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## Redaktion

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## Electronic supplementary material

The online version of this article (<https://doi.org/10.1007/s00393-018-0504-8>) contains supplementary material, which is available to authorized users.

## Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammatory changes in the synovial membranes and articular structures leading to bone structure damage, causing disability and a decreased quality of life [9, 21]. Alzheimer's disease (AD) is a complex, progressive, and irreversible neurodegenerative disease of the brain, which is also the most common form of dementia, a general term for memory loss and other cognitive impairments that interfere with daily life [18]. Although the etiologies of RA and AD are not fully understood, it is known that both diseases undergo an inflammatory process, while the pathogenesis of Alzheimer's disease is multifactorial [13]. The deregulation of multiple genetic and environmental factors involved in the inflammatory cascade is a common feature in both RA and AD [11, 12].

An inverse relationship between RA and AD has been previously reported, but the evidence is inconsistent [27].

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# Causal association between rheumatoid arthritis and a decreased risk of Alzheimer's disease

## A Mendelian randomization study

Meta-analyses of observational studies have shown that RA was inversely associated with the risk of AD [27]. RA and/or nonsteroidal anti-inflammatory drugs (NSAIDs) used for the treatment of RA have been considered to be one of the risk factors for AD [23]. However, observational studies are prone to bias such as reverse causation and residual confounding, thereby precluding a clear understanding of the protective effect of RA on AD [17, 22].

Mendelian randomization (MR) is a technique that uses genetic variants as instrumental variables (IVs) to assess whether an observational association between a risk factor or exposure and an outcome is consistent with a causal effect [6]. A two-sample MR estimates causal effects, in which the exposure and outcome data are measured in different samples [20]. It is very useful in situations where it is difficult to measure the

exposure and outcome in the same set of individuals [20]. The aim of this study was to examine whether RA is causally associated with AD using a two-sample MR analysis.

## Materials and methods

### Data sources and selection of genetic variants

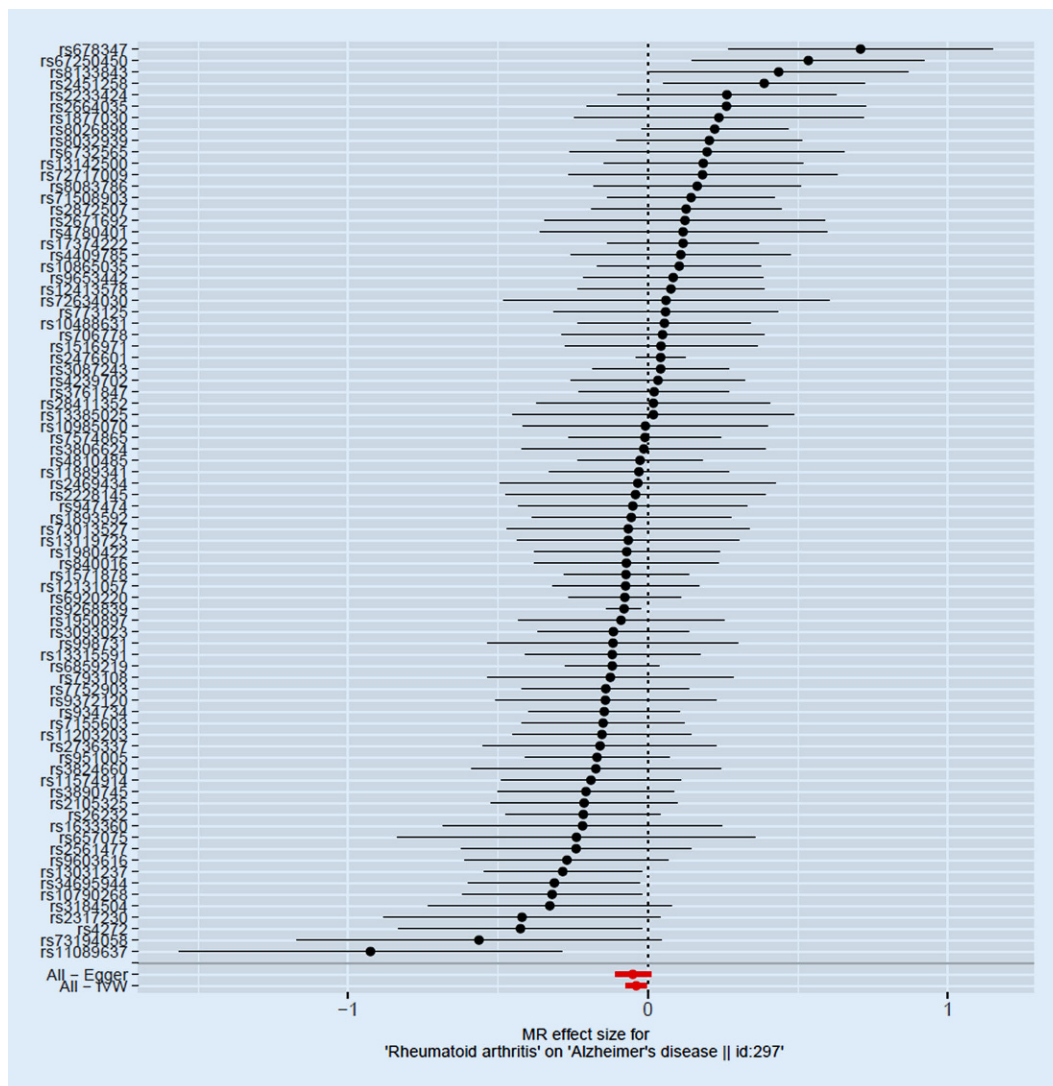
We used publicly available summary statistics datasets consisting of three-stage trans-ethnic, European-specific, and Asian-specific genomewide association study (GWAS) meta-analyses, from which we evaluated 10 million single nucleotide polymorphisms (SNPs) in 29,880 RA cases and 73,758 controls assigned as exposures [24]. The summary statistics from a meta-analysis of four GWAS datasets consisting of 17,008 AD cases and 37,154 controls of European

