Unravelling genetic causality of haematopoiesis on bone metabolism in human

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Abstract

Objective: Haematopoiesis was shown to regulate bone metabolism in in vivo studies. However, whether haematopoiesis has causal effects on bone health has never been investigated in human. We aimed to evaluate the causal relationships of blood traits with bone mineral density (BMD) and fracture.

Design and Methods: Using two-sample Mendelian randomization, causal relationship of 29 blood traits with estimated BMD (eBMD), total body BMD (TBBMD), lumbar spine BMD (LSBMD), femoral neck BMD (FNBMD) and fracture were evaluated by inverse-variance weighted (IVW) method and multiple sensitivity analyses. Relevant genetic data were obtained from largest possible publicly available genome-wide association studies.

Results: Eight genetically determined red blood cell traits showed positive causal effects on eBMD, with beta estimates ranging from 0.009 (mean corpuscular haemoglobin) to 0.057 (haemoglobin concentration), while three white blood cell traits, including lymphocyte count (beta:-0.020; 95% CI: -0.033 to -0.007), neutrophil count (beta:-0.020; 95% CI:-0.035 to -0.006) and white blood cell count (beta:-0.027; 95% CI:-0.039 to -0.014), were inversely associated with eBMD. Causal effects for six of these blood traits were validated on TBBMD, LSBMD, FNBMD and/or fracture. The association of reticulocyte count (beta:0.040; 95% CI:0.016 to 0.063), haemoglobin (beta:0.058; 95% CI:0.021 to 0.094) and mean corpuscular haemoglobin concentration (beta:0.030; 95% CI:0.007 to 0.054) with eBMD remained significant in multivariable IVW analyses adjusted for other blood traits.

Conclusion: This study provided evidence that haematopoietic system might regulate skeletal system in human and suggested the possible pathophysiology of bone diseases among people with haematological diseases.

Significance statement

We conducted a novel Mendelian randomization study investigating causal relationship of blood cells with bone mineral density. Red and white blood cell traits have positive and inverse causal relationship with bone mineral density respectively, suggesting a potential link of haematopoietic system with skeletal system in human. Current findings suggest individuals with related haematological diseases, such as anaemia and leukocytosis, may have a lifelong increased risk of osteoporosis and/or fracture. Given that complete blood count is commonly performed in clinical setting, whether complete blood count can be used to predict fracture risk warrants further investigation.

Introduction

In the last decade, bone cells were shown to be a novel regulator of haematopoiesis, such as providing and interacting with haematopoietic stem cell niche (1-3), as well as secreting erythropoietin (EPO) (4). Bone tissue also forms part of the osteoimmune system, at which progenitors of myeloid and lymphoid cells, and mature immune cells are maintained (5). Meanwhile, haematopoiesis itself generates various types of blood cells, including white blood cells, which contribute to innate and acquired immune responses. As the key player of the immune system, the count of total white blood cells, as well as the count of each type of white blood cells, serve as the major diagnostic tools for disorders of the immune system, inflammatory and infectious diseases (6). The blood cell traits therefore enabled, at least in part, investigation of the human immune system. Although it is well accepted that there is interplay among haematopoietic, immune and skeletal systems in the field of osteoimmunology, whether haematopoiesis and immune systems are causally linked to bone metabolism remains largely unknown.

In terms of cell lineage, it is well known that osteoclasts are members of the monocyte/macrophage lineage. While osteoblasts are generally derived from mesenchymal stem cells, a recent in vivo study demonstrated that haematopoietic stem cells can give rise to osteoblasts in a murine model (7). However, the relationship between blood traits and bone metabolism in human is unclear. Although a few studies have evaluated the relationship of blood traits with bone mineral density (BMD), conflicting results were observed for white blood cells (8-10), red blood cells (8-13), and platelets (8, 9), which could be due to reverse causation and unmeasured confounding that are commonly encountered in conventional observational studies.

Mendelian randomization (MR) has been gaining popularity in recent years as its study design might overcome such limitations of epidemiological observational studies. In this two-sample MR study, we aimed to evaluate the causal relationship of 29 blood traits with BMD at heel estimated by ultrasound (eBMD), BMD at total body (TBBMD), lumbar spine (LSBMD) and femoral neck (FNBMD) measured by dual energy X-ray absorptiometry (DXA), as well as fracture, in the univariable analysis. Genetic correlation among the blood traits were calculated, and subsequent multivariable MR analyses were performed to evaluate the independent causal effects of blood traits on BMD.

Methods

Study design

The study design and key assumptions of univariable MR analysis is shown in Fig. 1. Genetic variants associated with the exposure were utilized as instrumental variables (IVs) to infer causality with the outcome. Two-sample MR approach was adopted to evaluate causal relationship of various blood traits on BMD measured at different skeletal sites, including eBMD, TBBMD, LSBMD and FNBMD, as well as fracture. A detailed description of data sources for all the exposures and outcomes are presented in Table 1.

