

Effects of Blood Pressure and Antihypertensive Drugs on Rheumatoid Arthritis: A Mendelian Randomization, Network Pharmacology, and Experimental Validation

Fulin Yan¹, Weishan Wu², Mengxiong Luo³, Sidian Yang¹, Jiguang Yin², Xiangpeng Wang¹

¹The First Clinical Medical School, Shandong University of Traditional Chinese Medicine, Jinan, 250000, People's Republic of China; ²Department of Joint Orthopedics, Affiliated Hospital of Shandong Traditional Chinese Medicine University, Jinan, 250000, People's Republic of China; ³Shandong University of Traditional Chinese Medicine, Jinan, 250000, People's Republic of China

Correspondence: Jiguang Yin, Department of Joint Orthopedics, Affiliated Hospital of Shandong Traditional Chinese Medicine University, Jinan, 250000, People's Republic of China, Email yjg070715@163.com; Xiangpeng Wang, The First Clinical Medical School, Shandong University of Traditional Chinese Medicine, Jinan, 250000, People's Republic of China, Email drwangxiangpeng@163.com

Background: We conducted a Mendelian randomization study in conjunction with a network pharmacology study to provide an analytical elucidation of the potential causal relationship between blood pressure and antihypertensive drugs and rheumatoid arthritis.

Methods: First, a Mendelian randomization analysis was performed using large-scale GWAS data to assess the causal associations of systolic/diastolic blood pressure and 12 antihypertensive drug classes with rheumatoid arthritis. The analytical model of inverse variance weighting was primarily utilized. Second, we constructed a “drug-component-target-disease” regulatory network graph and analyzed Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment. Third, we validated some of the mined data through molecular docking technology using BIOVIA Discovery Studio 2019. Finally, animal experiments were performed to verify.

Results: The results indicated that elevated diastolic blood pressure was significantly associated with an increased risk of rheumatoid arthritis. Among antihypertensive drugs, adrenergic neuron blockers, central antihypertensives, and potassium-sparing diuretics/aldosterone antagonists reduced the risk of rheumatoid arthritis. Sensitivity analyses demonstrated the absence of pleiotropy or heterogeneity. In addition, after network pharmacology research, the molecular docking of its active ingredients with their targets was carried out, and the link potential was relatively ideal. Construction of a rat model of rheumatoid arthritis, Western blotting assay for Endothelin Receptor Type B (EDNRB), Angiotensin II Receptor Type (AGTR) expression level. Western blot analysis showed elevated EDNRB and AGTR levels in the model group, with spironolactone group significantly reducing these levels, though still higher than methotrexate group.

Conclusion: The findings of our study indicate that elevated diastolic blood pressure is associated with an increased risk of rheumatoid arthritis. However, three classes of antihypertensive medications, namely adrenergic neuron blockers, central antihypertensives, and potassium-sparing diuretics and aldosterone antagonists, have been demonstrated to exert beneficial effects on rheumatoid arthritis.

Keywords: rheumatoid arthritis, hypertension, antihypertensive drugs, Mendelian randomization, network pharmacology

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that results in progressive joint damage and deformity. It is a systemic inflammatory disease that primarily affects the cartilage and underlying bones of small and medium-sized joints, as well as blood vessels and various internal organs. It affects approximately 1% of the global population.^{1,2} The etiology of RA remains poorly understood. However, emerging evidence suggests that the onset of RA is intricately linked to a complex interplay between genetic and environmental factors.³ Hypertension is the most common comorbidity in patients with rheumatoid arthritis, with a prevalence

of approximately 62.5%.⁴ However, the mechanism that leads to the development of hypertension in patients with RA is not clear and may be related to chronic systemic inflammation in RA patients.⁵ Chronic inflammatory diseases, including RA, have been shown to affect the cardiovascular system, promoting atherosclerosis and altered vascular function, which ultimately leads to increased blood pressure.^{6,7} This partly explains the high prevalence of hypertension in RA patients.

Hypertension represents a significant risk factor for cardiovascular disease in the general population,⁸ that affects approximately 1 billion people worldwide and causes more than 10.8 million deaths worldwide each year.^{9,10} Hypertension that is not adequately controlled is strongly associated with an increased risk of early onset of a number of diseases, including coronary heart disease, cerebrovascular disease, and peripheral vascular disease.^{11,12} Using data from the Framingham Heart Study and the Third US National Health and Nutrition Examination Survey, Singh et al estimated that for every 20 mm Hg increase in systolic blood pressure (SBP), there would be an increase of 1572 ischemic heart disease events and 602 strokes in RA patients.¹³ This demonstrates the importance of controlling blood pressure in patients with RA. Angiotensin-converting enzyme inhibitors (ACE-I) and Angiotensin II Receptor Blockers (ARB) may have beneficial effects in RA.¹⁴ Patients with RA often exhibit increased sympathetic activity and activation of the renin-angiotensin-aldosterone system, which not only drive hypertension-associated vascular remodeling but also exacerbate the inflammatory progression of RA.^{15,16} The inherent chronic systemic inflammation and immune dysregulation of RA further amplify these mechanisms, creating a vicious cycle that accelerates the co-progression of RA and hypertension. It is precisely this interplay of complex mechanisms that poses significant challenges for managing hypertension and treating RA disease in patients. Nevertheless, there is a paucity of randomized controlled trials that inform the management of hypertension in patients with rheumatoid arthritis.⁵ Furthermore, clinical studies are vulnerable to confounding factors and reverse causality.¹⁷ Mendelian randomization (MR) offers a novel approach to address these issues.

MR uses genetic variation as a tool to identify causal relationships between exposures and outcomes, relying on the fact that alleles are randomly assigned at conception. This method avoids confounding factors and mimics a randomized controlled trial.¹⁸ For example, Zhang et al conducted a MR study, which found a causal relationship between genetic susceptibility to gestational diabetes and the risk of coronary artery disease, with type 2 diabetes and hypertension identified as the primary mediators.¹⁹ Drug-targeted MR applies this principle by using genetic variations linked to drug targets to simulate their effects. Network pharmacology is an emerging field that integrates multidisciplinary knowledge from systems biology, multi-omics, pharmacology, and computational network analysis, with the goal of studying the global, systemic, and networked nature of drug actions.²⁰ Additionally, animal studies were conducted to validate the effects of selected antihypertensive drugs on RA progression, further strengthening the causal relationships identified in our MR analysis. While MR has traditionally been used to identify causal relationships between genetic variations and diseases, the integration of MR with network pharmacology is an emerging area of research. For example, studies by Yanan Xu have successfully combined MR with network pharmacology to explore the genetic determinants of Systemic lupus erythematosus and identify potential therapeutic targets.²¹ These studies highlight the value of combining genetic analysis with systems biology to understand disease mechanisms and guide drug development. This study aims to assess the causal relationship between blood pressure and RA using MR analysis in conjunction with network pharmacology and explore the potential therapeutic effects of various antihypertensive drugs on RA.

Methods and Design

Research Design

We used a two-sample MR method. First, we assessed the causal relationship between SBP and diastolic blood pressure (DBP), RA. Then, we assessed the effects of 12 antihypertensive drugs on RA using published genetic variants of antihypertensive drug targets as instrumental variables. These drugs included: β -adrenoceptor blockers, ACE-I, ARB, α -adrenoceptor blockers, calcium channel blockers (CCBs), centrally-acting antihypertensive

agents, loop diuretics, potassium-preserving diuretics, aldosterone antagonists, renin inhibitors, thiazides and related diuretics, vasodilators. To verify the reliability of the results, heterogeneity test, sensitivity analysis, and multiplicity analysis were used to exclude result bias. The following three key assumptions were used to reach MR satisfaction: (i) there is a strong correlation between the instrumental variables and the exposure factors; (ii) the instrumental variables are not correlated with any confounders associated with the exposure factor outcome variables; and (iii) the instrumental variables can only affect the outcome through their association with the exposure factors.

Selection of Data Sources and Instrumental Variables

Systolic and Diastolic Blood Pressure

We obtained SBP (SBP: ukb-a-360) and DBP (DBP: ukb-a-359) phenotypic data from the IEU Open GWAS database, which consists of 317,756 samples from European populations (<https://gwas.mrcieu.ac.uk/datasets/ukb-a-360/>, <https://gwas.mrcieu.ac.uk/datasets/ukb-a-359/>). Permissions to access the Genome Wide Association Study (GWAS) databases were not required, as the data used in this study are publicly available. We selected single nucleotide polymorphisms (SNPs) strongly associated with SBP and DBP ($p < 5 \times 10^{-8}$) and excluded polymorphisms that were chained in disequilibrium ($r^2 < 0.001$, genetic distance = 10 000kB). This is to avoid pseudo-associations and genetic confounding. We then excluded instrumental variables located in palindromic sequences to prevent causal inference bias and to improve the accuracy and reliability of the analysis; finally, we excluded genetic instrumental variables associated with confounders by using the LDlink database, focusing on SNPs with correlations with phenotype and outcome. We calculated the F value for each remaining SNP using the formula $F = \text{Beta}^2 / \text{SE}^2$.²² We removed SNPs with F values less than 10 to exclude weak instrument bias and used the remaining SNPs as final instrumental variables.

Antihypertensive Drugs

SNPs for 12 classes of antihypertensive drugs were selected from published data. In this study, the authors first extracted the protein targets and genes for these 12 classes of antihypertensive drugs from the DrugBank database, and then screened for the “best SNP” for the corresponding genes in each tissue using data from the GTEx project. After two-sample MR and quality control, the investigators chose SNPs that significantly affected SBP as the SNPs for the final analysis.²³ Subsequently, we used the LDlink database to exclude genetic instrumental variables associated with confounders, with a focus on excluding SNPs that correlated with phenotype and outcome.

RA Data Source

We obtained genetic data on RA from the IEU Open GWAS database from the GWAS study by Saori Sakaue’s team,²⁴ which includes 417,256 samples from European populations with 24,175,266 SNPs (<https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018910/>).

Statistical Analysis

MR was conducted using the “TwoSampleMR” package in R 4.2.0. In the present study, random effects inverse variance weighting (IVW), weighted median, MR-Egger regression, and weighted modeling were employed for the MR analyses, with IVW serving as the primary analysis to assess the risk between exposures and outcomes. The fundamental premise is to regard each genetic variant as an autonomous instrumental variable and to aggregate the estimation of causal effects of instrumental variables through meta-analysis. The IVW method is the most efficacious evaluation instrument for causal effects when all genetic variants are valid instrumental variables. The IVW and MR-Egger methods were employed to assess heterogeneity. When $P > 0.05$, it was concluded that there was no heterogeneity among the SNPs. The MR_leaveoneout_plot function was employed to conduct a sensitivity analysis and visualize the results. This involved the exclusion of individual SNPs to ascertain their potential influence on the final results. The MR_pleiotropy_test function and the MR-PRESSO method were employed for pleiotropy analysis. When $P > 0.05$, this indicated the absence of pleiotropy.

Bioinformatics Analysis

Drug Information and Target Screening

Antihypertensive drug classes identified through the UK National Formulary and API information was obtained, and the DrugBank database (<https://www.drugbank.ca/>) and the Therapeutic Target Database (TTD) (<https://db.idrblab.net/ttd/>) were used to identify the drug target targets and corresponding genes. The drug classes selected for this study included adrenergic neuron blockers, central antihypertensives, potassium-sparing diuretics (PSDs), and aldosterone antagonists. Search terms used in the DrugBank and TTD databases included drug names such as spironolactone, lisinopril, losartan, and metoprolol.

Rheumatoid Arthritis Disease Target Screening

Disease databases such as TTD, DisGeNET database (<https://disgenet.com/>), and Genecards database (<https://www.genecards.org/>) were searched with the keyword “Rheumatic Arthritis” as the keyword. To ensure high-confidence targets, only genes with a relevance score ≥ 2.0 were selected as higher-confidence candidates. This cutoff was chosen because a relevance score ≥ 2.0 in the Genecards database is classified as indicating a strong association with the disease. Duplicates were removed, and the final list of rheumatoid arthritis disease targets was obtained.

Mapping the Gene Regulation Network of “Drug-Component-Target-Disease”

Mapping of drug action targets and disease targets, and filling the drug, disease and other related information to create a network table. Import the obtained network table into Cytoscape 3.10.2 and draw a network diagram mapping drug components to target genes.

