Rheumatoid arthritis and risk of atrial fibrillation: evidence from pooled cohort studies and Mendelian randomization analysis

Supplementary files

Supplementary files:

Table S1. PRISMA 2020 checklist.

Table S2. Search strategy.

Table S3. STROBE-MR-checklist.

Table S5. Quality and bias of the included studies.

Figure S1. Funnel plot showed no significant heterogeneity among single SNPs.

Figure S2. Leave-one-out analysis for genetically predicted RA and AF.

Table S1. PRISMA 2020 checklist.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 4
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 4 and Table S2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 4,5
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 4,5
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 4,5

Section and Topic	Item #	Checklist item	Location where item is reported
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	NA
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Page 4,5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 4,5
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 5
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	NA
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Page 5
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 5
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Page 5
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NA
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 5
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 2

Section and Topic	Item #	Checklist item	Location where item is reported
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	NA
Study characteristics	17	Cite each included study and present its characteristics.	Page 7
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table S3
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Figure 3
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 7
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Figure 3
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Page 7
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Figure 3
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Figure 3
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 8,9
	23b	Discuss any limitations of the evidence included in the review.	Page 10
	23c	Discuss any limitations of the review processes used.	NA
	23d	Discuss implications of the results for practice, policy, and future research.	Page 8,9,10
OTHER INFORMATION			
Registration and	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	NA

Section and Topic	Item #	Checklist item	Location where item is reported
protocol	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 4
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 11
Competing interests	26	Declare any competing interests of review authors.	Page 11
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Page 11

Table S2. Search strategy.

PubMed	**Terms**
#1	atrial fibrillation [MeSH Terms]
#2	atrial fibrillation
#3	#1 or #2
#4	rheumatoid arthritis [MeSH Terms]
#5	rheumatoid arthritis
#6	#4 or #5
#7	#3 and #6
Embase	
#1	'atrial fibrillation':ti,ab,kw
#2	'rheumatoid arthritis':ti,ab,kw
#3	#1 and #2
Web of Science	
#1	atrial fibrillation (Topic)
#2	rheumatoid arthritis (Topic)
#3	#1 and #2

Table S3: STROBE-MR checklist of recommended items to address in reports of Mendelian randomization studies

Item No.	Section	Checklist item	Page No.	Relevant text from manuscript
1	**TITLE and ABSTRACT**	Indicate Mendelian randomization (MR) as the study's design in the title and/or the abstract if that is a main purpose of the study	Page 1	Rheumatoid arthritis and risk of atrial fibrillation: results from pooled cohort studies and Mendelian randomization analysis
	INTRODUCTION			
2	**Background**	Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question	Page 3	Rheumatoid arthritis (RA) is a chronic, systemic, destructive autoimmune disease that involves primarily joints and can affect multiple organs, including cardiovascular systems.
3	**Objectives**	State specific objectives clearly, including pre-specified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects	Page 3	The Mendelian randomization (MR) analysis can eliminate these limitations and has emerged as a powerful tool to identify more reliable associations than traditional observational studies by leveraging the random assortment of alleles during meiosis.
	METHODS			
4	**Study design and data sources**	Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:	Table S5	
		a) Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available.		

		b)	Participants: Give the eligibility criteria, and the sources and methods of selection of participants. Report the sample size, and whether any power or sample size calculations were carried out prior to the main analysis	Page 6	The power online analysis platform (https://shiny.cnsgenomics.com/mRnd/) was used to calculate power for MR.
		c)	Describe measurement, quality control and selection of genetic variants		
		d)	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	Page 5-6	Data sources and selection of instrumental variables (IVs)
		e)	Provide details of ethics committee approval and participant informed consent, if relevant		
5	**Assumptions**		Explicitly state the three core IV assumptions for the main analysis (relevance, independence and exclusion restriction) as well assumptions for any additional or sensitivity analysis	Page 5	Figure 1 provides an overview of the MR design
6	**Statistical methods: main analysis**		Describe statistical methods and statistics used		
		a)	Describe how quantitative variables were handled in the analyses (i.e., scale, units, model)		
		b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected		
		c)	Describe the MR estimator (e.g. two-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples		
		d)	Explain how missing data were addressed		
		e)	If applicable, indicate how multiple testing was addressed		
7	**Assessment of assumptions**		Describe any methods or prior knowledge used to assess the assumptions or justify their validity	Page 5	The random-effects inverse variance weighted (IVW) method was used as the main MR method, other methods (MR Egger, Weighted median, Simple mode, Weighted mode) were used as supplementary analyses.

