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ORIGINAL ARTICLE

Causal relationship between type 2 diabetes mellitus and bone mineral density: a Mendelian randomization study in an East Asian population

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Abstract

Summary It remains unclear whether the relationship between type 2 diabetes mellitus (T2DM) and bone mineral density (BMD) reflects causality in East Asian populations. Herein, a Mendelian randomization study conducted in East Asian population enhances the current clinical cognition that T2DM is not associated with reduction in BMD.

Purpose A Mendelian randomization (MR) approach was utilized to investigate the relationship between type 2 diabetes mellitus (T2DM) and bone mineral density (BMD) in East Asian populations.

Methods Genome-wide association study summary data from BioBank Japan were used to identify genetic variants strongly related to T2DM risk (36,614 cases and 155,150 controls) and osteoporosis (7788 cases and 204,665 controls). Heel BMD GWAS data of 1260 East Asian people from ieu open gwas project was considered as a second outcome. Inverse variance-weighted (IVW) analysis was mainly applied; MR-Egger and the weighted median were also used to obtain robust estimates. A series of sensitivity analyses including Cochran's Q test, MR-Egger regression, and leave-one-out analysis were used to detect pleiotropy or heterogeneity.

Results In the main analysis, IVW estimates indicated that T2DM significantly associated with the risk of osteoporosis (odds ratio = 0.92, 95% CI: 0.86–0.99, $p = 0.016$) and with higher BMD (OR: 1.25, 95% CI: 1.06–1.46, $p = 6.49 \times 10^{-3}$). Results of comprehensive sensitivity analysis were consistent with the main causality estimate. Horizontal pleiotropy and heterogeneity were absent in our MR study.

Conclusions T2DM is not associated with reduction in BMD in terms of genetic polymorphism in East Asian populations.

Keywords Bone mineral density · Mendelian randomization · Osteoporosis · Type 2 diabetes mellitus

Introduction

Osteoporosis is a metabolic bone disease characterized by loss of bone mass, damage to the microstructure of bone tissue, and a decline in bone quality, ultimately giving rise to increased bone fragility and the risk of fracture [1]. Approximately 50% of women and 20% of men are expected to have an osteoporotic fracture in their lifetime [2]. Owing to population aging, osteoporosis has become a serious public health problem. Among all osteoporotic fractures, hip fractures consistently account for the highest morbidity, mortality, and cost. By 2050, it is estimated that approximately 4.5 million to 6.26 million cases of osteoporotic hip fracture will occur worldwide, half of which will occur in Asia [3, 4]. In Asian, up to one in four patients who sustain a hip fracture die within a year and 6–8% of hip fracture patients will suffer second fracture within 2 years [5, 6]. Osteoporotic

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fractures are associated with higher rate of hospitalization and disability, decline in physical and cognitive function, and significant medical burden—projected to increase to 47.4 billion in 2030 [7]. Together, these outcomes impose a heavy social and economic burden on society.

In recent years, researchers have widely acknowledged the link between type 2 diabetes mellitus (T2DM) and osteoporosis. A meta-analysis conducted by Ma. LL et al. included 15 observational studies (3437 diabetics and 19,139 controls) with conflicting results, and concluded that people with T2DM had higher bone mineral density (BMD) than those without T2DM, regardless of the measured bone site, sex, age, body mass index (BMI), or medication use [8]. Observational studies conducted in China have also shown that T2DM was associated with the value of BMD [9–11]. However, Majima et al. found decreased BMD in the distal radius but not in the lumbar spine or femoral neck among 145 Japanese patients with T2DM [12]. In China, Chen et al. found that older men with T2DM are at greater risk of low BMD than those without T2DM [13]. These results might be owing to limitations such as small study samples, inconsistent measurement location of BMD, and confounding factors, such as metabolic diseases like hypertension and hyperlipidemia.

Mendelian randomization (MR) is a genetic epidemiological method that can uncover causal relationships between one or more genetic variations related to health outcomes, typically single-nucleotide polymorphisms (SNPs) and exposure factors [14]. MR studies have advantages over traditional observational epidemiological studies. First, confounding factors can be mitigated by the random assortment of alleles owing to Mendel's Second Law [15]. Second, reverse causality can be avoided, with alleles randomly assigned to offspring and therefore unlikely to be interfered with by confounders; genotypes are not affected by disease, which also avoids reverse causality bias [16]. Third, because of the high accuracy of gene variation sequencing, regression dilution bias caused by measurement error can be avoided [15].

