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ORIGINAL RESEARCH

# Causal Association Between Anemia and Cardiovascular Disease: A 2-Sample Bidirectional Mendelian Randomization Study

Ting Gan, MD*; Jing Hu, MD*; Wenhu Liu, MD; Cui Li, MS; Qian Xu, MS; Ya Wang, MD; Shuai Lu, MD; Anwer Khalid Okab Aledan, MS; Yan Wang, MD* Zhaohui Wang, MD*

**BACKGROUND:** Although previous observational studies have shown an association between anemia and cardiovascular disease (CVD), the underlying causal relationship between anemia and CVD remains uncertain.

**METHODS AND RESULTS:** We conducted a 2-sample bidirectional Mendelian randomization (MR) study to assess the causal association between anemia and CVD. We extracted summary statistics data for anemia, heart failure (HF), coronary artery disease (CAD), atrial fibrillation, any stroke, and any ischemic stroke (AIS) from relevant published genome-wide association studies. After rigorous quality control steps, independent single-nucleotide polymorphisms for each disease were selected as instrumental variables. Inverse-variance weighting was used as the primary method to estimate the causal association between anemia and CVD in the 2-sample MR analysis. Simultaneously, we performed a series of multiple methods analyses (median weighting, maximum likelihood [MR robust adjusted profile score]), sensitivity analyses (Cochran's *Q* test and MR-Egger intercept, leave-one-out test [MR pleiotropy residual sum and outlier]), instrumental variable strength evaluations (*F* statistic), and statistic power estimates to verify the robustness and reliability of our results. Furthermore, the associations between anemia and CVD from different studies, including the UK Biobank and FinnGen studies, were combined by meta-analysis. The MR analysis showed that genetically predicted anemia was significantly associated with HF risk at the Bonferroni-corrected significance level (odds ratio [OR], 1.11 [95% CI, 1.04–1.18]; *P*=0.002) and was suggestively associated with CAD risk (OR, 1.11 [95% CI, 1.02–1.22]; *P*=0.020). However, the associations between anemia and atrial fibrillation, any stroke, or AIS were not statistically significant. In the reverse MR analysis, we found that genetic susceptibility to HF, CAD, and AIS was significantly associated with anemia risk. The ORs of HF, CAD, and AIS were 1.64 (95% CI, 1.39–1.94; *P*=7.60E-09), 1.16 (95% CI, 1.08–1.24; *P*=2.32E-05), and 1.30 (95% CI, 1.11–1.52; *P*=0.001), respectively. Genetically predicted atrial fibrillation was suggestively associated with anemia (OR, 1.06 [95% CI, 1.01–1.12]; *P*=0.015). Sensitivity analyses found weak evidence of horizontal pleiotropy and heterogeneity, which ensured the robustness and reliability of the results. Meta-analysis also showed the statistically significant association between anemia and HF risk.

**CONCLUSIONS:** Our study supports bidirectional causality between anemia and HF and significant associations between genetic predisposition to CAD and AIS with anemia, which contributes to the clinical management of both diseases.

**Key Words:** cardiovascular disease ■ anemia ■ Mendelian randomization ■ heart failure ■ coronary artery disease ■ atrial fibrillation ■ stroke ■ any ischemic stroke

Correspondence to: Zhaohui Wang, MD, and Yan Wang, MD, Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Dadao, Jianghan District, Wuhan City, Hubei Province, China. Email: wuxiaohongtian@163.com; tomato82@126.com

*Drs Gan, Hu, Y. Wang, and Z. Wang contributed equally.

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## CLINICAL PERSPECTIVE

### What Is New?

- Although previous observational studies have shown an association between anemia and cardiovascular diseases, the underlying causal relationship between anemia and cardiovascular diseases remains uncertain.
- We conducted a 2-sample bidirectional Mendelian randomization study to assess the causal association between anemia and cardiovascular diseases.

### What Are the Clinical Implications?

- These findings support detection and treatment of anemia for preventing heart failure.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **AIS** | any ischemic stroke |
| **ARIC** | Atherosclerosis Risk in Communities |
| **AS** | any stroke |
| **IV** | instrumental variable |
| **IVW** | inverse-variance weighting |
| **MR** | Mendelian randomization |
| **PRESSO** | pleiotropy residual sum and outlier |

Cardiovascular diseases (CVDs) remains the leading cause of disease burden in the world,[1] with an 18.7% increase in deaths attributable to CVD from 2010 to 2020, making it the number 1 cause of death.[2] In addition to the well-recognized common CVD risk factors (hypertension, hyperlipidaemia, obesity, diabetes, smoking, and alcohol consumption), accumulating observational studies have suggested that anemia is associated with an increased risk of CVD.

Anemia, defined by the World Health Organization as a hemoglobin concentration of <13 g/dL in men and <12 g/dL in women, affects nearly one-third of the global population and is a clear public health burden and a public health issue.[3] Anemia is common among patients with CVD and is estimated to be prevalent in ≈10% to 43% of patients with acute coronary syndrome and 30% to 70% of patients with heart failure (HF).[4] The results of a prospective cohort study of 14 410 subjects without CVD at baseline showed that patients with anemia had a 41% increased risk of CVD compared with those without anemia during a mean follow-up of 6.1 years.[5] Several observational studies have suggested that anemia is related to an increased risk of adverse prognostic events in patients with HF, coronary artery disease (CAD), atrial fibrillation (AF), and stroke.[6–9] However, one study showed that the relationship between anemia and CAD was not statistically significant after adjusting for traditional CAD factors and covariates.[10] Notably, these observational studies may be affected by potential confounding factors (eg, diabetes, obesity, and hyperlipidemia), which makes it difficult to ascertain the causality between anemia and CVD.

Under assumptions, Mendelian randomization (MR) can estimate the causal relationship between an exposure and outcome by using genetic variants as instrumental variables (IVs), which are strongly associated with the exposure and strengthen the causal inference by controlling for nonheritable environmental confounders and reverse causation.[11] Summary statistics data obtained from genome-wide association studies (GWASs) provide reliable IVs for MR analyses. Previous MR analyses showed positive associations of hemoglobin concentration with the risk of CAD and venous thromboembolism,[12] which may be related to vasoconstriction attributable to scavenging of NO by hemoglobin and promotion of platelet adhesion or activation.[13] To date, there has been no genetic study on the relationship between anemia and CVD.

In the present study, we performed a 2-sample bidirectional MR study to explore the causal relationship between anemia and major CVD as well as the causal effect of CVD on anemia.

