


# Evaluation of the Effect of Sodium-Glucose Cotransporter 2 Inhibition on Fracture Risk: Evidence From Mendelian Randomization and Genetic Association Study

Huajie Dai,<sup>1,2</sup> Longyi Zheng,<sup>3</sup> Zheng Zhu,<sup>1,2</sup> Xin Geng,<sup>4</sup> Tianzhichao Hou,<sup>1,2</sup> Qi Wang,<sup>1,2</sup> Yijie Zhu,<sup>1,2</sup> Hong Lin,<sup>1,2</sup> Shuangyuan Wang,<sup>1,2</sup> Ruizhi Zheng,<sup>1,2</sup> Zhiyun Zhao,<sup>1,2</sup> Mian Li,<sup>1,2</sup> Jieli Lu,<sup>1,2</sup> Yu Xu,<sup>1,2</sup> Tiange Wang,<sup>1,2</sup> Jianmin Liu,<sup>1,2</sup>  Guang Ning,<sup>1,2</sup> Weiqing Wang,<sup>1,2</sup> Yufang Bi,<sup>1,2</sup> Jie Zheng,<sup>1,2,5</sup> and Min Xu<sup>1,2</sup> 

<sup>1</sup>Department of Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

<sup>2</sup>Shanghai National Clinical Research Center for Metabolic Diseases, Key Laboratory for Endocrine and Metabolic Diseases of the National Health Commission of the P.R. China, Shanghai Key Laboratory for Endocrine Tumor, State Key Laboratory of Medical Genomics, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

<sup>3</sup>Department of Endocrinology, Changhai Hospital, Naval Medical University, Shanghai, China

<sup>4</sup>National Center for Liver Cancer, International Cooperation Laboratory on Signal Transduction, Third Affiliated Hospital of Naval Medical University, Naval Medical University, Shanghai, China

<sup>5</sup>MRC Integrative Epidemiology Unit, Bristol Medical School, University of Bristol, Bristol, UK

## ABSTRACT

This study aims to evaluate the causal effect of sodium-glucose cotransporter 2 (SGLT2) inhibition on bone mineral density (BMD), osteoporosis, and fracture risk using genetics. Two-sample Mendelian randomization (MR) analyses were performed utilizing two sets of genetic variants as instruments (six and two single-nucleotide polymorphisms [SNPs]) associated with *SLC5A2* gene expression and glycated hemoglobin A1c levels. Summary statistics of BMD from the Genetic Factors for Osteoporosis consortium (BMD for total body,  $n = 66,628$ ; femoral neck,  $n = 32,735$ ; lumbar spine,  $n = 28,498$ ; forearm,  $n = 8143$ ) and osteoporosis (6303 cases, 325,717 controls) and 13 types of fracture ( $\leq 17,690$  cases,  $\leq 328,382$  controls) data from the FinnGen study were obtained. One-sample MR and genetic association analyses were conducted in UK Biobank using the individual-level data of heel BMD ( $n = 256,286$ ) and incident osteoporosis (13,677 cases, 430,262 controls) and fracture (25,806 cases, 407,081 controls). Using six SNPs as the instrument, genetically proxied SGLT2 inhibition showed little evidence of association with BMD of total body, femoral neck, lumbar spine, and forearm (all  $p \geq 0.077$ ). Similar results were observed using two SNPs as instruments. Little evidence was found for the SGLT2 inhibition effect on osteoporosis (all  $p \geq 0.112$ ) or any 11 major types of fracture (all  $p \geq 0.094$ ), except for a nominal significance for fracture of lower leg ( $p = 0.049$ ) and shoulder and upper arm ( $p = 0.029$ ). One-sample MR and genetic association analysis showed that both the weighted genetic risk scores constructed from the six and two SNPs were not causally associated with heel BMD, osteoporosis, and fracture (all  $p \geq 0.387$ ). Therefore, this study does not support an effect of genetically proxied SGLT2 inhibition on fracture risk. © 2023 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

**KEY WORDS:** MENDELIAN RANDOMIZATION ANALYSIS; SGLT2 INHIBITION; BONE MINERAL DENSITY; OSTEOPOROSIS; FRACTURE

## Introduction

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are widely accepted oral hypoglycemic agents in type 2 diabetes patients.<sup>(1)</sup> These drugs have gained popularity in recent years

because of their beneficial impact on glycemic control and cardiovascular and kidney outcomes.<sup>(2-6)</sup> Despite these apparent benefits, the effects of SGLT2 inhibitors on bone mineral metabolism and fracture risk attracted much attention and have been underexplored.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Received in original form May 18, 2023; revised form June 28, 2023; accepted July 6, 2023.

Address correspondence to: Min Xu, MD, PhD, Shanghai National Clinical Research Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China. E-mail: [della.xumin@shsmu.edu.cn](mailto:della.xumin@shsmu.edu.cn)

Additional Supporting Information may be found in the online version of this article.

HD, LZ, ZZhu, and XG are joint first authors.

*Journal of Bone and Mineral Research*, Vol. 38, No. 11, November 2023, pp 1645–1653.

DOI: 10.1002/jbmr.4880

© 2023 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

The previous reports from clinical trials showed controversial findings. In the CANVAS (Canagliflozin Cardiovascular Assessment Study), which included 4330 type 2 diabetes patients, with a median follow-up of 195.9 weeks, the rate of all fractures was higher in canagliflozin treatment (a dose of 300 or 100 mg) than placebo (16.9 versus 10.9 participants with fracture per 1000 patient-years; hazard ratio [HR] = 1.55; 95% confidence interval [CI] 1.21–1.97).<sup>(3)</sup> Canagliflozin has been linked to increased bone turnover markers and decreased hip bone mineral density (BMD) in older patients with type 2 diabetes.<sup>(7)</sup> However, this risk was not observed in other trials with canagliflozin, including the CANVAS-R (Canagliflozin Cardiovascular Assessment Study-Renal) and the CRENCE (Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation).<sup>(4)</sup> Additionally, in trials evaluating the effects of other SGLT2 inhibitors, such as dapagliflozin,<sup>(5,8)</sup> empagliflozin,<sup>(6,9)</sup> and ertugliflozin,<sup>(10)</sup> no significant increased risk of bone fractures was reported. In real-world studies, treatment with SGLT2 inhibitors showed a similar fracture or fall risk compared with other hypoglycemic drugs currently in use, such as dipeptidyl peptidase 4 (DPP-4) inhibitors,<sup>(11–15)</sup> sulphonylurea,<sup>(16)</sup> and glucagon-like peptide 1 (GLP-1) receptor agonists.<sup>(17–19)</sup>

Although meta-analyses of randomized controlled trials (RCTs) and observational studies have also shown that the overall risk was not increased in SGLT2 inhibitor users,<sup>(20–25)</sup> these results do not eliminate concerns about the potential increased risk of fractures caused by SGLT2 inhibitors in clinical practice. The findings from the observational studies may be limited by confounding factors and not long enough follow-up duration. RCTs are crucial in establishing a causal relationship between SGLT2 inhibitors and bone health outcomes. However, conducting RCTs to investigate the long-term effects of drugs on outcomes with low incidence rate could be challenging and costly.