Results of the National Osteoporosis Risk Assessment have shown that Asian populations have a significantly increased likelihood of osteoporosis compared with white populations [17]. Several ethnicity phenotyping studies have provided a more detailed understanding of ethnic distinctions in the pathophysiology, prevalence, and clinical and health care system factors of T2DM and osteoporosis [18–22]. Ethnic disparities may be related to genetic risk factors, but further study is necessary. Ahmad et al. used MR to study the effects of T2DM on BMD in the European population, but there are no relevant reports in Asian populations [23]. Initially, we utilized genome-wide association study (GWAS) summary data on osteoporosis to investigate the relationship between osteoporosis and T2DM. However, in the Japanese cohort, the diagnosis of osteoporosis was dependent on physicians' diagnoses at cooperating hospitals, rather than bone mineral density (BMD) measurements. Consequently, we attempted to confirm our findings by using GWAS data on heel BMD from the East Asian population. In this study, we aimed to provide a clinical reference for early prevention of osteoporosis and reduction of osteoporotic fracture complications.

Methods

GWAS data sources

We obtained GWAS summary data for both T2DM and osteoporosis from BioBank Japan (BBJ). GWAS summary data of T2DM were obtained from a meta-analysis of 36,614 cases and 155,150 controls with Japanese ancestry [24]. GWAS summary data of osteoporosis were obtained for 7788 cases and 204,665 controls with Japanese ancestry [25]. As BBJ project registered not only patients with newly developed diseases but also patients who were diagnosed and treated before starting the project, some participants were enrolled several years after disease onset or diagnosis. Patients with T2DM or osteoporosis were diagnosed by physicians at the cooperating hospitals [26, 27]. We also include another GWAS summary data for heel BMD of 1260 East Asian people from ieu open gwas project (https://gwas.mrcieu.ac.uk/) (Dataset: ukb-e-3148_EAS) as a second outcome.

Instrumental variables selection process

Candidate genetic instrumental variables (IVs) robustly associated with the exposure of interest ($p < 5 \times 10^{-8}$) were obtained from GWAS of T2DM [28]. Linkage disequilibrium clumping with a clumping window of 10 MB was applied to eliminate SNPs with larger p values at a threshold of linkage disequilibrium $R^2 > 0.001$, using the Asian population reference to ensure independence among IVs. SNPs that were significantly associated with the outcome ($p < 5 \times 10^{-8}$) were discarded. For missed SNPs in the outcome GWAS dataset, proxies were identified at the cutoff of $R^2 > 0.8$. If no suitable proxy was available, SNPs were discarded. The F-statistic was used to verify the strength of IVs, using the following formula: $R^2 \times (N-2)/(1-R^2)$. Here, R^2 indicates the proportion of variance in educational attainment explained by a given SNP and N indicates sample size. More specifically, R^2 was calculated with the following formula: $R^2 = [2 \times \mathrm{Beta}^2 \times (1-\mathrm{EAF}) \times \mathrm{EAF}]/[2 \times \mathrm{Beta}^2 \times (1-\mathrm{EAF}) \times \mathrm{EAF} + 2 \times \mathrm{SE}^2 \times N \times (1-\mathrm{EAF}) \times \mathrm{EAF}]$. Here, Beta indicates the genetic effect of SNP on educational attainment, EAF is effect allele frequency, SE is standard

error, and N is sample size; only strong IVs (F-statistic > 10) for each of the exposures of interest were retained [29, 30]. Fourth, we excluded ambiguous and palindromic SNPs (minor allele frequency > 0.42) for which the effect cannot be corrected in the harmonizing process. The MR-pleiotropy residual sum and outlier (MR-PRESSO) test was conducted to discard SNPs with potential pleiotropy.

