Non-causal Effect of Circulating Vitamin D Levels on the Risk of Rheumatoid Arthritis: A Two-sample Mendelian Randomization Study

Kun Xiang  
Anhui Medical University

Yu-Qian Hu  
Anhui Medical University

Yi-Sheng He  
Anhui Medical University

Peng Wang  
Soochow University

Sha-Sha Tao  
Anhui Medical University

Yue Chen  
Anhui Medical University

Ya-Ting Feng  
Anhui Medical University

Xiao-Ke Yang  
First Affiliated Hospital of Anhui Medical University

Hai-Feng Pan (panhaifeng1982@sina.com)  
Anhui Medical University  https://orcid.org/0000-0001-8218-5747

Research

Keywords: Mendelian randomization, Vitamin D, Rheumatoid arthritis, Causal effect

Posted Date: May 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-479402/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

Background: Recently, many studies have indicated the potential roles of vitamin D in rheumatoid arthritis (RA). In the present study, the published available GWAS summary data was used to perform two-sample Mendelian randomization (MR) to infer the causal effect between circulating vitamin D levels and RA risk.

Methods: Single nucleotide polymorphisms (SNPs) which significantly associated with exposure were selected as instrumental variables from larger-scale genome-wide association study (GWAS). The robust analytical methods including MR Egger, inverse variance weighted (IVW), weighted median and weighted mode were conducted to infer the causal links.

Results: The IVW method suggested that there was no causal relationship of genetically predicted circulating vitamin D on RA (Asian: odds ratio (OR)=0.911, 95% confidence interval (CI), 0.542-1.534, P=0.727; European: OR=0.897, 95% CI, 0.633-1.269, P=0.538). MR Egger (Asian: OR=0.937, 95% CI, 0.373-2.352, P=0.895; European: OR=0.853, 95% CI, 0.469-1.553, P=0.639), weighted median (Asian: OR=0.895, 95% CI, 0.499-1.606, P=0.711; European: OR=0.885, 95% CI, 0.620-1.264, P=0.503) and weighted mode (Asian: OR=0.904, 95% CI, 0.515-1.588, P=0.738; European: OR=0.898, 95% CI, 0.618-1.306, P=0.604) demonstrated that vitamin D was not directly related to RA as well.

Conclusions: The MR analysis indicates that there is no evidence of causal effect of genetically predicted circulating vitamin D levels on RA risk.

1. Introduction

Rheumatoid arthritis (RA) is a systemic chronic autoimmune disease, mainly manifested by the proliferation of synovial lining cells and the erosion of cartilage and bone tissue. RA appears mostly in women and may occur at any age, with a peak incidence between 50 and 60 years old [1]. In general, progressive disability and shortened life expectancy are almost universal in RA patients [2], furthermore, physical pain also seriously affects the quality of life [3]. The exact pathogenesis of RA is not yet known [4], but it is believed that environmental, genetic, and infectious factors may interact in susceptible individuals and the heritability of RA is approximately 65% [5].

Vitamin D is a fat-soluble steroid that with the function of promoting the absorption of calcium and phosphorus, mainly including vitamin D2 and D3. It is generally believed that vitamin D is closely related to calcium homeostasis and bone formation. In addition, vitamin D has the potential to modulate immune cells, particularly T lymphocytes [6]. Vitamin D deficiency is common in autoimmune rheumatic diseases [7] which is accepted to be an established risk factor of numerous autoimmune diseases [8]. Vitamin D is involved in innate and adaptive immune responses and therefore insufficient vitamin D levels may be related to the loss of immune tolerance [9,10]. The inherent anti-inflammatory functions of vitamin D highlight its potential therapeutic value in autoimmune diseases [11-13]. However, no consensus is reached on whether there are causal effects between vitamin D and RA.

Two-sample Mendelian randomization (MR) is a technique which integrates large-scale genome-wide association study (GWAS) summary data, and single nucleotide polymorphisms (SNPs) significantly associated with exposure are used as instrumental variables to infer the causal links between exposure and outcome. MR method must follow the three assumptions. First, instrumental variables are independent of confounding factors. Second, instrumental variables are significantly related to exposure. Third, instrumental variables affect the outcome only through exposure [14]. The advantage of MR is that the causal inference is not interfered by common confounding factors such as environment and lifestyle.