**Table 1** The MR estimates from each method of the causal effect of rheumatoid arthritis on the risk of Alzheimer's disease

MR method	Number of SNPs	Beta ( $\beta$ )	SE	Association P-value	Cochran Q statistic	Heterogeneity P-value
Inverse-variance weighted	80	-0.039	0.017	0.021	107.3	0.019
MR-Egger	80	-0.050	0.030	0.096	108.4	0.013
Weighted median	80	-0.078	0.024	0.001	108.5 <sup>a</sup>	0.015 <sup>a</sup>

MR Mendelian randomization, SNP single nucleotide polymorphism,  $\beta$   $\beta$  coefficient, SE standard error

<sup>a</sup>Maximum likelihood method



**Fig. 1** ◀ Forrest plot of the causal effects of rheumatoid arthritis-associated single nucleotide polymorphisms on Alzheimer's disease. MR Mendelian randomization

ancestry were genotyped and imputed with 7,055,881 SNPs as outcomes [19]. For our two-sample MR study, we used genetic variants associated with RA as IVs to improve inference. We obtained summary statistics ( $\beta$  coefficients and standard errors [SE]) for 80 SNPs associated with RA as IVs from three gout GWAS datasets [24]. In addition, we utilized summary data for 80 AD-associated SNPs from GWAS as outcomes [19].

### Statistical analysis for Mendelian randomization

MR analysis requires genetic variants to be related to, but not potential confounders of, an exposure [5]. First, we assessed the independent association of

80 SNPs with RA. Second, we examined the association between each SNP and risk of AD. Third, we combined these findings to estimate the uncompounded causal association between RA and risk of AD using MR analysis. We performed a two-sample MR, which is a method used to estimate the causal effect of an exposure (RA) on outcomes (AD) using summary statistics from different GWAS datasets [15]. In order to assess the causal relationships between RA and Alzheimer's disease risk, we used summary data from GWAS on RA and AD along with their respective 80 SNPs, which were used as IVs.