Enrichment Analysis of Gene Ontology (GO) Functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways

The target gene was entered into the DAVID database (<https://david.ncifcrf.gov/>), the identifier was selected as “OFFICIAL GENE SYMBOL”, and the species was selected as “homo sapiens” for submission. Select “gene list” to output the table and perform GO and KEGG enrichment analysis for key gene targets. The top 5 results of biological process (BP), cellular component (CC) and molecular function (MF) were filtered out and added into GO analysis by the descending order of Fold Enrichment value, $P < 0.05$. Sorted in descending order of P-value with $P < 0.05$, 15 signaling pathways that could be key pathways were obtained as data for the correlation analysis of KEGG pathway.^{25–27} For KEGG, pathways with $P < 0.05$ were considered significant. The above data were imported into SRplot (<http://www.bioinformatics.com.cn/>) to visualize the GO function with KEGG pathway enrichment analysis data and form the corresponding enrichment bubble diagram.

Molecular Docking

Molecular docking technology is used to verify the reliability of the docking of the effective active ingredients of the antihypertensive drug with key target molecules. The following identifiers were used to acquire protein structures from the RCSB PDB: ACE (1O86), ADRA1A (8THK), AGTR1 (6OS2), ADRB2 (4LDO), ADRA2B (6K41), CA1 (1HCB), CA2 (5AML), SLC6A2 (8HFI), and EDNRB (5XPR). These protein structures were prepared for docking by removing water molecules and original ligands, and adding hydrogen atoms. Molecular docking was then performed using BIOVIA Discovery Studio 2019 software. The effective active ingredients are docked into the spatial cavity of the target for analysis at the best conformation of the binding.

Experimental Verification

Research Objective

The objective of the animal study was to evaluate the therapeutic effects of spironolactone on RA progression and compare its efficacy with methotrexate, a standard treatment for RA. Specifically, the study aimed to assess the expression levels of Endothelin Receptor Type B (EDNRB) and Angiotensin II Receptor Type (AGTR)

proteins in synovial tissue, which are implicated in the pathophysiology of RA, to determine whether spironolactone could modulate these protein levels and thus exert therapeutic effects.

Animals and Materials

Thirty male four-week zero SPF grade SD rats with a body mass of 220 ± 20 g were used in this experiment, purchased from Jinan Pengyue Laboratory Animal Breeding Co. Ltd (Production Certificate No. SYXK20180015, Jinan). The experiment was approved by the Animal Ethics Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (Ethics Approval SDSZYYAWE20240827001). All experimental animals were housed in the Animal Experiment Center of Shandong University of Traditional Chinese Medicine, and the experimental environmental conditions were room temperature of $22 \pm 2^\circ\text{C}$, humidity of $55 \pm 5\%$, a light-dark cycle of 12 h, and provision of free drinking water and diet. Complete Freund's adjuvant (CFA, Cat. No. F5881) and incomplete Freund's adjuvant (IFA, Cat. No. F5506) were purchased from Sigma, USA; Bovine Collagen type II (BCII, Cat. No. 180559) was purchased from Chondrex, USA; methotrexate tablets (Shanghai ShangPharma Xinyi Pharmaceutical Co); spironolactone tablets (Shanghai ShangPharma Xinyi Pharmaceutical Co).

All experimental animals were anesthetized with 3% pentobarbital sodium at the end of the study, followed by immediate euthanasia. The method of euthanasia involved intravenous injection of 3% pentobarbital sodium until the animals lost consciousness and ceased breathing and heartbeat. Specimens were collected after re-anesthetization.

BCII-CFA and BCII-IFA Emulsion Preparation

Bovine type II collagen was dissolved in 0.1 mol/L acetic acid solution to make a 4 mg/mL solution and placed in a 4°C refrigerator overnight. On the day of the experiment, equal volumes of CFA or IFA and bovine II collagen were fully ground on ice using a mortar and pestle to fully emulsify the 2 solutions, ie, BCII-CFA emulsion and BCII-IFA emulsion, which were prepared before clinical use.

Modeling, Grouping and Administration

After 7 days of acclimatization, 30 rats were injected with CII-CFA emulsion at 3 points on the back, the root of the tail, and the paw of the right hind foot using a microsyringe at multiple points subcutaneously with an injection volume of 0.1 mL. 7 d after the 1st stimulation, the immunization was strengthened by injecting CII-IFA emulsion at the same site at multiple points subcutaneously with an injection volume of 0.2 mL,²⁸ and the model of collagen-induced arthritis was established. Two weeks after the first immunization, rats with an arthritis score ≥ 4 were considered successful models. Arthritis scoring criteria: Normal: 0 points; Redness and swelling in one toe joint: 1 point; Redness and swelling in multiple toe joints: 2 points; Swelling in the foot/paw below the ankle joint: 3 points; Partial swelling of the foot/paw: 4 points. Rats with successful modeling were randomly divided into the spironolactone group, model group and methotrexate group, 10 rats in each group. All rat groups were assigned using a random number table to ensure no bias between groups. The dosage of spironolactone tablets and methotrexate tablets was calculated according to the "Methods of Pharmacological Experiments" based on the method of body surface area of human and rat (the dose of 200 g of rat was 0.018 times of the dose of 70 kg of human), and the routine dosage of spironolactone tablets and methotrexate tablets for rats was 7 mg/(kg-d) and 1 mg/(kg-d), respectively, while the control group was given an equal volume of sodium chloride injection by gavage every day and continued to be given for 4 weeks. The model group was gavaged daily with an equal volume of sodium chloride injection for 4 weeks.

Western Blotting for Protein Expression

At the end of drug administration, the synovial tissue of rats in each group was extracted and stirred with a mill under low temperature, then protein lysate was added and centrifuged at 4°C for 15 min (12,000 r/min, centrifugation radius of 8 cm), and the protein supernatant was aspirated, and the protein concentration was determined by using the BCA kit. Electrophoresis was performed with a 12% SDS-PAGE gel, which was then transferred to a PVDF membrane. The bands were transferred to the corresponding EDNRB (1:1000), AGTR

(1:1000), and β -actin (1:10,000) primary antibody solutions, and the membrane was washed with Tris Buffered Saline with Tween-20 at 4 °C overnight. The washed primary antibody reaction membrane was incubated in secondary antibody (1:10,000) working solution for 40 min and then developed.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (version 9.5). Quantitative data are presented as mean $\pm$ standard deviation. Differences among groups were assessed by one-way analysis of variance (ANOVA); in cases of unequal variance, the Brown–Forsythe test and Welch ANOVA were applied. A p-value less than 0.05 was considered statistically significant.

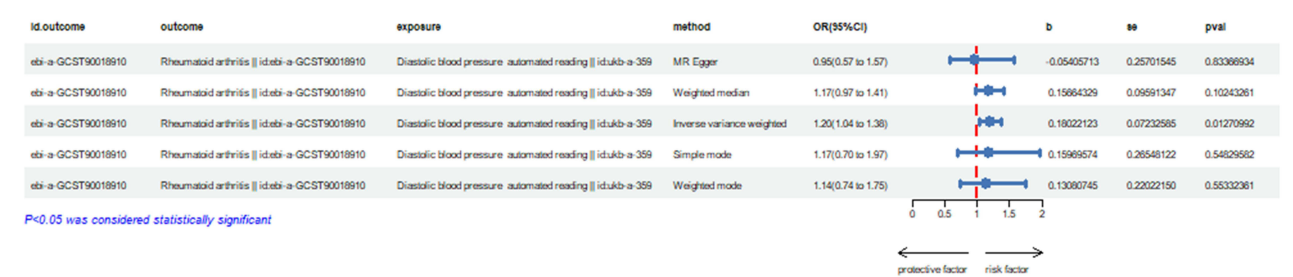


Figure 1 MR analysis results of diastolic pressure and RA.

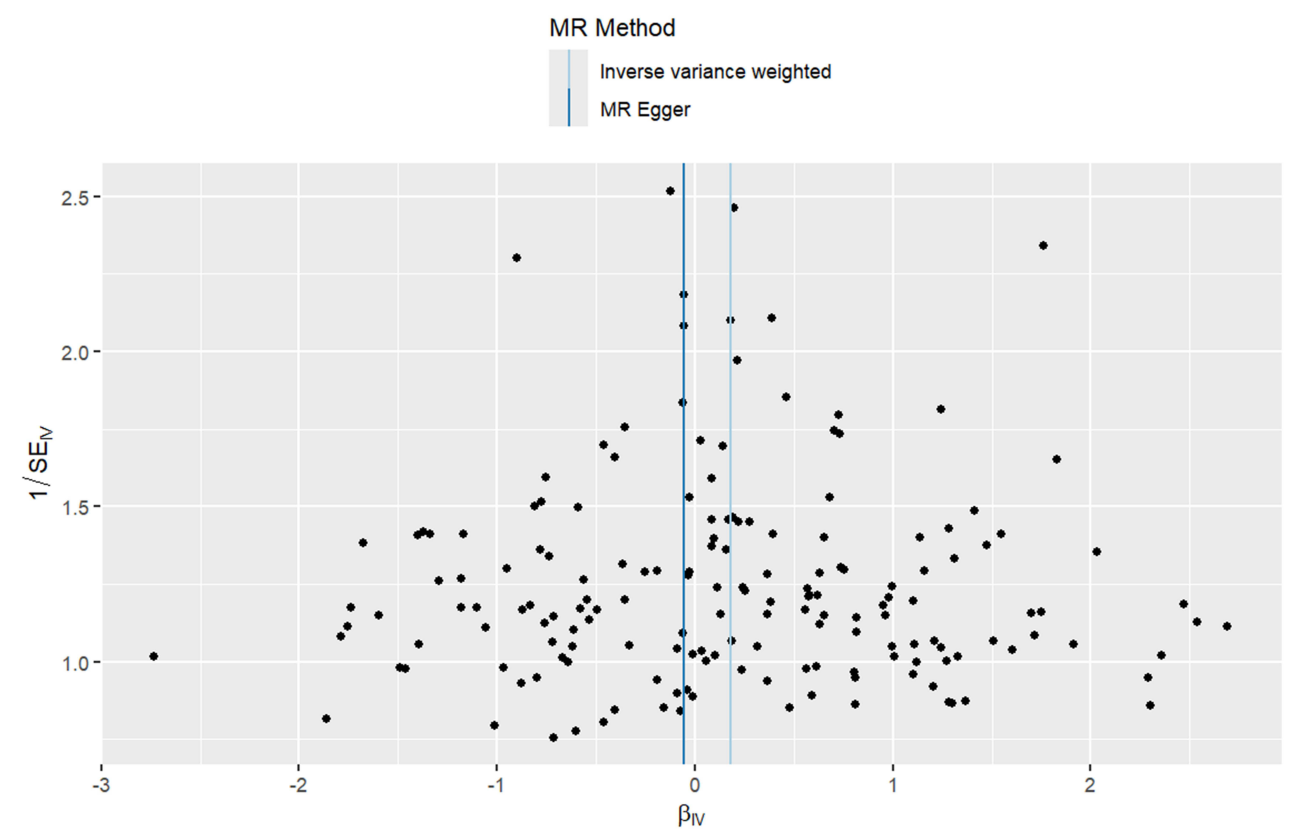


Figure 2 Funnel plot of MR results.

