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Mendelian randomization in the bone field

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Abstract

Identification of causative risk factors amenable for modification is essential for the prevention and treatment of osteoporosis. Observational studies have identified associations between several potentially modifiable risk factors and osteoporosis. However, observational studies are susceptible to confounding, reverse causation bias, and measurement error, all of which limit their ability to provide causal estimates of the effect of exposures on outcomes, thereby reducing their ability to inform prevention and treatment strategies against bone loss and fractures. In addition, not all risk factors are suitable for an analysis in a randomized clinical trial. Mendelian randomization is a genetic epidemiological method that exploits genetic variants as unbiased proxies for modifiable exposures (e.g., biomarkers, adiposity measures, dietary factors, and behaviors) to determine the causal relationships between exposures and health outcomes. This technique has been used to provide evidence of causal associations of serum estradiol concentrations, smoking, body mass index, and type 2 diabetes with bone mineral density and the lack of associations of serum thyroid stimulating hormone, urate, C-reactive protein, and 25-hydroxyvitamin D concentrations with bone mineral density in generally healthy populations. This review will briefly explain the concept of Mendelian randomization, the advantages and potential limitations of this study design, and give examples of how Mendelian randomization has been used to investigate questions relevant to osteoporosis.

Keywords

Bone mineral density; Fracture; Genome-wide association studies; Mendelian randomization; Osteoporosis; Single nucleotide polymorphisms

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Disclosure

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1 Introduction

Osteoporosis is a multifactorial disorder influenced by genetic predisposition, age, sex, and modifiable exposures [1]. Identification of causative risk factors amenable for modification is essential for the prevention and treatment of osteoporosis. Observational epidemiological studies have identified associations of several potentially modifiable risk factors such as sex-steroid deficiency, low body mass index (BMI), physical inactivity, smoking, heavy alcohol consumption, and low calcium, vitamin D, and antioxidant intake with bone mineral density (BMD) and fracture risk [1]. However, observational studies are susceptible to various biases such as confounding (where the association between the risk factor and outcome is driven by the association with another correlated risk factor) and reverse causality (where disease affects the risk factor, and not vice versa), thereby reducing their ability to inform prevention and treatment strategies against bone loss and fractures. Furthermore, regression dilution bias due to measurement error limits their ability to detect associations. Well-conducted randomized controlled trials (RCTs) are the gold standard for inferring causality but RCTs are expensive, resource-intensive, examine mainly short-term exposures (normally < 5 years), and may not be feasible to conduct.

An alternative approach is to use genetic variants as unbiased proxies of the modifiable exposure of interest to allow estimation of the long-term causal effect of the exposure on the outcome, a technique known as Mendelian randomization [2–6]. In this review we will briefly explain the concept of Mendelian randomization, discuss the advantages and limitations of this method, and provide examples of how Mendelian randomization has been used to evaluate questions relevant to osteoporosis.

2 Underlying principles and assumptions

Mendelian randomization is a genetic epidemiologic method that utilizes one or multiple genetic variants, typically single nucleotide polymorphisms (SNPs), robustly associated with the modifiable exposure (e.g., a biomarker, adiposity measure, dietary factor, or behavior) to estimate the causal relationship between the exposure and the health outcome (e.g., a disease, BMD, or fracture risk) [2]. The basis of Mendelian randomization is that if a genetic variant influences the exposure or imitates its biological effects, then the genetic variant should be associated with the outcome to the extent predicted by its association with the exposure and the impact of the exposure on the outcome. Mendelian randomization builds on Mendel's second law or the law of independent assortment, that is, each pair of alleles segregates independently of the other pairs of alleles during gamete formation [8]. This natural randomization processes implies that a genetic variant affecting the level of a specific modifiable exposure will generally be unrelated to other traits (exposures), such as other biomarkers, dietary and lifestyle factors, and socioeconomic status. The random allocation of alleles during conception reduces potential confounding in Mendelian randomization studies in a similar way as in an RCT in which participants are randomly assigned to an exposure group (Fig. 1).

In order to obtain valid causal estimates in a Mendelian randomization study, the genetic variant (or variants) that serves as a proxy for the exposure must be: (1) reliably associated

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with the exposure, that is, there should be strong evidence that the genetic variant affects the exposure; (2) unrelated to confounding factors of the exposure-outcome relationship; and (3) associated with the outcome solely through the exposure and not through any other causal pathway (Fig. 2). The first assumption can be tested directly, whereas the other two can be tested but not firmly established.