The inverse-variance weighted (IVW) method uses a meta-analysis approach to combine the Wald ratio estimates of the causal effect obtained from different

SNPs and provides a consistent estimate of the causal effect of the exposure on the outcome when each of the genetic variants satisfy the assumptions of an IV [26]. Although the inclusion of multiple variants in an MR analysis results in increased statistical power, it has the potential to include pleiotropic genetic variants that are invalid IVs [15]. To explore and adjust for pleiotropy (association of genetic variants with more than one variable), the weighted median and MR-Egger regression methods were performed. MR-Egger regression analysis accounts for the presence of unbalanced pleiotropy by introducing a parameter for this bias, thereby incorporating summary data estimates of causal effect from multiple individual variants, which is robust to invalid instruments [2]. MR-Eg-

S.-C. Bae · Y. H. Lee

**Causal association between rheumatoid arthritis and a decreased risk of Alzheimer's disease. A Mendelian randomization study****Abstract****Objective.** This study aimed to examine whether rheumatoid arthritis (RA) is causally associated with Alzheimer's disease (AD).**Methods.** We performed a two-sample Mendelian randomization (MR) analysis using the inverse-variance weighted (IVW), weighted median, and MR-Egger regression methods. We used the publicly available summary statistics datasets from three-stage trans-ethnic genome-wide association studies (GWAS) meta-analyses of 29,880 RA cases and 73,758 controls as exposures and a meta-analysis of 4 GWAS datasets consisting of 17,008 AD cases and 37,154 controls of European descent as outcomes.**Results.** We selected 80 single nucleotide polymorphisms (SNPs) from GWAS data on RA as instrumental variables (IVs), 60 of which were associated with RA on a genome-wide significance level. The IVW method showed evidence to support an inverse causal association between RA and AD ( $\beta = -0.039$ , standard error [SE] = 0.017,  $P = 0.021$ ). MR-Egger regression revealed that directional pleiotropy was unlikely to be a source of bias in the results (intercept = 0.002;  $P = 0.649$ ). The MR-Egger analysis showed no causal association between RA and AD ( $\beta = -0.050$ , SE = 0.030,  $P = 0.096$ ). However, the weighted median approach showed that RA and ADwere causally linked ( $\beta = -0.078$ , SE = 0.024,  $P = 0.001$ ). The funnel plot did not show heterogeneity between IV estimates based on the individual variants.**Conclusions.** The MR analysis supports that RA was causally associated with a reduced risk of AD.**Keywords**

Rheumatoid arthritis · Alzheimer's disease · Mendelian randomization · Causal association · Susceptibility

**Kausalzusammenhang zwischen rheumatoider Arthritis und vermindertem Risiko für M. Alzheimer. Eine Mendel-Randomisierungsstudie****Zusammenfassung****Ziel.** Die vorliegende Studie zielte darauf ab zu untersuchen, ob rheumatoide Arthritis (RA) in einem kausalen Zusammenhang mit M. Alzheimer stehe.**Methoden.** Dazu wurde die Analyse einer 2-Stichproben-Mendel-Randomisierung (MR) unter Einsatz von Verfahren mit inverser Varianzgewichtung (IVW), gewichtetem Mittel und der MR-Egger-Regression durchgeführt. Die Autoren verwendeten die Metaanalysen der öffentlich zugänglichen zusammenfassenden statistischen Datensätze von dreistufigen transethnischen genomweiten Assoziationsstudien (GWAS) mit 29.880 RA-Fällen und 73.758 Kontrollen als Exposition und eine Metaanalyse von 4 GWAS-Datensätzen mit 17.008 AD (Alzheimer's disease)-Fällen und

37.154 Kontrollen europäischer Abstammung als Endpunkte.

**Ergebnisse.** Als instrumentelle Variablen (IV) wurden 80 Einzelnukleotidpolymorphismen (SNPs) aus den GWAS-Daten zur RA ausgewählt, von denen 60 auf einem genomweiten Signifikanzniveau mit RA assoziiert waren. Die IVW-Methode erbrachte Belege für die Stützung eines inversen Kausalzusammenhangs zwischen RA und AD ( $\beta = -0,039$ ; Standardfehler, „standard error“, SE: 0,017,  $p = 0,021$ ). Anhand der MR-Egger-Regression zeigte sich, dass es unwahrscheinlich war, dass die directionale Pleiotropie eine Quelle für Bias in den Ergebnissen darstellte („intercept“ = 0,002;  $p = 0,649$ ). Die MR-Egger-Analyse ergab keinen Kausalzusammenhangzwischen RA und AD ( $\beta = -0,050$ ; SE = 0,030;  $p = 0,096$ ). Allerdings zeigte der Ansatz unter Verwendung des gewichteten Mittels, dass RA und AD kausal verknüpft waren ( $\beta = -0,078$ ; SE = 0,024;  $p = 0,001$ ). Der Funnel Plot ergab keine Heterogenität zwischen IV-Schätzwerten auf der Basis der individuellen Varianten.**Schlussfolgerung.** Die MR-Analyse lieferte Hinweise darauf, dass die RA kausal mit einem verminderten Risiko für M. Alzheimer verknüpft sei.**Schlüsselwörter**