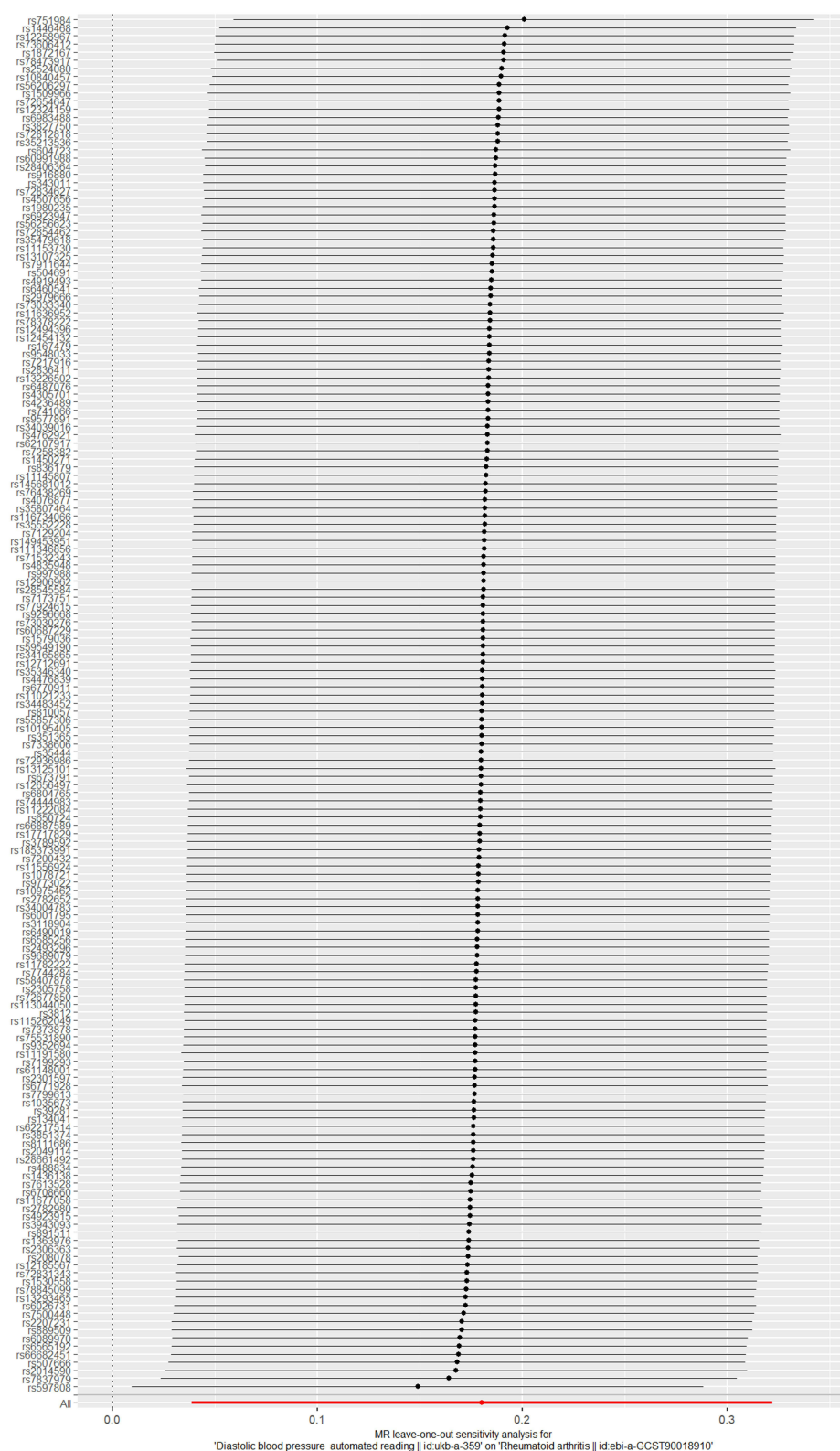


Figure 3 Results of “Leave-one-out” sensitivity analysis.

Results

Instrumental Variable

After using the clumped function to remove linkage disequilibrium and after LDlink to remove genetic instrumental variables associated with confounders, 176 SNPs remained in DBP, 168 SNPs remained in SBP and 289 SNPs remained in antihypertensive medications. In addition, 6 palindromic SNPs in SBP were removed as well as 1 incompatible allele SNP, and 7 palindromic SNPs in DBP were removed. Finally, 169 SNPs for diastolic BP and 161 SNPs for systolic BP, as well as the remaining 289 SNPs for antihypertensive drugs, were utilized for MR analysis. The F values corresponding to single SNPs in this study were all >10 , and there was no weak instrumental variable bias, so the present results were reliable.

MR Analysis of Blood Pressure and RA

The results of the analysis of DBP with RA, conducted using the IVW method, yielded a statistically significant outcome (OR: 1.197, 95% CI 1.039–1.380, $P=0.0127$). This finding suggests that elevated DBP may increase the risk of developing RA. The results of the MR analysis are presented in Figure 1. Meanwhile, the heterogeneity test revealed an association between DBP and RA with $P=5.02 \times 10^{-5}$ for the IVW method and $P=5.13 \times 10^{-5}$ for the MR-Egger method. This suggests the presence of heterogeneity among SNPs, which may indicate the potential for bias or an unrecognized complexity. To address this, we employed the random-effects IVW model as the primary method to provide a more conservative estimate that accounts for this between-SNP variance. However, it is also possible that this heterogeneity reflects normal variation within the study population. Consequently, we conducted a sensitivity analysis and a multiplicity analysis. The funnel plot exhibited a roughly symmetrical shape, providing visual support for the absence of major pleiotropic bias (Figure 2). Following the sensitivity analysis, which proposed each SNP individually using the MR_leaveoneout_plot

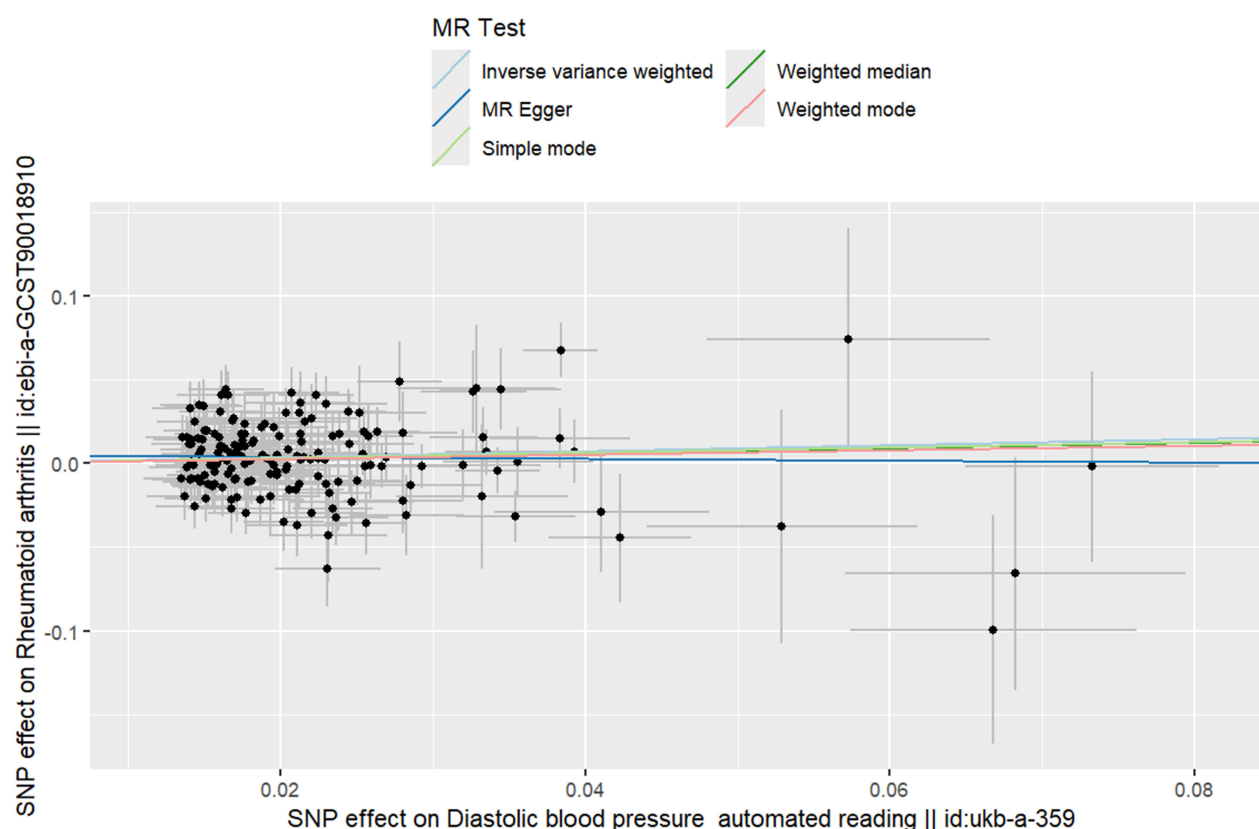


Figure 4 Plot of results of MR analysis.

function, the results of the IVW analysis of DBP and RA were found to be similar to those of the analysis that included all SNPs. Additionally, no SNPs with a significant impact on the estimation value of the causal association were identified (Figure 3). The data visualization analyses indicated that the overall SNP effect of the exposure factor DBP was elevated by all four methods (except MR Egger), as was the SNP effect of the outcome variable RA. Furthermore, when the SNP effect of the exposure factor was zero, it was also zero for the outcome variable, indicating that this result was not affected by horizontal pleiotropy (Figure 4). The results of the MR_pleiotropy_test function for DBP and RA in the pleiotropy analysis indicated a P-value of 0.34 for the MR-PRESSO method, and 0.602 for the MR-Egger method. These results suggest that the present study was not affected by horizontal pleiotropy. The overall consistency across these pleiotropy-robust methods, despite the statistical heterogeneity, strengthens the credibility of the observed causal relationship between DBP and RA. Nevertheless, no substantial evidence was found to suggest that SBP exerts a significant influence on rheumatoid arthritis.

Antihypertensive Drugs and RA

In the analysis of antihypertensive drugs and RA, adrenergic neuron blockers (OR: 0.156, 95% CI 0.047–0.514, $P=0.0023$), central antihypertensives (OR: 0.416, 95% CI 0.185–0.934, $P=0.0335$) and PSDs and aldosterone antagonists (OR: 0.183, 95% CI 0.065–0.512, $P=0.0012$) were statistically significant in the IVW method of three antihypertensive medications, while the IVW method of the remaining nine antihypertensive medications was not statistically significant (Figure 5). Among the heterogeneity tests, the IVW method of adrenergic neuron blockers with RA $P=0.69$ and MR-Egger method $P=0.49$; the IVW method of central antihypertensives with RA $P=0.60$ and MR-Egger method $P=0.64$; the IVW method of PSDs and aldosterone antagonists with RA $P=0.94$ and MR-Egger method $P=0.93$, none of which detected significant heterogeneity. In the pleiotropy analysis, the

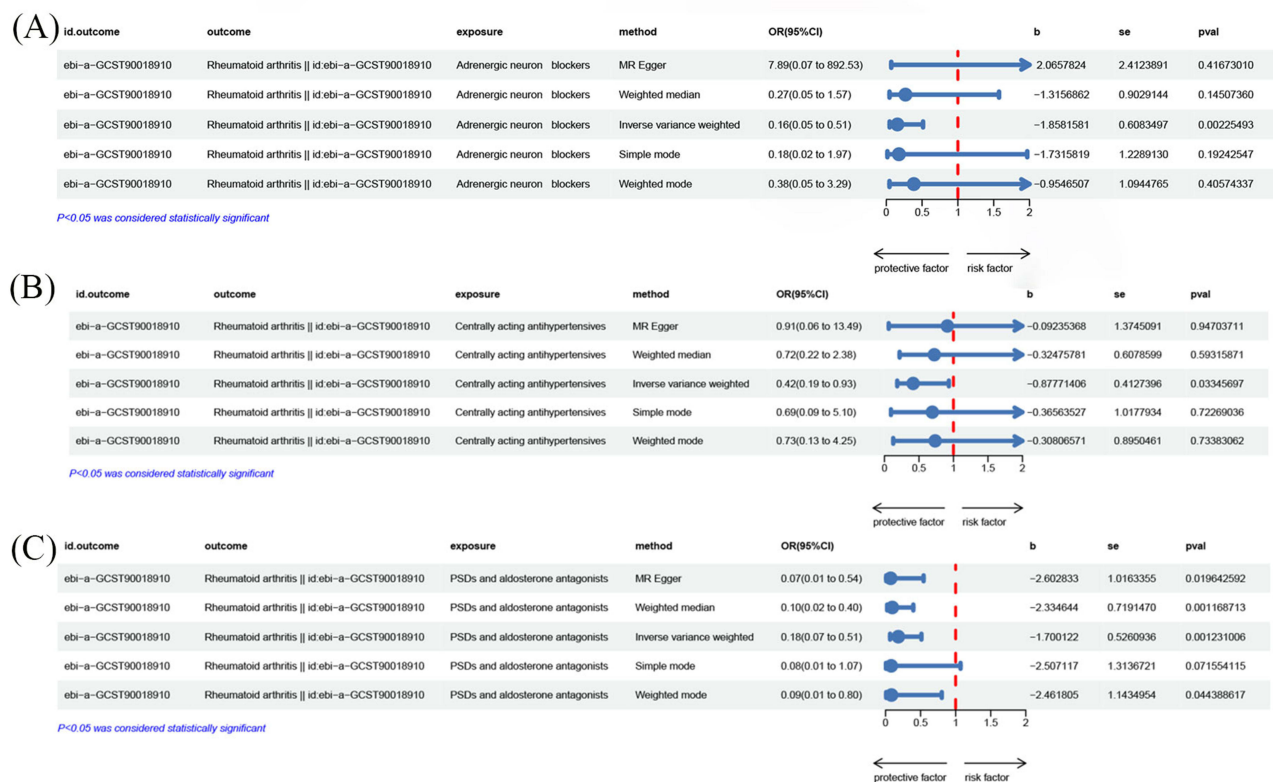


Figure 5 (A–C) Results of MR analysis of the association of adrenergic neuron blockers, central blood pressure lowering drugs, PSD and aldosterone antagonists with RA.

results of the MR_pleiotropy_test function of adrenergic neuron blockers with RA suggested $P = 0.13$, MR PRESSO method $P = 0.62$; and the results of the MR_pleiotropy_test function of central antihypertensives with RA suggested $P = 0.56$, MR PRESSO method $P = 0.68$; The results of MR_pleiotropy_test function of PSDs and aldosterone antagonists with RA suggested $P = 0.31$, MR PRESSO method $P = 0.95$, and the results all suggested that the present study was not affected by horizontal pleiotropy. In addition, scatter plots, leave-one-out analysis, and funnel plots obtained after MR analysis of the three antihypertensive drugs with RA further proved the reliability of our results (Figures 6–8).