Therefore, more evidence is required to evaluate whether a causal relationship between SGLT2 inhibitors and bone fracture exists. Mendelian randomization (MR) is an alternative approach to overcoming these challenges.<sup>(26)</sup> MR is a genetic epidemiological method that utilizes genetic variants as instrumental variables to investigate the causal relationship between exposure and outcome. By using genetic variants as proxies for the exposure of interest, MR can provide unbiased estimates of causality and overcome some of the limitations of traditional observational studies. Drug-target MR is an extension of MR using genetic variants that simulate the pharmacological inhibition of drug-target genes.<sup>(27)</sup> New methods, such as genetic colocalization<sup>(28)</sup> and expression quantitative trait loci (eQTLs) (genetic variants associated with drug target mRNA expression),<sup>(29–31)</sup> can be used to create reliable MR instruments for drug exposure.

In the present analysis, we aim to investigate the causal relationship between genetically proxied SGLT2 inhibition and bone health-related outcomes, including BMD, osteoporosis, and bone fractures using two-sample drug-target MR. We also conducted the one-sample MR and genetic association analyses using the individual-level data in UK Biobank as a validation. The findings from this study may provide reliable evidence of the long-term effects of SGLT2 inhibition on bone health, which could inform clinical decision-making.

## Materials and Methods

### Study design

Figure 1 illustrates the design of this study. We aimed to evaluate the causal role of SGLT2 inhibition on bone health using a

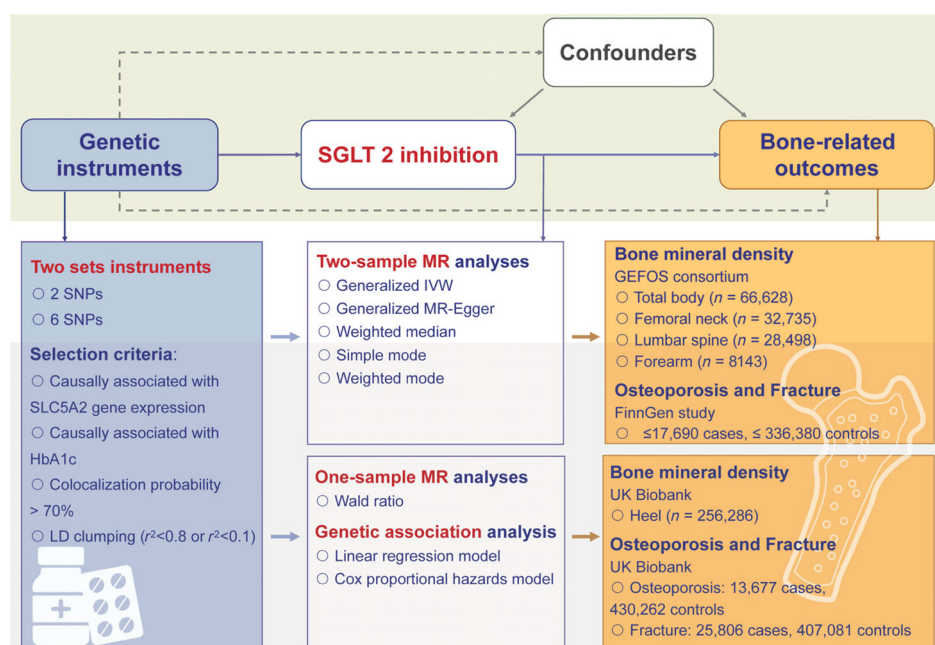
two-sample drug-target MR approach and one-sample MR together with the genetic association analysis. First, we selected two sets of genetic variants that were proxies of the effect of SGLT2 inhibition. Three bone health-related outcomes, including BMD, osteoporosis, and bone fracture, were then selected. The generalized inverse variance-weighted (IVW) approach was used as the primary method, with several sensitivity analyses, including generalized MR-Egger, weighted median, simple mode, and weighted mode method, conducted to validate the results. Second, we performed the one-sample MR and genetic association analyses in participants with heel BMD or incident cases of osteoporosis and fractures in the UK Biobank to validate the findings in the two-sample MR.

### Selection and validation for genetic predictors of SGLT2 inhibition

To generate genetic instruments that can proxy the lifelong glucose-lowering effect of SGLT2 inhibition, we used a four-step instrument selection pipeline that has been previously described.<sup>(31,32)</sup> First, genetic variants within a  $\pm 250$ Kb window of the *SLC5A2* gene (the target gene for SGLT2 inhibition) were selected and associated with the mRNA expression level of the *SLC5A2* gene in one or more of the 50 tested tissues ( $p < 0.001$ , a significance level in a genomic region) using data from Genotype-Tissue Expression (GTEx,  $n \leq 706$ )<sup>(33)</sup> and eQTLGen consortium (whole blood,  $n \leq 31,684$ ).<sup>(34)</sup> Second, the association of each *SLC5A2* variant with HbA1c level was estimated as an indicator of glucose-lowering effect via SGLT2 inhibition, which was used as proxies in some previous anti-diabetic drug target MR studies,<sup>(35,36)</sup> and only variants that show regional-wide association with HbA1c ( $p$  value  $< 1 \times 10^{-4}$ ) were selected using data from a subgroup of unrelated individuals of European ancestry without diabetes in the UK Biobank ( $n = 344,182$ ). Third, genetic colocalization was used to validate whether the expression of *SLC5A2* and HbA1c share the same causal variant in the *SLC5A2* region (a genetic colocalization probability  $> 0.7$  was used as the evidence of colocalization).<sup>(28,37)</sup> Finally, a standard clumping process was conducted using correlation among variants  $< 0.8$  as the threshold to remove variants with very high correlation and remained six single-nucleotide polymorphisms (SNP), or  $< 0.1$  permitting the variants to be in weak linkage disequilibrium (LD), which is a commonly used pairwise squared correlation between nearby variants for drug target MR,<sup>(38)</sup> and remained two SNPs. After these selection steps, two sets of genetic variants were selected as instruments of SGLT2 inhibition (Supplemental Table S1).