Data source

Exposure for MR analyses were 29 blood cell traits, with summary statistics retrieved from a genome-wide association study (GWAS) comprising 408,112 UK Biobank participants of European ancestry as the discovery cohort (14). Independent genome-wide significant genetic

variants associated with blood traits identified by the GWAS were selected as IVs (details in Supplementary Methods A, https://osf.io/k4m37/ (15)). The outcome was eBMD in primary analyses, with summary statistics obtained from the largest GWAS of BMD to-date that comprised 426,824 UK Biobank participants (16). Secondary MR analyses evaluating the causal effects of blood traits on other bone traits (including TBBMD, LSBMD and FNBMD and fracture) were conducted to validate the results from primary analysis (details described in Supplementary Methods B, https://osf.io/k4m37/ (15)).

Univariable MR analysis

The main analysis for the MR study was the inverse-variance weighted (IVW) method, which assumed all IVs are valid (17). Prior to main analysis, radial MR analysis was used to identify outliers which had large contribution to Cochran's Q statistics that might imply heterogeneity, including horizontal pleiotropy (18). MR Steiger filtering method was adopted to calculate and compare the variance explained by all IVs in exposure and outcome, and infer the direction of causality for each IV. IVs identified to have the expected causal direction from exposure (blood traits) to outcome (bone traits) were kept (19). Using the same principle, MR Steiger directionality test was also applied to orient the overall causal effect of all IVs (19). Online calculators were utilized for power calculation (20) and measuring strength of IVs. Summary of the IVs adopted in various analyses are presented in Supplementary Tables 1-5 (https://osf.io/k4m37/ (15)). Sensitivity analyses, including weighted median (21), MR-Egger (22) and contamination mixture (23) methods, were applied (details in Supplementary Methods C, https://osf.io/k4m37/ (15)). A causal relationship was only considered genuine if significant association was demonstrated in IVW [multiple testing corrected by false discovery date (FDR) q-value <0.05], weighted median, and contamination mixture methods, while MR-Egger

intercept test was insignificant. To ensure the second assumption (independence assumption, Fig. 1) holds, supplementary analysis was performed by excluding IVs associated with potential confounding factors if significant causal relationship was identified for the primary analysis of eBMD (details in Supplementary Methods D, https://osf.io/k4m37/ (15)).

Genetic correlation and multivariable MR

In view of the presence of calculated and compound blood traits, genetic correlation between traits were calculated to derive the minimal representative set of blood traits (Supplementary Methods E, https://osf.io/k4m37/ (15)). If any of the representative blood traits was shown to casually affect eBMD in univariable analysis, multivariable IVW analysis was performed by adjusting for the beta estimates of other representative blood traits (Supplementary Methods F, https://osf.io/k4m37/ (15)).

Results

Primary univariable analyses

The characteristics of the IVs are presented in Supplementary Table 1 (https://osf.io/k4m37/ (15)). Out of 29 blood traits examined, 11 showed possible causal relationships with eBMD in IVW analysis (FDR q-value<0.05), with similar results yielded in sensitivity analyses. Eight of the 11 traits were red blood cell traits having positive causal effects on eBMD. Four were immature red blood cell traits, including high light scatter reticulocyte count [IVW: per SD increase in genetically determined blood trait, beta estimate in eBMD (in SD): 0.024; 95% CI: 0.013 to 0.035], high light scatter reticulocyte percentage (0.022; 95% CI: 0.011 to 0.034), reticulocyte count (0.031; 95% CI: 0.019 to 0.042) and reticulocyte percentage 0.029; 95% CI: 0.018 to 0.040) (Table 2a and Supplementary Fig. 1, https://osf.io/k4m37/ (15)). The four

mature red blood cell traits were haematocrit (0.046; 95% CI: 0.031 to 0.062), haemoglobin concentration (0.057; 95% CI: 0.041 to 0.073), mean corpuscular haemoglobin (0.009; 95% CI: 0.001 to 0.018), and mean corpuscular haemoglobin concentration (0.036; 95% CI: 0.020 to 0.051) (Table 2b and Supplementary Fig. 2, https://osf.io/k4m37/ (15)). Three white blood cell related traits, including lymphocyte count (-0.020; 95% CI: -0.033 to -0.007), neutrophil count (-0.020; 95% CI: -0.035 to -0.006) and white blood cell count (-0.027; 95% CI: -0.039 to -0.014), showed inverse causal effects on eBMD (Table 2c and Supplementary Fig. 3, https://osf.io/k4m37/ (15)). Evidence of association was unavailable for other red blood cells, white blood cells and platelet-related traits (Supplementary Table 6 and Supplementary Fig. 1-4, https://osf.io/k4m37/ (15)). The potential bias and type I error rate incurred by the sample overlap was minimal as presented in Supplementary Table 1 (https://osf.io/k4m37/ (15)).

