual effect is independent of confounders.

Results: BMD was positively associated with reticulocyte-related traits, including high light scatter reticulocyte count and percentage, immature reticulocyte fraction, reticulocyte count and percentage, with causal effect estimate (beta) ranging from 0.023 to 0.064. Conversely, inverse association of BMD with hematocrit, hemoglobin, and red blood cell count was observed, with beta ranging from −0.038 to −0.019. The association remained significant in MVMR analysis after adjustment for confounders. For white blood cells, BMD was inversely associated with neutrophil count (beta: 0.029 to −0.019) and white blood cell count (beta: 0.024 to −0.02). Results across TBBMD, LSBMD, and FNBMD were consistent.

Conclusions: This study suggested bone metabolism had a causal effect on hematopoietic system in humans. Its causal effect on red blood cell traits was independent of confounders. Further studies on how improving bone health can reduce risk of hematological disorders are warranted.

1. Introduction

Bone has been recognized as an organ affecting hematopoiesis and immune system [1,2]. The cross-talk between bone, immune system, and hematopoiesis was coined osteoimmunology [3], which primarily involves the studies of the interaction of bone and immune system. Indeed, bone has also been shown to affect non-immune blood cells, such as erythrocyte. For example, osteoblast regulates erythropoiesis via erythropoietin production [4]. However, most, if not all, of these studies were conducted using *in vitro* and *in vivo* models. Although there were human observational studies investigating the cross-sectional relationship between bone mineral density (BMD) and blood cells [5–9], inconsistent results were observed, and no causality can be inferred due to its cross-sectional nature. Thus, whether bone could affect blood cells in humans remains largely unknown.

Mendelian randomization (MR) is now considered one of the approaches for inferring exposure-outcome causal relationship. MR study design, if conducted appropriately, should be free from confounding and reverse causation. Genetic variation was used as instrumental variable that could exert a life-long effect. This is particularly useful to infer

Peer review under responsibility of The Korean Society of Osteoporosis.
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https://doi.org/10.1016/j.afos.2024.10.001
Received 29 April 2024; Received in revised form 9 September 2024; Accepted 13 October 2024
Available online 28 October 2024

genetic causal relationship between traits (eg, BMD and blood cells), when their causality is difficult or impossible to be inferred using conventional epidemiological studies. In this study, we aimed to evaluate the genetic causality of BMD on blood cells using a MR approach.

2. Methods

A two-sample MR study design was purposed for evaluating causal relationship of BMD with different blood cells. The assumptions and study design were illustrated in Fig. 1. Gene-exposure and gene-outcome statistics were retrieved from published genome-wide association study (GWAS)/GWAS meta-analysis of predominantly European ancestry. Dual-energy X-ray absorptiometry (DXA) derived BMD at lumbar spine (LSBMD) (N = 32961; ~70% European) [10], femoral neck (FNBMD) (N = 32961; ~70% European) [10] and total body (TBBMD) (N = 56284; ~86% European) [11] were used as the exposures in the MR analysis.

The outcome of interest were 29 blood traits, including 6 immature red blood cell (RBC) traits, 8 mature RBC traits, 11 white blood cell traits, and 4 platelet traits. Summary statistics of the genetic association for the blood traits were retrieved from the published GWAS comprising 408,112 participants from the UK Biobank [12].

2.1. Genetic instruments

From the GWAS meta-analysis of LSBMD/FNBMD comprising 17 cohorts, 96 single nucleotide polymorphisms (SNPs) were genome-wide significant (P-value $< 5 \times 10^{-8}$) in the discovery dataset using linear mixed-effect model [10]. Among the 64 SNPs that remained genome-wide significant in the meta-analysis of discovery and replication cohorts, the SNPs were considered independent if they were separated by at least 1 Mb from the top SNPs [10]. A total of 48 and 49 independent genome-wide SNPs were selected as the instruments for LSBMD and FNBMD, respectively [10]. For TBBMD, genome-wide complex trait analysis (GCTA) was used to perform conditional analysis after meta-analyzing the European cohorts [11]. A total of 81 independent genome-wide SNPs were identified [11] and they were selected as the instruments for TBBMD. All the data sources were retrieved from published studies. Participants' consent and ethic approval were available from original studies.