Rheumatoide Arthritis · M. Alzheimer · Mendel-Randomisierung · Kausalzusammenhang · Anfälligkeit

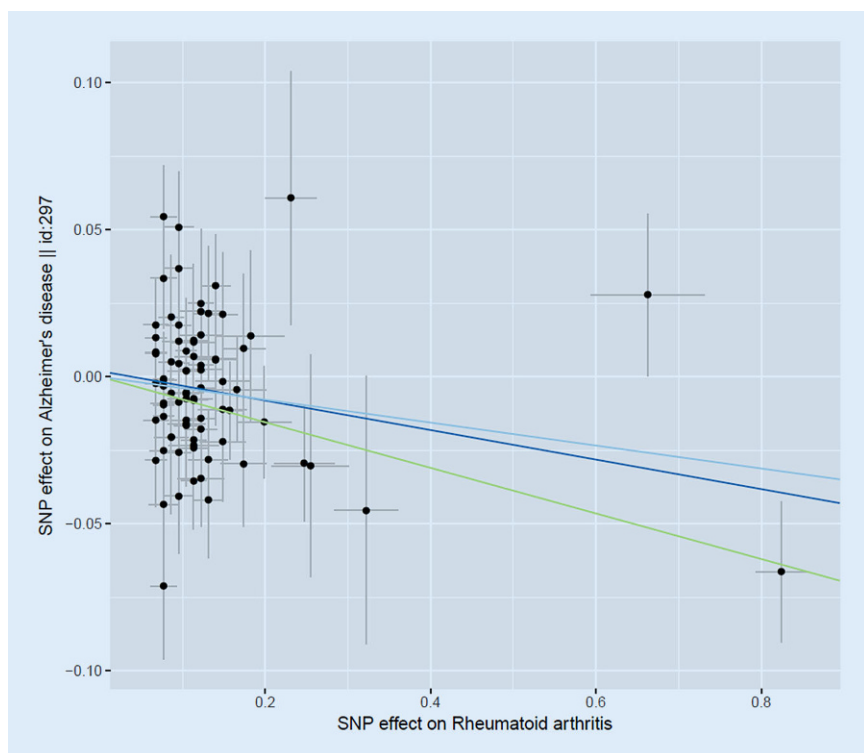
ger is a weighted linear regression of the gene-outcome coefficients on the gene-exposure coefficients [2]. The slope of this regression represents the estimate of the causal effect and the intercept can be interpreted as an estimate of the average horizontal pleiotropic effect across the genetic variants [7]. The weighted median estimator provides a consistent estimate of the causal effect even when up to 50% of the information contributing to the analysis comes from genetic variants that are invalid IVs [3]. The weighted

median estimator has an advantage of retaining greater precision of the estimates over the MR-Egger analysis [3]. Tests were considered statistically significant when  $P < 0.05$ . All MR analyses were performed with MRBase platform [16].

**Heterogeneity and sensitivity test**

We assessed the heterogeneities between SNPs using Cochran's Q statistics and funnel plots [10]. We also performed a "leave one out" analysis to investigate

the possibility of causal association being driven by a single SNP. We performed a subgroup analysis using only IV SNPs at a genome-wide significance for sensitivity test.



**Fig. 2** ▲ Scatter plots of genetic associations of rheumatoid arthritis against those of Alzheimer's disease. The slopes of each line represent the causal association for each method. *Blue line* represents the inverse-variance weighted estimate, *green line* represents the weighted median estimate, and *dark blue line* represents the MR(Mendelian randomization)-Egger estimate. *SNP* single nucleotide polymorphism

## Results

### Studies included in the meta-analysis

#### Instrumental variables for Mendelian randomization

We selected 80 SNPs as IVs from the GWAS data on RA and 60 of those which were associated with RA had genome-wide significance (supplementary data). Forty-six of all SNPs were inversely associated with AD, and the association with 10 SNPs was statistically significant (supplementary data).