Given the current results of the association between vitamin D and RA was inconsistent, and the causal effect between vitamin D and RA had not yet been established, the two-sample MR was performed to infer whether there were causal effects of circulating vitamin D levels on RA risk.

2. Methods

2.1 GWAS summary data

Summary data for the circulating vitamin D levels were selected form a larger-scale GWAS analysis, including 79,366 European ancestry [15]. The GWAS summary data for RA were obtained from a larger dataset with 19,234 individuals (4,873 Asian and 14,361 European ancestry) [16]. The selection of large sample GWAS was conducive to obtaining more comprehensive and reliable results. The corresponding information of SNPs including effect allele, other allele, effect sizes, standard error and effect allele frequency (EAF) was collected.
2.2 Instrumental variables selection

The following quality control steps were used to select the optimal instrumental variables to avoid the impact of bias on the results. First, SNPs significantly associated with exposure were served as potential instrumental variables ($P<5\times10^{-6}$). Second, the clumping process ($R^2<0.001$, clumping distance=10,000 kb) was performed to avoid the underlying bias caused by strong linkage disequilibrium (LD). Third, the SNPs which with minor allele frequency (MAF) less than 0.01 were excluded from the present study. Fourthly, if the information of vitamin D related SNPs was not present in the outcome GWAS, the proxy SNPs with high LD ($R^2>0.80$) were chosen to replace the variants of interest. Finally, to ensure that the effect of the selected SNPs on circulating vitamin D levels and the effect of the same SNPs on the outcomes corresponded to the same allele, the palindromic SNPs were excluded.

One of the assumptions of MR analysis is that the included SNPs must be highly correlated with exposure. In the present study, the $F$ statistic ($F=R^2(n-k-1)/k(1-R^2)$) was used to explore whether there were instrumental variables that were weakly correlated with exposure. In this equation, $R^2$ represents the cumulative explained variance of the selected instrumental variables on circulating vitamin D levels, $k$ is the number of selected instrumental variables and $n$ represents the sample size. When $F>10$, the correlation between instrumental variables and exposure is considered to avoid the bias caused by weak instrumental variables.

2.3 Statistical analysis

In this study, MR methods with robust testing power including inverse variance weighted (IVW), MR Egger, weighted median and weighted mode were used to infer the causal effects of vitamin D with RA. The IVW conducts a meta-analysis approach to divide the $\beta$ coefficient of the SNPs of RA by the $\beta$ coefficient of the SNPs of vitamin D to obtain an overall estimate of the effect of vitamin D on RA [17]. In the absence of horizontal pleiotropy, or when the horizontal pleiotropy is balanced, IVW linear regression enable to obtain unbiased causal estimates [18]. Directional pleiotropic test, causal effect test and causal effect estimation are the three components of MR Egger [19]. Under the Instrument Strength Independent of Direct Effect (InSIDE) condition, even if all instrumental variables are invalid, MR Egger can also give a consistent causal effect estimate [20]. Weighted median is suitable for the situations where up to 50% of the information comes from invalid instrumental variables. If most of the instrumental variables in the model do not meet the requirements of using MR for causal inference, the weighted mode approach is effective [21]. Weighted mode is usually used as a supplementary analysis. MR-Egger regression was conducted to test whether the instrumental variables had potential pleiotropic effects. Cochran Q statistic was performed to quantify the heterogeneity among selected SNPs. Leave-one-out sensitivity analysis was used to test whether the overall estimates were affected by single SNP.

The TwoSampleMR package in R software (version 4.0.3) was used to conduct statistical analysis.

3. Results

3.1 Genetic variants selection

In the analysis of Asian population, after the SNPs with strong LD were excluded, 8 SNPs were selected as potential instrumental variables. After excluding a palindromic SNP (rs8018720), 7 variants of interest were selected as instrumental variables (Table 1). For European population, 5 SNPs were served as instrumental variables after excluding a palindromic SNP (rs8018720) (Table 1). The remained SNPs explained 2.2% (Asian) and 1.8% (European) of the variance of circulating vitamin D levels. $F$ statistics were greater than 10, suggesting weak instrumental variables were less likely to exist.