3 Advantages of the Mendelian randomization design

Mendelian randomization studies have several advantages over conventional observational epidemiological studies. First, confounding is mitigated due to the Mendel's second law of random assortment of alleles. Second, reverse causality is prevented because genetic variants are fixed at conception and cannot be affected by disease processes. This further mitigates against confounding, as genetic variants cannot be influenced by confounding factors that operate after conception. Third, regression dilution bias due to measurement error is avoided because genetic variants are measured with high precision. A further advantage is that genetic differences in exposure can reflect the effect of lifelong exposure on the outcome. Hence, Mendelian randomization analyses can provide unbiased estimates of exposure-outcome relationships, provided the fundamental assumptions are fulfilled (Fig. 2).

4 Limitations of the Mendelian randomization design

Although Mendelian randomization studies are profoundly less prone to confounding than conventional observational epidemiological studies, confounding of the genotype-outcome relationship can occur due to linkage disequilibrium, population stratification, and pleiotropy.

Linkage disequilibrium is the non-random association of alleles at different genetic loci. Loci that are close on the chromosome tend to be inherited together. Confounding can occur if the genetic variant that is used as proxy for the exposure is in linkage disequilibrium with a gene that directly affects the outcome under study.

Population stratification arises when allele frequencies and exposure or outcome distributions vary substantially between different subgroups of the population and produce an association between the exposure and outcome at the overall population level that is not present in any of the subgroups. This bias can be mitigated by restriction of the analysis to ethnically homogenous groups. For example, the frequency of the genetic variant underlying lactase persistence (i.e., maintenance of lactase expression after weaning) differs widely across populations. Hence, a Mendelian randomization study on milk consumption, using the genetic variant related to lactase persistence as an instrumental variable, could potentially be biased by population stratification if ancestry is not accounted for.

Pleiotropy refers to the phenomenon in which a single locus affects multiple phenotypes [9]. Depending of type, pleiotropy may be unproblematic or lead to confounded estimates. One type of pleiotropy is ‘vertical pleiotropy’ (also known as ‘mediated pleiotropy’ or ‘type II pleiotropy’ [5]), which refers to association with other phenotypes downstream the exposure of interest. MR analyses exploit vertical pleiotropy, as they assume that associations of the genetic variants with the outcome or any other variable are due to the causal pathway

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through the exposure. For example, genetic variants that influence BMI may also be associated with downstream phenotypes (mediators) such as estradiol and glucose concentrations and type 2 diabetes, which are associated with higher BMD [10,11]. These associations are expected and would not invalidate the results from a Mendelian randomization study of the association between BMI and BMD. However, 'horizontal pleiotropy' (also known as 'biological pleiotropy' or 'type I pleiotropy' [5]), which arises when a genetic variant associates with more than one phenotype on separate pathways, can invalidate the results from Mendelian randomization analyses. Whether pleiotropy may be of concern can be explored by assessing the association between the genetic variant(s) and potential confounders. Since not all genetic variants used as proxies for the exposure of interest may fulfill the 'no pleiotropy' assumption, several approaches have been developed to detect and adjust for pleiotropy, such as the MR-Egger [12,13], MR-PRESSO [14], and multivariable MR methods [15,16] (Box 1).

Another shortcoming is that genetic variants generally have small effects on the exposure (i.e., explain only a small proportion of the variance) and therefore large sample sizes are necessary to obtain statistically significant results. The power can be increased by the use of multiple genetic variants (explaining more of the variance in the exposure), which can be combined into a weighted genetic risk score. A further potential limitation is canalization, which refers to compensatory processes during development that can atone for disrupting environmental or genetic forces and mitigate the results. Furthermore, since genetic variants reflect lifelong exposure, the MR approach cannot be used to assess whether an exposure at a certain induction period in the life course is related to the outcome. Finally, Mendelian randomization can only be used if one or more suitable genetic variants associated with the exposure of interest are available.