Bioinformatics Analysis

Construction of “Drug-Component-Target-Disease” Regulatory Network Diagrams

After integrating adrenergic neuron blockers, central antihypertensives, as well as PSDs and aldosterone antagonists with a total of 12 drug types and a total of 107 API ingredient information, 70 drug targets were obtained by searching through the DrugBank database and the TTD, and the drug therapeutic targets and the integrated 2722. After mapping the drug therapeutic targets with the integrated 2722 targets, 15 common targets for diseases and drug components were obtained. Cytoscape software was utilized to construct the “drug-component-target-disease” regulatory network diagram (Figure 9). Core targets were selected based on objective network topology analysis, focusing on nodes with high degree centrality (number of connections) and betweenness centrality (role as a bridge in the network). These metrics indicate the pivotal regulatory roles of the selected targets.

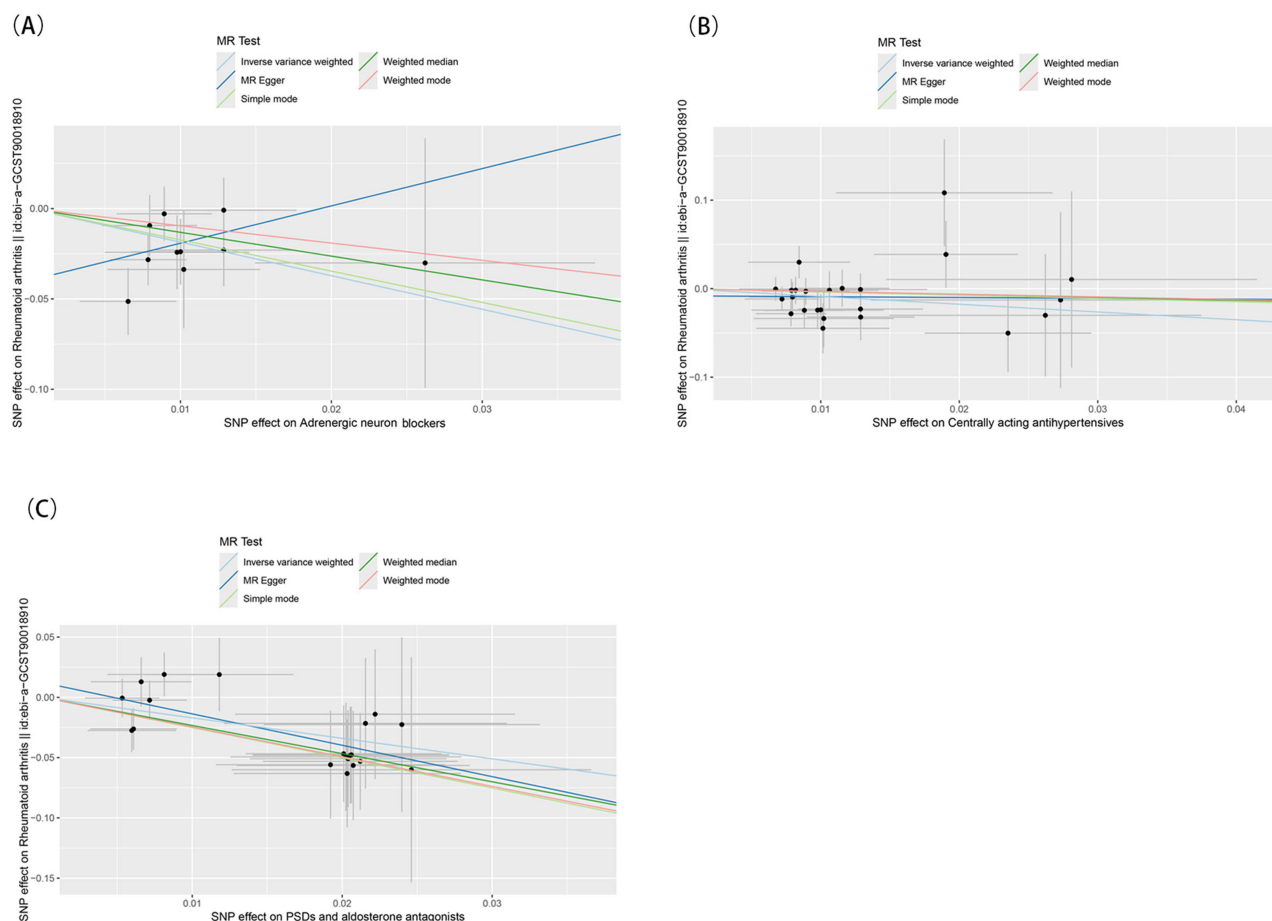


Figure 6 Scatter plot of single nucleotide polymorphism. (A) Adrenergic neuron blockers with SNP-RA. (B) Central antihyper-tensives with SNP-RA. (C) PSDs and aldosterone antagonists with SNP-RA.

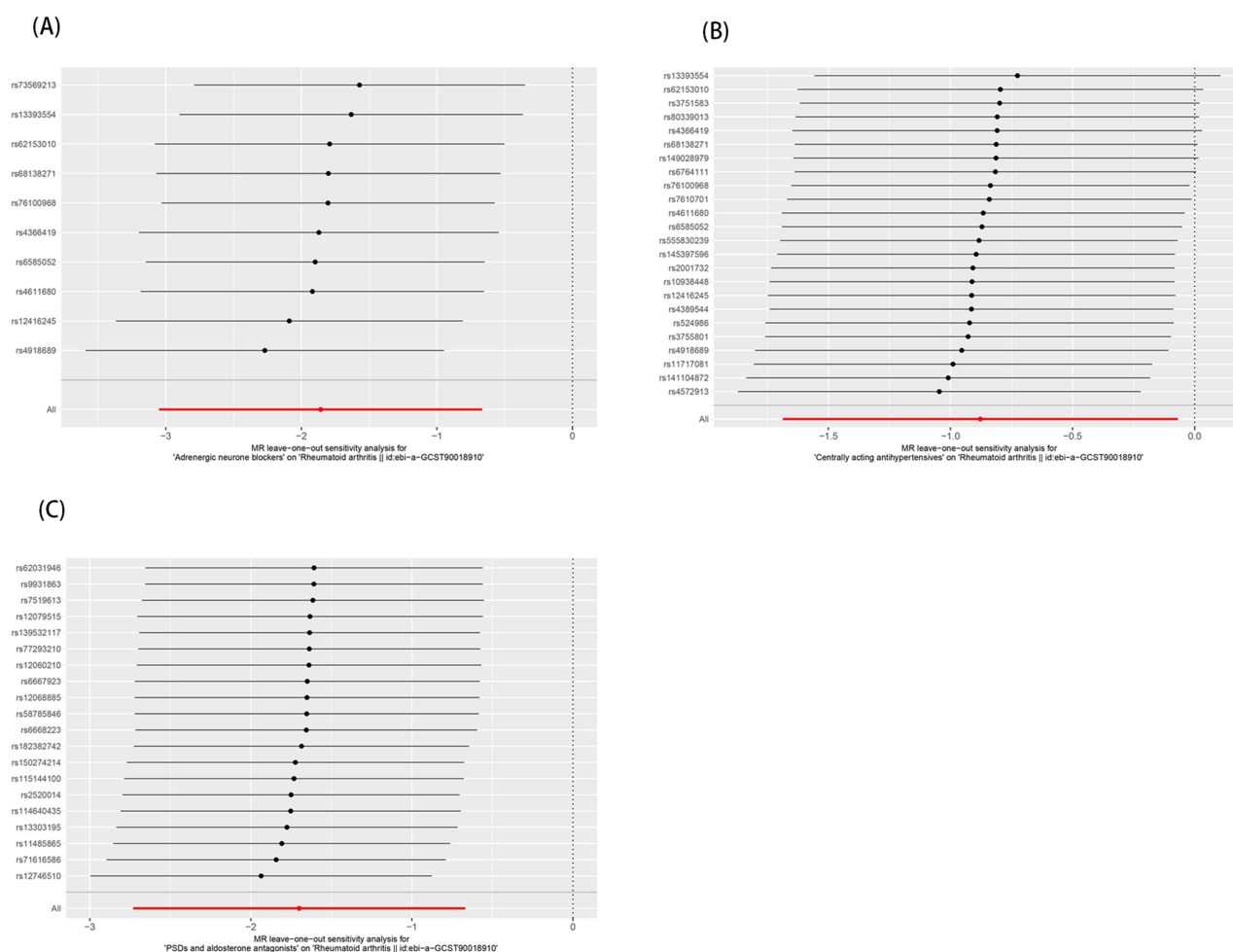


Figure 7 Leave-one-out plot of the effect of antihypertensive drugs on RA. **(A)** Adrenergic neuron blockers on RA **(B)** Central antihypertensive drugs on RA. **(C)** PSDs and aldosterone antagonists on RA.

GO Function and Enrichment Analysis of Kyoto Genes and Genomic Encyclopedia Pathways

Based on the 15 common targets, GO enrichment analysis yielded a total of 68 GO-enriched entries, including 53 entries for BP, 4 entries obtained for CC, and 11 entries obtained for MF, which were plotted in graphs by Fold Enrichment size. Related to BP were renin-angiotensin regulation of aldosterone production, angiotensin maturation, angiotensin activation signaling pathway, endothelin receptor signaling pathway, and adrenergic receptor signaling pathway; related to CC were caveolae-like invaginations of the plasma membrane, apical portion of the cell, receptor complex, and cytoplasmic membrane; and related to MF were cyanamide hydratase activity, bradykinin receptor binding, endothelin receptor activity, norepinephrine binding, and aromatic ester activity (Figure 10). KEGG pathway enrichment showed that the drug closely interacted with several signaling pathways such as renin secretion, cGMP-PKG signaling pathway, calcium signaling pathway, neuroactive ligand-receptor interaction, and vascular smooth muscle contraction (Figure 11).

Molecular Docking

To further study the relationship between antihypertensive drugs and rheumatoid arthritis, we used molecular docking to reliably study their active ingredients and potential targets. The binding between small molecule compounds and ligands can reduce the conformations that lead to more stable conformations, thereby increasing the possibility of their interactions. In this study, we employed the -CDOCKER score to estimate binding affinity between ligands and targets. Lower CDOCKER binding energies typically indicate stronger ligand-receptor

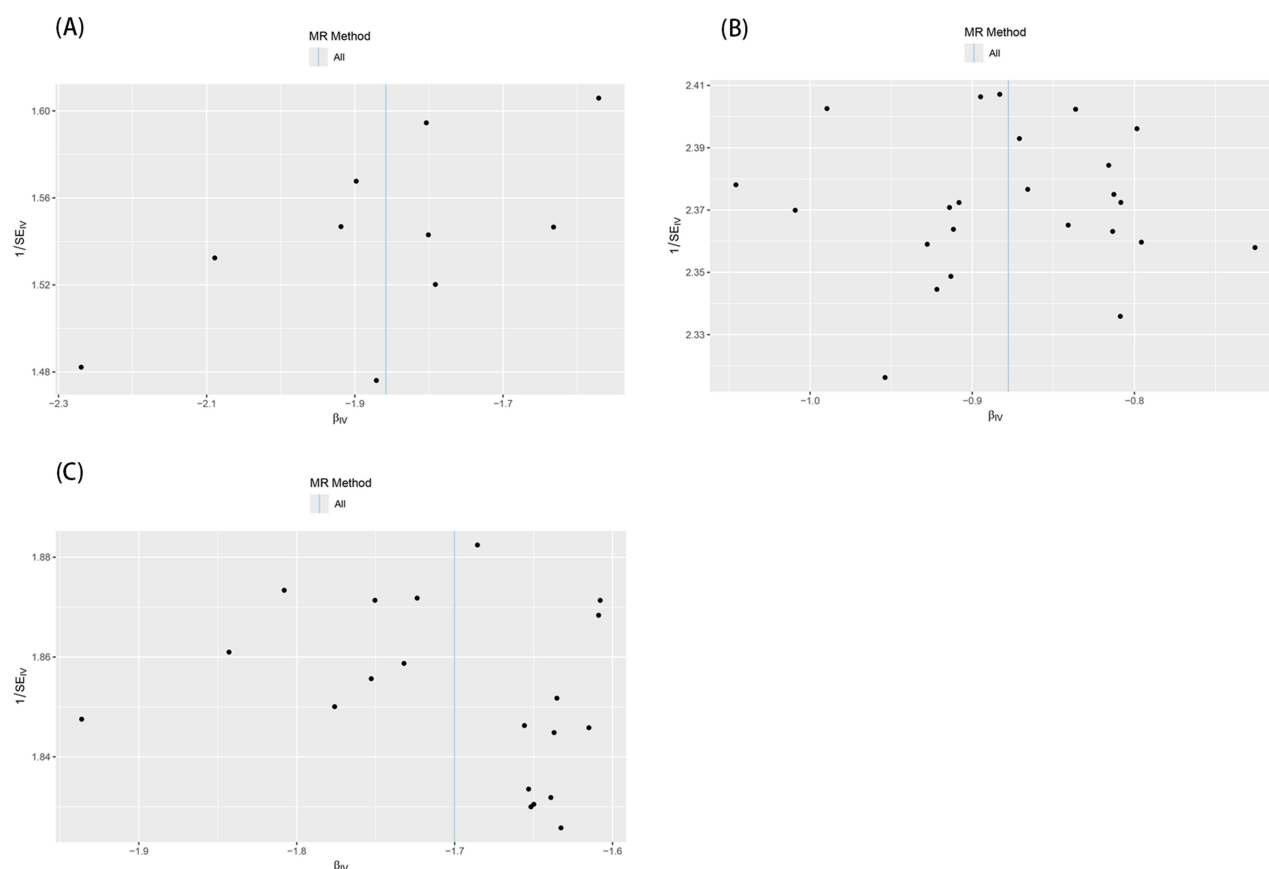


Figure 8 Funnel plot of the effect of antihypertensive drugs on RA. (A) Adrenergic neuron blocker on RA (B) Central antihypertensive drug on RA. (C) PSDs and aldosterone antagonists on RA.