### Study outcomes

The primary outcomes include BMD, osteoporosis, and fractures. For the following MR analysis, we leveraged the summary statistics derived from relevant genomewide association studies (GWAS) for these outcomes. The summary data of BMD were obtained from Genetic Factors for Osteoporosis (GEFOS) consortium (BMD for total body,  $n = 66,628$ ; femoral neck,  $n = 32,735$ ; lumbar spine,  $n = 28,498$ ; forearm,  $n = 8,143$ ).<sup>(39,40)</sup> The summary data of osteoporosis (6303 cases, 325,717 controls) and 13 types of fracture, including osteoporosis with pathological fracture (1433 cases), fracture of lower leg (17,690 cases), forearm (17,201 cases), shoulder and upper arm (10,273 cases), wrist and hand level (9950 cases), rib, sternum, and thoracic spine (7845 cases), femur (7671 cases), foot (6548 cases), skull and facial



**Fig. 1.** Study design. We conducted two-sample Mendelian randomization (MR) analyses of the effect of SGLT2 inhibition on bone health-related outcomes. Two sets of genetic variants were selected as proxies of the effect of SGLT2 inhibition. Bone health-related outcomes, including bone mineral density, osteoporosis, and bone fracture, were selected as outcomes. Summary data of exposure and outcomes were obtained from related meta-analyses of genomewide association analyses. The generalized inverse variance-weighted approach was applied as the primary method to estimate the causal effect on selected outcomes. Several sensitivity analyses were conducted. We also conducted one-sample MR and genetic association analyses using the UK Biobank individual-level data to validate our results in two-sample MR. BMD = bone mineral density; GEFOS = Genetic Factors for Osteoporosis; IVW = inverse variance weighted; LD = linkage disequilibrium; SGLT2 = sodium-glucose cotransporter 2; SNP = single-nucleotide polymorphism.

bones (6051 cases), lumbar spine and pelvis (5364 cases), and neck (1288 cases), were obtained from the FinnGen study<sup>(41)</sup> (Supplemental Table S2).

## Statistical analysis

### MR analyses of SGLT2 inhibition and BMD, osteoporosis, and fracture risk

The summary data of the genetic associations of the six- or two-instrument SNPs were looked up and extracted from the GWAS for the respective outcome and harmonized to ensure that the effect of an SNP on the exposure and outcome corresponding to the same allele before conducting causal estimation. We used the generalized inverse variance weighted (IVW) method<sup>(42)</sup> to increase the power of the analysis. This method takes into account the correlation between the two or six genetic predictors of SGLT2 inhibition, which allowed for a relaxed clumping threshold. An LD matrix was obtained for each pair of variants in the 1000 Genomes data set, and the IVW method was used to estimate the MR effect while considering the LD matrix of genetic variants. All MR estimates were scaled to standard deviation (SD) unit to reflect the equivalent of a one SD unit (0.62%) reduction in HbA1c. We calculated the statistical power of our analyses by utilizing an online tool (<https://shiny.cnsgenomics.com/mRnd/>). The findings indicate that our analyses possess more than 80% power when the  $\beta$  value for SGLT2 inhibition on BMD surpasses  $-0.25$  or the odds ratio on osteoporosis or fracture exceeds 1.5, except for the fracture at neck because of the small sample size of cases.

### Validation of MR assumptions and sensitivity analyses

Reports of the study findings were according to the STROBE-MR (Strengthening the Reporting of Mendelian Randomization Studies) guidelines.<sup>(43)</sup> The three critical key assumptions of MR were tested through a set of sensitivity analyses. The relevance assumption was validated by estimating the strength of the genetic predictors using  $R^2$  and  $F$ -statistics.  $F$ -statistics above 10 was considered as the evidence against weak instrument bias. The exclusion restriction assumption was tested using several sensitivity analyses, including MR-Egger regression, weighted median analysis, and simple and weighted mode analyses. Cochran's  $Q$  test was used to estimate the heterogeneity between instruments.

### One-sample MR and genetic association analysis in UK Biobank

To corroborate results in two-sample MR, we conducted one-sample MR and genetic association analysis using individual-level data from UK Biobank. Weighted genetic risk scores (wtGRS) for six and two SNPs were constructed respectively for each participant in UK Biobank weighted by their effect size ( $\beta$ ) for HbA1c. We first conducted one-sample MR analyses using wtGRS of SGLT2 inhibition. We then performed genetic association analysis of wtGRS with heel BMD at baseline ( $n = 256,286$ ). Heel BMD is assessed using ultrasound measurements of the calcaneus. The estimation of BMD is based on the Quantitative Ultrasound Index, which is derived by combining measurements of the speed of sound and broadband ultrasound attenuation (<https://biobank.ndph.ox.ac.uk/showcase/refer.cgi?id=100248>).

We also performed genetic association analysis of wtGRS with the risk of incident osteoporosis or fracture (13,677 osteoporosis and 25,806 fracture cases followed up for an average of 11.8 years). In detail, after excluding participants with osteoporosis or fracture at baseline, the remaining participants were followed up and new-onset osteoporosis or fractures were recorded from electronic medical records. Osteoporosis and fracture were defined based on the occurrence of one or more International Classification of Disease, 10th Revision (ICD10) codes (Supplemental Table S3). Cox proportional hazard models were used to evaluate the associations between wtGRS and the risk of incident osteoporosis or fracture. The models adjusted for age, sex, genotype measurement batch, assessment center, and the top 10 genetic principal components. We further conducted stratification analyses according to age ( $\geq 60$  versus  $<60$  years), sex, type 2 diabetes, and chronic kidney disease status.

All the analyses were performed on the SAS (version 9.4, SAS Institute, Cary, NC, USA) and R platform (version 4.2.1). The “TwoSampleMR,” “Mendelian Randomization” packages were used for statistical analyses.

For all analyses, Bonferroni corrections were applied to establish multiple testing-adjusted thresholds, which are 0.013 (0.05/4) in the analysis of BMD and 0.004 (0.05/14) in the analysis of osteoporosis and fracture.

The study protocol was approved by the Institutional Review Board of Ruijin Hospital affiliated to the Shanghai Jiao Tong University School of Medicine and the UK Biobank scientific committee (project number 64754). UK Biobank study obtained informed consent from the study participants and approval from its institutional review board.