5 Estimating the causal effect of exposure on outcome

Mendelian randomization studies can make use of summary-level data from genome-wide association studies (GWAS). Publicly available summary-level data for BMD and fracture are provided by the GEnetic Factors for OSteoporosis Consortium (GEFOS) (http://www.gefos.org/). The conventional and easiest way to estimate the causal effect of an exposure on an outcome in a Mendelian randomization study based on summary-level data is the ratio method where the coefficient for the effect of the genetic variant on the outcome is divided by the coefficient for the effect of the genetic variant on the exposure (see example in Fig. 3). This method can be used for a single genetic variant or multiple genetic variants in combination. Other approaches for estimating causal associations in Mendelian randomization studies have been described in detail elsewhere [13,17–21].

6 Mendelian randomization studies in the bone field

Over the past decade, GWAS have made an important contribution to the identification of genetic variants associated with numerous potential risk factors for health-related outcomes. The GWAS results have facilitated the use of Mendelian randomization to evaluate causal relationships between modifiable exposures and outcomes. This has resulted in a growing number of Mendelian randomization studies.

We searched PubMed until August 2018 for Mendelian randomization studies assessing the causal role of potentially modifiable risk factors for osteoporosis. We used the search terms "Mendelian randomization" combined with "bone mineral density", "fracture" or "osteoporosis" and identified several studies of which all but one were performed during the last 2–3 years [10,11,22–31] (Table 1). The Mendelian randomization approach has been used to show that increased serum estradiol concentrations have a causal effect on increasing BMD [10] and that decreased BMD, earlier menopause, and late puberty are associated with increased fracture risk [31]. The use of multiple SNPs in Mendelian randomization analyses has also established that increased BMI are associated with increased BMD in children [25]; that type 2 diabetes and higher fasting glucose concentrations are associated with increased BMD [11] but not with reduced fracture risk in adults [31]; that decreased grip strength is associated with an increased fracture risk [31]; and that smoking is associated with decreased BMD [28]. Furthermore, the Mendelian randomization method has been used to show that genetically higher serum 25−hydroxyvitamin D concentrations are not associated with increased BMD [10,24] or fracture risk [31] in generally healthy populations, and that serum thyroid stimulating hormone [29,31], homocysteine [31], urate [23], and C-reactive protein (an inflammatory marker) [22,26] concentrations are not associated with BMD or fracture risk. A recent Mendelian randomization study showed no association between genetically higher alcohol consumption and BMD [28]. Other Mendelian randomization studies found no association of genetically predicted milk consumption, using an SNP (rs4988235) located upstream from the lactase gene as an instrumental variable, with BMD [27,30] or fracture risk [30,31]. However, the lactase persistence genotype has been found to be associated with higher BMI [27,32] and height [33] and with lower fruit and vegetable consumption [33]. No association has been observed between genetic predisposition to type 1 diabetes, coronary artery disease, rheumatoid disease, and inflammatory bowel disease and risk of fracture [31]. While those diseases are unlikely causally associated with fracture risk, the MR study design cannot be used to assess whether complications or treatment of the diseases influence fracture risk.

Most of the exposures that have been assessed for association with BMD or fracture risk using the MR study design would have been unfeasible to test in RCTs. These include for example the effect of alcohol consumption, smoking, estrogen levels, and higher BMI as these exposures have adverse effects on other health outcomes. Other modifiable exposures that would unlikely to be properly evaluated for associations in future RCTs include long-term intake of calcium (may increase the risk of cardiovascular disease [34,35]), other nutrients and dietary compounds, heavy metals, and coffee (difficult to prevent individuals in the placebo group from consuming coffee) as well as comorbidities, body height and other anthropometric measures.

7 Conclusions

The use of the Mendelian randomization study design to infer causality has been evolving over the last decade. This technique has been used to provide evidence of possible causal associations of serum estradiol concentrations, earlier menopause, late puberty, BMI, grip strength, and smoking with BMD and/or fracture risk and the lack of associations of 25−hydroxyvitamin D, serum thyroid stimulating hormone, homocysteine, urate, and C-

reactive protein concentrations with BMD and/or fracture risk. If used prudently, Mendelian randomization can provide an important contribution to our understanding of the etiology of osteoporosis and can be valuable to prioritize exposures for evaluation in RCTs.