## METHODS

### Study Design

All data and materials used in this MR study are publicly available, and specific sources can be found in the article. An overview of the bidirectional MR study design is shown in Figure 1; we conducted the bidirectional MR study based on the 3 assumptions.[14] Informed consent and ethical approval were obtained for the original GWASs. This MR study was performed following the latest STROBE-MR (Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomisation) guideline.[15]

### Data Sources and Single-Nucleotide Polymorphism Selection for Anemia

Single-nucleotide polymorphisms (SNPs) were used as our IVs to conduct the 2-sample bidirectional MR study.[16] Summary-level data for anemia were obtained from FinnGen (FREEZE 7; https://r7.finngen.fi/), including 19 885 cases and 76 464 controls, with samples mainly from European individuals. Anemia was defined according to the *International Classification of Diseases, Tenth Revision* (*ICD-10*), codes (D50–D53, D55–D59, and D60–D64).

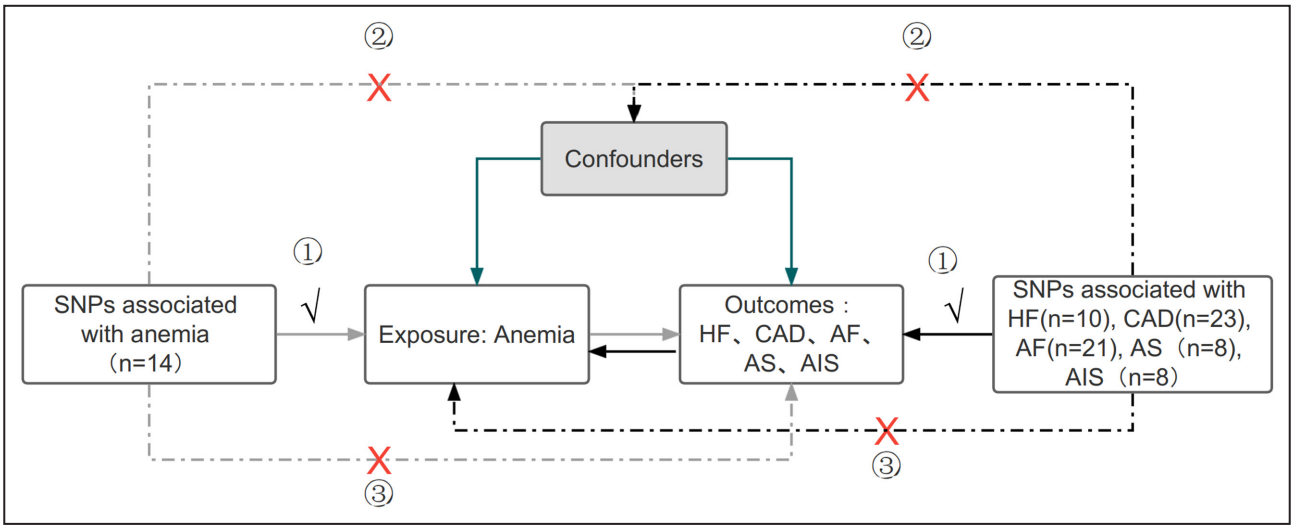


**Figure 1. Assumptions and study design flowchart of the MR study.**
The MR method is based on 3 hypotheses: (1) instrumental variables directly affect the exposure; (2) instrumental variables are independent of any confounding factor; and (3) instrumental variables affect the results only via exposure but not through other pathways. Gray lines show the relationship across instrumental variables, exposure, and outcomes in the MR study examining the effects of anemia on cardiovascular diseases, and black lines show these relationships in the reverse MR study. AF indicates atrial fibrillation; AIS, any ischemic stroke; AS, any stroke; CAD, coronary artery disease; HF, heart failure; MR, Mendelian randomization; and SNP, single-nucleotide polymorphism.

A series of screening procedures were conducted to select eligible IVs. First of all, because few SNPs identified by GWASs for anemia reached the genome-wide significance levels of $P<5\times10^{-8}$, SNPs with suggestive genome-wide significance ($P<5\times10^{-6}$) were selected as the IVs for anemia. Second, the clumping process ($r^2<0.001$ and clump window=10 000-kb) was conducted to select independent SNPs without linkage disequilibrium as the IVs (Table S1). Third, we retrieved the secondary phenotype of each SNP in the PhenoScanner database with a threshold of $P<1\times10^{-5}$ and removed SNPs associated with outcome confounders (Table S2) to avoid potential pleiotropic effects.[17] Fourth, SNPs with palindromic alleles and SNPs that were not available in the outcome GWASs were eliminated. Finally, we evaluated variance ($R^2$) and the $F$ statistic to detect whether the selected IVs were weakly correlated with the exposure.[18,19] $R^2$ indicates variability explained by genetic instruments. The $R^2$ was calculated using the formula[20]: $R^2=\beta^2(1-EAF)\times2EAF$. EAF is the frequency of mutated genes. $F$ statistics were calculated using the formula[19]: $F=R^2(N-K-1)/[K(1-R^2)]$. $K$ is the number of SNP-exposure association, and N is the sample size of the GWAS for the SNP-exposure association. SNPs with $F$ statistics >10 were defined as reliable and valid IVs, which could prevent the MR results from being influenced by weak instrument bias.

## Data Sources and SNP Selection for CVDs

Summary statistical data for 5 CVD outcomes were extracted from large genetic consortia.[21–24] Notably, we selected independent data sets for 2-sample MR analysis to avoid bias caused by overlapping samples. A description of the demographics and GWASs included in this study is shown in Table 1 and Table S1. Summary statistical data for HF (47 309 cases and 930 014 controls) were obtained from the Heart Failure Molecular Epidemiology for Therapeutic Targets Consortium.[21] Summary statistical data for CAD were extracted from a published GWAS meta-analysis of 48 studies, including 60 801 cases and 123 504 controls.[22] A summary data set for AF was retrieved from the Atrial Fibrillation Genetics Consortium, including 60 620 cases and 970 216 controls.[23] Summary-level data sets for any stroke (AS; 40 585 cases and 406 111 controls) and any ischemic stroke (AIS; 34 217 cases and 406 111 controls) were derived from the MEGASTROKE Consortium.[24] Ten SNPs associated with HF, 23 SNPs associated with CAD, 21 SNPs associated with AF, 8 SNPs associated with AS, and 8 SNPs associated with AIS reached the genome-wide significance level ($P<5\times10^{-8}$) and were selected as IVs for the corresponding exposure factors in the reverse-direction MR analysis.