## Results

### Strength of the genetic predictors for SGLT2 inhibition

The characteristics of two sets of instruments (two variants: rs9930811 and rs35445454; six variants: rs4488457, rs8057326,

rs11865835, rs9930811, rs34497199, and rs35445454) used to proxy pharmacological targets of SGLT2 are listed in Supplemental Table S1. Both sets of instruments showed strong strength  $F$ -statistics = 34.0 or 24.1, which were over the common threshold of 10, suggesting that weak instrument bias is unlikely to be an issue in this study.

### Effect of genetically proxied SGLT2 inhibition on BMD

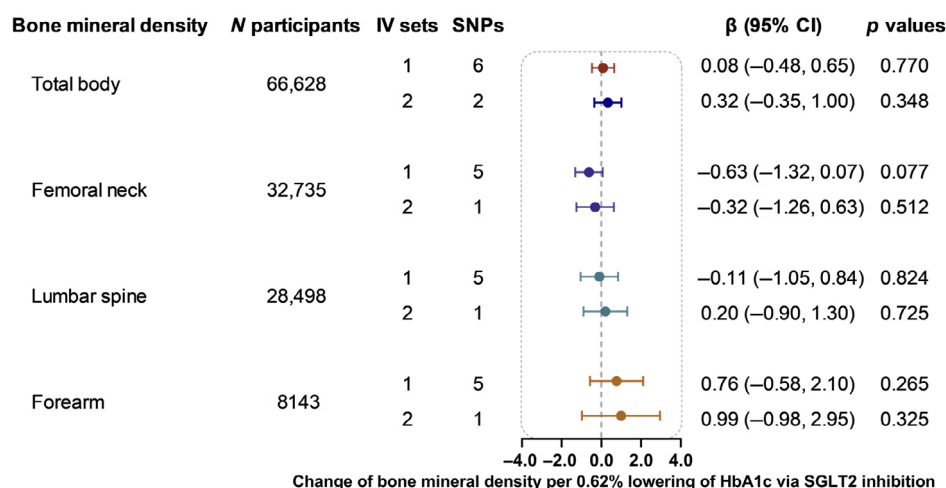
Genetically proxied SGLT2 inhibition using six SNPs as the instrument showed little evidence of association with BMD of total body ( $\beta$  0.08, 95% CI  $-0.48$ – $0.65$ ,  $p = 0.770$ ), femoral neck ( $\beta$   $-0.63$ , 95% CI  $-1.32$ ,  $0.07$ ,  $p = 0.077$ ), lumbar spine ( $\beta$   $-0.11$ , 95% CI  $-1.05$ ,  $0.84$ ,  $p = 0.824$ ), and forearm ( $\beta$   $0.76$ , 95% CI  $-0.58$ ,  $2.10$ ,  $p = 0.265$ ) (Fig. 2). The analyses using two SNPs as the instrument yield similar results (Fig. 2). The heterogeneity test using the Cochran  $Q$  test for IVW and the pleiotropy test using the MR-Egger intercept term implied little evidence of existence of heterogeneity or directional pleiotropy (Supplemental Table S4).

The sensitivity analyses, including MR-Egger, weighted median, and simple and weighted mode analyses, also showed little evidence of the association between genetically proxied SGLT2 inhibition and BMD (Supplemental Table S4).

### Effect of genetically proxied SGLT2 inhibition on osteoporosis and fractures

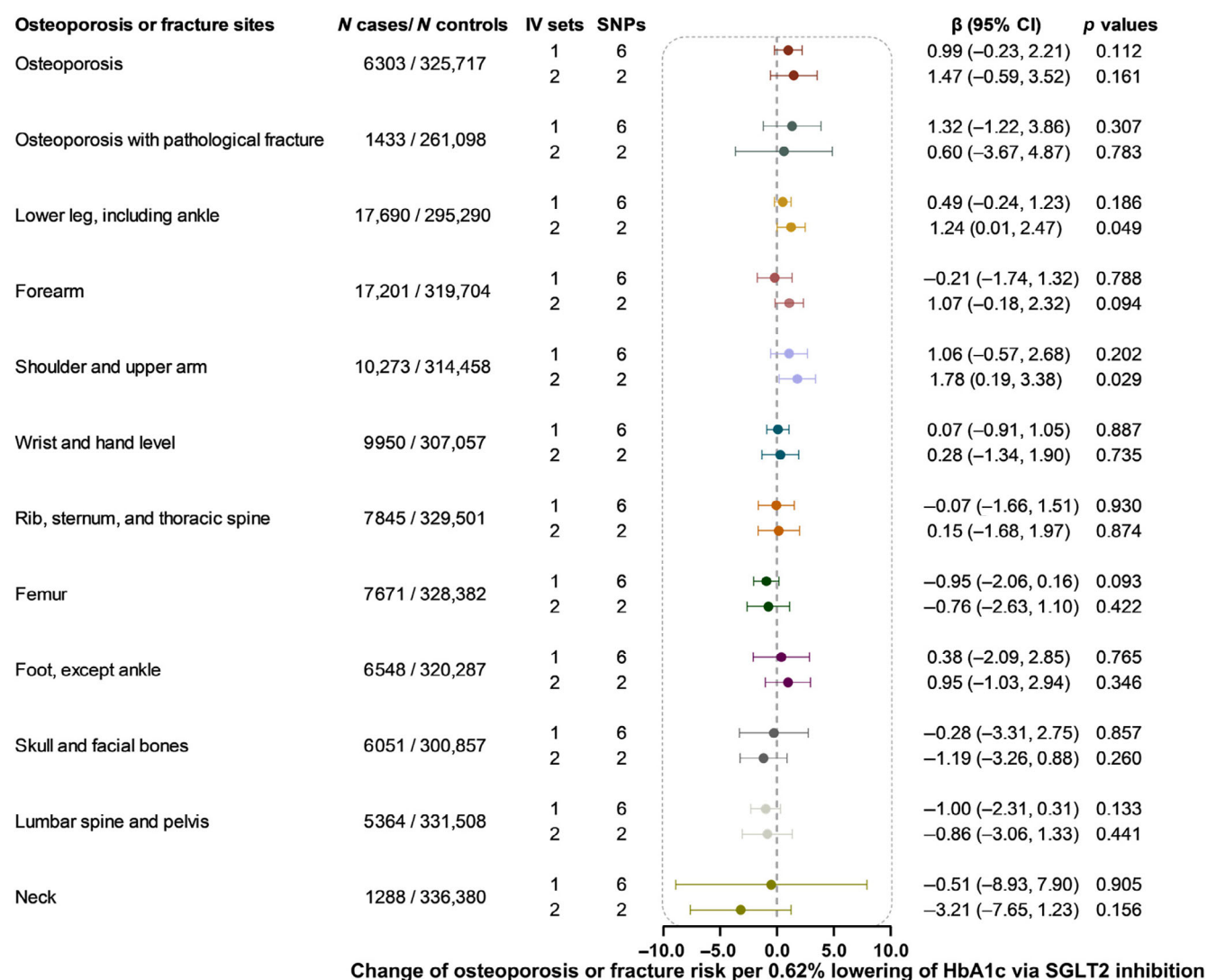
Using six SNPs as instrumental variables, we did not detect the causal effect of genetically proxied SGLT2 inhibition on osteoporosis or any of the 13 fracture subtypes (Fig. 3). Among the 14 outcomes examined using two SNPs as instruments, genetically proxied SGLT2 inhibition was merely nominally associated with a higher risk of fracture of lower leg ( $\beta$  1.24, 95% CI  $0.01$ ,  $2.47$ ,  $p = 0.049$ ) and shoulder and upper arm ( $\beta$  1.78, 95% CI  $0.19$ ,  $3.38$ ,  $p = 0.029$ ), but the results did not pass the multiple testing (threshold  $p = 0.004$ ) (Fig. 3).