Mendelian randomization

To perform robust and reliable causal inference of the effect of T2DM on BMD, in the main analysis, we performed multiplicative random-effect inverse variance-weighted (MRE-IVW) analysis [31]. Sensitivity analyses were performed using weighted median [32] and MR-Egger methods [33]. MR-Egger regression is not restricted to a zero intercept, which can determine a genotype–outcome dose–response relationship in which pleiotropic effects are taken into account [34]. However, the MR-Egger method is more sensitive than other methods for detecting unobserved associations of genetic variants with confounders of the exposure–outcome association, and a larger sample size is required for the same underlying exposure variants [33]. The weighted median method can provide consistent effect estimates when at least 50% of the information in the analysis comes from valid instruments. The Cochran Q test for the IVW method was used to detect heterogeneity [35]. No heterogeneity was detected if the p value of Cochran's Q was > 0.05. The intercept term derived from MR-Egger regression was used to examine horizontal pleiotropy. The leave-one-out test was then performed to assess whether the IVW estimate was biased by the influence of single SNPs.

We looked up each SNP in Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/) to assess whether the estimate was violated by potential risk factors, including age, BMI, sex, history of fragility fracture, unhealthy lifestyle (smoking, alcohol, high-sodium diet), endocrine factors (estrogen, glucocorticoids, vitamin D, parathyroid hormone, calcitonin), concomitant chronic diseases (liver and kidney diseases, cerebrovascular diseases, rheumatoid diseases), drugs (chemotherapy drugs, steroids), and malnutrition. All the statistical analyses were performed using R software (version 4.0.2, TwoSampleMR package 0.5.5).

Gene ontology (GO) enrichment analysis

To further explore the biological role underlying T2DM on the osteoporosis development, we performed a gene ontology (GO) and KEGG enrichment analysis using the nearest genes for each lead SNP. Comprehensive gene list annotation and analysis resource were performed in Metascape (http://metascape.org/gp/index.html), a customer-friendly web-based portal [36]. Enrichment dot bubble was plotted by https://www.bioinformatics.com.cn, a free online platform for data analysis and visualization.

Results

Eighty-two SNPs robustly associated with T2DM were remained after clumping method. And as shown in Supplementary table S1, 18 SNPs were found to correlate with potential risk factors and removed. In the harmonizing process, palindromic SNP (rs2057565) was removed. Thus, after rigorous SNP filtering steps in quality control, 63 IVs remained for further analysis (see Supplementary table S2). All the SNPs used in our analyses together explained 1.66% of the variance in type 2 diabetes mellitus. No weak IVs (F-statistic ≤ 10) for our exposure of interest were detected. No potentially pleiotropic variants were identified in the MR-PRESSO outlier test. As seen in Table 1, in the main analysis, IVW estimates indicated that T2DM significantly decreased the risk of osteoporosis (odds ratio [OR] = 0.92, 95% confidence interval [CI]: 0.86–0.99, $p = 0.016$). Results of MR-Egger and weighted median were consistent with IVW, though insignificant. The p value derived from MR-Egger intercept was 0.729 (Egger_intercept = 0.0025), suggesting absence of horizontal pleiotropy. The value of Cochran's Q was 65.106, indicating an absence of heterogeneity ($p = 0.369$).

Three missed SNPs without available proxies (rs149692182, rs4793326, and rs5874792) in the Heel BMD GWAS dataset were discarded, leaving 60 IVs remained for further analysis between T2DM and BMD (see Supplementary table S2). As shown in Table 1, based on IVW method, T2DM was identified to be causally associated with higher BMD (OR: 1.25, 95% CI: 1.06–1.46, $p = 6.49 \times 10^{-3}$). The causal association remained significant based on weighted median method (OR: 1.43, 95% CI: 1.11–1.83, $p = 5.50 \times 10^{-3}$). Results of MR-Egger were consistent with IVW, though insignificant (OR: 1.20, 95% CI: 0.76–1.88, $p = 0.440$). The p value derived from MR-Egger intercept was 0.846 (Egger_intercept = 0.0034), suggesting absence of horizontal pleiotropy. The value of Cochran's Q was 56.633, indicating an absence of heterogeneity ($p = 0.563$).