#### Mendelian randomization results

The IVW method supported an inverse causal association between RA and AD ( $\beta = -0.039$ ,  $SE = 0.017$ ,  $P = 0.021$ ; [Table 1](#); [Figs. 1 and 2](#)). The intercept represents the average pleiotropic effect across the genetic variants (the average direct effect of a variant with the outcome). If the intercept differs from zero (the MR-

Egger test), then there is evidence for directional pleiotropy. MR-Egger regression revealed that directional pleiotropy was unlikely to have a bias on the results (intercept = 0.002;  $P = 0.649$ ). The MR-Egger analysis showed no causal association between RA and AD ( $\beta = -0.050$ ,  $SE = 0.030$ ,  $P = 0.096$ ; [Table 1](#); [Figs. 1 and 2](#)). However, weighted median approach supported a causal association between RA and AD ( $\beta = -0.078$ ,  $SE = 0.024$ ,  $P = 0.001$ ; [Table 1](#); [Fig. 2](#)). The inverse association between RA and AD was not consistent with MR-Egger and weighted median methods. The IVW and weighted median methods suggest a negative causal effect of RA on AD risk, whereas the MR-Egger method suggests a null causal effect. Considering that the weighted median estimator has the advantage of retaining greater precision of the estimates compared to the MR-Egger analysis [3], the results of MR analysis may support an inversely causal association between RA and AD.

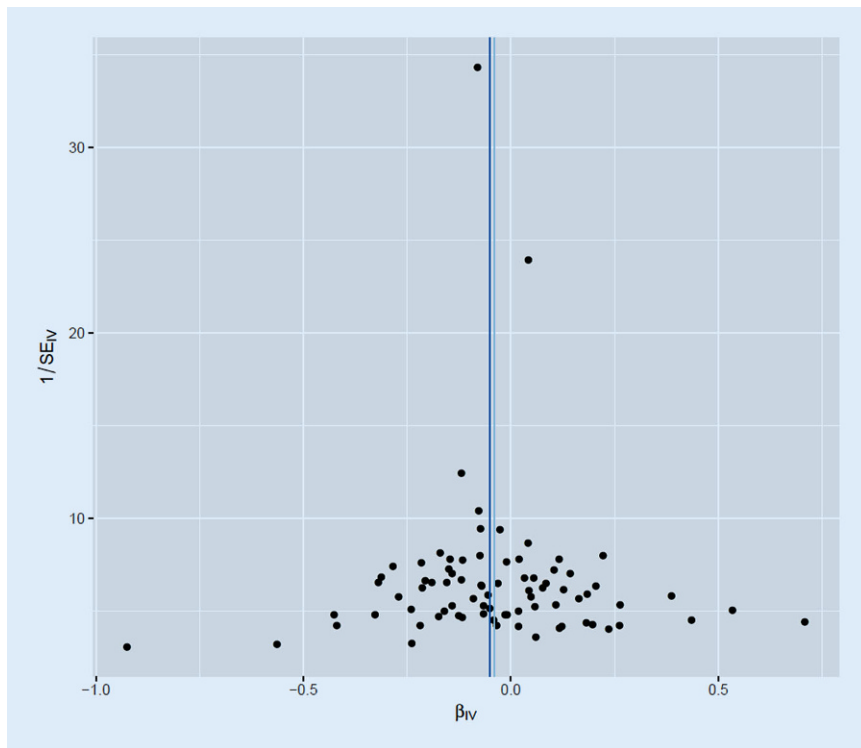
## Heterogeneity and sensitivity test

Cochran's Q test indicated evidence of heterogeneity between IV estimates based on the individual variants, but funnel test showed a symmetry, indicating no evidence of heterogeneity ([Table 1](#); [Fig. 3](#)). Results from a "leave one out" analysis demonstrated that no single SNP was driving the IVW point estimate, except for rs9268839. When the MR analysis was limited to 60 SNPs at genome-wide significance, the results remained significant (weighted median approach  $\beta = -0.078$ ,  $SE = 0.004$ ,  $P = 0.004$ ).

## Discussion

RA has been reported to be a protective factor against the development of AD [23, 27]. It remains unclear whether RA has a causal relationship with AD, as it was previously reported that association of AD with factors like smoking may be a result of bias or confounding factors inherent to observational studies, including nonsteroidal anti-inflammatory drugs (NSAID) use, reverse causation, small study numbers and sizes, and selection biases [17, 22]. We carried out three different estimating methods (inverse variance weighting method, weighted median method, and MR-Egger regression) for the MR analyses. Although the MR estimates using IVW, MR-Egger, and weighted median analysis were not consistent, IVW and weighted median analysis supported an inverse causal association between RA and AD. Considering that the weighted median estimator has the advantage of retaining greater precision in the estimates compared to the MR-Egger analysis [3], this MR analysis indicated a causal role of RA on the risk of Alzheimer's disease. Thus, our study corroborates the inverse association found in previous observational studies [23, 27].