The heterogeneity test showed no heterogeneity for all analyses except for the analysis of genetically proxied SGLT2



**Fig. 2.** Causal effect of genetically proxied SGLT2 inhibition on bone mineral density. Data are presented as the change of bone mineral density per 0.62% (6.75 mmol/mol) reduction of HbA1c via SGLT2 inhibition estimated by the generalized inverse variance weighted method. CI = confidence interval; IV = instrumental variable; SGLT2 = sodium-glucose cotransporter 2; SNP = single-nucleotide polymorphism.





**Fig. 3.** Causal effect of genetically proxied SGLT2 inhibition on osteoporosis and fracture. Data are presented as the change of osteoporosis or fracture risk per 0.62% (6.75 mmol/mol) reduction of HbA1c via SGLT2 inhibition estimated by generalized inverse variance weighted method. CI = confidence interval; IV = instrumental variable; SGLT2 = sodium-glucose cotransporter 2; SNP = single-nucleotide polymorphism.

inhibition on fracture of forearm and foot (except ankle) ( $p = 0.001$ ). The MR-Egger intercept term test showed little evidence of directional pleiotropy ( $p \geq 0.053$ ) (Supplemental Table S5). In the sensitivity analyses, most results were nonsignificant, except for the effect of genetically proxied SGLT2 inhibition on the fracture of shoulder and upper arm ( $\beta$  1.97, 95% CI 0.67, 3.27,  $p = 0.003$  in the weighted median method).

#### One-sample MR and genetic association analysis in UK Biobank

The wtGRS of SGLT2 inhibition constructed from six or two SNPs was not significantly associated with heel BMD either for the one-sample MR or the association analysis (Table 1). After a mean of 11.8 years of follow-up, 13,677 osteoporosis and 25,806 fracture cases were recorded. No significant associations between the wtGRSs and osteoporosis or fracture risk were found (Table 1).

We further stratified the participants according to age (<60 or  $\geq 60$  years), sex, and the presence of type 2 diabetes and chronic kidney disease. No significant association between genetically proxied SGLT2 inhibition and BMD, osteoporosis, or fracture risk was detected either (Table 1).

#### Discussion

In the present large-scale MR and genetic association study, using the currently available comprehensive genetic data for bone density, osteoporosis, and fracture, we did not observe a causal effect of genetically proxied SGLT2 inhibition on BMD, osteoporosis, and fracture risk.

The results of clinical trials to study whether SGLT2 inhibitors increase fracture risk showed controversial results. The main evidence supporting an increased risk comes from the CANVAS study, which included 4330 participants with type 2 diabetes,

**Table 1.** The One-Sample MR and Genetic Associations Between Genetically Proxied SGLT2 Inhibition and Bone Health Outcomes in General Population

Stratification factor	wtGRS	Heel bone mineral density			Osteoporosis			Fracture		
		N participants	β (95% CI)	p Values	N cases/ N controls	HR (95% CI)	p Values	N cases/ N controls	HR (95% CI)	p Values
One-sample MR Total	6 SNPs	256,286	−0.13 (−1.19, 0.94)	0.867	13,677/430,262	0.46 (0.01, 23.46)	0.762	25,806/407,081	0.28 (0.02, 4.78)	0.408
	2 SNPs		−0.16 (−1.17, 0.85)	0.820		1.51 (0.03, 65.20)	0.885		0.42 (0.03, 5.71)	0.565
Genetic association analyses Total	6 SNPs	256,286	0.03 (−0.24, 0.31)	0.809	13,677/430,262	1.23 (0.44, 3.39)	0.696	25,806/407,081	1.39 (0.66, 2.92)	0.387
	2 SNPs		0.02 (−0.11, 0.15)	0.756		0.95 (0.59, 1.52)	0.831		1.12 (0.79, 1.58)	0.516
Age (years) <60	6 SNPs	145,029	−0.11 (−0.48, 0.26)	0.553	4061/242,965	1.83 (0.28, 11.92)	0.527	11,304/229,756	1.66 (0.54, 5.09)	0.378
	2 SNPs		−0.05 (−0.22, 0.12)	0.595		0.92 (0.39, 2.18)	0.842		1.28 (0.76, 2.16)	0.350
≥60	6 SNPs	98,122	0.23 (−0.23, 0.68)	0.324	8864/165,919	1.10 (0.31, 3.89)	0.885	13,144/157,109	1.13 (0.40, 3.20)	0.815
	2 SNPs		0.12 (−0.09, 0.33)	0.268		1.00 (0.56, 1.79)	0.990		1.01 (0.63, 1.64)	0.959
Sex Men	6 SNPs	117,088	0.08 (−0.38, 0.55)	0.726	2268/201,082	0.46 (0.04, 5.61)	0.544	9426/187,912	0.52 (0.15, 1.77)	0.295
	2 SNPs		0.03 (−0.19, 0.24)	0.804		0.61 (0.19, 1.95)	0.405		0.85 (0.48, 1.49)	0.563
Women	6 SNPs	139,228	−0.03 (−0.36, 0.30)	0.858	11,409/229,180	1.49 (0.49, 4.56)	0.481	16,380/219,169	2.46 (0.97, 6.26)	0.058
	2 SNPs		0 (−0.15, 0.16)	0.962		1.04 (0.62, 1.74)	0.883		1.33 (0.86, 2.04)	0.197
T2D Yes	6 SNPs	11,106	−0.49 (−2.12, 1.14)	0.553	675/18,798	0.49 (0, 47.99)	0.760	1609/17,282	4.30 (0.22, 86.07)	0.340
	2 SNPs		−0.01 (−0.77, 0.74)	0.972		0.62 (0.07, 5.27)	0.664		0.90 (0.22, 3.58)	0.877
No	6 SNPs	245,180	0.06 (−0.23, 0.34)	0.694	13,002/411,444	1.26 (0.44, 3.59)	0.662	24,197/389,779	1.27 (0.59, 2.73)	0.540
	2 SNPs		0.02 (−0.11, 0.15)	0.757		0.96 (0.59, 1.56)	0.876		1.12 (0.79, 1.60)	0.516
CKD Yes	6 SNPs	17,018	0.47 (−0.63, 1.57)	0.399	1208/30,316	0.10 (0, 3.03)	0.187	2045/27,574	0.20 (0.01, 2.74)	0.228
	2 SNPs		0.23 (−0.28, 0.73)	0.378		0.24 (0.05, 1.16)	0.075		0.66 (0.20, 2.21)	0.500
No	6 SNPs	239,268	0.004 (−0.28, 0.29)	0.979	12,469/401,046	1.55 (0.53, 4.52)	0.419	23,761/379,507	1.64 (0.76, 3.56)	0.210
	2 SNPs		0.01 (−0.13, 0.14)	0.934		1.08 (0.66, 1.78)	0.747		1.17 (0.82, 1.68)	0.381