To ensure the independence assumption holds for the IVs, a supplementary analysis was conducted by excluding IVs which are associated with potential confounders (listed in Supplementary Table 7, https://osf.io/k4m37/ (15)). Among the 11 blood traits significantly associated with eBMD in primary analysis, significant causal effects of high light scatter reticulocyte count [IVW: per SD increase in genetically determined blood trait, beta estimate in eBMD (in SD): 0.068; 95% CI: 0.038 to 0.098], reticulocyte count (0.073; 95% CI: 0.042 to 0.104) and haematocrit (0.092; 95% CI: 0.064 to 0.12) with eBMD were consistently observed in IVW and other sensitivity analysis, with larger effect sizes (Supplementary Table 8, https://osf.io/k4m37/ (15)). For other blood cell traits, the causal associations with eBMD were attenuated, or inconsistent associations between IVW and sensitivity analyses were found, which could be attributed to the reduced statistical power upon exclusion of IVs (Supplementary Table 9, https://osf.io/k4m37/ (15)).

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175 *Genetic correlation and multivariable analyses*

176 Based on the pairwise genetic correlation of the blood traits (Supplementary Table 10,
177 https://osf.io/k4m37/ (15)), a minimal set of 21 traits were selected to represent all the 29 blood
178 traits (Supplementary Table 11, https://osf.io/k4m37/ (15)). Among the 21 representative traits,
179 reticulocyte count, haemoglobin concentration, mean corpuscular haemoglobin, mean
180 corpuscular haemoglobin concentration, lymphocyte count and white blood cell count were
181 causally associated with eBMD in univariable analyses (Table 2). Thus, multivariable MR
182 analyses were performed for these traits to evaluate whether their causal effects on eBMD were
183 independent of other blood traits, by conditioning on the other 20 representative blood traits.

184

185 Multivariable IVW analyses demonstrated little change of causal estimates for reticulocyte
186 count (0.040; 95% CI: 0.016 to 0.063), haemoglobin (0.058; 95% CI: 0.021 to 0.094) and mean
187 corpuscular haemoglobin concentration (0.030; 95% CI 0.007 to 0.054) on eBMD. The
188 multivariable MR-Egger test yielded similar positive causal estimates (Table 3). Nevertheless,
189 the causal relationship of mean corpuscular haemoglobin, lymphocyte count and white blood
190 cell count with eBMD were attenuated in the multivariable analysis. All the multivariable MR-
191 Egger intercept tests were insignificant (Table 3).

192

193 *Secondary analyses*

194 Secondary analyses were performed to evaluate the causal relationship of blood traits with
195 other bone-related traits, including TBBMD, LSBMD, FNBMD and fracture. Characteristics
196 of the IVs adopted in the secondary analyses are presented in Supplementary Tables 2-5

(https://osf.io/k4m37/ (15)). Among the 11 blood traits which were shown to have causal effects on eBMD in the univariable analyses (Table 2), the association was validated for two white and two red blood cell traits with DXA-derived BMD. Genetically increased white blood cell count was inversely associated with TBBMD (Supplementary Table 12, https://osf.io/k4m37/ (15)) while neutrophil count was inversely associated with LSBMD (Supplementary Table 13, https://osf.io/k4m37/ (15)). Both traits were associated with reduced FNBMD (Supplementary Table 14, https://osf.io/k4m37/ (15)). For red blood cell traits, reticulocyte percentage and high light scatter reticulocyte percentage were positively associated with FNBMD (Supplementary Table 14, https://osf.io/k4m37/ (15)). Similarly, three of the 11 causal relationships were validated for fracture, including genetically increased haematocrit and haemoglobin with reduced risk of fracture; and increased neutrophil count with increased fracture risk (Supplementary Table 15, https://osf.io/k4m37/ (15)). The potential bias and type I error rate incurred by the sample overlap between the GWAS of blood traits and fracture was minimal as presented in Supplementary Table 5 (https://osf.io/k4m37/ (15)).

Discussion

Our study findings provide evidence on the potential link between the haematopoietic and skeletal systems in human and the pathophysiology of bone diseases among people with haematological diseases. In primary analyses, genetically increase in red and white blood cell traits were observed to have positive and inverse causal effects on eBMD respectively (Supplementary Fig. 5, https://osf.io/k4m37/ (15)). Genetically increased haemoglobin also had positive causal effect on eBMD independent of other blood traits, while consistent association with reduced fracture risk was observed.