interactions (more negative docking scores often correspond to more stable binding conformations). Due to variations across target systems and computational conditions, no universal absolute energy threshold exists for all systems. Therefore, this study primarily relies on relative changes in docking results within the same system to identify high-affinity ligands. A total of 9 core targets were selected in this study: ACE, ADRA1A, AGTR1, ADRB2, ADA2B, CA1, CA2, SLC6A2 and EDNRB. The docking results are as follows (Figures 12 and 13).

Protein Expression of Rat EDNRB, AGTR, and β -Actin

Western blot results (Figure 14) revealed significantly elevated expression levels of EDNRB and AGTR proteins in synovial tissue from the RA model group compared to rats receiving standard treatment (methotrexate) ($P < 0.001$). This indicates that the RA disease state is accompanied by abnormal upregulation of these two proteins.

A key finding was that EDNRB and AGTR protein expression levels were significantly downregulated in the spironolactone monotherapy group compared to the model group ($P < 0.01$), directly demonstrating spironolactone's therapeutic activity. However, protein levels in the spironolactone group remained higher than those in the methotrexate group ($P < 0.05$), suggesting that the intensity or mechanism of action may differ from that of standard drugs.

Discussion

We used large-scale GWAS data to examine the effects of blood pressure and antihypertensive medications on rheumatoid arthritis. Our results show that there is no evidence that SBP has any effect on RA, whereas elevated DBP can increase the risk of RA. In addition, we identified a causal relationship between adrenergic neuron blockers, central antihypertensives, and PSDs and aldosterone antagonists three antihypertensive medications and RA, and that the use of all three of them reduces the risk of RA, which has a protective effect.

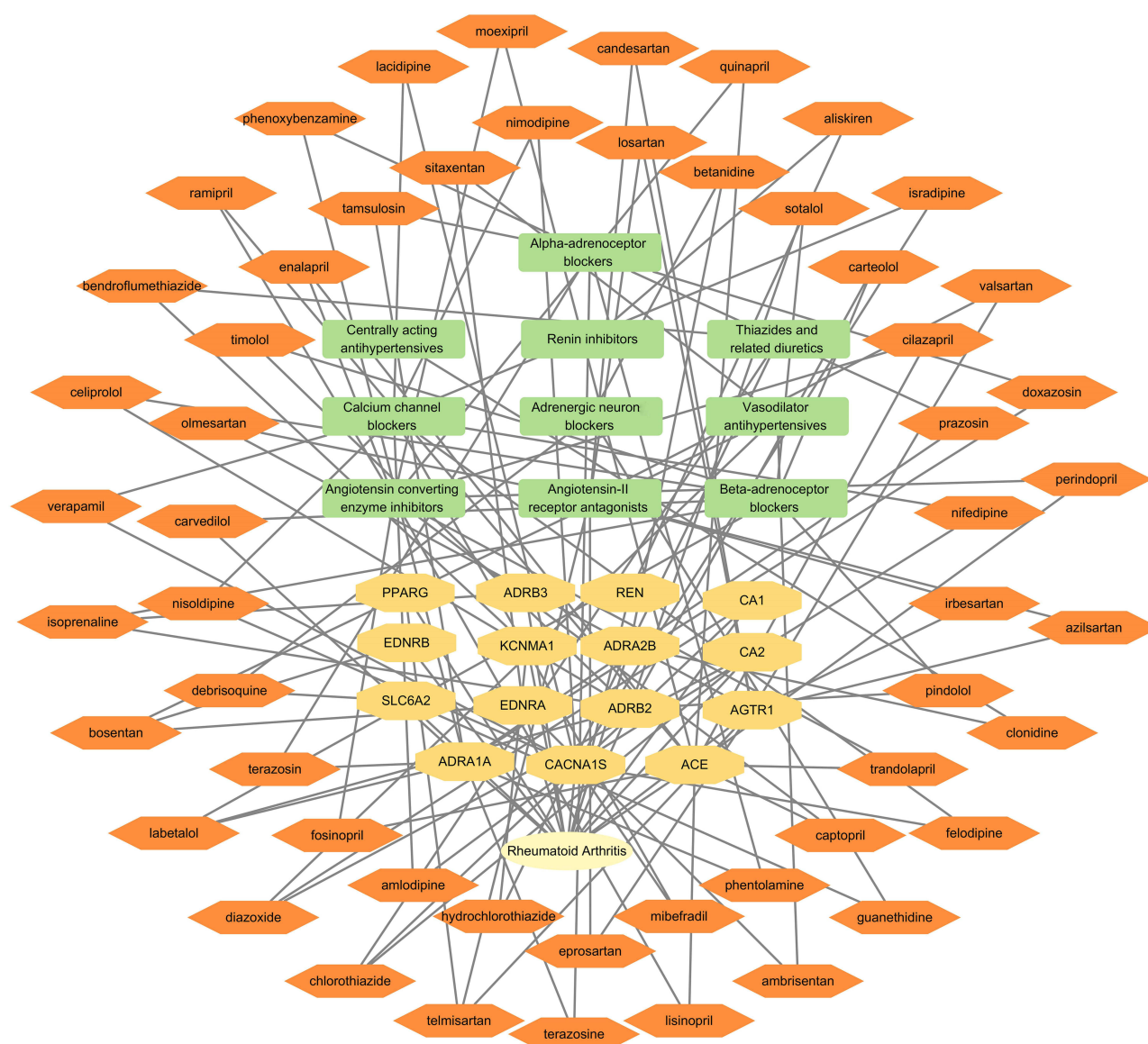


Figure 9 “Drug-component-target-disease” regulation network diagram.

The relationship between hypertension and RA has been widely studied, with several large cohort studies demonstrating that RA patients have a higher prevalence of hypertension compared to the general population. For instance, Han et al reported that 34% of RA patients were hypertensive, compared to 23.4% in a control group.²⁹ The potential causal relationship between the two has also been the subject of investigation by researchers, with some studies suggesting a possible association between systemic inflammation and hypertension. In a study by Claudia et al, elevated SBP, pulse pressure, and mean arterial pressure were found to be significantly correlated with intercellular adhesion molecule-1. Additionally, DBP and interleukin-6 (IL-6) demonstrated a significant correlation.³⁰ Soluble intercellular adhesion molecule-1 and IL-6 are two biomarkers that are indicative of an inflammatory response.³¹ The findings of this study indicate that elevated blood pressure may act as a stimulus for the onset of inflammation. Further evidence by Sung KC et al demonstrated that elevated C-reactive protein levels in hypertensive individuals may indicate an increased inflammatory burden, a feature commonly observed in RA.³² Furthermore, Dai et al proposed that hypertension induces high shear stress in the vasculature,

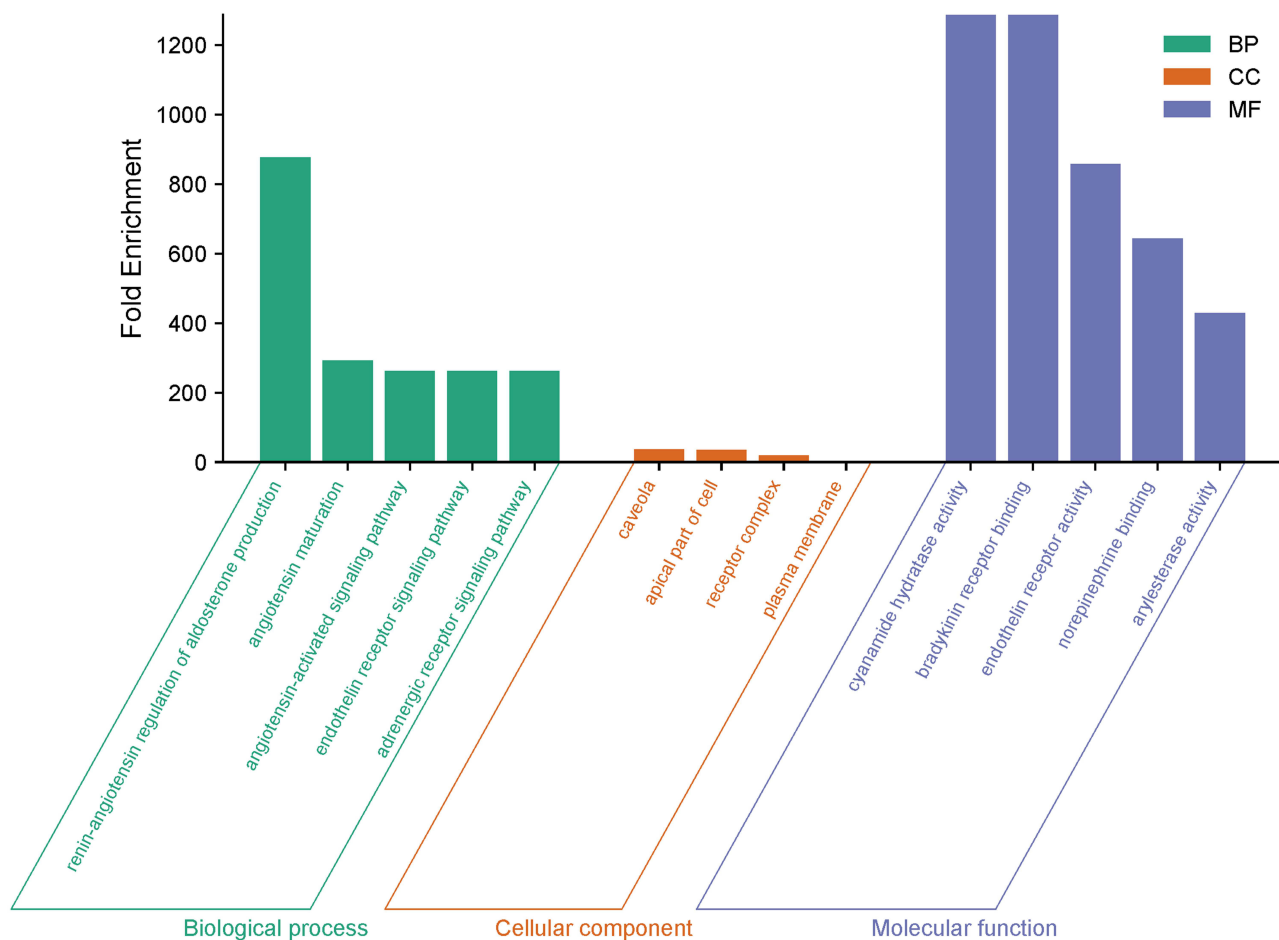


Figure 10 GO enrichment analysis.

activating inflammatory pathways through endothelial cells, which may contribute to both arterial inflammation and the onset of RA.³³

In our study, three antihypertensive drugs, adrenergic neuron blockers, central antihypertensives, and PSDs and aldosterone antagonists, were verified to be causally associated with RA. Nevertheless, other antihypertensive medications have also been demonstrated to be potentially linked to RA, as their therapeutic efficacy may originate, at least in part, from the reduction of inflammation associated with hypertension. Angiotensin II has been demonstrated to elevate the levels of adhesion molecules, cytokines, and chemokines, thereby eliciting proinflammatory effects on leukocytes, endothelial cells, and vascular smooth muscle cells. ARB, conversely, have been demonstrated to impede the activity of inflammatory mediators, including reactive oxygen species and C-reactive protein.¹⁴ Additionally, ARB attenuate the chronic low-grade inflammatory state associated with hypertension by modulating immune cell activity. This includes inhibition of T cell activation and reduction of inflammatory secretions from monocytes.^{34,35} The mechanism of action of ARB on inflammatory processes is comparable to that of ACEIs. ARB exert anti-inflammatory effects by inhibiting the proinflammatory actions of angiotensin II through the selective blockade of angiotensin II receptors, particularly AT1 receptors.³⁶ In an open study, 15 patients with RA were treated with captopril for 48 weeks, and 10 of them reported improvement in rheumatic symptoms, which may be related to the presence of sulfhydryl groups in captopril, similar to d - penicillamine.^{37,38} CCBs reduce the activity of T and B cells by blocking the entry of calcium ions into cells.³⁹ The inhibition of pro-inflammatory cytokine release is a key mechanism of action for both CCB and NSAIDs.

