T2D and Osteoporosis Risk: A Mendelian Randomisation Study

Zhenming Zeng[1], Zhenzhen Wang[1], Xiahai Zheng[1], Tao Jiang[2], Min Shao[1] and Yong Lian[3]

[1]*Department of Rehabilitation, The Third Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China*
[2]*Department of Orthopaedics, Guangdong Provincial Second Hospital of Traditional Chinese Medicine, Guangzhou, China*
[3]*Department of Orthopaedics, Third School of Clinical Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou,China*

ABSTRACT

Objective: To investigate the causal effect of Type-II Diabetes (T2D) on osteoporosis (OP) risk using a two-example Mendelian randomisation (MR).

Study Design: Descriptive study.

Place and Duration of the Study: Third School of Clinical Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou, China, from February to July 2024.

Methodology: The data of the whole gene association study (GWAS) were gathered from two separate databases and the same East Asia descent, including 433,540 genetic variants in T2D and 212,453 genetic variants in osteoporosis. These genetic variants were used as instrumental variables, subsequently, these variables were analysed by several methods, including inverse variance weighted (IVW)-random effects, MR-Egger regression, and weighted median estimator (WM).

Results: The outcomes indicated that a potential causal association existed between T2D and OP in the primary MR analysis (IVW: Odds ratio (OR) = 1.0934, 95% confidence interval (95% CI): 0.8735-0.9643, p = 0.0007). Meanwhile, the secondary MR also revealed that T2D was causally associated with OP (MR-Egger: OR = 1.1142, 95% CI: 0.9511-0.9966, p = 0.0004, and WME: OR = 1.1422, 95% CI: 0.8611-0.9666, p = 0.0004). The accuracy and robustness of the above results were confirmed using sensitivity tests.

Conclusion: A causal relationship was found between T2D and OP, which also revealed that T2D increased the risk of OP.

Key Words: *Mendelian randomisation, Type-II diabetes, Osteoporosis, Causal inference*.

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INTRODUCTION

Osteoporosis (OP) is a common and highly prevalent chronic bone metabolic disease characterised by a decrease in both bone density and bone strength,[1] which predisposes to fragility fractures or death in the elderly.[2] The international osteoporosis study reported that one in three women and one in five men over the age of 50 years are likely to have OP globally.[3-5] This disease imposes heavy health and economic burdens on individuals, families, and health systems.

Diabetes mellitus (DM) is a global chronic metabolic disease that affects 537 million adults worldwide and is increasing in prevalence every year, and this number will reach 643 million according to a survey from the International Diabetes Federation (IDF).[6]

Correspondence to: Dr. Yong Lian, Department of Orthopaedics, Third School of Clinical Medicine, Guangzhou University of Traditional Chinese Medicine, Liwan District, Guangzhou, China
E-mail: liany5132@163.com

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Some studies have reported that patients with Type II Diabetes (T2D) are prone to bone loss, decreased bone strength, and brittle fractures.[7] In contrast, it has also been reported in some literature that T2D does not increase the risk of OP but rather elevates bone mineral density (BMD), which is a protective factor for OP.[8] However, most of these results are mixed with a number of confounding factors, which created a major problem for the study of the association between T2D and OP.

Mendelian randomisation (MR) analysis, designed by George in 2003, is a novel epidemiological approach to estimate the causal effect of a modifiable exposure on an outcome by leveraging genetic variants (typically single-nucleotide polymorphisms, SNPs).[9] MR, the mimic design of random control trials, has advantages over traditional epidemiology. It is not constrained by time, place, manpower, or ethics, also, it can minimise the effects of confounding factors and avoid inversion of causality.[10] MR techniques to explore the potential causal relationship between T2D and OP, and to assess whether the reported associations are influenced by pleiotropic effects of genetic variants or other unmeasured confounders.

Given the complexities and mixed findings surrounding the relationship between T2D and OP, it is imperative to adopt a robust analytical approach to untangle these associations. By utilising

genetic variants as instrumental variables, MR can provide a more accurate estimation of the causal effect of T2D on OP, accounting for potential confounding factors that have plagued previous observational studies. This approach holds the potential to clarify whether T2D truly increases the risk of OP, or if it exerts opposite effects by influencing bone mineral density, thereby guiding clinical interventions and public health strategies more effectively.

The objective of this study was to investigate the causal effect of T2D on OP risk using a two-example Mendelian randomisation (MR).