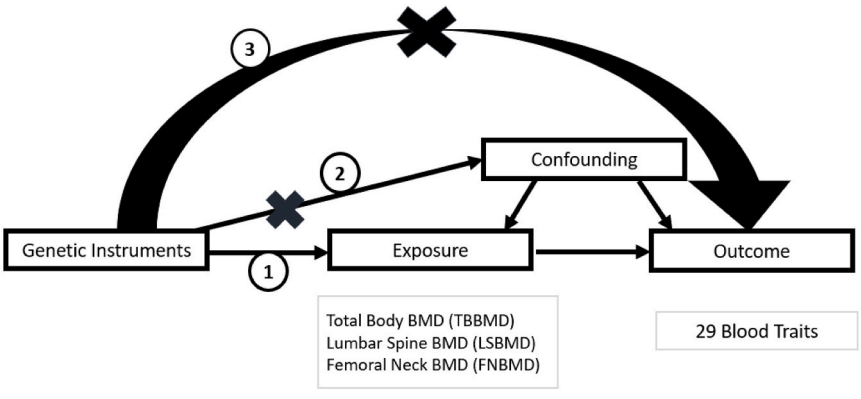
The instruments were matched with the GWAS summary statistics of the 29 blood cell traits [12] and confounders (for multivariable MR analysis). Palindromic instruments with minor allele frequency > 0.3 or instruments being unavailable from the GWAS of blood cell traits were replaced with proxies identified from the European population of the 1000 Genomes Project. Only proxies with high linkage disequilibrium with the original instruments ($r^2 \geq 0.8$) and showed genome-wide significant association with the exposure were chosen for the analysis.

2.2. Power calculation

For each analysis, proportion of variance (R^2) explained by instruments on the exposure was estimated by the effect estimate (beta) and allele frequency of instruments. Causal estimate on blood traits required to achieve 80% statistical power and F-statistics evaluating strength of genetic instruments were computed using the online calculators [13,14].

2.3. MR statistical analysis

Radial regression was performed before the statistical analysis for detection of influential data points in inverse-variance weighted (IVW) method [15]. After removing the influential instruments, the IVW method was conducted as the main analysis. The IVW method assumed that all instruments were valid [16]. Cochran's Q statistics was computed to evaluate heterogeneity of instruments [15]. For traits with heterogeneous instruments, the causal estimates were calculated using the random-effect model. Weighted median (WM), MR-Egger and contamination mixture methods were performed as sensitivity analyses. WM method provided a consistent measure if more than 50% of instruments were valid [17]. MR-Egger method provided a less biased estimate even in the presence of pleiotropy, yet the power of this method is considerably low [17,18]. Its regression intercept was used to estimate the mean pleiotropic effect [18]. Contamination mixture method evaluated the causal effect with the lowest mean square error compared to other methods, even in the presence of invalid instruments [19]. Multiple testing correction for the casual relationships between each bone site and the 29 blood cell traits was performed on the main analysis results using false discovery rate (FDR) q-value [20]. A causal relationship would be considered significant only if the significant association was observed in the IVW, weighted median and contamination mixture methods, while insignificant P-value was observed in the MR-Egger intercept test [21]. The insignificance of MR-Egger intercept test indicated the absence of horizontal pleiotropy. Results were presented as changes in blood cell traits in standard deviation (SD) per SD increase in BMD at different sites.



Assumptions of Mendelian Randomization Analysis
1. Relevance assumption: Genetic instruments are associated with exposure
2. Independence assumption: Genetic instruments are not associated with any confounding that affect the exposure-outcome relationship
3. Exclusion assumption: Genetic instruments are assumed to affect the outcome only via the exposure

Fig. 1. Assumptions in MR analysis and study design.

2.4. Sensitivity analysis by excluding SNPs associated with potential confounders

Potential confounders between exposure and outcome might bias the causal effect estimate. Risk factors for osteoporosis, including low body mass index (BMI) and renal disease, were also associated with blood cells [22]. Besides, the correlation between blood cell traits might also bias the association with BMD. To address the independence assumption of the MR study design (Fig. 1), analyses with significant associations between BMD and blood traits were repeated by excluding instruments that were genome-wide significant (P-value $< 5 \times 10^{-8}$) with potential confounders (including obesity, renal-related markers and blood cell traits) identified by PhenoScanner [23].