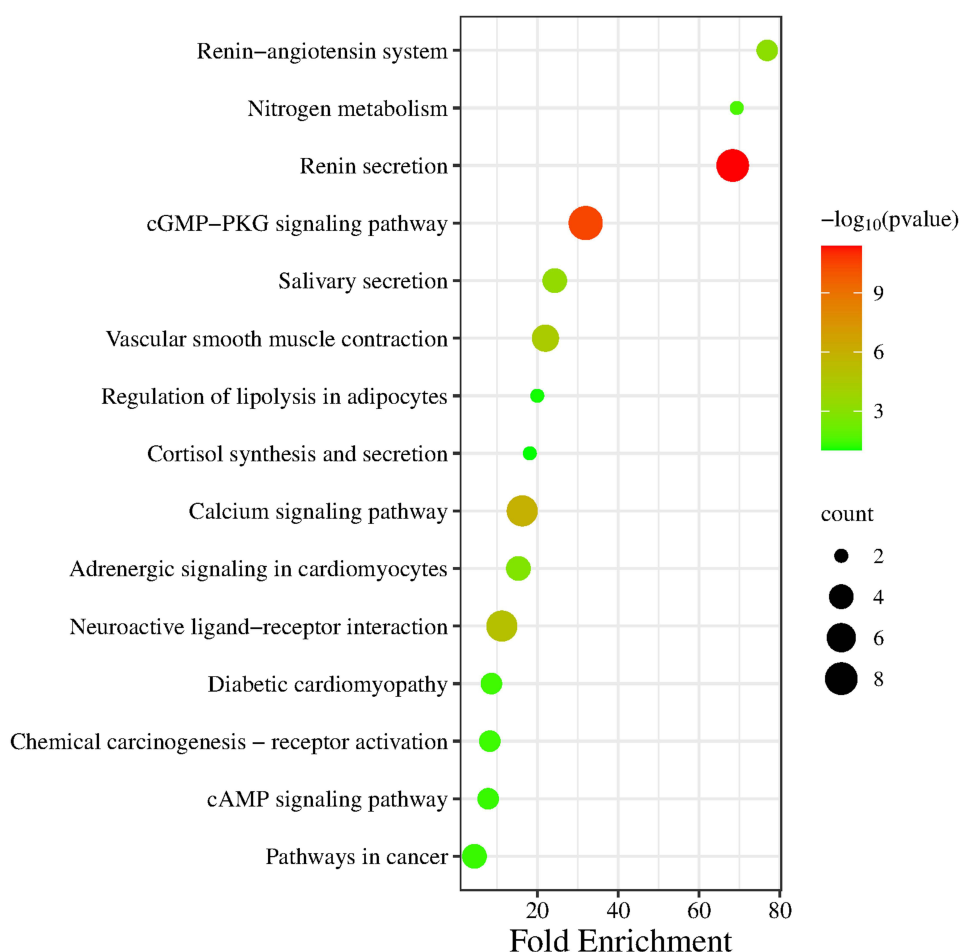


Figure 11 Bubble diagram of KEGG signaling pathway.

CCB reduces inflammation levels by inhibiting calcium ion inward flow and decreasing the production of pro-inflammatory cytokines, including tumor necrosis factor alpha and IL-6.³⁶

RA is a chronic systemic inflammatory disease, and adrenergic neuron blockers, especially beta-blockers, can reduce sympathetic nervous system (SNS) overactivation by blocking beta-adrenergic receptors.⁴⁰ The sympathetic nervous system regulates the immune response primarily by innervating lymphoid organs and releasing norepinephrine and epinephrine.⁴¹ In a model of rheumatoid arthritis, the SNS mediates proinflammatory effects through a β_2 -adrenergic mechanism.⁴² Activation of SNS enhances ADRB expression in CD8+ T cells and other immune cells by inhibiting ADRB, β -blockers can modulate these stress responses and immune cell mobilization, reducing the inflammatory response.³⁶ This suggests a direct anti-inflammatory action of beta-blockers by modulating immune responses, independent of blood pressure reduction. In addition, central antihypertensive drugs lower blood pressure mainly by stimulating central α_2 -adrenergic receptors causing peripheral sympathetic inhibition.⁴³ The central antihypertensive drugs are also known to reduce blood pressure. Thus, central antihypertensives reduce the inflammatory response indirectly through blood pressure control, rather than through a direct immune modulation mechanism. In previous studies it was indicated that aldosterone plays an active role in proinflammation.⁴⁴ The anti-inflammatory potential of spironolactone, a drug used in the treatment of aldosteronism, is indicated by its significant inhibitory effect on the transcription and release of numerous pro-inflammatory cytokines, including tumor necrosis factor, interferon-gamma neutralizing antibody, and granulocyte-macrophage colony-stimulating factor. These effects have been observed in blood cells from RA patients,

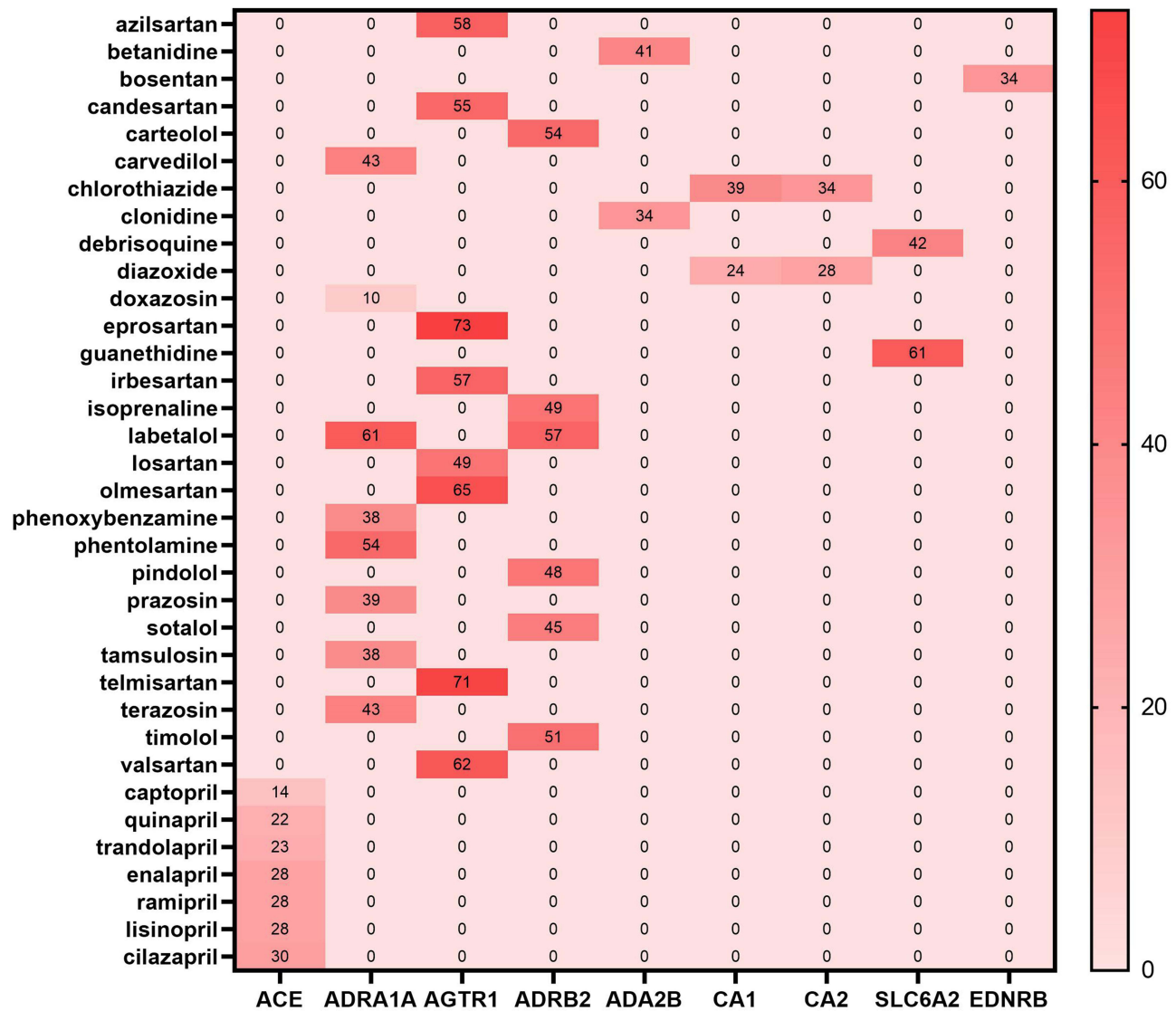


Figure 12 Molecular docking results of drug ingredients and the corresponding protein of the gene targets.

indicating that spironolactone’s anti-inflammatory action is likely direct. Regular spironolactone treatment may therefore inhibit these cytokines, reducing the inflammatory response in RA patients.⁴⁵

The KEGG pathway enrichment analysis revealed that the drugs exhibited a high degree of interaction with multiple signaling pathways, including renin secretion, cGMP-PKG signaling, calcium signaling, neuroactive ligand-receptor interaction, and vascular smooth muscle contraction. The renin inhibitor aliskiren has been demonstrated to effectively inhibit macrophage infiltration and the expression of chemokines (eg, OPN and MCP-1) by decreasing renin activity and lowering Ang II levels in podocytes, thereby reducing inflammatory responses.⁴⁶ cGMP-PKG signaling pathway regulates multiple physiological processes such as inflammation, vascular smooth muscle contraction, and apoptosis through the cGMP-PKG phosphorylation function.⁴⁷ Furthermore, evidence indicates that the cGMP signaling pathway plays a role in modulating the inflammatory response by inhibiting T cell activation.⁴⁸ The calcium signaling pathway plays a pivotal role in maintaining calcium concentration homeostasis and cell signaling in vivo. It also regulates selective lipopolysaccharide induced macrophage function by activating protein kinase C, which participates in host defense and inflammation. Additionally, protein kinase C promotes IκBα re-synthesis, temporarily inhibiting NF-κB activity and thus participating in the inflammatory response.^{49,50} Several network pharmacology studies have indicated that