3.2 Two-sample MR analysis

For the Asian population, IVW result indicated that there was no causal effect between circulating vitamin D levels and the risk of RA (odds ratio (OR)=0.911, 95% confidence interval (CI), 0.542-1.534, $P=0.727$) (Table 2). Furthermore, MR Egger (OR=0.937, 95% CI, 0.373-2.352, $P=0.895$), weighted median (OR=0.895, 95% CI, 0.499-1.606, $P=0.711$) and weighted mode (OR=0.904, 95% CI, 0.515-1.588, $P=0.738$) obtained consistent results (Table 2) (Fig. 1a). Leave-one-out sensitivity analysis showed that the results were reliable and stable (Fig. 2a). The results of MR-Egger regression showed that there was no horizontal pleiotropy between instrumental variables and RA ($P=0.946$) (Fig. 3a). Heterogeneity tests demonstrated that there was no significant heterogeneity exist ($P=0.914$) (Fig. 4a).

In the analysis of the European population, the results of IVW (OR=0.897, 95% CI, 0.633-1.269, $P=0.538$), MR Egger (OR=0.853, 95% CI, 0.469-1.553, $P=0.639$), weighted median (OR=0.885, 95% CI, 0.620-1.264, $P=0.503$) and weighted mode (OR=0.898, 95% CI, 0.618-1.306, $P=0.604$) indicated that no causal effect was found between circulating vitamin D levels and RA risk (Table 2) (Fig. 1b). Leave-one-out
sensitivity analysis showed that the results were reliable and stable (Fig. 2b). The results of MR-Egger regression indicated no significant evidence of horizontal pleiotropy ($P=0.850$) (Fig. 3b). In addition, the results showed that there was no heterogeneity among the included instrumental variables ($P=0.338$) (Fig. 4b).

4. Discussion

In the current study, the two-sample MR was performed to infer the potential causal links between vitamin D and RA. The results demonstrated that there was no causal effect of genetically predicted circulating vitamin D on RA risk.

In addition to its inherent functions of regulating calcium and phosphorus metabolism and promoting bone development, the immunomodulatory properties of vitamin D have also been extensively studied. However, existing evidence on the association between vitamin D and RA was contradictory. A study found that the serum 25OH vitamin D level of early RA patients was significantly lower than that of healthy controls [22]. A study focusing on serum and synovial fluid vitamin D metabolites in RA patients found no significant difference in concentrations of 25(OH)D3, 24,25(OH)2D3, 1,25(OH)2D3 and 25(OH)D2 when compared with healthy controls, except for 3-epi-25(OH)D3 [23]. Beyer et al. suggested that most RA patients did not suffer from hypovitaminosis D and it was not related to the differences in the severity of RA as well [24]. However, a recent study showed that vitamin D deficiency was associated with RA activity and severity, and might predict the disability and imaging progression of early RA patients within 1 year [25]. There were some researches on the vitamin D-related gene polymorphisms and RA. A study demonstrated that the serum vitamin D level of RA patients might be normal, but the polymorphisms of the vitamin D receptor (VDR) gene restricted the anti-inflammatory effect of vitamin D by changing the 1,25(OH)2 D3 binding site [26]. A meta-analysis found an association between VDR gene polymorphisms and RA susceptibility [27]. However, the study pointed out that some mutations would make carriers susceptible to RA, while others would reduce the susceptibility of RA.

The reasons for the inconsistent conclusions might be that the sample size was different and the studies with small sample size had a high contingency. In addition, the circulating vitamin D levels of participants were closely related to their eating habits and lifestyles, therefore, it was unreliable to estimate the association between vitamin D and RA without adjusting these confounding factors. However, some data indicated that hypovitaminosis D status was related to the progression of RA and vitamin D supplementation might alleviate the clinical symptoms of RA [28]. A study showed that treatment with vitamin D supplementation in RA patients with secondary osteoporosis could effectively increase bone mineral density [29]. Nevertheless, a study involving 1180 individuals indicated that vitamin D supplementation did not provide other benefits for anti-rheumatic therapy [30]. The specific roles of vitamin D in the pathology of RA and whether vitamin D supplementation is effective remains to be confirmed in the future.