2.5. Multivariable MR (MVMR)

To further explore the independent causal effects of BMD on blood traits, a MVMR analysis was performed to evaluate the direct effect of risk factor on the outcome. MVMR was conducted by adjusting for the beta estimate of each confounding variable separately. BMI, phosphate and calcium were known for their correlations with bone metabolism [24–26]. BMI is a known predictor for BMD while calcium and phosphate are the main component of bone. These factors might also play a role in hematopoiesis [27–29]. Furthermore, chronic kidney disease (CKD) patients were reported to have an elevated risk of anaemia [30] and fracture [31]. Kidney function is linked to bone metabolism through several mechanisms, including the regulation of hormones. On the other hand, it is also linked to RBC production by regulating the production of erythropoietin. To measure kidney function, estimated glomerular filtration rate (eGFR) is one of the parameters used in the diagnosis of CKD. BMI, phosphate, calcium, eGFR and CKD were included as confounding factors in the MVMR analysis, aiming to examine the direct effect of exposure on the outcome by keeping these confounding factors constant [32]. Summary statistics of the genetic instruments were extracted from the respective GWAS of phosphate [33], calcium [33], BMI [34], eGFR [35] and CKD [35]. Several MVMR methods were applied in this study, such as MVMR-IVW, median-based method (MVMR-Median) and MVMR-Egger, which were developed based on IVW, WM and MR-Egger in the univariable MR analysis, respectively [36]. Multivariable MR-Egger intercept test could also help detect residual pleiotropic effect [36].

Multiple testing with FDR was applied for the main analysis. A two-sided q-value < 0.05 was considered statistically significant. For the remaining analysis, a two-sided P-value < 0.05 was considered statistically significant. All statistical analysis was conducted using R (version 4.1.3). The packages "MendelianRandomization" and "RadialMR" were employed for MR analysis and Radial regression, respectively.

3. Results

Variance explained by instruments ranged from 7% to 9% for TBBMD (Table S1), 4% to 5% for LSBMD (Table S2) and 3% to 4% for FNBMD (Table S3), respectively. Random-effect model was applied in the IVW analyses as heterogeneous instruments were discovered from Cochran's Q test statistics (Tables S1–S3).

For RBC traits, TBBMD, LSBMD and FNBMD were positively associated with five immature RBC traits (q-value < 0.05), including high light scatter reticulocyte count [IVW beta for TBBMD (95% CI): 0.042 (0.024 to 0.06); LSBMD: 0.044 (0.02 to 0.067); FNBMD: 0.062 (0.037 to 0.088)], high light scatter reticulocyte percentage [TBBMD: 0.044 (0.027 to 0.062); LSBMD: 0.046 (0.021 to 0.07); FNBMD: 0.064 (0.036 to 0.092)], immature reticulocyte fraction [TBBMD: 0.038 (0.02 to 0.055); LSBMD: 0.052 (0.028 to 0.076); FNBMD: 0.048 (0.021 to 0.075)], reticulocyte count [TBBMD: 0.023 (0.005 to 0.041); LSBMD: 0.033 (0.013 to 0.052); FNBMD: 0.045 (0.019 to0.071)] and reticulocyte percentage [TBBMD: 0.038 (0.022 to 0.055); LSBMD: 0.04 (0.021 to 0.059); FNBMD: 0.053 (0.025 to 0.08)], respectively (Fig. 2 & Tables S4–S6). Consistent results were observed with weighted median and contamination mixture methods (P-value < 0.05). Conversely, BMD was inversely associated (q-value < 0.05 for the IVW method and P-value < 0.05 for both weighted median and contamination mixture methods) with three of the mature RBC traits including hematocrit [TBBMD: 0.027 (−0.042 to −0.011); LSBMD: 0.035 (−0.057 to −0.013); FNBMD: 0.037 (−0.064 to −0.011)], hemoglobin [TBBMD: 0.019 (−0.035 to −0.002); LSBMD: 0.033 (−0.054 to −0.011); FNBMD: 0.028 (−0.053 to −0.004)] and RBC count [TBBMD: 0.03 (−0.046 to −0.014); LSBMD: 0.034 (−0.055 to −0.013); FNBMD: 0.038 (−0.066 to −0.01)] (Fig. 3 & Tables S4–S6).