Figure 1 shows the scatter plot of the above three methods for the association of T2DM with risk of osteoporosis (Fig. 1A) and BMD (Fig. 1B). And in the leave-one-out test, no SNPs were found to violate the causality estimates (Supplemental Fig. S1). GO and KEGG pathway enrichment analysis found significant enrichment of top 20 crucial regulation pathways (Fig. 2). Among them, several pathways may be involved in osteoporosis pathogenesis, like repression of WNT target genes, diacylglycerol metabolic process, and regulation of Notch signaling pathway.

Table 1 MR estimates of the causal association and compensatory sensitivity analyses between T2DM and osteoporosis and BMD

Exposure	Outcome	Main analysis		Compensatory sensitivity analyses							
		MRE-IVW		Weighted median		MR-Egger		MR-Egger regression		Cochran's *Q* test	
		OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	Egger_intercept	*p*	*Q* value	*p*
T2DM	Osteoporosis	0.92 (0.86, 0.99)	0.016	0.92 (0.83, 1.03)	0.144	0.89 (0.74, 1.07)	0.221	0.0025	0.729	65.107	0.369
	BMD	1.247 (1.064, 1.463)	6.49×10^{-3}	1.425 (1.11, 1.83)	5.50×10^{-3}	1.196 (0.762, 1.879)	0.440	0.0034	0.846	56.633	0.563

MR Mendelian randomization, *MRE-IVW* multiplicative random effects-inverse variance-weighted, *OR* odds ratio, *CI* confidence interval, *T2DM* type 2 diabetes mellitus, *BMD* bone mineral density

Discussion

To the best of our knowledge, this is the first MR study among East Asian individuals using multiple genetic variants for T2DM and analyzing their effect on osteoporosis/BMD. This study provides robust genetic evidence in support of the hypothesis that T2DM is not associated with reduction in BMD in terms of genetic polymorphism. No evidence of directional pleiotropy or heterogeneity was observed in our study.

Type 2 diabetes mellitus (T2DM) is well-known to be associated with normal or elevated bone density but, concurrently, low bone turnover and increased risk for fracture [37–39]. Through observational studies, there is still no unified conclusion regarding the effect of T2DM on BMD in Asian populations. Some observational studies have indeed found that patients with T2DM have higher BMD compared to non-diabetics [9, 40], while others have found no association [41] or the opposite [12, 13, 42, 43]. The conflicting results may be due to differences in study design, use of medications, and confounding factors, such as BMI. Therefore, in our study, we adopted an MR method to avoid bias so as to explain the contradiction in previous observational studies and confirm that T2DM is not associated with reduction in BMD. Results from the National Osteoporosis Risk Assessment have shown that Asian populations are associated with a significantly increased likelihood of osteoporosis compared with white populations [17]. In an MR study, Ahmad et al. reported that genetically influenced increases in T2DM risk and fasting plasma glucose have weak positive effects on BMD [23]. Another MR study among non-diabetic individuals of European descent by Mitchell et al. demonstrated that a genetically predicted 1-mmol/L increment in fasting glucose was associated with a 4% higher total hip BMD, albeit without reaching statistical significance ($p=0.06$) [44]. A two-sample MR study using GWAS summary statistics obtained from the Meta-analyses of the Glucose and Insulin-related traits Consortium and Genetic Factors for Osteoporosis Consortium showed that lumbar spine BMD increased by 0.49 g/cm^2 (95% CI: 0.01–0.97) in response to a per-unit increase in fasting insulin, revealing a higher BMD in patients with T2DM than in those without diabetes [45]. Consistent with studies conducted among individuals of European descent, the observed association with an elevated BMD was confirmed in our study among individuals of Asian descent.