Several observational studies have been conducted to evaluate the relationship of blood cell traits with BMD. Positive (8, 10-12, 24), null (13), and inverse (9, 11) association were observed for different blood cell traits. The discrepancies could be explained by the cross-sectional nature of study design, as well as differences in study participants, such as the general population (13), non-anaemic population (12), post-menopausal women (8-10, 24) and old men (11). The discrepancies could also be due to the definition of blood cell traits. For example, three studies (8-10) examined "white blood cell counts", which consisted of a mixed population of white blood cells. Conversely, the MrOS study (11) investigated counts of different white blood cells individually and showed that association of high neutrophil, low lymphocyte, and low monocyte were associated with rapid bone loss. In addition, analysis bias and selection bias might explain the different results. Unmeasured confounders, such as comorbidities, might cause biases (9). Meanwhile, MR approach was reported to be less subjected to residual confounding and reverse causation when compared to conventional observational studies. We adopted the two-sample MR approach using GWAS data of 29 blood traits to investigate the problem and demonstrated that red blood cell and white blood cell traits had positive and inverse causal effects on eBMD respectively.

Due to the sample overlapping problem in the primary analyses, we performed secondary analyses in samples with minimal overlap. Some of the causal relationships observed for eBMD (including reticulocyte percentage, high light scatter reticulocyte percentage, neutrophil count and white blood cell count) could be validated in IVW analyses for DXA-derived BMD. For the non-reproducible traits, one plausible reason was the relatively small sample size of the GWAS of DXA-derived BMD, and hence low statistical power. Assume the causal estimates

for DXA-derived BMD were the same as that observed for eBMD in primary analyses, minimum power of 26.6% and 12.7% was observed for mean corpuscular haemoglobin with TBBMD and LSBMD/FNBMD respectively (Supplementary Table 16, https://osf.io/k4m37/ (15)). Another reason could be the intrinsic difference between eBMD and DXA-derived BMD. eBMD was positively and modestly correlated with DXA-derived TBBMD, LSBMD and FNBMD (r~0.4-0.6) (16). While most of the susceptibility loci of DXA-derived BMD were also identified in the GWAS of eBMD, a few eBMD-associated loci were reported to have opposite direction of effects when compared to the DXA-derived BMD traits (25). In addition, genetically increased haematocrit and haemoglobin were shown to reduce fracture risk, whereas neutrophil count elevated fracture risk. Effects of these blood traits on fracture were directionally consistent with their effects on BMD, although not all causal relationships could be validated. This might be explained by the relatively small sample size of the fracture GWAS and hence limited power (Supplementary Table 5, https://osf.io/k4m37/ (15)). Another reason might be the multifactorial nature of fracture, such as non-bone related factors like muscle strength and propensity of falls. Nevertheless, these do not affect the findings from our MR analyses that lifelong increase in several red blood cell traits might be protective to bone, while lifelong increase in certain white blood cell traits might be harmful to bone health.

The protective and independent effects of red blood cell traits on bone metabolism are supported by laboratory studies and clinical observations. Haematopoietic progenitors secrete osteolectin that is important for bone mass maintenance during adulthood (26, 27). Clinically, patients with sickle cell disease are more likely to have low bone mass and fragility fracture (28). In addition, a very recent study showed that erythroferrone, which is secreted by bone marrow erythroblasts, is a positive regulator of bone metabolism (29).

On the other hand, our univariable MR analyses demonstrated that genetically increased counts of lymphocyte, neutrophil and white blood cell had adverse effects on BMD, and increased neutrophil count might even elevate the fracture risk. Cautious interpretation may be required, as the causal association of these white blood cell traits with eBMD was no longer significant in the supplementary analysis excluding IVs associated with potential confounders. Nevertheless, due to removal of a substantial portion (up to 68.2%) of IVs, the variance explained by the IVs on the exposure decreased, reducing the statistical power of the analysis. We only have 37.2% to 65.3% power in detecting a 0.02 SD change in eBMD per SD increase in white blood cell traits (Supplementary Table 9, https://osf.io/k4m37/ (15)). The analysis should be re-visited when the power issue is resolved, such as having GWAS of larger sample size. Lymphocyte and neutrophil account for over 90% of the five main types of white blood cells in human. Inflammation, which is characterized by increased white blood cell counts, is a well-established risk factor of osteoporosis, due to its stimulation of osteoclastogenesis. Autoantibodies and cytokine would stimulate the osteoclast differentiation. For example, cytokines released from T-lymphocyte such as granulocyte macrophage-colony stimulating factor (GM-CSF) could facilitate mature osteoclast formation (30, 31). CCL2 (C-C motif chemokine ligand 2) and CXCL1 (C-X-C motif chemokine ligand 1), two chemokines secreted from neutrophil, are mediators of osteoclastogenesis that can accelerate osteoclast maturation (32). Nevertheless, lymphocyte count and white blood cell count were selected in the minimal set of 21 representative blood traits. Multivariable analyses by adjustment for other representative blood traits attenuated the association with eBMD, implying that total effects of these two traits on eBMD as observed in univariable analyses could be, in part, explained by its correlation with other blood traits.