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Box 1

MR methods to explore and adjust for pleiotropy

MR-Egger regression. This method can (1) test for directional pleiotropy, (2) test for a causal effect, and (3) provide an estimate of the causal effect of the exposure on the outcome. While the standard inverse-variance weighted method for Mendelian randomization assumes that all genetic variants fulfill the instrumental variable assumptions, the MR-Egger method can test whether the genetic variants have pleiotropic effects on the outcome that differ on average from zero (directional pleiotropy). Provided that pleiotropic effects of genetic variants are uncorrelated with the associations of genetic variants with the exposure, MR-Egger can consistently estimate the causal effect even when genetic variants are pleiotropic. However, MR-Egger estimates typically have low precision, and can be strongly influenced by outlying genetic variants.

MR-PRESSO (Mendelian randomization pleiotropy residual sum and outlier). This method can (1) identify horizontal pleiotropy, (2) adjust for horizontal pleiotropy via outlier removal, and (3) test the significant differences in the causal estimates before and after removal of outliers. The method is best used to identify inconsistencies between genetic associations for different genetic variants, and removing outlying genetic variants. However, if the method removes several variants, then the user should be suspicious about whether consistency in the remaining variants is really meaningful.

Multivariable MR analysis uses multiple genetic variants associated with two or more measured risk factors to simultaneously estimate the causal effect of each risk factor on the outcome. This approach is similar to the concurrent evaluation of several treatments in a factorial randomized controlled trial. The method is most useful when there are related risk factors with shared genetic predictors, but it is difficult to find specific genetic predictors of each individual trait.

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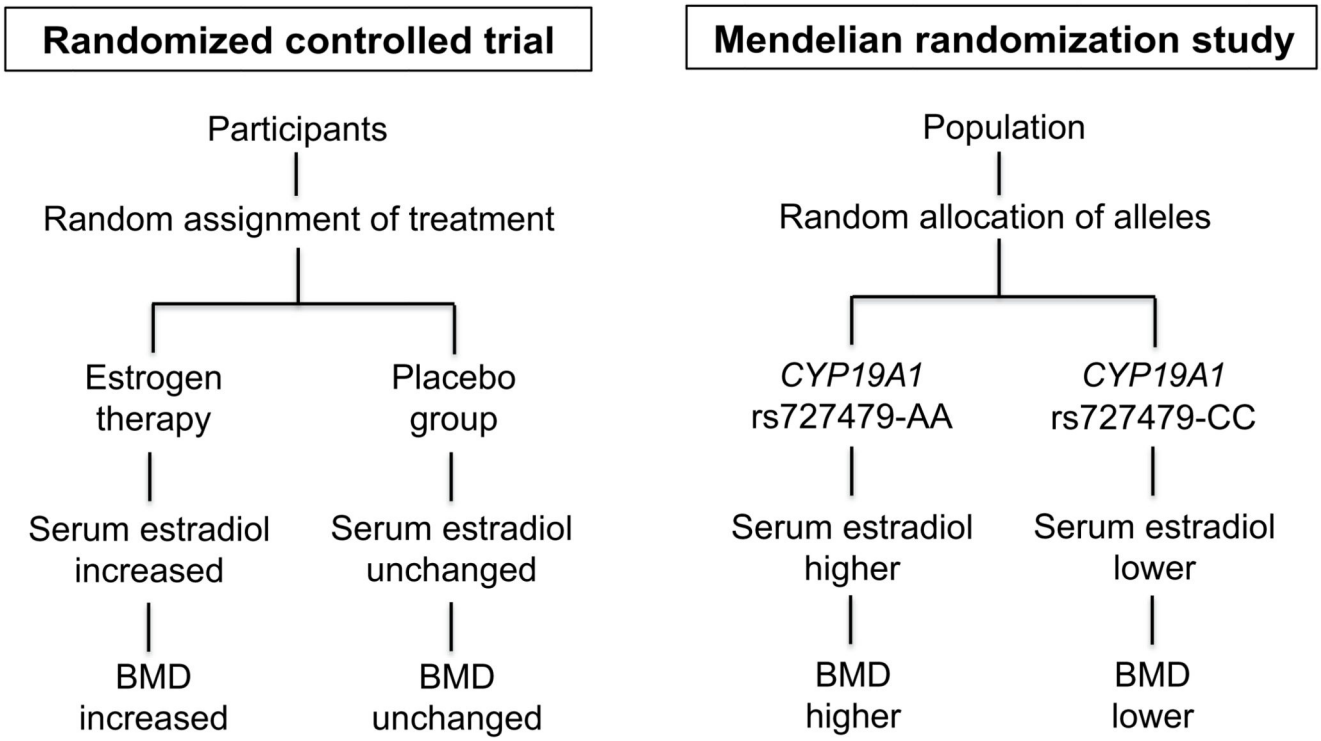

Fig. 1.