Despite a normal to high BMD compared with non-diabetic individuals, patients with T2DM more often have an increased fracture risk [37–39]. Fragility fractures in patients with T2DM may be explained by the presence of impaired structural properties, including abnormalities in

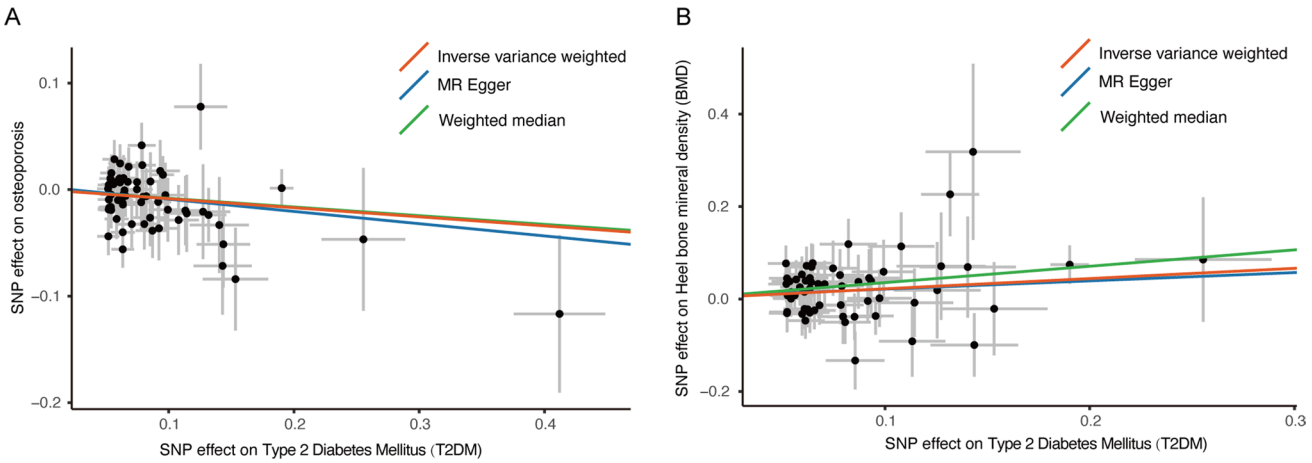

Fig. 1 Scatter plot of Mendelian randomization analyses for the association of T2DM with risk of osteoporosis (A) and heel bone mineral density (B). MR, Mendelian randomization; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus

dynamic, material, and microarchitectural bone properties, which ultimately lead to bone fragility [46–48]. In a study among Japanese men, those with T2DM had a hazard ratio of 2.76 for fragility fractures compared with normoglycemic men [49]. Both longer diabetes duration and poor glycemic control are associated with a higher fracture risk [50]. Losada-Grande et al. investigated the association between insulin use and fracture risk and found that insulin use appeared to be associated with a 38% excess fracture risk among patients with T2DM in the early stages of disease [38]. Studies have shown that higher glycated hemoglobin (HbA1C)-poor blood glucose control is positively correlated with higher BMD in patients with diabetes [8]. Long-term high blood glucose levels lead to retinopathy and peripheral neuropathy, which further affect the patient's balance and result in an increased risk of falling [51].

Given the cross-talk between osteoblasts and osteoclasts with abnormal microstructural repair in these T2DM patients, it is expected that reduction in bone resorption follows with resultant low bone turnover — the dynamic process of resorption followed by replacement by new bone [52, 53]. A balance between osteoclast-dependent bone resorption and osteoblast-dependent bone formation is essential for the maintenance of bone material quality. High glucose levels may interfere with osteoclast and matrix differentiation and inhibit osteoclast-mediated bone matrix degradation, resulting in elevated BMD [54]. Another possible explanation is that patients with T2DM have an increased number of osteoblast precursor cells and elevated levels of Dickkopf-related protein-1 (DKK-1), a regulator that inhibits osteoblast maturation; this phenomenon can increase the number of immature osteoblasts in patients with T2DM in comparison with controls [55]. β-Cell failure and low levels of insulin-like growth factor-1 negatively affect osteoblast function [56]. The possibility of a preventive or therapeutic role for thiazides and statins in osteoporosis has been emphasized in some studies because users of these anti-diabetic drugs have significantly greater bone mineral content than non-users [57, 58]. The reductase inhibitor 3-hydroxy-3-methylglutaryl coenzyme, the main component of statins, can stimulate bone formation by increasing the expression of the bone morphogenetic protein-2 gene in bone cells. Thiazides can significantly elevate the levels of circulating calcium, thus promoting bone formation. Furthermore, high circulating insulin levels in patients with insulin resistance could explain the high BMD in patients with T2DM because insulin is known to exert anabolic effects on bone [59, 60]. It is speculated that low turnover of bone in diabetes may lead to defective microfracture repairs and, hence, to their accumulation, contributing to decreased bone quality.