## Statistical Analysis

In the MR analysis, we used inverse-variance weighting (IVW) as the primary analysis method, which calculated and combined the Wald ratio of each SNP through meta-analysis to estimate the overall effect of the exposure on the outcome.[25] Simultaneously, we conducted the following multiple analysis methods to assess the

**Table 1. Details of Data Sources Included in the Study**

| Diseases | Data source | Population | Sample size (cases/controls) | Covariates adjusted in GWAS |
|---|---|---|---|---|
| Anemia | FinnGen | European | 19885/76464 | Age, sex, and genotyping batch |
| Heart failure | HERMES | European | 47309/930014 | Age, sex, and principal components |
| Atrial fibrillation | AFGen | European | 60620/970216 | Birth year, sex, and genotype batch |
| Coronary artery disease | CARDIoGRAMplusC4D | Mixed | 60801/123504 | Sex, age, and generation |
| Any stroke | MEGASTROKE | European | 40585/406111 | Age, sex |
| Any ischemic stroke | MEGASTROKE | European | 34217/406111 | Age, sex |

AFGen indicates Atrial Fibrillation Genetics; CARDIoGRAMplusC4D, Coronary Artery Disease Genome-Wide Replication and Meta-Analysis Plus the Coronary Artery Disease Genetics; FinnGen, FinnGen consortium; GWAS, genome-wide association study; HERMES, Heart Failure Molecular Epidemiology for Therapeutic Targets; and MEGASTROKE, MEGASTROKE consortium.

robustness and reliability of the results: (1) the median weighting method, which could provide robust estimates and draw a reliable conclusion when more than half of the SNPs were valid IVs[26]; (2) the MR-Egger method, which could identify horizontal pleiotropy and correct the multiple effects, although it consumes statistical power[27]; (3) the maximum-likelihood method, which could combine data on multiple genetic variants into a single causal estimate, which has better finite-sample type 1 error rates and is complementary to the MR-Egger regression method[28]; (4) the MR robust adjusted profile score, which could correct horizontal pleiotropy and reduce the bias caused by pleiotropy effects[29]; and (5) the MR pleiotropy residual sum and outlier (MR-PRESSO) method, which could detect horizontal pleiotropy and correct horizontal pleiotropy via outlier removal.[30] Collectively, we used comprehensive methods to investigate the causal relationship between anemia and CVD to ensure the robustness and reliability of the results.

## Sensitivity Analyses

We conducted sensitivity analyses using a variety of methods. First, the Cochran *Q* test was performed to assess the heterogeneity between the SNP-specific estimates. If the heterogeneity was not statistically significant in the results ($P>0.05$), the fixed-effects model of IVW was used for MR analysis; otherwise, the random-effects model was used.[31] Second, the MR-Egger intercept method was conducted to assess the horizontal pleiotropy of SNPs.[27] Third, the leave-one-out sensitivity analysis was applied to detect whether the results were influenced by any single SNP. Fourth, we plotted funnel plots to visually judge the pleiotropy of SNPs and scatterplots to show associations between the exposure and outcome. Finally, we extracted genetic variants associated with anemia from the UK Biobank study and performed 2-sample MR analysis. The associations between anemia and CVDs from different databases, including the UK Biobank and FinnGen studies, were combined by meta-analysis.

The Bonferroni correction method was used to identify false-positive results caused by multiple tests. Associations with $P<0.005$ (0.05 divided by 10) were considered statistically significant, whereas associations with $P>0.005$ and $P<0.05$ were defined as suggestive associations. All statistical analyses were conducted using the "TwoSampleMR," "mr.raps," and "MR-PRESSO" packages in R software, version 4.2.0.

# RESULTS

## Characteristics of the Selected SNPs

We derived IVs that were significantly associated with anemia from the GWAS and removed some SNPs (*rs2476601*, *rs707926*, *rs3129761*, and *rs7184768*) after searching the PhenoScanner database, because they were associated with potential confounders, including "diabetes type 1," "systolic blood pressure," "self-reported type 1 diabetes," and "whole body fat-free mass" (Table S2). In addition, we removed palindromic SNPs (ie, A/T or G/C) and SNPs that were not available in the outcome. Finally, the screened SNPs with *F*-statistics all >10 (Table S1), which showed no weak tool bias, were selected as the IVs for the MR analyses (Tables S3–S8).

## Causal Effects of Anemia on CVD

The results of MR analyses are presented in Figure 2 and Figures S1 through S3. The results showed that genetically predicted anemia was significantly associated with an increased risk of HF (odds ratio [OR], 1.11 [95% CI, 1.04–1.18]; $P=0.002$) in the fixed-effects IVW model. The result was consistent in the maximum likelihood, weighted median, MR-PRESSO, and robust