Comparison of a randomized controlled trial and a Mendelian randomization study. In a randomized controlled trial, participants are randomly assigned to receive treatment (e.g., estrogen therapy) or placebo, thereby avoiding potential confounding between treatment and other risk factors. A Mendelian randomization study generates a similar scenario. For example, the A allele of rs727479 in the *CYP19A1* gene is associated with higher estradiol concentrations, which are related to higher bone mineral density (BMD). Alleles are inherited largely independently of environmental exposures, and individuals who inherit the A allele of rs727479 are assigned to an average higher estradiol concentration than those who inherit the C allele. As in a randomized controlled trial, groups defined by genotype will experience an average difference in exposure to estradiol but no difference in other exposures (confounding factors). Hence, a Mendelian randomization analysis of genotype is similar to an intention-to-treat analysis in a randomized controlled trial.

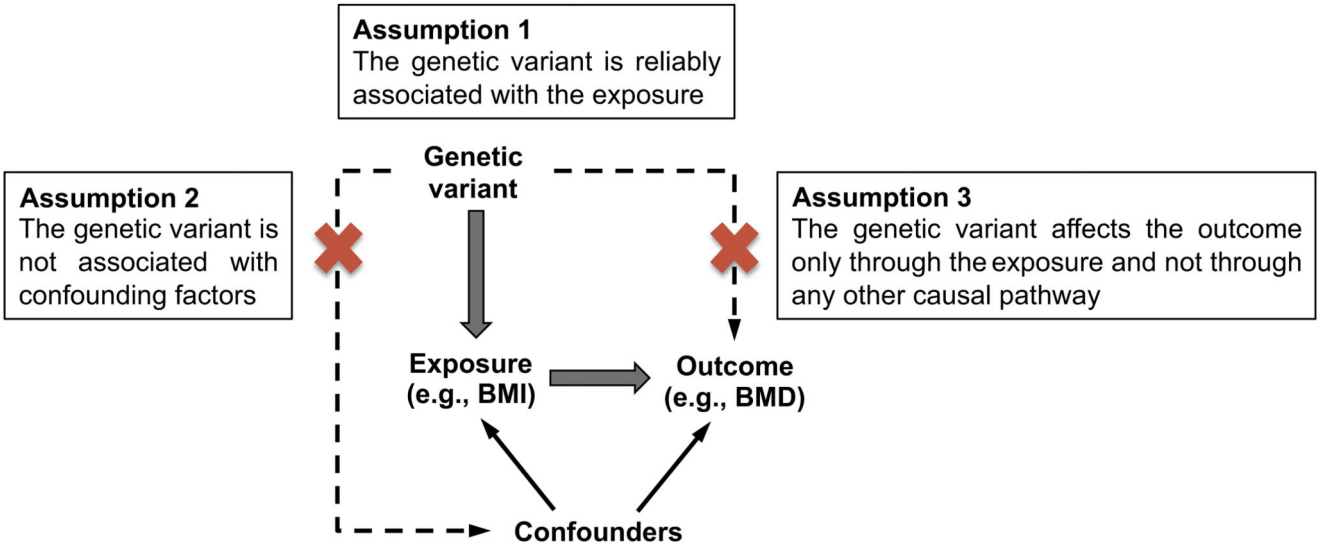

Fig. 2.

Assumptions underlying a Mendelian randomization study. BMD, bone mineral density; BMI, body mass index.