GO and KEGG pathway enrichment analysis also found significant enrichment of several crucial regulation pathways, which observed to be involved in osteoporosis. Among them, diacylglycerol (DAG) metabolic process and regulation of Notch signaling pathway might be remarkable. Recent lipidomics studies have shown significant dysregulation of lipids in aging-related bone mineral density loss and the occurrence of osteoporosis, including alterations in DAG [61, 62]. In response to stimulations, activated phospholipase C (PLC) hydrolyzes PIP2 to DAG. DAG activates protein kinase C (PKC)[63], leading to GSK-3β inactivation and NFATc1 induction, which has a key role in the RANKL-induced osteoclast differentiation [64]. Furthermore, Notch signaling is implicated in governing cell fate determination, proliferation, differentiation, and apoptosis of skeletal cells, including osteocytes, chondrocytes, osteoblasts, and osteoclasts [65]. The deletion of Notch receptors in mouse bone marrow macrophages increased osteoclastogenesis as well as osteoclast precursor proliferation, exerting its effect both

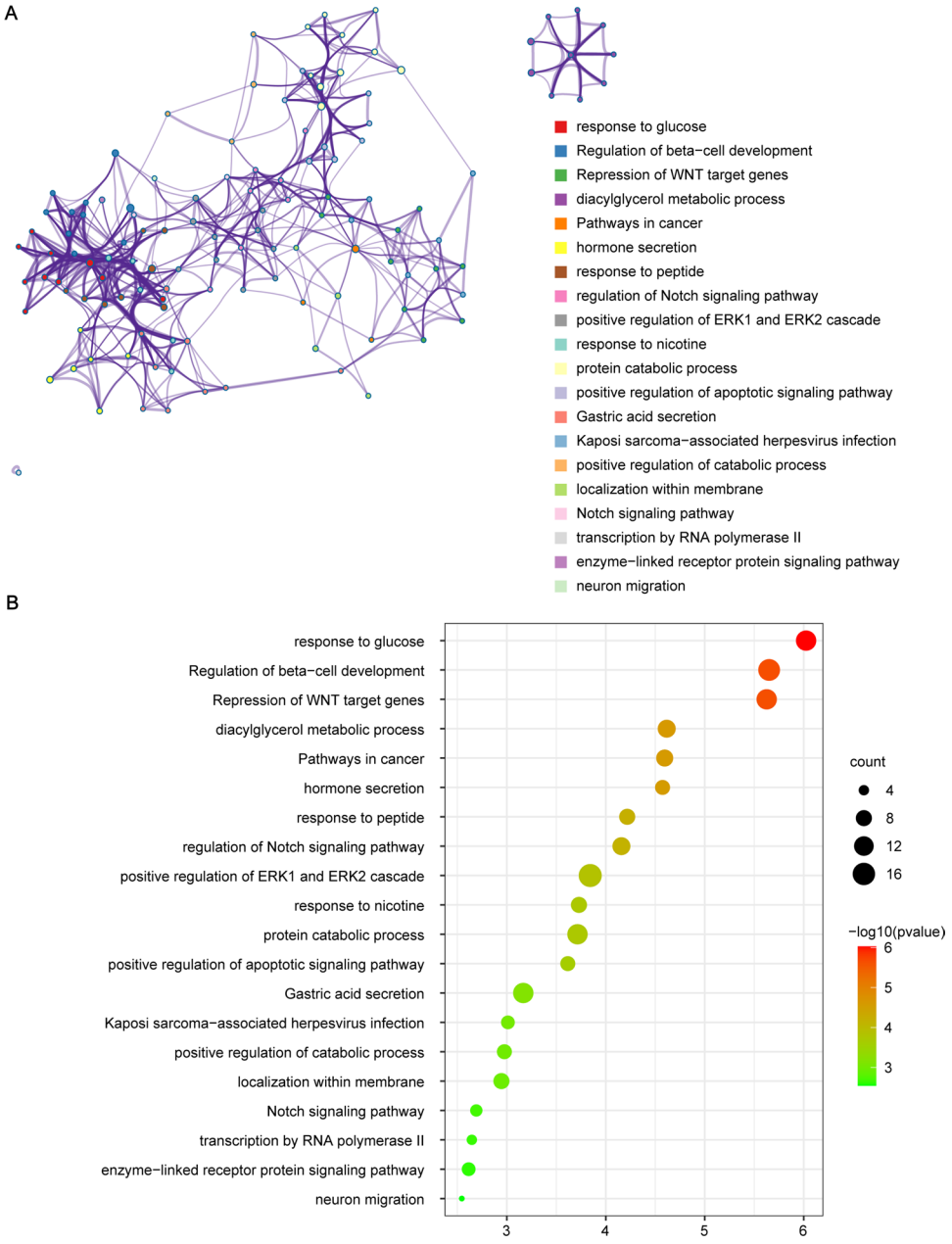

Fig. 2 Gene ontology enrichment analysis of nearest genes for single-nucleotide polymorphisms used on significant MR analysis results (A) enriched ontology clusters, where every cluster is represented in a single color and shown as a circle; (B) GO enrichment dot bubble plot where count means enriched number of genes

in osteoclast precursors and indirectly via osteoblast lineage cells directly, which raise caution that Notch signaling inhibition may be one of the osteoporosis pathogenesis [66].

T2DM is often combined with obesity, which can also have detrimental effects on bone. Bioactive lipids play important structural and functional roles and directly relate to bone homeostasis. The increased bone marrow fat (BMF) is generating growing interest as a possible explanation to the bone loss in diabetes patients. Patsch and his colleagues [67] found that altered bone marrow fat composition (specifically the proportion of saturated versus unsaturated lipid) is linked with fragility fractures and diabetes. Stem cell differentiation to adipocytes involves the transcription factor known as peroxisome proliferator-activated receptor (PPARγ2) and is viewed as competing with osteoblastogenesis [68]. Adipose tissue can release a number of adipokines (e.g., leptin, adiponectin, and resistin), which can regulate bone formation by affecting osteoblasts or osteoclasts [69].

Our study has the following limitations.