				The results were presented as odds ratio (OR) with 95% confidence intervals (CIs).
8	**Sensitivity analyses and additional analyses**	Describe any sensitivity analyses or additional analyses performed (e.g. comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations)	Page 5	Sensitivity analyses including heterogeneity test, funnel plot, pleiotropy test, and leave-one-out sensitivity test were employed to evaluate the robustness of the results. Heterogeneity was assessed with the Cochran's Q test. Pleiotropy was accessed with the MR-PRESSO test.
9	**Software and pre-registration**			
	a)	Name statistical software and package(s), including version and settings used	Page 5	R version 4.2.1 and TwoSampleMR package version 0.5.6.
	b)	State whether the study protocol and details were pre-registered (as well as when and where)	NA	
	RESULTS			
10	**Descriptive data**			
	a)	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram	NA	
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	NA	
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	NA	
	d)	For two-sample MR: i. Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples	NA	

		ii. Provide information on the number of individuals who overlap between the exposure and outcome studies		
11	**Main results**			
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	Page 7	These SNPs explain approximately 27.44% of the variation in RA patients.
	b)	Report MR estimates of the relationship between exposure and outcome, and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference	Page 7	The IVW method showed no statistically significant difference in the genetic predisposition risk for RA and AF (OR = 1.009, 95% CI: 0.986 ~ 1.032, P = 0.449).
	c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA	
	d)	Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	Figure S1	
12	**Assessment of assumptions**			
	a)	Report the assessment of the validity of the assumptions		
	b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2, Q statistic or E-value)	Page 7	No significant heterogeneity in SNP effects was observed by Cochran's Q test (P = 0.584) and funnel plot (Figure S1).
13	**Sensitivity analyses and additional analyses**			
	a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	Page 7	No significant heterogeneity in SNP effects was observed by Cochran's Q test (P = 0.584) and funnel plot (Figure S1).

	b)	Report results from other sensitivity analyses or additional analyses	Page 7	The MR-Egger test (intercept = -2.546 × 10−3, SE = 3.348 × 10−3, P = 0.455) showed there is no detectable directional pleiotropy
	c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	NA	
	d)	When relevant, report and compare with estimates from non-MR analyses		
	e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	Page 7	No single SNP was found to strongly or reversely influence the overall effect of RA on AF in the leave-one-out analysis (Figure S2).
	DISCUSSION			
14	**Key results**	Summarize key results with reference to study objectives	Page 8	However, as opposed to the meta-analysis, the causality between genetically predisposed RA and AF risk was not supported by the MR analysis.
15	**Limitations**	Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them	Page 9	. Furthermore, after correction for heart failure and type 2 diabetes, the results remained consistent, further supporting our MR analysis. Similarly, MR analysis may yield different results due to variations in data sources and statistical methods.
16	**Interpretation**			
	a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	Page 9-10	The disparities between observational studies and MR analysis conclusions lead us to consider several potential reasons.
	b)	Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-	Page 9-10	

		environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions	
		c) Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	Page 9-10
17	**Generalizability**	Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure	NA
	OTHER INFORMATION		
18	**Funding**	Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based	Page 11
19	**Data and data sharing**	Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article, or report whether the code is publicly accessible and if so, where	
20	**Conflicts of Interest**	All authors should declare all potential conflicts of interest	Page 11

Table S5. Quality and bias of the included studies.