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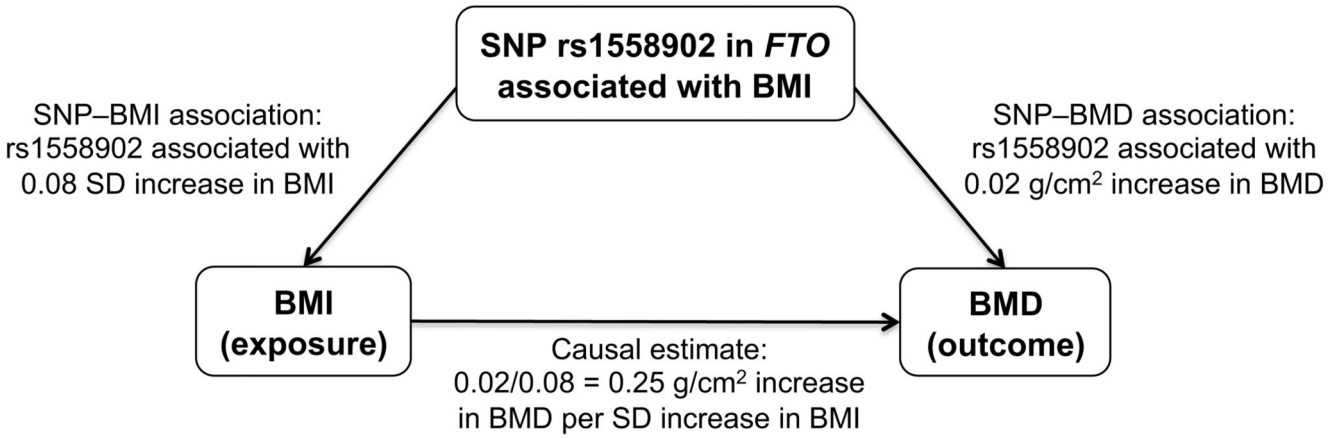

Fig. 3.

Example of a Mendelian randomization study assessing the causal association between body mass index (BMI) and bone mineral density (BMD). In this example, the genetic variant (single-nucleotide polymorphism, SNP) rs1558902 in the *FTO* gene is used as proxy for BMI. The A-allele of rs1558902 is associated with 0.02 g/cm^2 higher BMD ($p = 1.8 \times 10^{-9}$) [36] and 0.08 standard deviation (SD) higher BMI ($p = 7.5 \times 10^{-153}$) in individuals of European ancestry [37]. The causal estimate is derived by dividing the beta coefficient for the SNP–outcome association with the coefficient for the SNP–exposure association: 0.02/0.08 = 0.25, i.e., BMD is increased by 0.25 g/cm^2 per SD increase in genetically predicted BMI.

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Table 1

Examples of Mendelian randomization studies in the bone field.

Exposure	Genetic variants[a]	Outcome	Sample size and data sources for the outcome data	Unit	Estimate (95% CI)	*p*-Value	Interpretation	Ref.
FN-BMD	43	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD decrease in FN-BMD	Fracture: 1.55 (1.48 to 1.63)	< 0.001	Supports a causal association between decreased FN-BMD and increased fracture risk	[31]
LS-BMD	40	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD decrease in LS-BMD	Fracture: 1.43 (1.37 to 1.50)	< 0.001	Supports a causal association between decreased LS-BMD and increased fracture risk	[31]
Earlier menopause	54	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD change, i.e.,3.9 years earlier menopause	Fracture: 1.10 (1.00 to 1.21)	0.05	Supports a causal association between earlier menopause and increased fracture risk	[31]
Late puberty	106	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD change, i.e.,1.4 years late puberty	Fracture: 1.06 (1.00 to 1.13)	0.04	Supports a causal association between late puberty and increased fracture risk	[31]
Estradiol	1	BMD	32,965 individuals of European descent, GEFOS Consortium	SD change in BMD and g/cm^2 in eBMD per 10% increase in estradiol	FN-BMD: 0.038 (NA) LS-BMD: 0.031 (NA) eBMD: 0.030 (NA)	4.6×10^{-6} 0.001 $6.0 \times 10^{\times -18}$	Supports a causal association between serum estradiol levels and increased BMD	[10]
Vitamin D	4	BMD	1824 Chinese postmenopausal women	1 g/cm^2 change in BMD per 1 ln-ng/mL increase in 25OHD	FN-BMD: −0.04 (−0.13 to 0.03) LS-BMD: 0.05 (−0.16 to 0.06) TH-BMD: −0.04 (−0.13 to 0.04)	0.26 0.38 0.33	No support for a causal association of increased serum 25OHD levels with increased BMD	[24]
	5	BMD	32,965 individuals of European descent, GEFOS Consortium and 142,487 individuals from the UK Biobank	SD change in BMD and g/cm^2 change in eBMD per 1 SD increase in 25OHD	FN-BMD: 0.02 (−0.03 to 0.07) LS-BMD: 0.02 (−0.04 to 0.08) eBMD: −0.03 (−0.05 to −0.01)	0.37 0.49 0.02	No support for a causal association of increased serum 25OHD levels with increased BMD	[10]
	4	Fracture	264,973 individuals of predominantly European ancestry, GEFOS and GENOMOS consortia and UK	OR of fracture per 1 SD decrease in 25OHD	Fracture: 0.84 (0.70 to 1.02)	0.07	No support for a causal association of decreased serum 25OHD levels with increased fracture risk	[31]