There are clinical implications in the current study. Our findings suggested that people with related haematological diseases, such as anaemia and leukocytosis, may have a lifelong increased risk of osteoporosis and/or fracture. Yet, the observed effect estimates of blood traits on BMD were small, which could be due to the following reasons. First, eBMD was used in the primary analysis, which may not accurately reflect the effect size for DXA-measured BMD. Although DXA-measured BMD was examined in the secondary analysis, the GWAS were of small sample size and had insufficient power, potentially leading to inaccurate estimation of effect size. Second, the GWAS of blood traits adopted in this MR study were measured in healthy people without serious haematological disorders. The current MR findings could only reflect the effect of blood traits on BMD in people without haematological disorders, while it is expected that the effect of haematological disorders on BMD could be even stronger. Third, the estimates used in the analyses were adjusted for several covariates in the original GWAS, thus the effect estimates observed in the study may reflect the sole effect of blood traits on BMD, which is small but statistically significant. It should be noted that, interventions that affect the blood traits, especially the pharmacological ones, may also affect the skeletal systems. Future studies examining the relationship of interventions affecting the blood traits with BMD are warranted. On the other hand, given that complete blood count is commonly performed in clinical setting, whether complete blood count can be used to predict long-term bone health in terms of BMD and fracture risk warrants further investigation.

The strength of this study was the inclusion of summary statistics from the largest GWAS meta-analysis and large number of IVs in primary analyses that provided ample power. The primary analyses had at least 80% power in detecting a causal estimate of as low as 0.008 SD change in eBMD per SD change in several blood traits (Supplementary Table 1, https://osf.io/k4m37/ (15)). Thus, even if a causal effect is present for these traits on eBMD, the genuine effect size

may be too small to be detected in the present study. The study design of MR allowed causal inference between traits, which was often infeasible to be evaluated using randomized controlled trial. Adoption of a stringent definition of potentially genuine finding in this study reduces false positive rate.

Nevertheless, there were limitations. For the causal association revealed to be potentially genuine in the primary analyses, MR-Egger intercept tests were all insignificant, implying horizontal pleiotropy was unlikely, although it cannot be completely ruled out. Yet, a consistent conclusion could be drawn from the supplementary analysis excluding IVs associated with potential confounder and multivariable MR analysis that several red blood cell traits were independently associated with BMD. Furthermore, both GWAS of blood cell traits and eBMD comprised study participants from the UK Biobank, possibly leading to biases towards the direction of the observational association due to the weak instrument bias (33). In particular, the IVs were discovered in the data adopted in MR analysis, which was reported to worsen the issue of weak instrument bias due to winner's curse (33). Nevertheless, due to the relatively high F-statistics of the IVs (≥97.06, average per IV; Supplementary Table 1, https://osf.io/k4m37/ (15)), weak instrument bias was not expected despite sample overlap. A recent simulation study also suggested that two-sample MR methods could be safely applied within biobanks of large sample size (>300,000) (34). With the assumption that sample overlap between the exposure and outcome datasets was 100% and the observational estimate was 0.1 SD change in eBMD per SD increase in the blood cell traits, the bias and Type 1 error rate due to sample overlap under the null hypothesis were 0.001 SD in eBMD and 0.05 respectively (Supplementary Table 1, https://osf.io/k4m37/ (15)). Moreover, we conducted secondary analyses to assess the causal effects of blood traits on DXA-derived BMD and fracture, which had no or minimal sample overlap with the UK Biobank cohort. The secondary analyses

validated the causal effects of six blood traits on bone health. Therefore, the potentially genuine causal relationships observed in this study were likely to be true. Lastly, GWAS/GWAS meta-analysis of DXA-derived BMD and fracture had relatively small sample size. The lack of evidence of association might be due to insufficient statistical power.

In conclusion, this study suggested that both red blood cell and white blood cell parameters might causally influence BMD and fracture risk. While the current study evaluated the causal relationship of haematopoiesis with bone health using genetic data from the general population, future investigation on the pathophysiology between haematological diseases and bone health is warranted.

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Ethical approval: Ethical approval was available from original genome-wide association studies from which the genetic data was extracted for the current analysis.

Informed consent: Informed consent from participants was available from original genome-wide association studies from which the genetic data was extracted for the current analysis.

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Table 1 Data source used in Mendelian randomization analysis