Diagnoses of these diseases were based on the physicians' diagnoses at cooperating hospitals. First of all, diagnosis of osteoporosis in the Japanese cohort was based on the physicians' diagnoses at cooperating hospitals without mention of BMD measurement or history of fracture, implying that the findings might have lacked precision and could be somewhat unreliable. Somehow, measurement

error in using the outcome of "osteoporosis" as a measure for low BMD may limit the strength of these conclusions and prevent a causal interpretation. However, we also conducted additional analysis using heel bone mineral density GWAS, which will provide more solid evidence for the causal relationship between type 2 diabetes mellitus and bone mineral density in an East Asian population. This approach will help to overcome the limitations of the initial diagnosis and strengthen the validity of the findings. Second, the SNPs in this study were derived from a Japanese GWAS database, which does not completely represent all genetic characteristics of the Asian population. Third, summary-level data from the BBJ GWAS studies might be potentially overlapping, which has the potential to bias causal effect estimates in MR studies. Fourth, the number of SNPs as IVs was relatively small, which can only explain a limited causal relationship. Fifth, the use of publicly available data means that it was not possible to conduct subgroup analyses by age, sex, and disease duration. Despite these weaknesses, to the best of our knowledge, this is the first MR study with the largest GWAS dataset to focus on the effect of T2DM traits on the risk of osteoporosis in Asian individuals. Our study findings provide evidence for causal inference in the absence of a randomized, controlled trial. We also applied a variety of methods based on different assumptions in our study, making the causal findings more reliable.

Conclusion

Our MR study among East Asian populations provides further evidence that T2DM is not genetically associated with reduction in BMD. In clinical practice, it is important to regulate blood glucose levels to achieve a balance between increasing bone intensity and reducing diabetes-related complications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00198-023-06807-6.

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Data availability The data used to generate the results in this study were obtained from genome-wide association study summary statistics which were publicly released by genetic consortia.

Declarations

Ethical approval We used publicly available aggregate data in this study; therefore, no separate ethical approval was required.

Conflicts of interest None.

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