In general, there are several limitations in the present study should be noticed. First, since the absence of clinical parameters, and disease activity information in original GWAS, further subgroup analysis was impossible. Second, our study was unable to determine whether overlapping participants were involved in the exposure and outcome GWAS used in the two sample MR analyses. Nevertheless, the deviation from participants overlap could be minimized by the $F$ statistic [31].

In summary, the two-sample MR analysis suggest that there is no causal effect between circulating vitamin D levels and RA risk. However, further studies should utilize updated data from large-scale GWAS to obtain more reliable results.

Abbreviations

CI: Confidence interval; EAF: effect allele frequency; GWAS: Genome-wide association study; InSIDE: Instrument Strength Independent of Direct Effect; IVW: Inverse variance weighted; MAF: Minor allele frequency; LD: Linkage disequilibrium; MR: Mendelian randomization; OR: Odds ratio; RA: Rheumatoid arthritis; SNP: Single nucleotide polymorphism; VDR: Vitamin D receptor.

Declarations

Acknowledgements

None.

Authors' contributions

HFP conceived of the presented idea. KX performed the computations and manuscript writing. PW and SST were involved in acquisition of data. YQH, YSH, YC, YTF and XKY were involved in interpretation of data. All authors discussed the results and contributed to the final
manuscript.

**Funding**

This work was supported by the National Natural Science Foundation of China (81872687).

**Availability of data and materials**

The dataset generated or analyzed in this study is included with this article and can be made available from the corresponding author upon reasonable request.

**Ethics approval and consent to participate**

The article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no conflict of interest.

**Author details**

1. Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Meishan Road, Hefei, Anhui, China.
2. Inflammation and Immune Mediated Diseases Laboratory of Anhui Province, 81 Meishan Road, Hefei, Anhui, China. 3. Center for Genetic Epidemiology and Genomics, School of Public Health, Soochow University Medical College, Suzhou, China. 4. Department of Rheumatology and Immunology, the First Affiliated Hospital of Anhui Medical University, 218 Jixi Road, Hefei, Anhui, China.

**References**

1. Sparks JA. Rheumatoid Arthritis. Ann Intern Med. 2019;170(1):Itc1–16.
2. Zhang Y, Lu N, Peloquin C, Dubreuil M, Neogi T, Aviña-Zubieta JA, et al. Improved survival in rheumatoid arthritis: a general population-based cohort study. Ann Rheum Dis. 2017;76(2):408–13.
3. Lin JZ, Liu Y, Ma JD, et al. Reduced skeletal muscle independently predicts 1-year aggravated joint destruction in patients with rheumatoid arthritis. Ther Adv Musculoskelet Dis. 2020;12:1759720X20946220.
4. Cao F, Cheng MH, Hu LQ, Shen HH, Tao JH, Li XM, et al. Natural products action on pathogenic cues in autoimmunity: Efficacy in systemic lupus erythematosus and rheumatoid arthritis as compared to classical treatments. Pharmacol Res. 2020;160:105054.
5. Kim K, Bang SY, Lee HS, Bae SC. Update on the genetic architecture of rheumatoid arthritis. Nat Rev Rheumatol. 2017;13(1):13–24.
6. Cyprian F, Lefkou E, Varoudi K, Girardi G. Immunomodulatory Effects of Vitamin D in Pregnancy and Beyond. Front Immunol. 2019;10:2739.
7. Illescas-Montes R, Melguizo-Rodríguez L, Ruiz C, Costela-Ruiz VJ. Vitamin D and autoimmune diseases. Life Sci. 2019;233:116744.
8. Yamamoto E, Jørgensen TN. Immunological effects of vitamin D and their relations to autoimmunity. J Autoimmun. 2019;100:7–16.
9. Dankers W, Colin EM, van Hamburg JP, Lubberts E. Vitamin D in Autoimmunity: Molecular Mechanisms and Therapeutic Potential. Front Immunol. 2016;7:697.
10. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D. Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiol Rev. 2016;96(1):365–408.
11. Molto A, Gossec L, Poiraudon S, et al. Evaluation of the impact of a nurse-led program of systematic screening of comorbidities in patients with axial spondyloarthritis: The results of the COMEDSPA prospective, controlled, one year randomized trial. Semin Arthritis Rheum. 2020;50(4):701–8.
12. Lourido L, Ruiz-Romero C, Picchi F, et al. Association of serum anti-centromere protein F antibodies with clinical response to infliximab in patients with rheumatoid arthritis: A prospective study. Semin Arthritis Rheum. 2020,50(5):1101–8.
13. Abo-Shanab AM, Kholoussi S, Kandil R, Dorgham D. Cytokines, 25-OH vit D and disease activity in patients with juvenile-onset systemic lupus erythematosus. Lupus. 2020;961203320973068.

14. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362:k601.

15. Jiang X, O'Reilly PF, Aschard H, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nat Commun. 2018;9(1):260.

16. Okada Y, Wu D, Trynka G, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature. 2014;506(7488):376–81.

17. Wu F, Huang Y, Hu J, Shao Z. Mendelian randomization study of inflammatory bowel disease and bone mineral density. BMC Med. 2020;18(1):312.

18. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7:e34408.

19. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377–89.

20. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25.

21. Ooi BNS, Loh H, Ho PJ, Milne RL, Giles G, Gao C, et al. The genetic interplay between body mass index, breast size and breast cancer risk: a Mendelian randomization analysis. Int J Epidemiol. 2019;48(3):781–94.

22. Buondonno I, Rovera G, Sassi F, Rigoni MM, Lomater C, Parisi S, et al. Vitamin D and immunomodulation in early rheumatoid arthritis: A randomized double-blind placebo-controlled study. PLoS One. 2017;12(6):e0178463.

23. Li D, Jeffery LE, Jenkinson C, Harrison SR, Chun RF, Adams JS, et al. Serum and synovial fluid vitamin D metabolites and rheumatoid arthritis. J Steroid Biochem Mol Biol. 2019;187:1–8.

24. Beyer K, Lie SA, Kjellevold M, Dahl L, Brun JG, Bolstad Al. Marine ω-3, vitamin D levels, disease outcome and periodontal status in rheumatoid arthritis outpatients. Nutrition. 2018;55–56:116 – 24.

25. Mouterde G, Gamon E, Rincheval N, Lukas C, Seror R, Berenbaum F, et al. Association Between Vitamin D Deficiency and Disease Activity, Disability, and Radiographic Progression in Early Rheumatoid Arthritis: The ESPOIR Cohort. J Rheumatol. 2020;47(11):1624–8.

26. Mukhtar M, Sheikh N, Suqaina SK, Batool A, Fatima N, Mehmood R, et al. Vitamin D Receptor Gene Polymorphism: An Important Predictor of Arthritis Development. Biomed Res Int. 2019;2019:8326246.

27. Bagheri-Hosseinabadi Z, Imani D, Yousefi H, AbbasiFard M. Vitamin D receptor (VDR) gene polymorphism and risk of rheumatoid arthritis (RA): systematic review and meta-analysis. Clin Rheumatol. 2020;39(12):3555–69.

28. Gioia C, Lucchino B, Tarsitano MG, Iannuccelli C, Di Franco M. Dietary Habits and Nutrition in Rheumatoid Arthritis: Can Diet Influence Disease Development and Clinical Manifestations? Nutrients. 2020;12(5):1456.

29. Kwon OC, Oh JS, Park MC, Kim YG. Effect of Vitamin D Supplementation on Bone Mineral Density in Rheumatoid Arthritis Patients With Osteoporosis. Front Med (Lausanne). 2020;7:443.

30. Wu J, Dong J, Li S, Luo J, Zhang Y, Liu H, et al. The Role of Vitamin D in Combination Treatment for Patients With Rheumatoid Arthritis. Front Med (Lausanne). 2020;7:312.

31. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. Am J Epidemiol. 2013;178(7):1177–84.