Note: Weighted genetic risk scores were constructed for UK Biobank participants using the genetic instruments weighted based on their effect size for HbA1c. Linear regression and Cox proportional hazard models were used to evaluate the effect estimates of SGLT2 inhibition on heel bone mineral density and osteoporosis and incident fracture risk, respectively.

Abbreviation: CI = confidence interval; CKD = chronic kidney disease; HR = hazard ratio; T2D = type 2 diabetes; wtGRS = weighted genetic risk score.

of whom 350 experienced fracture events during follow-up.<sup>(3)</sup> However, no increased risk of fracture was observed in the CANVAS-R, a CANVAS-like clinical trial, which included 5812 participants and 146 participants with fracture events during follow-up.<sup>(3)</sup> A previous study attempted to determine why the CANVAS and CANVAS-R showed different effects on fracture risk<sup>(44)</sup> by evaluating differences in baseline characteristics, randomized treatment interactions with participant characteristics, dose effects, duration of follow-up, metabolic effects, and adverse events related to falls between these above two highly similar trials. However, the study yielded the conclusion that there was no evidence to clearly explain the heterogeneity in fracture risk between the two studies.<sup>(44)</sup> Considering the null result for fracture risk in the CREDENCE trial, which included 4401 participants and 135 participants with fracture events during follow-up, the researchers speculated the observed association in CANVAS might be a chance finding.<sup>(4,44)</sup> In other clinical trials of SGLT2 inhibitors, including DECLARE (Dapagliflozin Effect on Cardiovascular Events) (17,160 patients, 897 participants with fracture events during follow-up),<sup>(5)</sup> the EMPA-REG-OUTCOME (BI 10773 [Empagliflozin] Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) (7020 participants, 183 participants with fracture events during follow-up),<sup>(6)</sup> and VERTIS CV (Evaluation of Ertugliflozin Efficacy and Safety Cardiovascular Outcomes Trial) (8246 participants, 201 participants with fracture events during follow-up),<sup>(10)</sup> the risk of fracture in the intervention group was comparable to that in the placebo group. These results suggest that SGLT2 inhibition is unlikely to increase fracture risk.<sup>(45)</sup> However, one concern is that clinical trials usually suffer from limitations such as a small number of cases or short intervention or follow-up periods. The MR study design representing lifetime manipulation of the SGLT2 inhibitor's targets may partially compensate for these deficiencies.

In observational studies, the relative fracture risk for SGLT2 inhibitors was compared with other hypoglycemic drugs currently in use, such as DPP-4 inhibitors,<sup>(11–15)</sup> sulphonylurea,<sup>(16)</sup> and GLP-1 receptor agonists,<sup>(17–19)</sup> and most studies yielded negative results.<sup>(46)</sup> Compared with observational studies, which may be subject to potential confounders and draw relative risks, the MR study is less likely to be affected by confounders and directly evaluate the causal effect of SGLT2 inhibition on fracture risk.

Notably, although our primary analysis provided little evidence to support the effects of genetically proxied SGLT2 inhibition on BMD, osteoporosis, and most of the types of fracture, we observed a putative association between genetically proxied SGLT2 inhibition and fracture of shoulder and upper arm, although the results did not pass the multi-testing threshold. This association was not reported in previous investigations. Only a nested case–control study involving 210,042 type 2 diabetes patients found that those using SGLT2 inhibitors had a similar risk of shoulder and upper arm fractures as those using other non-insulin antidiabetic drugs.<sup>(47)</sup> Therefore, attention needs to be paid to the effect of SGLT2 inhibitor use on site-specific fractures in clinical practice. Besides, further analyzing fracture data at different sites in previous clinical trials may help to clarify the relationship between SGLT2 inhibitors and fracture risk.

Several limitations should be acknowledged. First, our MR analysis estimated the effect of SGLT2 inhibition based on on-target reductions in HbA1c levels rather than the direct effects of SGLT2 inhibitors. This is based on the assumption that the effect of SGLT2 inhibition on HbA1c levels is proportional to its overall impact, which may not be consistent with the actual

mechanism of SGLT2 inhibition. Additionally, pharmaceutical formulation, dosage, and administration of SGLT2 inhibitors can all affect their impact on fracture risk. These factors should be taken into account when interpreting the results of our study. Second, we did not conduct validation analyses using independent outcomes data in two-sample MR analyses. However, we used the largest data of bone-related outcomes currently available and employed various sensitivity analyses to assess the robustness of our findings. Additionally, we utilized one-sample MR analyses with individual-level data from the UK Biobank as a complementary method to support our main findings. The large sample size data and independent validation analysis may satisfy the statistical power to achieve a robust finding. Third, to mitigate the risk of obtaining negative results in our analyses due to insufficient statistical power, we conducted power calculations. Although we ensured adequate statistical power for most outcomes, we acknowledged the potential concern of insufficient statistical power for specific outcomes, particularly the fracture at the neck, due to its small sample size. Therefore, it is still essential to validate our findings through larger sample sizes and increased case numbers, especially for the low-incidence fracture outcomes. Lastly, our analysis was primarily performed in European populations. Thus, caution should be exercised to generalize the finding to other ethnic groups.