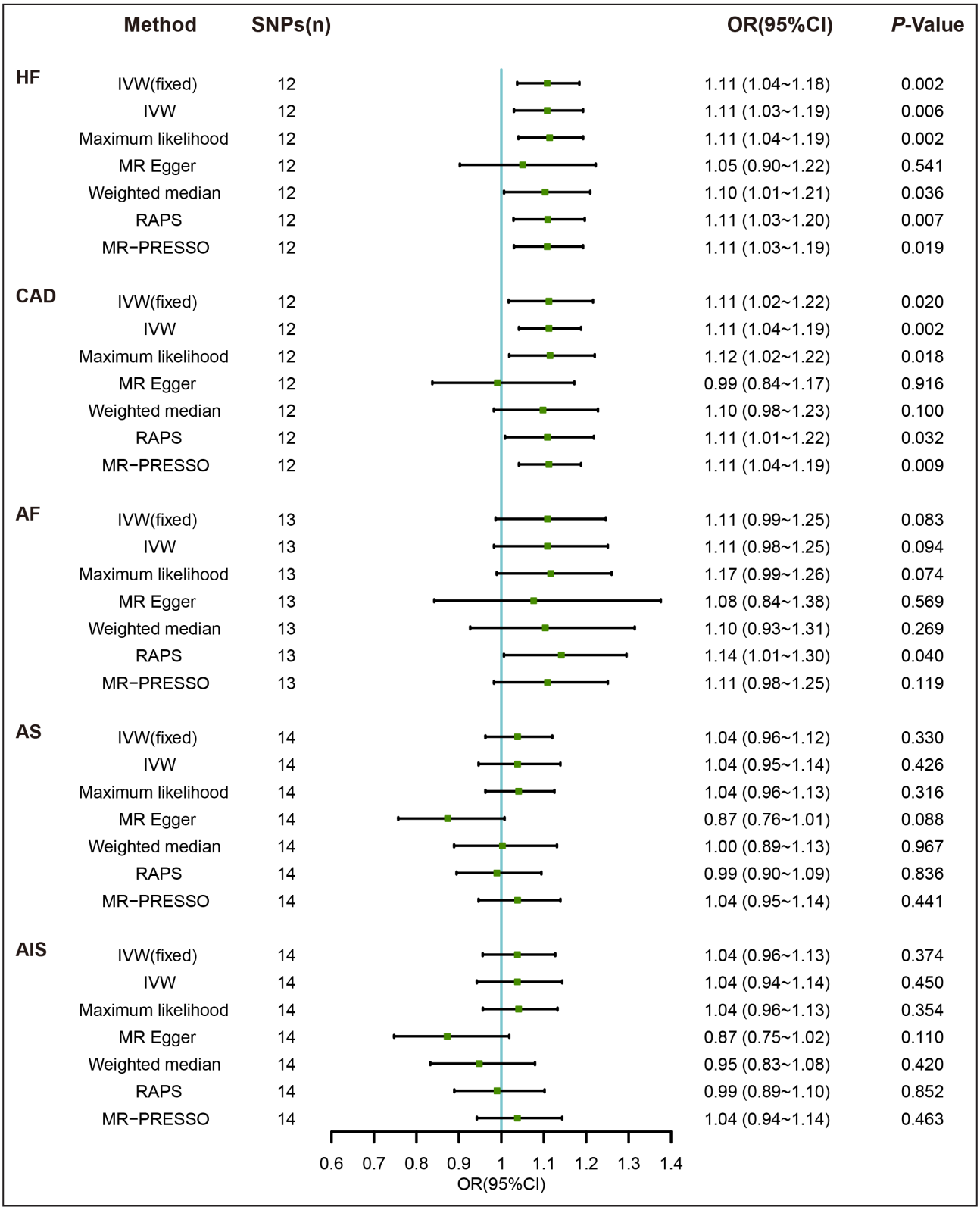


**Figure 2. Associations of genetically predicted anemia with risk of cardiovascular disease.**
AF indicates atrial fibrillation; AIS, any ischemic stroke; AS, any stroke; CAD, coronary artery disease; HF, heart failure; IVW, inverse-variance weighting; IVW(fixed), fixed-effects IVW; MR, Mendelian randomization; OR, odds ratio; PRESSO, pleiotropy residual sum and outlier; RAPS, robust adjusted profile score; and SNP, single-nucleotide polymorphism.

adjusted profile score analyses, although it was nonsignificant in the MR-Egger analysis. MR-PRESSO analysis detected no outliers, suggesting that the results were robust and reliable (Table S9). No heterogeneity among estimates of individual SNPs was detected in the Cochran *Q* test (*Q*=12.8; *P*=0.236), or no pleiotropy was observed in the MR-Egger regression analysis (intercept *P*=0.444; Table 2). Moreover, leave-one-out analysis suggested that the causal effect of anemia on HF was not driven by any single SNP (Figure S1).

Anemia was suggestively associated with CAD (OR, 1.11 [95% CI, 1.02–1.22]; *P*=0.020) in the fixed-effects IVW model. The heterogeneity (*P* value of Cochran *Q*>0.05), pleiotropy (*P* value for intercept >0.05), or outliers in the CAD analysis were not statistically significant (Table 2 and Table S9). Leave-one-out analysis showed that 1 outlier SNP was detected in the analysis of CAD (Figure S1).

There was no evidence of an association between genetic susceptibility to anemia and AF, AS, or AIS in the fixed-effects IVW analysis. However, robust adjusted profile score analysis suggested that anemia was suggestively associated with AF (OR, 1.14 [95% CI, 1.01–1.30]; *P*=0.040). The pleiotropy or heterogeneity was not statistically significant in the AF analysis (Table 2 and Table S9). Further studies are required to confirm the association between anemia and AF.

## Causal Effects of CVD on Anemia

After removing palindromic and unavailable SNPs, we selected 10 SNPs for HF, 23 SNPs for CAD, 21 SNPs for AF, 8 SNPs for AS, and 8 SNPs for AIS as the IVs in the analysis of the causal effects of CVDs on anemia (Tables S10–S15). The *F*-statistics of the screened SNPs were all >10 to avoid bias caused by weak IVs. The statistical results of reverse-direction MR are presented in Figure 3 and Figures S4 through S6. In the reverse-direction MR analyses, we found that genetic susceptibility to HF, CAD, and AIS was significantly associated with anemia. The ORs of HF, CAD, and AIS were 1.64 (95% CI, 1.39–1.94; *P*=7.60E-09), 1.16 (95% CI, 1.08–1.24; *P*=2.32E-05), and 1.31 (95% CI, 1.14–1.50; *P*=1.36E-04), respectively, in the fixed-effects IVW model. We observed that genetic susceptibility to AF was suggestively associated with anemia (OR, 1.06 [95% CI, 1.01–1.12]; *P*=0.015). The MR-PRESSO analysis detected no outliers in HF, CAD, or AF, and detected 1 and 2 outliers in AS and AIS, respectively (Table S16). The OR of AIS was 1.30 (95% CI, 1.11–1.52; *P*=0.001) in the fixed-effects IVW model after correction for 2 outliers in the MR-PRESSO analysis. Although genetic susceptibility to AS was suggestively associated with anemia in the fixed-effects IVW analysis (OR, 1.22 [95% CI, 1.05–1.42]; *P*=0.009), no association was observed after the removal of an outlier (OR, 1.13 [95% CI, 0.91–1.42]; *P*=0.275) in the random-effect analysis (Table S16). The heterogeneity among the estimates of individual SNPs was not statistically significant in the Cochran *Q* test, and the pleiotropy was not statistically significant in the MR-Egger regression analysis for 5 CVDs after correction for outliers in the MR-PRESSO analysis (Table 3 and Table S16). Moreover, leave-one-out analysis suggested that the causal relationships between HF, CAD, or AS with anemia were not driven by any single SNP, but 3 outlier SNPs were detected in the AIS analysis (Figure S4). In conclusion, the inverse MR analysis showed that higher genetic predictors of HF, CAD, and AIS were significantly and positively associated with increased risk of anemia after sensitivity and pleiotropy analyses at the Bonferroni-corrected significance level (*P*<0.005).