METHODOLOGY

This descriptive study was conducted at the Department of Orthopaedics, Third School of Clinical Medicine, Guangzhou University of Traditional Chinese Medicine, Guangzhou, China, from February to July 2024. The analyses used publicly available GWAS summary data. No primary data were collected for this manuscript, and therefore, no ethical issues were involved. As MR utilises genetic variants as instrumental variables, traditional randomisation or blinding protocols were not applicable.

In this study, T2D served as the exposure factor, while OP was the outcome variable. Single nucleotide polymorphisms (SNPs) that possessed a strong correction with the exposure were chosen as instrumental variables from the GWAS database. Under strict adherence to the principles of MR's three main assumptions, a two-sample Mendelian randomisation analysis was used to reveal a causal relationship between T2D and OP. Assumption one was the relevant assumption that SNPs are strongly associated with exposure. Assumption two was the independent assumption that SNPs are not related to any confounding factors. Assumption three was the exclusive assumption that SNPs are not associated with outcome. Multiple sensitivity analyses were used to detect the reliability and stability of these results.

The dataset of T2D was derived from the ebi GWAS database of the European Bioinformatics Institute (GWAS ID: ebi-a-GCST010118) and included 433,540 East Asians (7,346 cases and 432,386 controls) with 11,222,507 SNPs. The dataset of OP was originated from the Japan Biobank (GWAS ID: bbj-a-137) and consisted of 212,453 East Asians (5,565 cases and 321,693 controls) with 888,580,5 SNPs. Detailed GWAS data are provided in Table I.

Inclusion criteria were that (a) all the instrumental variables must satisfy the three major assumptions of MR, (b) SNPs with $p < 5e^{-8}$, (c) the parameters r^2 threshold to 0.001, (d) kilo base pair (kb) to 10,000, and (e) linkage disequilibrium (LD) <0.001. Exclusion criteria were that $R^2 > 0.001$ and range size = 10,000 kb.

The R^2 indicates the extent to which IVW explains exposure and its value was from the MR Steiger directionality test. The MAF here is the minor allele frequency, which is equivalent to the allele frequency (EAF), β is the allele effect value, and SD is the standard deviation. The R^2 was calculated using the Equation 1:

$$R^2 = 2 \times (1 - MAF) \times MAF \times \left(\frac{\beta}{SD}\right)^2$$

In addition, the authors assessed bias for weak instrumental variables (F values greater than 10 indicate that the SNP was strongly associated with the exposure factor) using the F-statistic, where N denotes the sample size of the exposure database and K is the number of SNPs. The F-statistic is specifically calculated as Equation 2:

$$F = \left(\frac{R^2}{1-R^2}\right)\left(\frac{N-k-1}{k}\right)$$

A two-sample Mendelian randomisation was carried out to explore the causal relationship between T2D and OP risk using these instrumental SNPs. During this process, several analytical methods were used to assess the effects, including inverse variance weighting (IVW)-random effects, IVW-fixed meta-analysis, maximum likelihood, weighted median (WM), MR-Egger regression, and penalised weighted median. These data were analysed in R (V.4.2.3) by using an R package of Two-Sample, MR at: (https://www.r-project.org/) and a p-value <0.05 was considered statistically significant.

Table I: Basic characteristics of exposure and outcome data.

Exposure/ outcome	ID	Sample size	Number of SNPs	Population	Database	Gender	Year
T2D	ebi-a-GCST010118	433540	11222507	East Asians	European bioinformatics	Males / females	2020
OP	bbj-a-137	212453	8885805	East Asians	Japan biobank	Males / females	2019

Table II: Results of Mendelian randomisation analysis and OR values.

Exposure	Methods	Outcome	OR	95% CI	p-value
T2D	IVW-mre	OP	1.0913	0.8735-0.9643	0.0007
	MR-Egger		1.1142	0.9511-0.9966	0.0004
	WME		1.1422	0.8611-0.9666	0.0004
	Weighted mode		1.0621	0.8666-1.0703	0.0463
	Simple mode		0.9605	0.7199-1.0464	0.0402

T2D: Type-II diabetes, OP: Osteoporosis, OR: Odds ratio, CI: Confidence interval, P: p-value, IVW-mre: Inverse variance weighted, MR-Egger: MR-Egger regression, WM: Weighted median estimator.