Traits	Description	Ancestry	Sample size*	Derivation of independent genome-wide significant variants
Blood Traits (14)	A GWAS of 29 blood cell phenotypes, with a discovery cohort conducted in 408,112 UK BioBank participants. The 29 traits included 6 immature red blood cell traits, 8 mature red blood cell traits, 4 platelet-related traits and 11 white blood cell traits. A meta-analysis was also conducted with 154,976 additional European participants from the Blood Cell Consortium for 15 of the blood cell traits only. The full set of summary statistics for the discovery cohort, but not the meta-analysis, was publicly available online.	European	408,112 (Discovery cohort)	A total of 16,900 conditionally independent blood trait-variant associations were identified from the discovery cohort. Briefly, for each of the 29 blood traits, the genome-wide significant variants were partitioned into the largest number of blocks, with pairwise block separation of ≥5Mb. In each block, only a parsimonious set of variants were kept to explain the signals in the block using a stepwise multiple linear regression approach. In constructing the model, a new variant in high LD (r^2>0.9) with a variant already in the parsimonious set would be ignored, as the new variant cannot represent an independent signal.
eBMD (16) (Primary analyses)	A GWAS of heel bone mineral density estimated by ultrasound (eBMD) in the white British UK Biobank cohort.	European	426,824	Conditional analysis was performed using GCTA, which used a standard stepwise selection model based on the LD pattern in the reference population. In brief, the analysis started with the variant with the strongest association in the meta-analysis. Other variants with minimum conditional p-value that fit all the selected variant(s) were subsequently added. Variants with large conditional p-values were excluded. The iteration stopped until no more variants were added or excluded. In this study, variants with r^2>0.9 were ignored, and the remaining variants situated >20Mbp away were defined as independent. 1,103 conditionally independent variants with genome-wide significance ($p < 6.6x10^{-9}$) were identified.
TBBMD (35) (Secondary analyses)	A GWAS meta-analysis of DXA-derived TBBMD from 30 cohorts across America, Europe, and Australia. The full set of summary statistics were available online.	86% European	66,628	Similar to the GWAS of eBMD, conditional analysis was performed using GCTA in the meta-analysis of cohorts with European ancestry only (N=56,284).

Traits	Description	Ancestry	Sample size*	Derivation of independent genome-wide significant variants
				81 independent genome-wide significant ($p < 5x10^{-8}$) variants associated with TBBMD were identified in the meta-analysis with European-specific cohort.
LSBMD and FNBMD (36) (Secondary analyses)	A GWAS meta-analysis of DXA-derived LSBMD and FNBMD, with the discovery stage performed in 17 GWAS of populations across North America, Europe, East Asia and Australia, which were part of GEnetic Factors for OSteoporosis consortium (GEFOS). In the follow-up replication stage, de-novo genotyping of 96 independent genome-wide significant SNPs obtained from the discovery cohorts were performed in 50,933 additional participants from 34 cohorts. A meta-analysis of these 96 SNPs comprising the discovery and replication cohorts were also conducted. The full set of summary statistics were only publicly available for the discovery cohort.	Predominantly European (~ 70%)	32,961 (Discovery cohort)	At the discovery stage, 96 SNPs with the strongest association reaching genome-wide significance ($p < 5x10^{-8}$) were selected for replication. Out of the 96 replicated SNPs, 64 remained genome-wide significant in the meta-analysis of discovery and replication cohorts. The SNPs were regarded as independent if they were separated by ≥1Mb from the top signal. There were 48 and 49 independent genome-wide significant SNPs associated with LSBMD and FNBMD respectively.
Fracture (37) (Secondary analyses)	A GWAS meta-analysis of fracture. The discovery stage comprised 23 cohorts recruited globally through the GEFOS, which were predominantly of European descent and from Europe, North America, Australia, and east Asia. Two additional GWAS from UK Biobank and EPIC Norfolk study were included as an extended discovery dataset. Selected variants were replicated in 147,200 cases and 150,085 controls from 23andMe. The full set of summary statistics were only publicly available online for the discovery data, but not the meta-analysis of the discovery and replication data.	Predominantly European (~80%)	264,973 (case: 37,857; control: 227,116; Discovery cohort)	A total of 15 variants from 11 chromosomes achieved genome-wide significance ($p < 5x10^{-8}$) in the meta-analysis of discovery and replication cohorts. For the variants on the same chromosome, they were separated by ≥9Mb, and were considered independent.

* The sample size refers to the cohort from which the full set of summary statistics were obtained from.

Table 2 Mendelian randomization results showing significant causal relationship of (a) immature red blood cell traits; (b) mature red blood cell traits; and (c) white blood cell traits with eBMD

(a)

					MR-Egger intercept test		Measurement for overlapping sample	
Methods	Estimate	95% CI	p-value	q-value	Estimate	p-value	Bias	Type I Error
High Light Scatter Reticulocyte Count								
IVW	0.024	0.013 - 0.035	2.16×10^{-05}	6.03×10^{-05}				
Weighted Median	0.019	0.006 - 0.031	0.004					
MR-Egger	0.014	-0.004 - 0.033	0.127		4.26×10^{-04}	0.191	0.001	0.05
Contamination Mixture	0.04	0.03 - 0.05	3.72×10^{-07}					
High Light Scatter Reticulocyte Percentage								
IVW	0.022	0.011 - 0.034	9.67×10^{-05}	2.03×10^{-04}				
Weighted Median	0.020	0.007 - 0.033	0.003					
MR-Egger	0.016	-0.003 - 0.034	0.097		2.99×10^{-04}	0.366	0.001	0.05
Contamination Mixture	0.04	0.03 - 0.05	2.15×10^{-07}					
Reticulocyte Count								
IVW	0.031	0.019 - 0.042	8.48×10^{-08}	4.74×10^{-07}				
Weighted Median	0.027	0.015 - 0.040	2.01×10^{-05}					
MR-Egger	0.023	0.005 - 0.041	0.013		3.25×10^{-04}	0.315	0.001	0.05
Contamination Mixture	0.04	0.03 - 0.05	5.29×10^{-10}					
Reticulocyte Percentage								
IVW	0.029	0.018 - 0.040	2.37×10^{-07}	9.93×10^{-07}				
Weighted Median	0.023	0.011 - 0.035	2.78×10^{-04}					
MR-Egger	0.020	0.002 - 0.038	0.027		4.01×10^{-04}	0.221	0.001	0.05
Contamination Mixture	0.04	0.03 - 0.04	6.35×10^{-10}					