In sensitivity analyses, we conducted the MR-Steiger test in 2-sample bidirectional MR analysis in FinnGen study, and the results showed that the directions of causal association between anemia and HF and between HF and anemia were statistically significant (Table S17). Furthermore, we also extracted IVs associated with anemia from the UK Biobank study (Table S18). MR analysis showed that genetically predicted anemia was not significantly associated with CVD, whereas genetically predicted HF, AS, and AIS were significantly associated with anemia (Tables S19–S22). We conducted a meta-analysis to synthesize results on the association between anemia and CVD from different data sources, including the FinnGen and UK Biobank studies, and the analysis showed a significant association between genetically predicted

**Table 2. Associations of Genetically Predicted Anemia With Risk of CVD in Sensitivity Analyses in FinnGen Study**

| | Heterogeneity test MR-Egger | | | Heterogeneity test IVW | | | Pleiotropy test | | |
|---|---|---|---|---|---|---|---|---|---|
| CVD | *Q* | *Q* df | *Q P* value | *Q* | *Q* df | *Q P* value | Egger intercept | SE | *P* value |
| Heart failure | 12.8 | 10 | 0.236 | 13.6 | 11 | 0.256 | 0.006 | 0.008 | 0.444 |
| Coronary artery disease | 3.4 | 10 | 0.969 | 6.0 | 11 | 0.875 | 0.014 | 0.009 | 0.142 |
| Atrial fibrillation | 12.7 | 11 | 0.312 | 12.8 | 12 | 0.383 | 0.003 | 0.012 | 0.788 |
| Any stroke | 11.7 | 12 | 0.474 | 19.5 | 13 | 0.109 | 0.022 | 0.008 | 0.016 |
| Any ischemic stroke | 11.3 | 12 | 0.506 | 18.0 | 13 | 0.158 | 0.022 | 0.009 | 0.024 |

CVD indicates cardiovascular disease; IVW, inverse-variance weighting; MR, Mendelian randomization; *Q*, heterogeneity statistic *Q*; and *Q* df, *Q* degree of freedom.

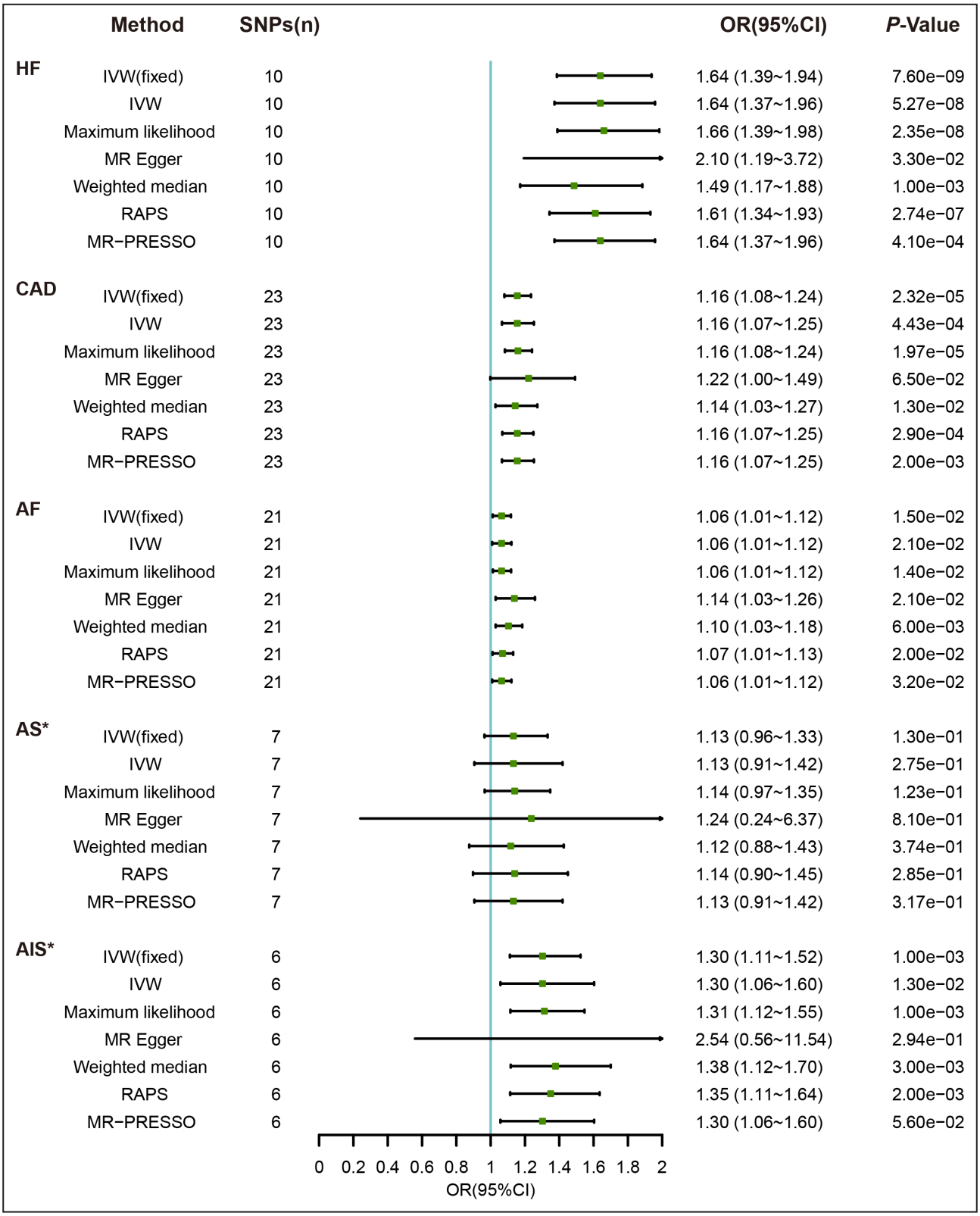


**Figure 3. Associations of genetically predicted cardiovascular disease with risk of anemia.**
AF indicates atrial fibrillation; AIS*, any ischemic stroke, "*rs2634074*" and "*rs4942561*" were removed as outliers in the MR-PRESSO analysis; AS*, any stroke, "*rs10774624*" was removed as an outlier in the MR-PRESSO analysis; CAD, coronary artery disease; HF, heart failure; IVW, inverse-variance weighting; IVW(fixed), fixed-effects IVW; MR, Mendelian randomization; OR, odds ratio; PRESSO, pleiotropy residual sum and outlier; RAPS, robust adjusted profile score; and SNP, single-nucleotide polymorphism.