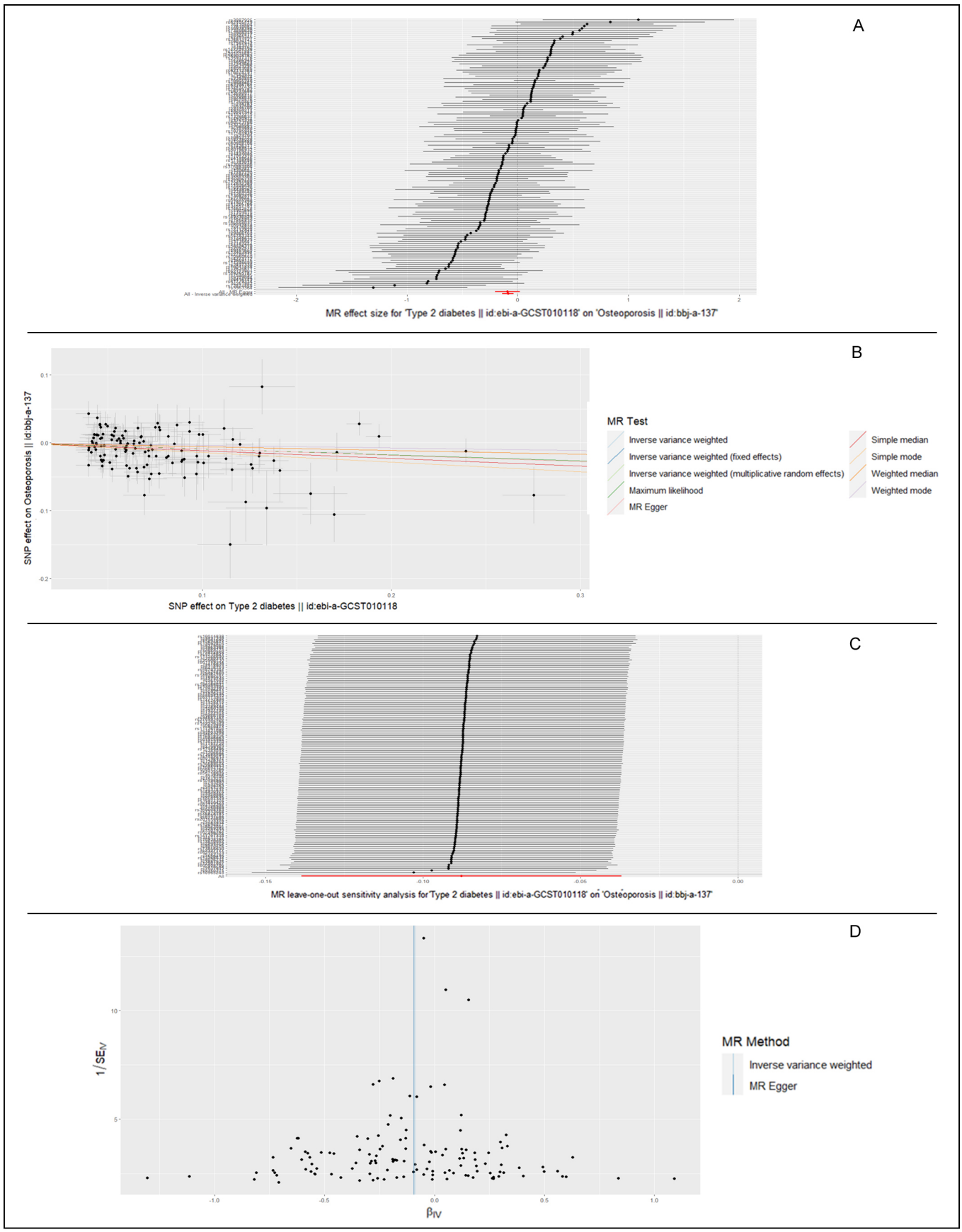

Figure 1: (A) Forest plot of the two-samples MR analysis (B) Scatter plot of the two-samples MR analysis. The slope of each line corresponding to the estimated MR effect in different models (C) Result of leave-one-out sensitivity analysis (D) Funnel plot of the two-sample MR analysis.

The reliability of the results is commonly assessed by the following tests. First, the MR-Egger intercept analysis method was utilised to assess the presence of horizontal pleiotropy, if the MR-Egger line was far from the origin 0 and greater than 0.05, the intercept term indicated the presence of horizontal pleiotropy, implying that the results of the MR analysis were unreliable and there may be false positives. Cochran's Q test was used to assess heterogeneity. Leave-one-out sensitivity test was applied to judge the stability and ensure the robustness of the MR results. In this method, each SNPs was removed one-by-one to see if the mean values of other remaining SNPs were all greater than or less than 0, then the MR results were said to be robust and reliable. Furthermore, an MR-presso method was also performed to pick out the SNPs that cause bias. The number of iterations (Nb Distribution) parameter was set at 10,000 in the MR-presso test, so that the results are more accurate, and if $p < 0.05$ in the global test, it meant heterogeneity. Additionally, funnel plot visualised the sensitivity whether the SNPs were symmetrically distributed on both sides of the IVW.