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Exposure	Genetic variants[a]	Outcome	Sample size and data sources for the outcome data	Unit	Estimate (95% CI)	*p*-Value	Interpretation	Ref.
			Biobank and EPIC-Norfolk study					
TSH	20	BMD	32,735 individuals of European descent, GEFOS Consortium	SD change in BMD per 1 SD decrease in serum TSH	FN-BMD: 0.003 (−0.053 to 0.048) LS-BMD: 0.010 (−0.069 to 0.049)	0.92 0.73	No support for a causal association of serum TSH levels with BMD	[29]
	20	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD decrease in serum TSH levels	Fracture: 0.99 (0.94 to 1.04)	0.78	No support for a causal association of serum TSH levels with fracture risk	[31]
Homocysteine	13	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD increase in homocysteine levels	Fracture: 0.98 (0.92 to 1.05)	0.78	No support for a causal association of homocysteine levels with fracture risk	[31]
Urate	5	BMD	2501 individuals, Framingham Heart Study	1 g/cm^2 change in BMD per 1 mmol/L increase in urate levels	TF-BMD: −0.36 (−0.73 to 0.07) FN-BMD: −0.33 (−0.68 to 0.03) LS-BMD: 0.04 (−0.39 to 0.46)	0.06 0.07 0.87	No support for a causal association of urate levels with BMD	[23]
CRP	20	BMD	32,965 individuals of European descent, GEFOS Consortium	1 g/cm^2 change in BMD per 1 ln-mg/L increase in CRP levels	FA-BMD: −0.02 (NA) FN-BMD: −0.04 (NA) LS-BMD: −0.04 (NA)	0.69 0.22 0.30	No support for a causal association between CRP levels and BMD	[26]
	29	Fracture	6386 individuals, The Rotterdam Study	OR of fracture per 1 SD increase in CRP levels	Fracture: 1.00 (0.99 to 1.00)	0.23	No support for a causal association between CRP levels and fracture risk	[22]
Fasting glucose	30	BMD	32,961 individuals of European descent, GEFOS Consortium	SD change in BMD per 1 mmol/L increase in fasting glucose levels	FN-BMD: 0.13 (0.01 to 0.25) LS-BMD: 0.08 (−0.04 to −0.21)	0.03 0.21	Supports a causal association of increased fasting glucose levels with increased BMD	[11]
	35	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD increase in fasting glucose levels	Fracture: 1.04 (0.97 to 1.12)	0.24	No support for a causal association of fasting glucose levels with fracture risk	[31]
T1D	19	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per doubling in odds of T1D susceptibility	Fracture: 1.00 (1.00 to 1.01)	0.57	No support for a causal association between T1D and fracture risk	[31]
T2D	32	BMD	32,961 individuals of European descent, GEFOS Consortium	SD change in BMD per increase in log-odds of T2D	FN-BMD: 0.034 (0.001 to 0.067) LS-BMD: 0.022 (−0.01 to −0.051)	0.04 0.13	Supports a causal association of T2D with increased BMD	[11]