**Table 3. Associations of Genetically Predicted CVD With Risk of Anemia in Sensitivity Analyses in FinnGen Study**

| CVD | Heterogeneity test MR-Egger | | | Heterogeneity test IVW | | | Pleiotropy test | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Q* | *Q* df | *Q P* value | *Q* | *Q* df | *Q P* value | Egger intercept | SE | *P* value |
| Heart failure | 9.2 | 8 | 0.327 | 10.1 | 9 | 0.339 | −0.017 | 0.019 | 0.389 |
| Coronary artery disease | 31.4 | 21 | 0.067 | 32.0 | 22 | 0.079 | −0.006 | 0.010 | 0.561 |
| Atrial fibrillation | 19.9 | 19 | 0.403 | 22.3 | 20 | 0.324 | −0.010 | 0.007 | 0.143 |
| Any stroke | 18.0 | 6 | 0.006 | 18.0 | 7 | 0.012 | 0.011 | 0.065 | 0.875 |
| Any stroke* | 11.5 | 5 | 0.042 | 11.6 | 6 | 0.072 | −0.006 | 0.058 | 0.920 |
| Any ischemic stroke | 23.5 | 6 | 0.001 | 24.0 | 7 | 0.001 | −0.028 | 0.086 | 0.757 |
| Any ischemic stroke† | 7.3 | 4 | 0.119 | 8.7 | 5 | 0.120 | −0.052 | 0.059 | 0.431 |

CVD indicates cardiovascular disease; IVW, inverse-variance weighting; MR, Mendelian randomization; *Q*, heterogeneity statistic *Q*; and *Q* df, *Q* degree of freedom.

*Any stroke, "*rs10774624*" was removed as an outlier in the MR pleiotropy residual sum and outlier analysis.

†Any ischemic stroke, "*rs2634074*" and "*rs4942561*" were removed as outliers in the MR pleiotropy residual sum and outlier analysis.

anemia and HF and between genetically predicted AS and anemia (Tables S23 and S24).

## DISCUSSION

We used a bidirectional 2-sample MR study to investigate the causal relationships between anemia and a broad range of CVDs for the first time. The results showed that a genetic predisposition to anemia was significantly associated with HF risk and was suggestively associated with CAD risk. However, there was no evidence of an association between anemia and AF, AS, or AIS. The reverse-direction MR analysis revealed significant positive associations of higher genetically predicted HF, CAD, and AIS with increased risk of anemia. Furthermore, genetic susceptibility to AF was suggestively associated with anemia risk. There is no MR evidence to support a potential causal effect of AS on anemia risk. Meta-analyses from different databases, including the FinnGen and UK Biobank studies, showed some differences but generally similar results, which may be related to differences in ethnic background. In general, there are genetic associations between anemia and CVD, and further exploration is needed to explore the underlying mechanisms.

The adverse effect of anemia on the development of HF has been consistently confirmed in observational studies.[32–34] A large cohort study of community-dwelling patients with congestive heart failure proposed that anemia was a common and independent prognostic factor for mortality.[6] Patients with severe anemia tend to be characterized by the exacerbation of HF, including more extensive left ventricular remodeling, higher levels of inflammatory markers, and worse renal function.[35] This may be related to the mechanism by which long-term chronic anemia can increase preload and decrease afterload, resulting in increased cardiac output, which can lead to undesirable left ventricular hypertrophy and left heart enlargement.[36] The MR analysis results verify a significant positive association of genetically predicted anemia with HF, which indicates that further studies are required to explore the biological mechanism of anemia on HF.

In patients with CAD, the relationships between anemia and adverse outcomes are less consistent, with some but not all studies showing an adverse effect of anemia on CAD outcomes.[37–39] The ARIC (Atherosclerosis Risk in Communities) study revealed that participants with anemia had a risk of CVD that was 41% higher than that of people without anemia.[5] A comprehensive analysis of 41 637 patients with acute coronary syndrome from 16 clinical trials found that cardiovascular mortality increased by 21% for every 1-g/dL decrease in hemoglobin below 14 g/dL over 30 days.[7] However, there was no significant association between anemia and CAD mortality after adjusting for traditional CVD risk factors and other covariates in the NHANES (National Health and Nutrition Examination Survey) II Mortality Study.[10] In our MR study, the results showed a suggestive association between anemia and CAD. Mechanistically, anemia may also exacerbate cardiac ischemia and contribute to the progression of CAD because of a decreased oxygen supply or increased demand. However, the effect and mechanism of anemia on CAD need to be further verified.

At present, a few observational studies have suggested that anemia is an independent risk factor for new-onset AF.[8] Anemia has been detected in 13% to 34% of patients with nonvalvular AF.[40,41] The treatment of patients with anemia with oral anticoagulants may increase the risk of bleeding because some patients may have hidden bleeding sites.[41,42] A retrospective study assessing the association of iron deficiency anemia with clinical outcomes in hospitalized patients with AF found that admission attributable to iron deficiency anemia was associated with a higher incidence of acute myocardial infarction but not with all-cause mortality.[43]

Our MR analysis showed no association between anemia and AF. The conclusion that anemia is associated with the risk of AF in observational studies may be influenced by confounders, such as age and concomitant diseases (eg, CAD and HF) because, similar to AF, the incidence of anemia increases with increasing age.[3]

Several observations and systematic analyses suggested an equivocal relationship between anemia and stroke. A systematic retrospective study showed that increased anemia severity was associated with increased in-hospital mortality in patients with AIS.[44] Another retrospective study found that high hemoglobin, not low hemoglobin, was related to an increased risk of recurrent stroke and composite vascular events.[45] This MR analysis provided no evidence that genetically predicted anemia was related to AS or AIS.

Notably, people may pay more attention to the cardiovascular harm caused by anemia but ignore that CVDs can also cause or aggravate anemia in clinical practice, which further promotes the occurrence of adverse cardiovascular outcomes. Therefore, we performed reverse MR analysis, and the results showed that higher genetically predicted HF, CAD, and AIS risks were significantly positively associated with anemia risk, and higher genetically predicted AF risk was suggestively associated with anemia risk. HF may increase the risk of anemia through several underlying pathophysiological mechanisms, including hemodilution, renal insufficiency, inflammatory activation, and depressed bone marrow function.[36] Patients with CAD and AIS may take antiplatelet drugs for a long time, which may lead to a decrease in the absorption capacity of iron in the stomach and increase the risk of anemia. Appropriate anticoagulant therapy reduces the risk of stroke attributable to AF by ≈70%,[46] and gastrointestinal tract bleeding from anticoagulants may be a possible mechanism of iron deficiency and anemia in patients with AF. Further studies are required to explore the mechanism by which CVDs promote anemia. Collectively, attention should be given to the more severe adverse cardiovascular outcomes associated with anemia attributable to CVDs.