For white blood cell traits, BMD was partially associated with neutrophil count [TBBMD: 0.021 (−0.037 to −0.006); LSBMD: 0.029 (−0.05 to −0.008); FNBMD: 0.019 (−0.041 to 0.004)] and white blood cell count [TBBMD: 0.021 (−0.037 to −0.005); LSBMD: 0.024 (−0.045 to −0.003); FNBMD: 0.02 (−0.042 to 0.003)] (Tables S4–S6). No evidence of association was found with platelet-related traits.

A sensitivity analysis was conducted upon exclusion of instruments associated with confounders, and the association of five immature and three mature RBC traits remained essentially unchanged (Tables S7–S8).

Since both the main analysis and sensitivity analysis revealed a significant causal relationship for BMD with RBC traits using univariable MR approach, a MVMR analysis was conducted to determine whether the results were affected by further adjustment for the beta estimates of the confounding factors. In the MVMR analysis upon adjustment for phosphate (Table S9), calcium (Table S10), BMI (Table S11), eGFR (Table S12) and CKD (Table S13) separately, the association was comparable to that in univariable MR analysis. Consistent and significant findings (P-value < 0.05) were observed between MVMR-IVW and MVMR-Median approaches.

4. Discussion

The present study attempted to investigate the role of bone metabolism on hematopoiesis. Genetically determined DXA-derived BMD was positively associated with reticulocyte measures but inversely associated with erythrocyte-related traits. Consistent and significant results across TBBMD, LSBMD, and FNBMD were observed. The results were consistently observed from both univariable MR and MVMR analyses. For the immune cell, BMD was inversely associated with the neutrophil and white blood cell counts. We also examined the causal relationship between BMD and other immune cells, but no significant association was observed. And there was insufficient evidence to prove the causation of BMD on platelet.

Our study suggested that BMD was positively associated with immature RBC traits but inversely associated with mature RBC traits. Reticulocytes are the transitional cells from erythroblasts to mature erythrocyte [37], while elevated reticulocyte is a sign of anaemia for clinical diagnosis [38]. In addition, osteopetrosis, a disease characterised by defective osteoclast and intensified BMD, can lead to anaemia and pancytopenia [39,40]. However, a previous observational study showed that high BMD loss was associated with an increased risk of anaemia [7]. This is contradictory to our findings. Notably, that study [7] showed a null association of BMD with the risk of anaemia. Thus, the high BMD loss could indeed represent a general health deterioration, instead of reflecting bone health or bone metabolism alone. Nevertheless, our findings, together with the clinical observation in the rare bone disease, suggest that higher bone mass may be causally associated with increased risk of anaemia.

On the other hand, bone marrow is also a storage for iron. Iron deficiency is a typical cause for anaemia. Bone metabolism was known for its interaction with iron homeostasis. For example, iron homeostasis is known to regulate bone metabolism [41]; while osteocyte regulates hepcidin secretion and iron homeostasis through fibroblast growth factor 23 production and cleavage [42]. In humans, a recent Korean

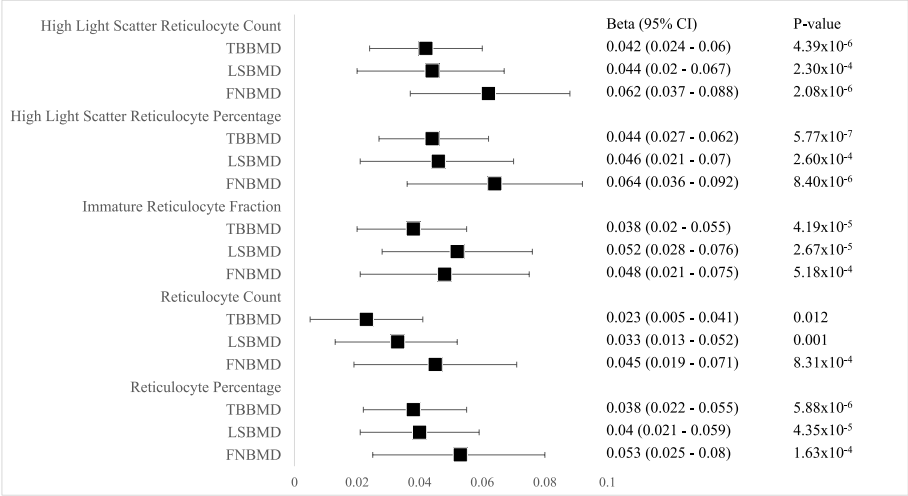


Fig. 2. Forest plot for MR IVW analysis of BMD at different sites on immature RBC traits with significant association.