In conclusion, we did not identify the causal effect of genetically proxied SGLT2 inhibition on BMD, osteoporosis, and fracture risk. Existing genetic evidence does not support that SGLT2 inhibition leads to the increased risk of fracture and related bone health outcomes, which provides guiding suggestions on SGLT2 inhibitor use during clinical practice.

## Acknowledgments

The authors thank all the participants of the UK Biobank. The UK Biobank study was approved by the National Health Service National Research Ethics Service (ref. 11/NW/0382), and all participants provided written informed consent. We thank the individual patients who provided the sample that made data available; without them, the study would not have been possible. The study also used GWAS summary data from the GEPOS consortium and the FinnGen study. We thank all the investigators who provided these data to support this study.

This work was funded by the National Natural Science Foundation of China (82270859, 81930021, 81970728, 91857205, and 82088102) and the Shanghai Municipal Education Commission–Gaofeng Clinical Medicine Grant Support (20152508 Round 2). MX, YB, and WW are members of an innovative research team of high-level local universities in Shanghai. The study sponsors were not involved in the design of the study; the collection, analysis, and interpretation of data; writing the report; and did not impose any restrictions regarding the publication of the report.

## Author Contributions

**Huajie Dai:** Conceptualization; methodology; formal analysis; writing – original draft; writing – review and editing; data curation; visualization. **Longyi Zheng:** Conceptualization; data curation; formal analysis; writing – original draft; methodology. **Zheng Zhu:** Data curation; formal analysis; writing – original draft; visualization; methodology. **Xin Geng:** Conceptualization; writing – review and editing; methodology. **Tianzhichao Hou:**

Writing – review and editing; data curation; methodology. **Qi Wang:** Writing – review and editing; data curation. **Yijie Zhu:** Writing – review and editing. **Hong Lin:** Writing – review and editing. **Shuangyuan Wang:** Writing – review and editing. **Ruizhi Zheng:** Writing – review and editing. **Zhiyun Zhao:** Writing – review and editing. **Mian Li:** Writing – review and editing. **Jieli Lu:** Writing – review and editing. **Yu Xu:** Writing – review and editing. **Tiange Wang:** Writing – review and editing. **Jianmin Liu:** Writing – review and editing. **Guang Ning:** Writing – review and editing; funding acquisition. **Weiqing Wang:** Writing – review and editing; funding acquisition. **Yufang Bi:** Writing – review and editing; funding acquisition. **Jie Zheng:** Conceptualization; writing – review and editing; methodology. **Min Xu:** Conceptualization; methodology; data curation; formal analysis; supervision; funding acquisition; writing – original draft; writing – review and editing; project administration.

## Data Availability Statement

UK biobank individual level data are available from the UK Biobank (<http://biobank.ndph.ox.ac.uk/showcase/>) (Application number: 64754). GWAS summary data for bone mineral density from GEFOS consortium is available at <http://www.gefos.org/>. GWAS summary data for osteoporosis and fracture from FinnGen study is available at [https://www.finnngen.fi/en/access\\_results](https://www.finnngen.fi/en/access_results).