In brief, this bidirectional MR analysis not only investigated the causal relationship between anemia and CVD but also analyzed the causal effect of CVD on anemia in reverse. To better understand and manage both diseases in clinical practice, further studies are needed to explore and elucidate the biological mechanism between anemia and CVD. Considering the poor prognosis associated with the causal relationship, it is recommended to further clarify the cause of patients with anemia in time and give intervention to prevent the occurrence of CVDs. At the same time, it is also necessary to strengthen the detection and prevention of anemia in patients with CVDs.

Our study has several strengths and limitations. First, this is the first study to investigate the bidirectional causal relationship between anemia and CVD through a 2-sample MR analysis that is not affected by confounders and reverse causation compared with observational studies. In addition, multiple sensitivity analyses, and IV strength evaluations, were conducted to ensure the robustness and effectiveness of the results. There are also some limitations. The MR analysis is based on specific assumptions that cannot be assessed. The present study was mainly conducted on individuals of European ancestry, which limits the generality of our results to other populations. Although we explored the association between anemia and CVD from a genetic perspective, the underlying mechanisms are not clear and require further investigation.

## CONCLUSIONS

In conclusion, we applied a bidirectional 2-sample MR study for the first time to investigate the causality between anemia and CVD. The present MR study supports that there is bidirectional causality between anemia and HF and demonstrates that CAD and AIS are potential risk factors for anemia. However, further original studies are still required to profoundly elucidate the exact association between anemia and CVD and the mechanism of such an association. Considering the poor prognosis associated with causality, it is recommended to strengthen the prevention and treatment of anemia in CVD.

## ARTICLE INFORMATION

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### Affiliations

Department of Cardiology, Union Hospital (T.G., W.L., C.L., Q.X., Y.W., A.K.A., Y.W., Z.W.)Hubei Key Laboratory of Biological Targeted Therapy, Union Hospital (T.G., W.L., C.L., Q.X., Y.W., A.K.A., Y.W., Z.W.)Hubei Provincial Engineering Research Center of Immunological Diagnosis and Therapy for Cardiovascular Diseases, Union Hospital (T.G., W.L., C.L., Q.X., Y.W., A.K.A., Y.W., Z.W.)Department of Infectious Diseases, Union Hospital (J.H.) and Department of Cardiac Surgery, Union Hospital (S.L.), Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

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### Disclosures

None.

### Supplemental Material

Table S1–S24
Figures S1–S6

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# Supplementary material

**Table S1** F statistics for the causal effect of anemia on CVD in MR analysis

| Diseases | Data sources | Sample size (cases/controls) | Ancestry | $R^2$(%) for anemia (Total) | F for anemia (Total) |
|---|---|---|---|---|---|
| Heart failure | HERMES | 47,309/930,014 | European | 3.5 | 279.8 |
| Coronary artery disease | CARDIoGRAMplusC4D | 60,801/123,504 | Mixed | 3.5 | 280.3 |
| Atrial fibrillation | AFGen | 65,446/522,744 | European | 3.7 | 273.4 |
| Any stroke | MEGASTROKE | 40,585/406,111 | European | 3.7 | 254.5 |
| Any ischemic stroke | MEGASTROKE | 34,217/406,111 | European | 3.7 | 254.5 |

FinnGen: the FinnGen Consortium; HERMES; Heart Failure Molecular Epidemiology for Therapeutic Targets; CARDIoGRAMplusC4D, Coronary Artery Disease Genome-wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics; AFGen, Atrial Fibrillation Genetics; MEGASTROKE, the MEGASTROKE Consortium.

Note: $R^2$: Variability explained by genetic instruments. The $R^2$ was calculated using the formula: $R^2=\beta^2(1\text{-}EAF)*2EAF$. $\beta$ is the association coefficient between the SNP and phenotype, and EAF is the minor allele frequency.

Furthermore, the F statistics were calculated using the formula: $F= R^2(N\text{-}K\text{-}1)/[K(1\text{-}R^2)]$. $R^2$ is the proportion of the variability of anemia explained by each instrument, K is the number of SNP-anemia association, N is the sample size of the GWAS for the SNP-anemia association.

**Table S2** Pleiotropic associations with used SNPs for anemia in FinnGen study

| SNP | Effect_allele | Other_allele | Traits | Direction |
|---|---|---|---|---|
| rs2476601 | A | G | Basophil percentage of granulocytes | + |
| | A | G | Basophil percentage of white cells | + |
| | A | G | Granulocyte count | - |
| | A | G | Lymphocyte count | - |
| | A | G | Myeloid white cell count | - |
| | A | G | Neutrophil count | - |
| | A | G | Sum basophil neutrophil counts | - |
| | A | G | Sum neutrophil eosinophil counts | - |
| | A | G | White blood cell count | - |
| | A | G | Amoxicillin clavulanate drug induced liver injury | NA |
| | A | G | Crohn disease | NA |
| | A | G | Drug induced liver injury | NA |
| | A | G | Drug induced liver injury all cholestatic DILI cases | NA |
| | A | G | Generalized vitiligo | NA |
| | A | G | Generalized vitiligo with concomitant other autoimmune disorder | NA |
| | A | G | Graves disease | NA |
| | A | G | Graves disease and Hashimotos thyroiditis | NA |
| | A | G | Hashimotos thyroiditis | NA |
| | A | G | Hypothyroidism | NA |
| | A | G | Myasthenia gravis | NA |
| | A | G | Myasthenia gravis among females | NA |
| | A | G | Rheumatoid arthritis | NA |
| | A | G | Rheumatoid arthritis | NA |
| | A | G | Rheumatoid arthritis | NA |
| | A | G | Rheumatoid arthritis ACPA positive | NA |
| | A | G | Rheumatoid arthritis cyclic citrullinated peptide CCP positive | NA |
| | A | G | Selective immunoglobulin A deficiency IgAD | NA |
| | A | G | Systemic lupus erythematosus SLE females | NA |
| | A | G | Type 1 diabetes | NA |
| | A | G | Crohns disease | - |
| | A | G | Crohns disease | NA |
| | A | G | Late onset myasthenia gravis | NA |
| | A | G | Systemic lupus erythematosus | + |
| | A | G | Systemic lupus erythematosus or rheumatoid arthritis | NA |
| | A | G | Type 1 diabetes and autoimmune thyroid diseases | + |
| | A | G | Diabetes diagnosed by doctor | + |
| | A | G | Insulin-dependent diabetes mellitus | + |
| | A | G | Long-standing illness, disability or infirmity | + |
| | A | G | Medication for cholesterol, blood pressure or diabetes: insulin | + |
| | A | G | Number of self-reported non-cancer illnesses | + |
| | A | G | Number of treatments or medications taken | + |
| | A | G | Other rheumatoid arthritis | + |
| | A | G | Other serious medical condition or disability diagnosed by doctor | + |
| | A | G | Self-reported diabetes | + |
| | A | G | Self-reported hyperthyroidism or thyrotoxicosis | + |
| | A | G | Self-reported hypothyroidism or myxoedema | + |