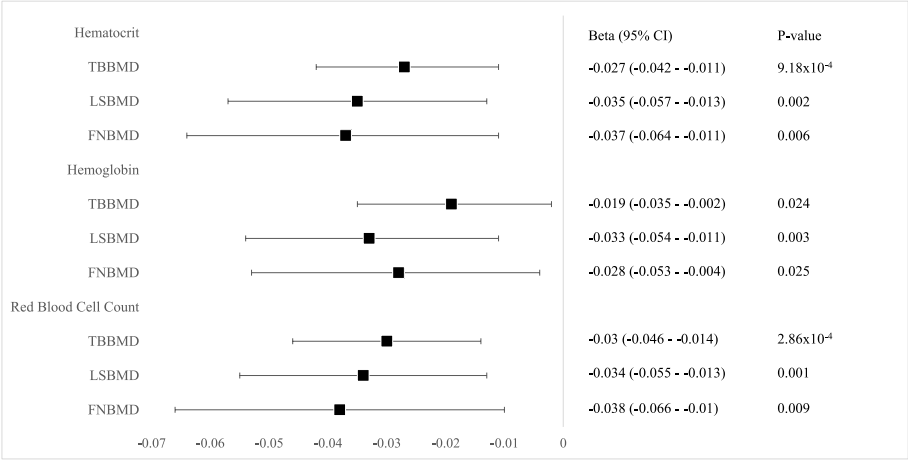


Fig. 3. Forest plot for MR IVW analysis of BMD at different sites on mature RBC traits with significant association.

study demonstrated that BMD was positively associated with total iron-binding capacity (TIBC) but inversely associated with serum iron level among postmenopausal women [43]. Elevated TIBC and reduced serum iron may indicate iron-deficiency anaemia in clinical practice. Results from the Korean study was in line with our observation that bone metabolism might be inversely linked with RBC production through interacting with iron homeostasis.

Although our observed relationship between BMD and RBC traits were consistent in the univariable MR analysis, there could be some unmeasured confounding factors that might affect the result. Bone matrix is mainly constituted by hydroxyapatite, a calcium phosphate compound. Not only do calcium and phosphate serve as a component of bone matrix, but they also regulate production of osteogenic cell and interfere production rate of bone matrix. In addition, they can also play a role in hematopoiesis. For example, phosphate can freely diffuse in bone marrow and regulate maintenance of hematopoietic stem cell [27]. Besides, elevated calcium concentration may enhance bone marrow hematopoiesis by creating unique stem cell-niche interaction [28]. Therefore, the causal relationship of BMD with blood cell traits could be confounded by the correlation with calcium and phosphate. Nevertheless, the association remained statistically significant in MVMR analysis (Tables S9–S10), demonstrating that there was an independent causal effect of BMD on the RBC traits.

Moreover, BMI is a marker for obesity, which is also known as a predictor for BMD. Increase in weight-related mechanical stress would increase osteoblast differentiation and hence improving bone density [44]. Obesity might also broadly affect hematopoiesis. For instance, obesity-related inflammation would elevate hepcidin concentration and limit the expression of duodenal ferroportin, reducing duodenal iron absorption and affecting erythroid lineage [29]. Nevertheless, the conclusion was essentially unchanged after adjusting for BMI in MVMR analysis, providing evidence that BMD could have an independent effect on the RBC traits (Table S11).

The kidneys play a crucial role in regulating both bone metabolism and blood cell production. The kidneys affect with bone metabolism through its regulation of bone-related hormones such as calcitriol and parathyroid hormone [45]. On the other hand, the kidney is responsible for the production of erythropoietin, which stimulates RBC production, in adults. Therefore, the association between BMD and RBC could be potentially confounded by renal function. Nevertheless, with the adjustment on eGFR (Table S12) and CKD (Table S13) in MVMR analysis, the results suggested that bone might have an independent effect on hematopoiesis.