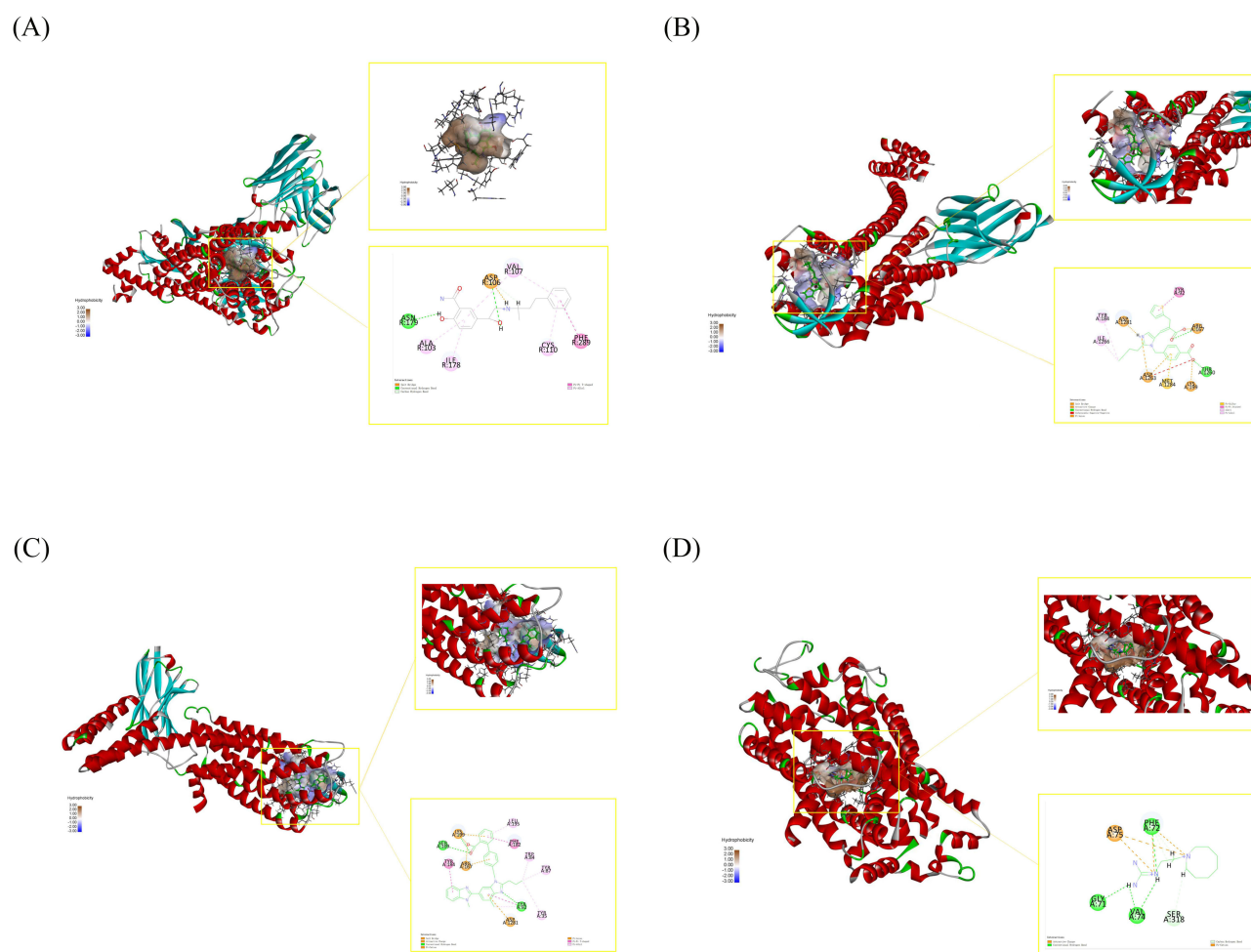


Figure 13 The results of molecular docking between the core targets and core ingredients (Four of the most strongly affinitive results). (A) ADRA1A-labetalol(-CDOCKER Interaction Energy: 61kcal/mol), (B) AGTR1-eprosartan(-CDOCKER Interaction Energy: 73kcal/mol), (C) AGTR1-telmisartan(-CDOCKER Interaction Energy: 71kcal/mol), (D) SLC6A2-guanethidine(-CDOCKER Interaction Energy: 61kcal/mol).

neuroactive ligand-receptor interactions are also important in RA, modulating both inflammation and analgesia.^{51–53} Furthermore, several studies have shown that the MAPK and PI3K/Akt pathways interact with vascular smooth muscle contraction signaling, with drugs like CCBs reducing inflammation and vascular reactivity, thus influencing both hypertension and RA.^{54,55}

Our molecular docking analysis identified three key targets—ADRA1A, AGTR1, and SLC6A2—that may play significant roles in the interaction between antihypertensive drugs and RA. ADRA1A, a subtype of the alpha-1 adrenergic receptor, regulates vascular smooth muscle contraction and is involved in sympathetic nervous system activation, which exacerbates inflammation in RA.^{56,57} Blocking ADRA1A with adrenergic neuron blockers may reduce this activation and modulate immune responses, potentially decreasing RA-related inflammation. AGTR1, a component of the renin-angiotensin system, is involved in both blood pressure regulation and inflammatory processes.⁵⁸ Targeting AGTR1 with ARB can lower blood pressure and inhibit proinflammatory pathways, offering a dual therapeutic effect for RA. SLC6A2, responsible for norepinephrine reuptake, regulates sympathetic tone.⁵⁹ By targeting SLC6A2, antihypertensive drugs may reduce excessive norepinephrine levels, attenuating inflammation in RA.

Our study has the following strengths: (1) The use of MR to study the effects of blood pressure and antihypertensive medications on RA largely reduces bias and confounders. (2) We used publicly available data in conjunction with our study design to screen instrumental variables, ensuring maximum reliability of instrumental variable estimates. (3) We

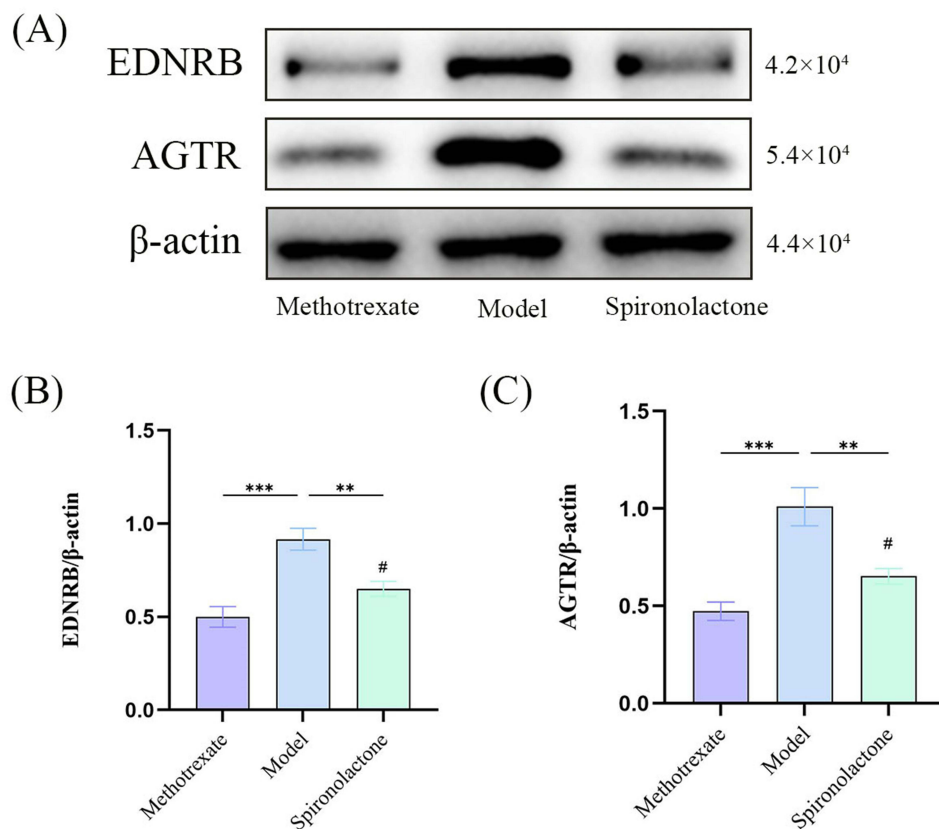


Figure 14 (A–C) Relative protein expression of EDNRB, AGTR, and β -actin in rat synovial tissue. Compared with the model group, *** $P < 0.001$, ** $P < 0.01$; compared with the methotrexate group, # $P < 0.05$.

further validated the reliability of the Mendelian findings through web-based pharmacological screening and molecular docking techniques.

It should be noted that this study is not without limitations. Firstly, the GWAS data used were limited to individuals of European ancestry, which precludes any exploration of the effects of blood pressure and anti-hypertensive medications on an ethnically diverse population. This limits the generalizability of the findings and may introduce bias due to population-specific genetic structures. Secondly, the assumption underlying MR is that genetic effects are independent of environmental factors. However, there is evidence to suggest that gene-environment interactions may influence disease onset and progression. Failure to consider these interactions may result in biased outcomes. Third, MR studies typically concentrate on the cumulative effects of lifetime exposures rather than the immediate effects of short-term exposures. However, the effects of drugs are typically observed within a relatively short period of time. As a result, the effect sizes observed in our study may not be directly comparable to those reported in clinical trials or observational studies. For instance, the genetic predisposition might lead to more permanent changes in blood pressure regulation, while the effects of medication could be reversible or less pronounced once the treatment is discontinued. Fourth, different classes of antihypertensive drugs have distinct mechanisms and may influence RA risk through various pathways. As a result, a single IVW analysis may not fully capture their effects on RA. Furthermore, despite employing rigorous sensitivity analyses (MR-Egger test, MR-PRESSO test) and finding no evidence of horizontal pleiotropy, the possibility of residual pleiotropy cannot be entirely ruled out. Undetected pleiotropic pathways may influence causal estimation results. Fifth, antihypertensive drugs primarily reduce cardiovascular risk by controlling blood pressure, whereas RA is a chronic inflammatory disease. Although some antihypertensive drugs may indirectly

impact RA progression through blood pressure control, they do not directly target RA's inflammatory mechanisms, which may account for the lack of significant statistical findings.

Conclusion

The objective of this study was to investigate the impact of blood pressure and antihypertensive medications on the development of rheumatoid arthritis. To achieve this, we employed the technique of MR. The results of our study indicate that elevated DBP is a significant risk factor for rheumatoid arthritis. Among the antihypertensive drugs, adrenergic neuron blockers, central antihypertensives, and PSDs and aldosterone antagonists have been identified as having beneficial effects on rheumatoid arthritis. Finally, the Mendelian results for the drugs and diseases were mined using network pharmacology, analyzed using GO and KEGG enrichment, and validated through molecular docking technology. These techniques were employed to further verify the reliability of the Mendelian results. These analyses suggest that these three classes of antihypertensive drugs may have potential therapeutic implications for RA, particularly in RA patients with concomitant hypertension. However, it is important to note that drug-target MR indicates association rather than clinical efficacy, and clinical trials would be necessary to confirm the therapeutic effectiveness of these drugs in RA treatment. We suggest exploring their combination with traditional RA treatments (such as anti-inflammatory drugs or immunosuppressants) to improve treatment outcomes, and conducting randomized controlled clinical trials to verify the actual efficacy of these drugs in RA patients, especially in terms of reducing disease activity and improving prognosis.

Abbreviations

RA, Rheumatoid arthritis; SBP, Systolic blood pressure; ACE-I, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin II Receptor Blocker; MR, Mendelian randomization; DBP, Diastolic blood pressure; CCBs, Calcium channel blockers; SNPs, Single nucleotide polymorphisms; GWAS, Genome Wide Association Study; IVW, Inverse variance weighting; TTD, Therapeutic Target Database; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, Biological process; CC, Cellular component; MF, Molecular function; PSDs, Potassium-sparing diuretics; IL-6, Interleukin-6; SNS, Sympathetic nervous system; EDNRB, Endothelin Receptor Type B; AGTR, Angiotensin II Receptor Type; CFA, Complete Freund's adjuvant; IFA, Incomplete Freund's adjuvant; BCII, Bovine Collagen type II.

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/additional Supplementary Material links (<https://gwas.mrcieu.ac.uk/datasets/ukb-a-360/>, <https://gwas.mrcieu.ac.uk/datasets/ukb-a-359/>, <https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018910/>). The dataset supporting the conclusions of this article is included within the article (and its additional file).

Ethics Approval and Consent to Participate

This study involved secondary analyses of de-identified, publicly available summary-level genetic data. The data sources include: GWAS data from the IEU Open GWAS database, and genetic variant data related to antihypertensive drug targets obtained from published literature. According to the Measures for the Ethical Review of Life Science and Medical Research Involving Human Subjects (issued by the National Health Commission of China, effective February 18, 2023), Article 32, Items 1 and 2, research using legally and publicly available human data, or data in which individual identities cannot be ascertained, is exempt from ethical review. This study meets the above criteria and is therefore exempt from the requirement for institutional ethical approval.

The experiment was approved by the Animal Ethics Committee of the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (Ethics Approval SDSZYYAWE20240827001). Animal care and experimental procedures complied with institutional and national guidelines for the care and use of laboratory animals. This study was conducted and reported in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. All

experimental procedures involving animals were designed, performed, and described following the ARRIVE checklist to ensure transparency, reproducibility, and animal welfare.

Acknowledgments

All data used in this study were obtained from openly available databases and consortiums. We gratefully acknowledge KEGG for granting permission to reproduce their pathway maps in this article. We express our sincere appreciation to them.

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of the article. This study was sponsored by the Natural Science Foundation of Shandong Province (ZR202112030177).