	Lindhardsen J, et al.	Kim SC, et al.	Bacani AK, et al.	Jang SY, et al.	Argnani L, et al.	Tilly MJ, et al.
Selection bias						
Representativeness of the exposed cohort	★	★		★	★	★
Selection of the nonexposed cohort	★	★	★	★	★	★
Ascertainment of exposure	★	★	★	★	★	★
Demonstration that the outcome of interest was not present at the start of the study	★	★	★	★	★	★
Comparability						
Comparability of cohorts on the basis of the design or analysis	★★	★★	★★	★★	★★	★★
Outcomes						
Assessment of outcome	★	★	★	★	★	★
Follow-up of sufficient duration for outcomes to occur	★		★	★	★	★
Adequacy of the follow-up of cohorts	★	★	★	★	★	★

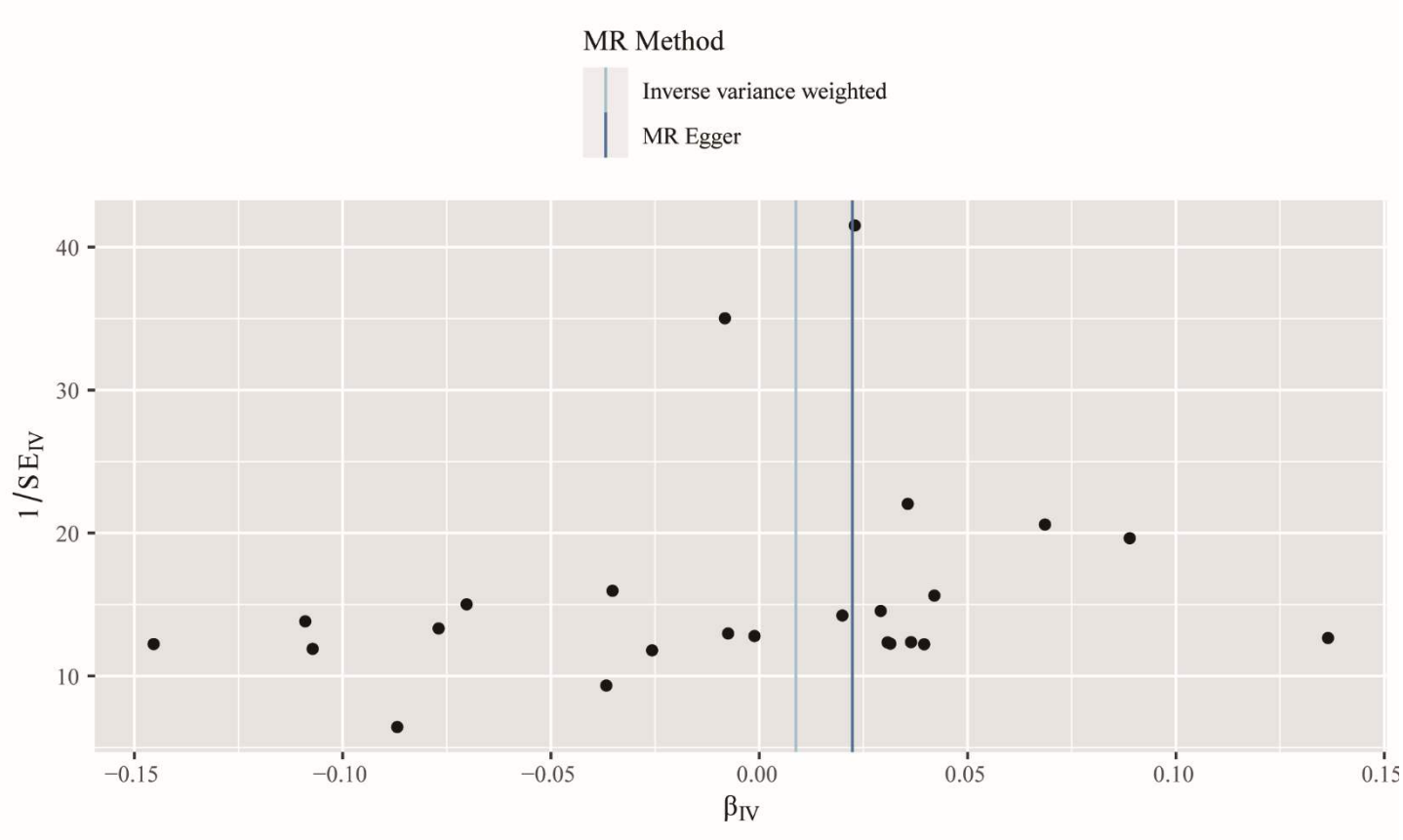

Figure S1. Funnel plot showed no significant heterogeneity among single SNPs.