RESULTS

Initially, 148 SNPs were included in this study, which reached a recognised threshold of genome-wide significance ($p < 5e^{-8}$, $r^2 < 0.001$, kb = 10,000). Subsequently, 19 alleles (SNPs) with intermediate allele frequencies were deleted after the harmonisation of T2D (ebi-a-GCST010118) and OP (bbj-a-137). Finally, 129 SNPs were included in this study. Meanwhile, the resulting F-statistics were all greater than 10, implying that there was no biased instrumental variable.

The primary IVW analysis (Figure 1A) revealed that T2D increases the risk of OP by approximately 9% (odds ratio (OR) = 1.0934, 95%, confidence interval (CI): 0.8735-0.9643, p = 0.0007). The result was corroborated by other secondary MR analyses (MR-Egger: OR = 1.1142, 95% CI: 0.9511-0.9966, p = 0.0004, and WME: OR = 1.1422,95% CI: 0.8611-0.9666, p = 0.0004). The detailed results were shown in Table II.

The robustness of the results of the study is as follows. First, Cochran's Q-test for IVW (Q = 161.2195, p = 0.023) and MR-Egger (Q = 161.2052, p = 0.0222) showed no heterogeneity in SNPs. Secondly, the outcome of the horizontal multivariate test showed that the Egger-intercept analysis results did not have an intercept with the origin 0 (Figure 1B). Therefore, the authors concluded that there was no horizontal multivariate validity of the SNPs (p = 0.91). Furthermore, the leave-one-out re-analysis, performed by removing one SNP at a time, showed that all results were stable to the left of the origin 0, indicating that the overall results are robust and with no SNPs with a large bias (Figure 1C). Additionally, the funnel plot visualises the symmetrical distribution of SNPs, indicating the absence of large bias (Figure 1D).

DISCUSSION

To date and to the best of the authors' knowledge, this research is the first to start researching on the causal association between T2D and OP under a two-sample MR analysis. Since genetic variables adhere to the Mendelian principle of random assortment, analysing instrumental variables that are closely associated with exposure can elucidate the causal relationships between exposure and disease. This allows current Mendelian studies to exclude the interference of confounding factors inherent in traditional epidemiology and to avoid reverse causality.[11]

In this study, a two-sample MR analysis was performed using two large samples of SNPs that were associated with T2D and OP from two different sources and the same East Asian population in the GWAS database. A definite causal relationship was observed between T2D and OP, with an average of 9% increased risk of OP per 1-SD increment in T2D. Moreover, the sensitive analysis of all the results suggested that no heterogeneity and horizontal pleiotropy existed.

Patients with T2D are complicated by chronic complications such as prolonged hypoglycaemia, muscle weakness, retinopathy, and neuropathy,[12] all of which can affect the process of bone resorption and bone formation and promote the progression of osteoporosis.[13] In addition to this, age, diet, medication, menopausal status, the internal environment in hyperglycaemic states, and their pathologic metabolites are all confounding factors that influence the experiment.[14-17]

To avoid the effects of confounding factors in traditional observational studies,[18] the authors performed a two-sample MR analysis to estimate the causal association between T2D and OP. Importantly, the randomness and fixity of alleles exclude the reverse causation bias, which is a common concern in observational studies.[19] Furthermore, the stability and accuracy of the results were enhanced by using the multiple MR analysis methods and sensitivity tests.

There are several limitations in this study. First, these SNPs represent only the genetic characteristics of East Asian populations, therefore, the results are not necessarily applicable to other human populations, which may lead to bias. Secondly, the exposure factor in this study was T2D, which is mainly characterised by blood glucose, and the outcome was OP, which is mainly characterised by BMD, however, other subgroup analyses including age, gender, and disease duration were not conducted. Finally, the association between exposure and outcome may be overestimated underlying the influence of other factors, such as BMI.

CONCLUSION

DM significantly increases the risk of OP, therefore, there is a need for early screening for OP in patients with DM, especially those with chronic hyperglycaemia or comorbidities.

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ETHICAL APPROVAL:
The analysis used publicly available GWAS summary data. No original data were collected for this manuscript; therefore, no ethical committee approval was required.

PATIENTS' CONSENT:
All participants provided written informed consent.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
ZZ, MS: Performed statistical analysis of the data.
ZW, XZ, TJ, YL: Contributed to the experimental work.
YL: Designed the study and prepared the first draft of the paper.
All authors approved the final version of the manuscript to be published.

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