Tables
| Ethnicity | SNP        | Effect allele | Other allele | Vitamin D Beta | SE | P value | RA Beta | SE | P value | Proxy SNP | Target effect allele | Target other allele |
|-----------|------------|---------------|--------------|----------------|----|---------|---------|----|---------|-----------|----------------------|---------------------|
| Asian     | rs10745742 | T             | C            | 0.017          | 0.002 | 1.88E-14 | -0.020 | 0.026 | 0.38    | rs1968487 | T                     | C                   |
|           | rs6839473  | A             | G            | 0.017          | 0.002 | 1.79E-15 | -0.062 | 0.167 | 0.73    | -         | -                    | -                   |
|           | rs2597193  | A             | G            | 0.019          | 0.002 | 6.26E-17 | 0.020  | 0.030 | 0.43    | -         | -                    | -                   |
|           | rs17216707 | C             | T            | -0.026         | 0.003 | 8.14E-23 | 0.010  | 0.056 | 0.89    | -         | -                    | -                   |
|           | rs10741657 | G             | A            | -0.031         | 0.002 | 2.05E-46 | 0.010  | 0.026 | 0.56    | -         | -                    | -                   |
|           | rs12785878 | T             | G            | 0.036          | 0.002 | 3.81E-62 | 0.010  | 0.028 | 0.58    | -         | -                    | -                   |
|           | rs2282679  | G             | T            | -0.089         | 0.002 | 1.00E-200| 0.010  | 0.028 | 0.73    | -         | -                    | -                   |
| European  | rs10745742 | T             | C            | 0.017          | 0.002 | 1.88E-14 | 0.010  | 0.018 | 0.59    | rs2117916 | A                     | G                   |
|           | rs6839473  | A             | G            | 0.017          | 0.002 | 1.79E-15 | -0.030 | 0.022 | 0.16    | -         | -                    | -                   |
|           | rs2597193  | A             | G            | 0.019          | 0.002 | 6.26E-17 | 0.020  | 0.020 | 0.32    | -         | -                    | -                   |
|           | rs12785878 | T             | G            | 0.036          | 0.002 | 3.81E-62 | -0.010 | 0.022 | 0.65    | -         | -                    | -                   |
|           | rs2282679  | G             | T            | -0.089         | 0.002 | 1.00E-200| 0.010  | 0.017 | 0.56    | -         | -                    | -                   |

Abbreviations: SNP, single nucleotide polymorphism; RA, rheumatoid arthritis
### Table 2. MR estimates of circulating vitamin D levels and the RA risk

| Ethnicity   | Methods      | Nsnp | Beta  | SE    | OR (95% CI)       | $P$ value | Horizontal pleiotropy | Heterogeneity | $F$ statistics |
|-------------|--------------|------|-------|-------|-------------------|-----------|----------------------|---------------|----------------|
|             |              |      |       |       |                   |           | Egger intercept | SE | $P$ value | Cochran's $Q$ | $P$ value |             |
| Asian       | MR Egger     | 7    | -0.065| 0.469 | 0.937 (0.373-2.352) | 0.895     | -0.002             | 0.021         | 0.946          | 1.494        | 0.914       | 255.02      |
|             | Weighted median | 7    | -0.111| 0.298 | 0.895 (0.499-1.606) | 0.711     |                   |               |               |             |            |             |
|             | Inverse variance weighted | 7    | -0.093| 0.266 | 0.911 (0.542-1.534) | 0.727     |                   |               |               |             |            |             |
|             | Weighted mode | 7    | -0.101| 0.287 | 0.904 (0.515-1.588) | 0.738     |                   |               |               |             |            |             |
| European    | MR Egger     | 5    | -0.159| 0.306 | 0.853 (0.469-1.553) | 0.639     |                   | 0.003         | 0.015          | 0.850        | 3.372       | 0.338       | 290.93      |
|             | Weighted median | 5    | -0.122| 0.182 | 0.885 (0.620-1.264) | 0.503     |                   |               |               |             |            |             |
|             | Inverse variance weighted | 5    | -0.109| 0.177 | 0.897 (0.633-1.269) | 0.538     |                   |               |               |             |            |             |
|             | Weighted mode | 5    | -0.107| 0.191 | 0.898 (0.618-1.306) | 0.604     |                   |               |               |             |            |             |

Abbreviations: SNP, single nucleotide polymorphism; RA, rheumatoid arthritis; OR, odds ratio

### Figures

![Figure A](image1)

![Figure B](image2)
Figure 1
Forest plots of the causal effects of circulating vitamin D levels on RA risk (A: Asian; B: European)

Figure 2
Sensitivity analyses of the causal effects of circulating vitamin D levels on RA risk (A: Asian; B: European)

Figure 3
Scatter plots of the causal effects of circulating vitamin D levels on RA risk (A: Asian; B: European)
Figure 4

Funnel plots of the causal effects of circulating vitamin D levels on RA risk (A: Asian; B: European)