There was evidence for the causal relationship of BMD with white blood cells. In the present study, genetically determined TBBMD and LSBMD were inversely associated with neutrophil and white blood cell count. Insignificant associations for FNBMD might be due to the lower statistical power (Table S3). Our findings aligned with an observational study [7] showing that lower BMD was associated with an increased risk of high neutrophil count. We previously showed that reduced FNBMD

was associated with infection and sepsis [46]. These findings suggested that bone metabolism also plays a role in immunity. However, given that neutrophil production increases during infection, the clinical significance of the change in neutrophil count within the normal range is unclear.

The intricate relationship between bone metabolism and hematopoiesis is well-documented. Numerous proteins associated with bone metabolism also play pivotal roles in hematopoiesis. For example, the chemokine C-X-C motif chemokine 12 (CXCL12), regulated by bone metabolism, is highly expressed in the bone marrow niche. This chemokine is essential for maintaining hematopoietic stem and progenitor cells within the bone marrow niche [47] and also regulates the differentiation of various immune cells [48]. Besides, Insulin-like Growth Factor 1 (IGF-1), which is stored in and secreted by osteoblast, was found to positively influence the erythropoiesis [49]. These biological interactions suggest a potential causal link between bone metabolism and hematopoiesis in humans. However, the clinical significance of this association remains to be determined. Therefore, further studies utilizing real-world data are warranted to elucidate this complex relationship.

The current study sheds light on the role of bone metabolism on hematopoiesis. This study also has important clinical implication. Bone biomarkers may provide valuable information for predicting or monitoring hematological disorders, such as anaemia, which is associated with poor functional outcomes and higher mortality in the older population. Current study may also provide insight for understanding the complex interaction between bone and blood cells that may help developing new therapy for bone and hematological disorders.

The strength of this study lies in the genetically determined causal effect of bone metabolism with hematopoiesis estimated by the MR analysis. A two-sample MR analysis with a non-overlapping population and adequately high F-statistics suggested that results were less likely to be influenced by weak instrumental bias. Moreover, consistent findings across TBBMD, LSBMD and FNBMD could suggest the causal effect of bone metabolism on hematopoiesis was likely to be true. Furthermore, coherent findings between univariable MR and MVMR analyses might advocate the effects of bone metabolism, independent of BMI, circulating calcium and phosphate levels and renal function on blood traits. Nevertheless, there are limitations. First, biases in the MR analysis could not be excluded completely. Although the insignificant MR-Egger intercept test suggested that horizontal pleiotropy was unlikely, there could still be a possibility of a potential pleiotropic effect with unmeasured phenotypes. Unmeasured phenotypes may include proteins that are regulated by bone metabolism, and their unexamined roles in hematopoiesis necessitate additional investigation. Second, unmeasured confounders typically lead to biased results in epidemiological studies. Yet, we did a sensitivity analysis by excluding instruments associated with potential confounders. The robust results suggested that the independence assumption of the MR setting was likely to be valid against unmeasured confounding. Third, predominant European population genetic data were utilized for the MR analysis. The generalizability of the findings on other populations is unclear. Fourth, although we adjusted for circulating calcium and phosphate levels in the MVMR analysis, the local concentration of calcium and phosphate in the bone environment could play a role. However, no such data is available in human. Fifth, the current two-sample MR approach assumes the causal effect is linear in nature. Nevertheless, we were unable to evaluate the non-linear relationship between bone and blood traits due to the lack of individual data. Further study evaluating the non-linear relationship between BMD and blood cells is warranted. Lastly, whether the causal effect observed from genetic evidence could be translated into clinical practice remains uncertain. Therefore, a further analysis of real-world cohorts and studies in other population is warranted.

5. Conclusions

This study showed that bone metabolism played an important role in hematopoiesis in humans. The causal effect of bone metabolism on RBC production was independent of confounding factors. Further study to examine whether improving bone health can reduce risk of hematological disorders is warranted.

Credit author statement

Shun-Cheong Ho: Methodology, Formal analysis, Writing - Original Draft, Visualization. **Gloria Hoi-Yee Li**: Methodology, Writing - Review & Editing. **Anskar Yu-Hung Leung**: Writing - Review and Editing. **Kathryn Choon-Beng Tan**: Writing - Review and Editing. **Ching-Lung Cheung**: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration.

Conflicts of interest

The authors declare no competing interests.

Data availability

Summary statistics of genetic instruments can be obtained from the websites listed on the referenced GWAS or GWAS meta-analysis.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.afos.2024.10.001.

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