(b)

Methods	Estimate	95% CI	p-value	q-value	MR-Egger intercept test		Measurement for overlapping sample	
					Estimate	p-value	Bias	Type I Error
Haematocrit								
IVW	0.046	0.031 - 0.062	6.48×10^{-09}	5.43×10^{-08}				
Weighted median	0.038	0.020 - 0.055	4.06×10^{-05}				0.001	0.05
MR-Egger	0.022	-0.008 - 0.052	0.153		7.41×10^{-04}	0.061		
Contamination Mixture	0.04	0.02 - 0.05	0.008					
Haemoglobin								
IVW	0.057	0.041 - 0.073	7.81×10^{-13}	1.31×10^{-11}				
Weighted median	0.055	0.037 - 0.073	1.66×10^{-09}				0.001	0.05
MR-Egger	0.038	0.009 - 0.067	0.011		6.07×10^{-04}	0.121		
Contamination Mixture	0.04	0.03 - 0.05	5.91×10^{-04}					
Mean Corpuscular Haemoglobin								
IVW	0.009	0.001 - 0.018	0.032	0.042				
Weighted median	0.017	0.007 - 0.028	0.001				0.001	0.05
MR-Egger	0.018	0.005 - 0.031	0.006		-4.95×10^{-04}	0.071		
Contamination Mixture	0.02	0.02 - 0.02	2.79×10^{-06}					
Mean Corpuscular Haemoglobin Concentration								
IVW	0.036	0.020 - 0.051	5.03×10^{-06}	1.68×10^{-05}				
Weighted median	0.041	0.023 - 0.059	8.38×10^{-06}				0.001	0.05
MR-Egger	0.053	0.027 - 0.080	8.62×10^{-05}		-7.44×10^{-04}	0.114		
Contamination Mixture	0.05	0.04 - 0.06	4.37×10^{-09}					

(c)

Methods	Estimate	95% CI	p-value	q-value	MR-Egger intercept test		Measurement for overlapping sample	
					Estimate	p-value	Bias	Type I Error
Lymphocyte Count								
IVW	-0.020	-0.033 - -0.007	0.002	0.004				
Weighted median	-0.025	-0.041 - -0.010	0.001					
MR-Egger	-0.021	-0.047 - 0.004	0.098		$3.26x10^{-05}$	0.929	0.001	0.05
Contamination Mixture	-0.04	-0.05 - -0.03	$2.60x10^{-04}$					
Neutrophil Count								
IVW	-0.020	-0.035 - -0.006	0.006	0.010				
Weighted median	-0.033	-0.050 - -0.015	$2.74x10^{-04}$					
MR-Egger	-0.016	-0.043 - 0.011	0.253		$-1.46x10^{-04}$	0.703	0.001	0.05
Contamination Mixture	-0.03	-0.04 - -0.02	$4.80x10^{-05}$					
White Blood Cell Count								
IVW	-0.027	-0.039 - -0.014	$4. 50x10^{-05}$	$1.08x10^{-04}$				
Weighted median	-0.039	-0.054 - -0.024	$4.88x10^{-07}$					
MR-Egger	-0.033	-0.057 - -0.009	0.007		$1.97x10^{-04}$	0.555	0.001	0.05
Contamination Mixture	-0.05	-0.06 - -0.04	$6.47x10^{-10}$					