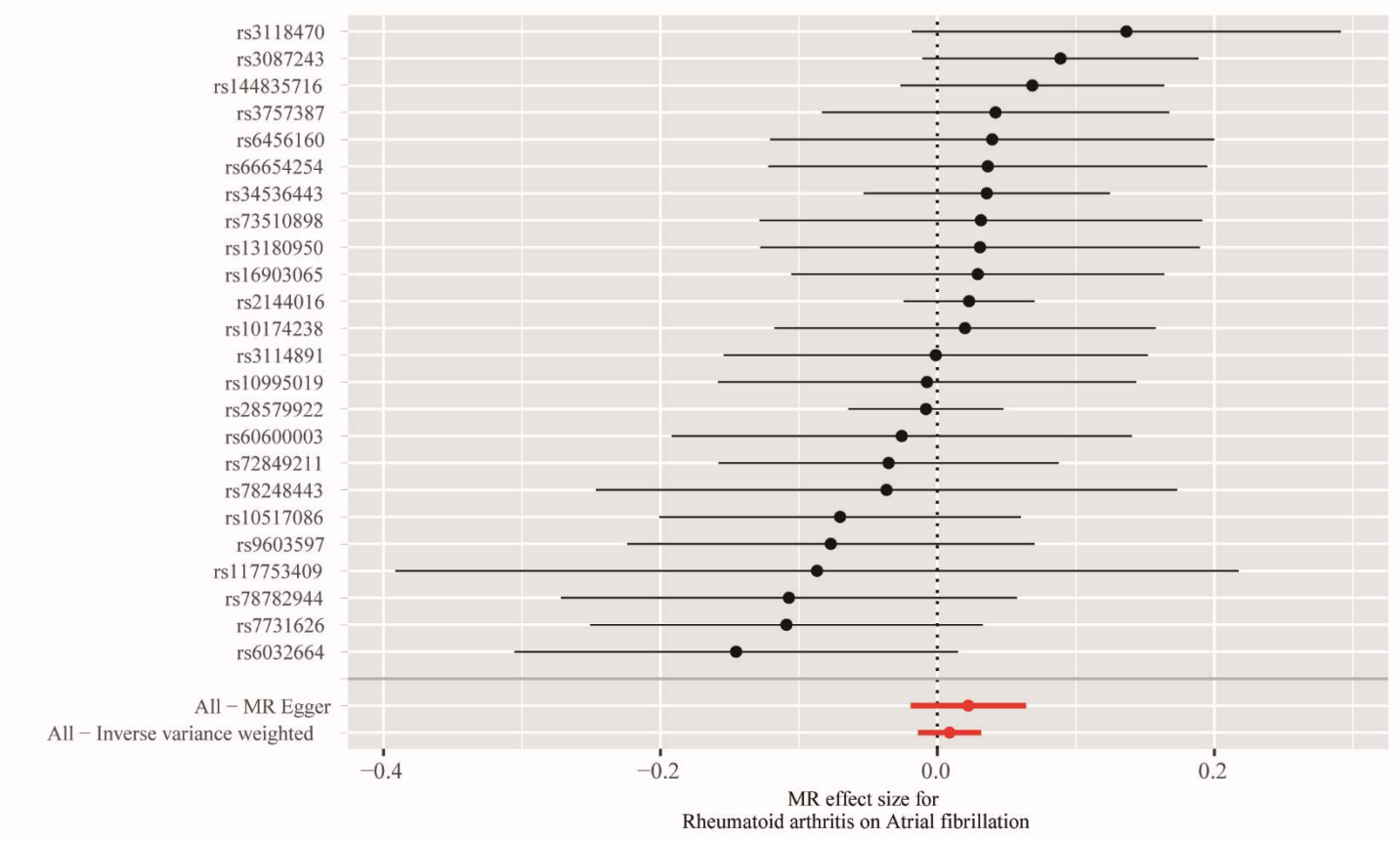

Figure S2. Leave-one-out analysis for genetically predicted RA and AF