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature*. 2003;423(6937):356–361. doi:10.1038/nature01661
2. Giles JT. Extra-articular manifestations and comorbidity in rheumatoid arthritis: potential impact of pre-rheumatoid arthritis prevention. *Clin Ther*. 2019;41(7):1246–1255. doi:10.1016/j.clinthera.2019.04.018
3. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. *J Autoimmun*. 2020;110:102400. doi:10.1016/j.jaut.2019.102400
4. Luque Ramos A, Redeker I, Hoffmann F, Callhoff J, Zink A, Albrecht K. Comorbidities in patients with rheumatoid arthritis and their association with patient-reported outcomes: results of claims data linked to questionnaire survey. *J Rheumatol*. 2019;46(6):564–571. doi:10.3899/jrheum.180668
5. Panoulas VF, Metsios GS, Pace AV, et al. Hypertension in rheumatoid arthritis. *Rheumatology*. 2008;47(9):1286–1298. doi:10.1093/rheumatology/ken159
6. Arosio E, De Marchi S, Rigoni A, Prior M, Delva P, Lechi A. Forearm haemodynamics, arterial stiffness and microcirculatory reactivity in rheumatoid arthritis. *J Hypertens*. 2007;25(6):1273–1278. doi:10.1097/HJH.0b013e3280b0157e
7. Franklin SS. Arterial stiffness and hypertension: a two-way street? *Hypertension*. 2005;45(3):349–351. doi:10.1161/01.HYP.0000157819.31611.87
8. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364(9438):937–952. doi:10.1016/S0140-6736(04)17018-9
9. Murray CJ, Aravkin AY, Zheng P, et al. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019. *Lancet*. 2020;396(10258):1223–1249. doi:10.1016/S0140-6736(20)30752-2
10. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365(9455):217–223. doi:10.1016/S0140-6736(05)17741-1
11. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360(9349):1903–1913.
12. MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990;335(8692):765–774. doi:10.1016/0140-6736(90)90878-9
13. Singh G, Miller JD, Huse DM, Pettitt D, D'Agostino RB, Russell MW. Consequences of increased systolic blood pressure in patients with osteoarthritis and rheumatoid arthritis. *J Rheumatol*. 2003;30(4):714–719.
14. Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. *J Hum Hypertens*. 2007;21(1):20–27. doi:10.1038/sj.jhh.1002101
15. Adlan AM, Paton JF, Lip GY, Kitas GD, Fisher JP. Increased sympathetic nerve activity and reduced cardiac baroreflex sensitivity in rheumatoid arthritis. *J Physiol*. 2017;595(3):967–981. doi:10.1113/JP272944

16. Ferreira MB, Fonseca T, Costa R, et al. Prevalence, risk factors and proteomic bioprofiles associated with heart failure in rheumatoid arthritis: the RA-HF study. *Eur J Intern Med.* 2021;85:41–49. doi:10.1016/j.ejim.2020.11.002
17. Shi AX, Zivich PN, Chu H. A comprehensive review and tutorial on confounding adjustment methods for estimating treatment effects using observational data. *Appl Sci.* 2024;14(9):3662. doi:10.3390/app14093662
18. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med.* 2008;27(8):1133–1163. doi:10.1002/sim.3034
19. Zhang Y, Yu S, Chen Z, et al. Gestational diabetes and future cardiovascular diseases: associations by sex-specific genetic data. *Eur Heart J.* 2024;45(48):5156–5167.
20. Zhao L, Zhang H, Li N, et al. Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula. *J Ethnopharmacol.* 2023;309:116306. doi:10.1016/j.jep.2023.116306
21. Xu Y, Wang Z, Jia T, Liang S. Exploring potential drug targets for SLE through Mendelian randomization and network pharmacology. *PLoS One.* 2025;20(1):e0316481. doi:10.1371/journal.pone.0316481
22. Xu H, Wu Z, Feng F, Li Y, Zhang S. Low vitamin D concentrations and BMI are causal factors for primary biliary cholangitis: a mendelian randomization study. *Front Immunol.* 2022;13:1055953. doi:10.3389/fimmu.2022.1055953
23. Walker VM, Kehoe PG, Martin RM, Davies NM. Repurposing antihypertensive drugs for the prevention of Alzheimer's disease: a Mendelian randomization study. *Int J Epidemiol.* 2020;49(4):1132–1140. doi:10.1093/ije/dyz155
24. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet.* 2021;53(10):1415–1424. doi:10.1038/s41588-021-00931-x
25. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci.* 2019;28(11):1947–1951. doi:10.1002/pro.3715
26. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res.* 2023;51(D1):D587–d592. doi:10.1093/nar/gkac963
27. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* 2000;28(1):27–30. doi:10.1093/nar/28.1.27
28. Tianyue M, Yimei F, Ganlin G, et al. 二四汤对牛II型胶原诱导大鼠类风湿性关节炎的干预机制 [Mechanisms of Ersi Decoction on Rheumatoid Arthritis Rats Induced by Bovine Type II Collagen-solution]. *Chinese Journal of Experimental Traditional Medical Formulae.* 2022;28(05):38–45. Chinese.
29. Han C, Robinson DW, Hackett MV, Paramore LC, Fraeman KH, Bala MV. Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *J Rheumatol.* 2006;33(11):2167–2172.
30. Chae CU, Lee RT, Rifai N, Ridker PM. Blood pressure and inflammation in apparently healthy men. *Hypertension.* 2001;38(3):399–403. doi:10.1161/01.HYP.38.3.399
31. Shen CK, Huang BR, Yeh WL, et al. Regulatory effects of IL-1 β in the interaction of GBM and tumor-associated monocyte through VCAM-1 and ICAM-1. *Eur J Pharmacol.* 2021;905:174216. doi:10.1016/j.ejphar.2021.174216
32. Sung KC, Suh JY, Kim BS, et al. High sensitivity C-reactive protein as an independent risk factor for essential hypertension. *Am J Hypertens.* 2003;16(6):429–433. doi:10.1016/S0895-7061(03)00566-1
33. Dai G, Kaazempur-Mofrad MR, Natarajan S, et al. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. *Proc Natl Acad Sci U S A.* 2004;101(41):14871–14876. doi:10.1073/pnas.0406073101
34. Boskabadi J, Askari VR, Hosseini M, Boskabadi MH. Immunomodulatory properties of captopril, an ACE inhibitor, on LPS-induced lung inflammation and fibrosis as well as oxidative stress. *Inflammopharmacology.* 2019;27(3):639–647. doi:10.1007/s10787-018-0535-4
35. Platten M, Youssef S, Hur EM, et al. Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity. *Proc Natl Acad Sci U S A.* 2009;106(35):14948–14953. doi:10.1073/pnas.0903958106
36. Felkle D, Jarczyński M, Kaleta K, Zięba K, Nazimek K. The immunomodulatory effects of antihypertensive therapy: a review. *Biomed Pharmacother.* 2022;153:113287. doi:10.1016/j.biopha.2022.113287
37. Martin MF, Surrall KE, McKenna F, Dixon JS, Bird HA, Wright V. Captopril: a new treatment for rheumatoid arthritis? *Lancet.* 1984;1(8390):1325–1328. doi:10.1016/S0140-6736(84)91821-X
38. Popa CD, van Riel PL. The use of captopril in rheumatoid arthritis: combining treatment targets! *Can J Cardiol.* 2013;29(5):639.e613.
39. Liu W, Matsumori A. Calcium channel blockers and modulation of innate immunity. *Curr Opin Infect Dis.* 2011;24(3):254–258. doi:10.1097/QCO.0b013e3283463e5b
40. Oberbeck R, Kobbe P. Beta-adrenergic antagonists: indications and potential immunomodulatory side effects in the critically ill. *Curr Med Chem.* 2009;16(9):1082–1090. doi:10.2174/092986709787581770
41. Scanzano A, Cosentino M. Adrenergic regulation of innate immunity: a review. *Front Pharmacol.* 2015;6:171. doi:10.3389/fphar.2015.00171
42. Gao D, Gao X, Yang F, Wang Q. Neuroimmune Crosstalk in Rheumatoid Arthritis. *Int J Mol Sci.* 2022;23(15):8158.
43. van Zwieten PA. Centrally acting antihypertensive drugs. Present and future. *Clin Exp Hypertens.* 1999;21(5–6):859–873. doi:10.3109/10641969909061015
44. Pacurari M, Kafoury R, Tchounwou PB, Ndebele K. The Renin-Angiotensin-aldosterone system in vascular inflammation and remodeling. *Int J Inflam.* 2014;2014:689360. doi:10.1155/2014/689360
45. Bendtzen K, Hansen PR, Rieneck K. Spironolactone inhibits production of proinflammatory cytokines, including tumour necrosis factor- α and interferon- γ , and has potential in the treatment of arthritis. *Clin Exp Immunol.* 2003;134(1):151–158. doi:10.1046/j.1365-2249.2003.02249.x
46. Choi DE, Jeong JY, Lim BJ, et al. Aliskiren ameliorates renal inflammation and fibrosis induced by unilateral ureteral obstruction in mice. *J Urol.* 2011;186(2):694–701. doi:10.1016/j.juro.2011.03.122
47. Siednienko J, Nowak J, Moynagh PN, Gorczyca WA. Nitric oxide affects IL-6 expression in human peripheral blood mononuclear cells involving cGMP-dependent modulation of NF- κ B activity. *Cytokine.* 2011;54(3):282–288. doi:10.1016/j.cyto.2011.02.015
48. Fischer TA, Palmesthofer A, Gambaryan S, et al. Activation of cGMP-dependent protein kinase I β inhibits interleukin 2 release and proliferation of T cell receptor-stimulated human peripheral T cells. *J Biol Chem.* 2001;276(8):5967–5974. doi:10.1074/jbc.M009781200
49. Han Y, Meng T, Murray NR, Fields AP, Brasier AR. Interleukin-1-induced nuclear factor-kappaB-IkappaB α autoregulatory feedback loop in hepatocytes. A role for protein kinase C α in post-transcriptional regulation of ikappaB α resynthesis. *J Biol Chem.* 1999;274(2):939–947. doi:10.1074/jbc.274.2.939

50. St-Denis A, Chano F, Tremblay P, St-Pierre Y, Descoteaux A. Protein kinase C- α modulates lipopolysaccharide-induced functions in a murine macrophage cell line. *J Biol Chem*. 1998;273(49):32787–32792. doi:10.1074/jbc.273.49.32787
51. Nandi A, Das A, Dey YN, Roy KK. The abundant phytocannabinoids in rheumatoid arthritis: therapeutic targets and molecular processes identified using integrated bioinformatics and network pharmacology. *Life*. 2023;13(3):700.
52. Zhang X, Dong X, Zhang R, et al. Compounds identification and mechanism prediction of YuXueBi capsule in the treatment of arthritis by integrating UPLC/IM-QTOF-MS and network pharmacology. *Heliyon*. 2024;10(7):e28736. doi:10.1016/j.heliyon.2024.e28736
53. Zhang Y, Wang D, Tan S, Xu H, Liu C, Lin N. A systems biology-based investigation into the pharmacological mechanisms of wu tou tang acting on rheumatoid arthritis by integrating network analysis. *Evid Based Complement Alternat Med*. 2013;2013:548498. doi:10.1155/2013/548498
54. Liu Y, Wei W, Hong C, et al. Calreticulin induced endothelial ICAM-1 up-regulation associated with tristetraproline expression alteration through PI3K/Akt/eNOS/p38 MAPK signaling pathway in rheumatoid arthritis. *Mol Immunol*. 2019;107:10–20. doi:10.1016/j.molimm.2019.01.005
55. Nong SX, Liang D, Ma X, et al. Differential expression and significance of peripheral blood genes in coronary artery heart disease. *J Thorac Dis*. 2022;14(9):3415–3428. doi:10.21037/jtd-22-991
56. Sandroni PB, Schroder MA, Hawkins HT, et al. The α 1A-adrenergic receptor regulates mitochondrial oxidative metabolism in the mouse heart. *J Mol Cell Cardiol*. 2024;187:101–117. doi:10.1016/j.yjmcc.2023.12.003
57. Tripathi A, Vana PG, Chavan TS, et al. Heteromerization of chemokine (C-X-C motif) receptor 4 with α 1A/B-adrenergic receptors controls α 1-adrenergic receptor function. *Proc Natl Acad Sci U S A*. 2015;112(13):E1659–1668. doi:10.1073/pnas.1417564112
58. Erichsen L, Thimm C, Bohndorf M, Rahman MS, Wruck W, Adjaye J. Activation of the renin-angiotensin system disrupts the cytoskeletal architecture of human urine-derived podocytes. *Cells*. 2022;11(7). doi:10.3390/cells11071095
59. Ryu V, Buettner C. Fat cells gobbling up norepinephrine? *PLoS Biol*. 2019;17(2):e3000138. doi:10.1371/journal.pbio.3000138

International Journal of General Medicine

Publish your work in this journal

The International Journal of General Medicine is an international, peer-reviewed open-access journal that focuses on general and internal medicine, pathogenesis, epidemiology, diagnosis, monitoring and treatment protocols. The journal is characterized by the rapid reporting of reviews, original research and clinical studies across all disease areas. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/international-journal-of-general-medicine-journal>

Dovepress
Taylor & Francis Group