Table 3 Multivariate Mendelian randomization results of blood traits with eBMD

Exposure	Methods	Estimate	95% CI	p-value	MR-Egger intercept test Estimate	MR-Egger intercept test p-value
Immature Red Blood Cell Trait						
Reticulocyte Count						
	IVW	0.040	0.016 - 0.063	0.001		
	MR-Egger	0.035	0.008 - 0.062	0.010	0.000	0.477
Mature Red Blood Cell Traits						
Haemoglobin						
	IVW	0.058	0.021 - 0.094	0.002		
	MR-Egger	0.037	-0.006 - 0.081	0.093	0.001	0.090
Mean Corpuscular Haemoglobin						
	IVW	0.004	-0.007 - 0.016	0.450		
	MR-Egger	0.012	-0.003 - 0.027	0.112	0.000	0.115
Mean Corpuscular Haemoglobin Concentration						
	IVW	0.030	0.007 - 0.054	0.011		
	MR-Egger	0.049	0.014 - 0.085	0.006	-0.001	0.163
White Blood Cell Traits						
Lymphocyte Count						
	IVW	-0.021	-0.045 - 0.003	0.087		
	MR-Egger	-0.018	-0.049 - 0.013	0.263	0.000	0.775
White Blood Cell Count						
	IVW	-0.017	-0.043 - 0.009	0.197		
	MR-Egger	-0.027	-0.060 - 0.007	0.120	0.000	0.379

Figure legend

Figure 1 Assumptions in Mendelian randomization analysis and study design.

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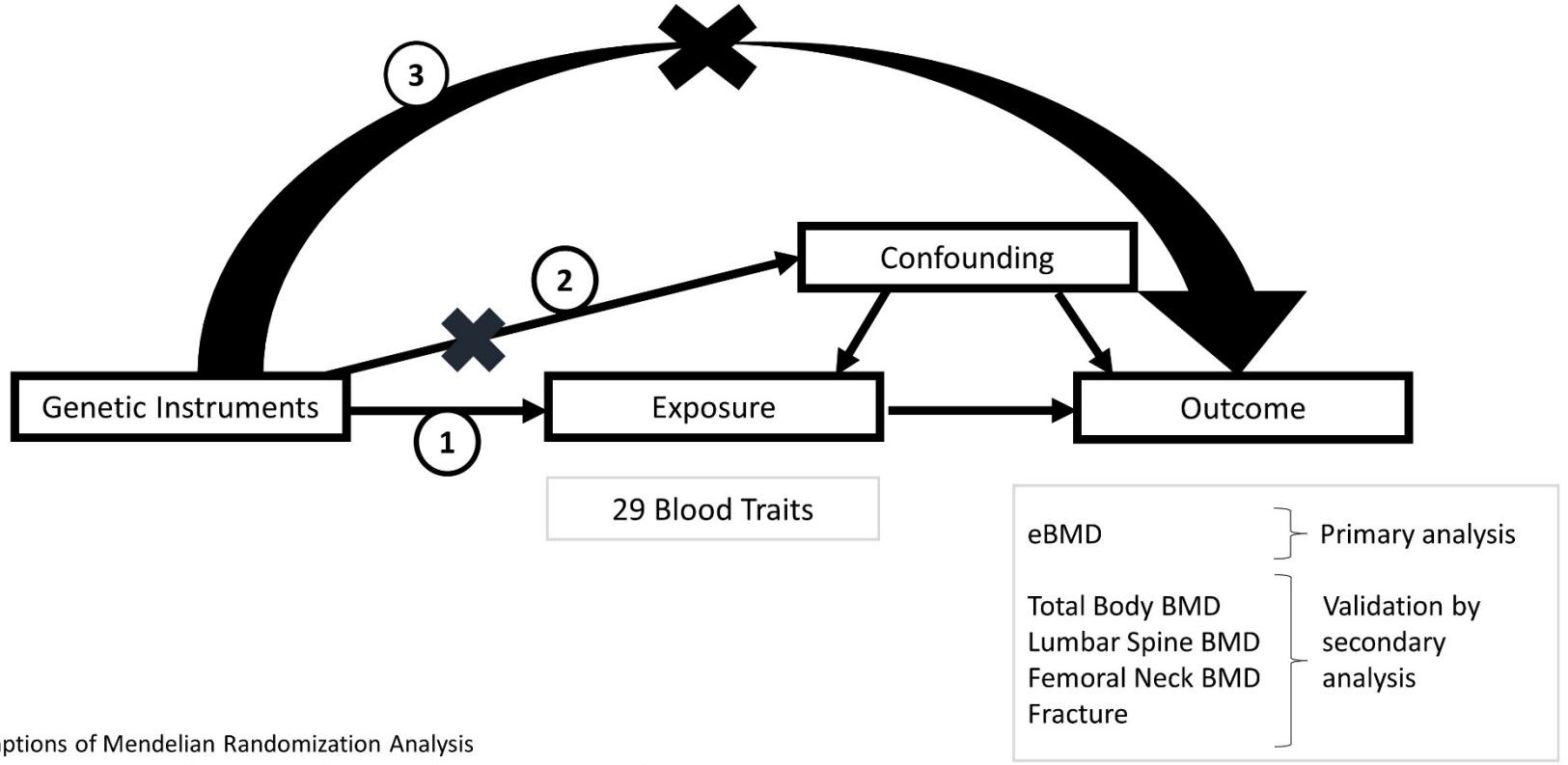

Assumptions of Mendelian Randomization Analysis

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 restriction: Genetic instruments are assumed to affect the outcome only via the exposure