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Exposure	Genetic variants[a]	Outcome	Sample size and data sources for the outcome data	Unit	Estimate (95% CI)	*p*-Value	Interpretation	Ref.
T2D	38	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per doubling in odds of T2D susceptibility	Fracture: 0.99 (0.99 to 1.01)	0.37	No support for a causal association between T2D and fracture risk	[31]
BMI	32	BMD	5221 children, Avon Longitudinal Study of Parents and Children	SD change in BMD per 1 SD increase in BMI	UL-BMD: 0.46 (0.31 to 0.61) LL-BMD: 0.55 (0.41 to 0.68) SP-BMD: 0.48 (0.33 to 0.63) SK-BMD: −0.02 (−0.20 to 0.15)	< 0.001 < 0.001 < 0.001 0.78	Supports a causal association between increased BMI and increased BMD in children	[25]
Grip strength	15	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD decrease in grip strength	Fracture: 2.14 (1.13 to 4.04)	0.01	Supports a causal association between decreased grip strength and fracture risk	[31]
Milk consumption	1	BMD	32,965 individuals of European descent, GEFOS Consortium	SD change in BMD per 1 SD increase in milk consumption	FA-BMD: 0.05 (−0.13 to 0.23) FN-BMD: 0.02 (−0.09 to 0.06) LS-BMD: 0.02 (−0.07 to 0.10)	NA NA NA	No support for a causal association between milk consumption and BMD	[27]
	1	BMD and fracture	105,256 European-descent individuals	1 g/cm^2 change in BMD and OR of fracture for TT and TC genotypes versus CC genotype	FN-BMD: 0.10 (0.02 to 0.18) TT FN-BMD: 0.06 (−0.04 to 0.17) TC Fracture: 0.86 (0.61 to 1.21) TT Fracture: 0.90 (0.68 to 1.21) TC	NA NA NA NA	No support for a causal association between milk consumption and BMD and fracture risk	[30]
	1	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per 1 SD decrease in milk consumption	Fracture: 1.01 (0.80 to 1.23)	0.94	No support for a causal association between milk consumption and fracture risk	[31]
Alcohol consumption	5–6	BMD	32,735 individuals from the GEFOS Consortium and 445,921 individuals of European descent from the UK Biobank	NA	FN-BMD: −0.008 (NA) LS-BMD: 0.067 (NA) FA-BMD: 0.194 (NA) eBMD: 0.010 (NA)	0.96 0.20 0.36 0.04	No support for a causal association between alcohol consumption and BMD	[28]
Smoking status	139–142	BMD	32,735 individuals from the GEFOS Consortium and 445,921 individuals of European descent from the UK Biobank	NA	FN-BMD: −0.139 (NA) LS-BMD: 0.003 (NA) FA-BMD: 0.264 (NA) eBMD: 0.053 (NA)	0.05 0.98 0.08 0.003	Supports a causal association between smoking and decreased BMD	[28]

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Exposure	Genetic variants[a]	Outcome	Sample size and data sources for the outcome data	Unit	Estimate (95% CI)	*p*-Value	Interpretation	Ref.
Coronary artery disease	38	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per doubling in odds of coronary artery disease susceptibility	Fracture: 1.00 (0.99 to 1.02)	0.76	No support for a causal association between coronary artery disease and fracture risk	[31]
Rheumatoid disease	30	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per doubling in odds of rheumatoid disease susceptibility	Fracture: 1.01 (1.10 to 1.02)	0.14	No support for a causal association between rheumatoid disease and fracture risk	[31]
Inflammatory bowel disease	151	Fracture	264,973 individuals of predominantly European ancestry, GEFOS Consortium, UK Biobank and EPIC-Norfolk study	OR of fracture per doubling in odds of inflammatory bowel disease susceptibility	Fracture: 1.00 (1.10 to 1.01)	0.92	No support for a causal association between inflammatory bowel disease and fracture risk	[31]

Abbreviations: 25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index; CRP, C-reactive protein; eBMD, estimated bone mineral density from ultrasound; EPIC, European Prospective Investigation of Cancer; FA, forearm; FN, femoral neck; GEFOS, Genetic Factors for Osteoporosis; LL, lower limbs; LS, lumbar spine; NA, not available; OR, odds ratio; SD, standard deviation; SK, skull; T1D, type 1 diabetes; T2D, type 2 diabetes; TF, total femur; TH, total hip; TSH, thyroid stimulating hormone; UL, upper limbs bone mineral density.

[a]Number of genetic variants used as instrumental variables in the Mendelian randomization analysis.