## Disclosures

All authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

## References

- Scheen AJ. Sodium-glucose cotransporter type 2 inhibitors for the treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2020;16:556–577.
- Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. *Circulation*. 2016;134:752–772.
- Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. 2017;377:644–657.
- Perkovic V, Jardine MJ, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. 2019;380:2295–2306.
- Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2019;380:347–357.
- Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117–2128.
- Bilezikian JP, Watts NB, Usiskin K, et al. Evaluation of bone mineral density and bone biomarkers in patients with type 2 diabetes treated with canagliflozin. *J Clin Endocrinol Metab*. 2016;101:44–51.
- Cahn A, Mosenzon O, Wiviott SD, et al. Efficacy and safety of dapagliflozin in the elderly: analysis from the DECLARE-TIMI 58 study. *Diabetes Care*. 2020;43:468–475.
- Monteiro P, Bergenstal RM, Toural E, et al. Efficacy and safety of empagliflozin in older patients in the EMPA-REG OUTCOME(R) trial. *Age Ageing*. 2019;48:859–866.
- Cannon CP, Pratley R, Dagogo-Jack S, et al. Cardiovascular outcomes with ertugliflozin in type 2 diabetes. *N Engl J Med*. 2020;383:1425–1435.
- D'Andrea E, Wexler DJ, Kim SC, Paik JM, Alt E, Paterno E. Comparing effectiveness and safety of SGLT2 inhibitors vs DPP-4 inhibitors in patients with type 2 diabetes and varying baseline HbA1c levels. *JAMA Intern Med*. 2023;183:242–254.
- Karasić A, Lanzinger S, Chia-Hui Tan E, et al. Empagliflozin cardiovascular and renal effectiveness and safety compared to dipeptidyl peptidase-4 inhibitors across 11 countries in Europe and Asia: results from the EMPagliflozin comparative effectiveness and SaFety (EMPRISE) study. *Diabetes Metab*. 2023;49:101418.
- Lui DTW, Wu T, Tang EHM, et al. Fracture risks associated with sodium-glucose cotransporter-2 inhibitors in type 2 diabetes patients across eGFR and albuminuria categories: a population-based study in Hong Kong. *Diabetes Res Clin Pract*. 2023;197:110576.
- Cowan A, Jeyakumar N, Kang Y, et al. Fracture risk of sodium-glucose cotransporter-2 inhibitors in chronic kidney disease. *Clin J Am Soc Nephrol*. 2022;17:835–842.
- Abrahami D, Douros A, Yin H, Yu OHY, Azoulay L. Sodium-glucose cotransporter 2 inhibitors and the risk of fractures among patients with type 2 diabetes. *Diabetes Care*. 2019;42:e150–e152.
- van Dalem J, Werkman NCC, van den Bergh JP, et al. Use of sodium-glucose co-transporter 2 inhibitors, changes in body mass index and risk of fracture: a population-based cohort study. *Diabetes Res Clin Pract*. 2022;190:109993.
- Al-Mashhadi ZK, Viggers R, Starup-Linde J, Vestergaard P, Gregersen S. SGLT2 inhibitor treatment is not associated with an increased risk of osteoporotic fractures when compared to GLP-1 receptor agonists: a nationwide cohort study. *Front Endocrinol (Lausanne)*. 2022;13:861422.
- Zhuo M, Hawley CE, Paik JM, et al. Association of sodium-glucose cotransporter-2 inhibitors with fracture risk in older adults with type 2 diabetes. *JAMA Netw Open*. 2021;4:e2130762.
- Patorno E, Pawar A, Bessette LG, et al. Comparative effectiveness and safety of sodium-glucose cotransporter 2 inhibitors versus glucagon-like peptide 1 receptor agonists in older adults. *Diabetes Care*. 2021;44:826–835.
- Li X, Li T, Cheng Y, et al. Effects of SGLT2 inhibitors on fractures and bone mineral density in type 2 diabetes: an updated meta-analysis. *Diabetes Metab Res Rev*. 2019;35:e3170.
- Chen HB, Meng RS, Yang YL, Yu TH. The risk of all-cause death with dapagliflozin versus placebo: a systematic review and meta-analysis of phase III randomized controlled trials. *Expert Opin Drug Saf*. 2023;22:133–140.
- Cao H, Rao X, Jia J, Yan T, Li D. Effects of sodium-glucose co-transporter-2 inhibitors on kidney, cardiovascular, and safety outcomes in patients with advanced chronic kidney disease: a systematic review and meta-analysis of randomized controlled trials. *Acta Diabetol*. 2023;60:325–335.
- Gong C, Shen SC, Zhang K, et al. Association of sodium-glucose cotransporter 2 inhibitors with cardiovascular outcome and safety events: a meta-analysis of randomized controlled clinical trials. *Front Cardiovasc Med*. 2022;9:926979.
- Tsai WC, Hsu SP, Chiu YL, et al. Cardiovascular and renal efficacy and safety of sodium-glucose cotransporter-2 inhibitors in patients without diabetes: a systematic review and meta-analysis of randomised placebo-controlled trials. *BMJ Open*. 2022;12:e006055.
- Kanie T, Mizuno A, Takaoka Y, et al. Dipeptidyl peptidase-4 inhibitors, glucagon-like peptide 1 receptor agonists and sodium-glucose cotransporter-2 inhibitors for people with cardiovascular disease: a network meta-analysis. *Cochrane Database Syst Rev*. 2021;10:CD013650.
- Burgess S, Timpson NJ, Ebrahim S, Davey SG. Mendelian randomization: where are we now and where are we going? *Int J Epidemiol*. 2015;44:379–388.
- Rosoff DB, Bell AS, Jung J, Wagner J, Mavromatis LA, Lohoff FW. Mendelian randomization study of PCSK9 and HMG-CoA reductase inhibition and cognitive function. *J Am Coll Cardiol*. 2022;80:653–662.
- Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet*. 2014;10:e1004383.
- Walker VM, Kehoe PG, Martin RM, Davies NM. Repurposing antihypertensive drugs for the prevention of Alzheimer's disease: a Mendelian randomization study. *Int J Epidemiol*. 2020;49:1132–1140.



30. Zheng J, Xu M, Walker V, et al. Evaluating the efficacy and mechanism of metformin targets on reducing Alzheimer's disease risk in the general population: a Mendelian randomisation study. *Diabetologia*. 2022;65:1664–1675.
31. Xu M, Zheng J, Hou T, et al. SGLT2 inhibition, choline metabolites, and cardiometabolic diseases: a mediation Mendelian randomization study. *Diabetes Care*. 2022;45:2718–2728.
32. Zheng J, Lu J, Cui B, et al. Effect of sodium-glucose cotransporter 2 inhibition on prostate cancer risk: evidence from Mendelian randomization and electronic health-care study. *JAMA Oncol*.
33. GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science*. 2020;369:1318–1330.
34. Vosa U, Claringbould A, Westra HJ, et al. Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat Genet*. 2021;53:1300–1310.
35. Au Yeung SL, Schooling CM. Impact of glycemic traits, type 2 diabetes and metformin use on breast and prostate cancer risk: a Mendelian randomization study. *BMJ Open Diabetes Res Care*. 2019;7:e000872.
36. Luo S, Schooling CM, Wong ICK, Au Yeung SL. Evaluating the impact of AMPK activation, a target of metformin, on risk of cardiovascular diseases and cancer in the UK Biobank: a Mendelian randomisation study. *Diabetologia*. 2020;63:2349–2358.
37. Zheng J, Haberland V, Baird D, et al. Phenome-wide Mendelian randomization mapping the influence of the plasma proteome on complex diseases. *Nat Genet*. 2020;52:1122–1131.
38. Yarmolinsky J, Bull CJ, Vincent EE, et al. Association between genetically proxied inhibition of HMG-CoA reductase and epithelial ovarian cancer. *JAMA*. 2020;323:646–655.
39. Medina-Gomez C, Kemp JP, Trajanoska K, et al. Life-course genome-wide association study meta-analysis of total body BMD and assessment of age-specific effects. *Am J Hum Genet*. 2018;102:88–102.
40. Zheng HF, Forgetta V, Hsu YH, et al. Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. *Nature*. 2015;526:112–117.
41. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613(7944):508–518.
42. Burgess S, Zuber V, Valdes-Marquez E, Sun BB, Hopewell JC. Mendelian randomization with fine-mapped genetic data: choosing from large numbers of correlated instrumental variables. *Genet Epidemiol*. 2017;41:714–725.
43. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA*. 2021;326:1614–1621.
44. Zhou Z, Jardine M, Perkovic V, et al. Canagliflozin and fracture risk in individuals with type 2 diabetes: results from the CANVAS program. *Diabetologia*. 2019;62:1854–1867.
45. Toyama T, Neuen BL, Jun M, et al. Effect of SGLT2 inhibitors on cardiovascular, renal and safety outcomes in patients with type 2 diabetes mellitus and chronic kidney disease: a systematic review and meta-analysis. *Diabetes Obes Metab*. 2019;21:1237–1250.
46. Dong B, Lv R, Wang J, et al. The extraglycemic effect of SGLT-2is on mineral and bone metabolism and bone fracture. *Front Endocrinol*. 2022;13:918350.
47. Schmedt N, Andersohn F, Walker J, Garbe E. Sodium-glucose cotransporter-2 inhibitors and the risk of fractures of the upper or lower limbs in patients with type 2 diabetes: a nested case-control study. *Diabetes Obes Metab*. 2019;21(1):52–60.