MR minimizes the possibility of bias inherent to observational studies due to residual confounding or reverse causality [29]. The genetic variant may be associated with multiple phenotypes, a phenomenon known as pleiotropy, which may result in a confounded estimate from



**Fig. 3** ▲ Funnel plot assessing heterogeneity. *Blue line* represents the inverse-variance weighted estimate, and *dark blue line* represents the MR-Egger estimate. *SE* standard error, *IV* instrumental variable,  $\beta$   $\beta$  coefficient

MR and may potentially lead to biased causal estimates [25]. Although the inclusion of multiple variants in MR analyses typically lead to an increased statistical power, it also results in the potential inclusion of pleiotropic genetic variants that are invalid IVs [30]. Therefore, approaches used in sensitivity analyses need to be applied to ensure the validity of conclusions drawn from the MR study. To eliminate pleiotropy, we employed a weighted median estimator, which provides valid estimates even in the case of 50% of SNPs being invalid instruments [3], as well as using an MR-Egger regression to test for unbalanced pleiotropy and to determine the causal estimate exposure's influence on the outcome [2]. The MR-Egger approach showed no evidence of unbalanced pleiotropy and no causal association between RA and AD. However, the MR-Egger method results in a loss of precision and power [28]. The weighted median estimator is greater in precision of the estimates compared to the MR-Egger analysis. Our weighted median estimator results were similar to those of the IVW estimator, thereby

strengthening our confidence in these associations. Our data support the previous observational studies, which have shown an inverse association between RA and AD. Current findings may provide an opportunity to find the underlying mechanisms of the effects of RA on the AD risk factors.

Certain plausible mechanisms could predict how RA is linked to the occurrence of AD. First, the inverse incidence rates between RA and AD cases might be explained by the deregulation of intrinsic cellular and molecular regulators of the inflammatory cascades [13]. Granulocyte macrophage colony stimulating factor (GM-CSF), highly produced in RA synovitis, can induce the differentiation of myeloid cells into specializing cells such as microglia [4]. GM-CSF overexpression could act on bone marrow-derived microglia, transforming them into a proper amyloid-clearing factory to remove amyloid deposition [4]. Moreover, GM-CSF seems to have a trophic effect on neuronal cells, such as enhancing cerebral performance including short-term memory skills [4]. Second, balance in

the pathways of interleukin-6 and microRNA-146a is likely to play a critical role in controlling the incidence of AD in treated RA patients [1, 11, 12, 14].

This MR study differs from a previous MR study on the causal relationship between RA and AD risk performed by Policicchio et al. [27], because our data were obtained from the trans-ethnic GWAS meta-analysis of European and Asian subjects, which increased statistical power due to an increased sample size. Our findings on the protective effects of RA on the occurrence of AD are not consistent with those of Policicchio and group. Therefore, this discrepancy indicates a need for further MR studies on this subject.

The present study has several limitations. First, genetic variants have a modest effect on RA, as they only explain a very small proportion of variance in a particular exposure [31]. This means that a very large number of cases are required to detect a causal relationship for the outcome of interest. Second, it is important to ensure that there is a strong relationship between the genetic variant and an exposure. "Weak instrument bias," which refers to a genetic variant that does not have a sufficiently strong association with the exposure, may affect this MR analysis [8]. However, the sensitivity test performed to limit our analysis of SNPs at a genome-wide significance did not change our results. Third, the AD study was based on participants of European ancestry. As causality may depend on ethnicity and selection bias, further MR studies are needed for other populations for a holistic understanding.

In conclusion, the MR analysis suggested the inverse association between RA and AD. Considering that the weighted median estimator has the advantage of retaining greater precision in the estimates compared to the MR-Egger analysis, the results of MR analysis supported that RA was causally associated with a reduced risk of AD.

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## Compliance with ethical guidelines

**Conflict of interest.** S.-C. Bae and Y.H. Lee declare that they have no competing interests.

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

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