| | | | | |
|---|---|---|---|---|
| | A | G | Self-reported pernicious anemia | + |
| | A | G | Self-reported rheumatoid arthritis | + |
| | A | G | Self-reported type 1 diabetes | + |
| | A | G | Started insulin within one year diagnosis of diabetes | + |
| | A | G | Taking other prescription medications | + |
| | A | G | Treatment with folic acid product | + |
| | A | G | Treatment with insulin | + |
| | A | G | Treatment with insulin product | + |
| | A | G | Treatment with levothyroxine sodium | + |
| | A | G | Treatment with methotrexate | + |
| | A | G | Treatment with thyroxine product | + |
| | A | G | Treatment with thyroxine sodium | + |
| | A | G | Rheumatoid arthritis | + |
| | A | G | Rheumatoid arthritis | + |
| | A | G | Arthritis rheumatoid | NA |
| | A | G | Arthritis rheumatoid | NA |
| | A | G | Arthritis rheumatoid | NA |
| | A | G | Crohn disease | NA |
| | A | G | Diabetes mellitus type 1 | NA |
| | A | G | Vitiligo | NA |
| | A | G | Coronary artery disease | + |
| rs707926 | A | G | Eosinophil count | + |
| | A | G | Eosinophil percentage of granulocytes | + |
| | A | G | Eosinophil percentage of white cells | + |
| | A | G | High light scatter percentage of red cells | + |
| | A | G | High light scatter reticulocyte count | + |
| | A | G | Immature fraction of reticulocytes | + |
| | A | G | Mean platelet volume | - |
| | A | G | Neutrophil percentage of granulocytes | - |
| | A | G | Reticulocyte count | + |
| | A | G | Reticulocyte fraction of red cells | + |
| | A | G | Sum eosinophil basophil counts | + |
| | A | G | Age-related macular degeneration | - |
| | A | G | Age-related macular degeneration | - |
| | A | G | Advanced age related macular degeneration | NA |
| | A | G | Primary sclerosing cholangitis | - |
| | A | G | Asthma | - |
| | A | G | Hearing difficulty or problems with background noise | + |
| | A | G | Intestinal malabsorption | - |
| | A | G | Mouth or teeth dental problems: mouth ulcers | + |
| | A | G | No blood clot, bronchitis, emphysema, asthma, rhinitis, eczema or allergy diagnosed by doctor | + |
| | A | G | Past tobacco smoking | - |
| | A | G | Peak expiratory flow | + |
| | A | G | Self-reported ankylosing spondylitis | + |
| | A | G | Self-reported asthma | - |
| | A | G | Self-reported malabsorption or coeliac disease | - |
| | A | G | Started insulin within one year diagnosis of diabetes | + |
| | A | G | Systolic blood pressure | - |

| | | | | |
|---|---|---|---|---|
| | A | G | Time spent using computer | + |
| | A | G | Treatment with insulin product | + |
| | A | G | Age at menopause | - |
| rs3129761 | C | G | Body mass index males | - |
| | C | G | Body mass index | - |
| | C | G | Hematocrit | - |
| | C | G | Hemoglobin concentration | - |
| | C | G | High light scatter percentage of red cells | - |
| | C | G | High light scatter reticulocyte count | - |
| | C | G | Lymphocyte count | - |
| | C | G | Mean corpuscular hemoglobin concentration | - |
| | C | G | Monocyte count | - |
| | C | G | Red blood cell count | - |
| | C | G | Reticulocyte count | - |
| | C | G | Reticulocyte fraction of red cells | - |
| | C | G | Crohns disease | - |
| | C | G | Inflammatory bowel disease | - |
| | C | G | Ulcerative colitis | - |
| | C | G | Asthma | + |
| | C | G | Asthma | + |
| | C | G | Diabetes diagnosed by doctor | + |
| | C | G | Doctor diagnosed asthma | + |
| | C | G | Eye problems or disorders: diabetes related eye disease | + |
| | C | G | Forced expiratory volume in 1-second | - |
| | C | G | Forced expiratory volume in 1-second, best measure | - |
| | C | G | Forced expiratory volume in 1-second, predicted percentage | - |
| | C | G | Forced vital capacity | - |
| | C | G | Forced vital capacity, best measure | - |
| | C | G | Illnesses of father: lung cancer | + |
| | C | G | Illnesses of siblings: diabetes | + |
| | C | G | Insulin-dependent diabetes mellitus | + |
| | C | G | Intestinal malabsorption | + |
| | C | G | Long-standing illness, disability or infirmity | + |
| | C | G | Medication for cholesterol, blood pressure or diabetes: insulin | + |
| | C | G | Nasal polyp | + |
| | C | G | No blood clot, bronchitis, emphysema, asthma, rhinitis, eczema or allergy diagnosed by doctor | - |
| | C | G | Number of self-reported non-cancer illnesses | + |
| | C | G | Number of treatments or medications taken | + |
| | C | G | Other disorders of pancreatic internal secretion | + |
| | C | G | Other rheumatoid arthritis | + |
| | C | G | Other serious medical condition or disability diagnosed by doctor | + |
| | C | G | Overall health rating | + |
| | C | G | Self-reported adrenocortical insufficiency or addisons disease | + |
| | C | G | Self-reported asthma | + |
| | C | G | Self-reported diabetes | + |
| | C | G | Self-reported diabetic eye disease | + |
| | C | G | Self-reported hyperthyroidism or thyrotoxicosis | + |
| | C | G | Self-reported hypothyroidism or myxoedema | + |

| | | | | |
|---|---|---|---|---|
| | C | G | Self-reported malabsorption or coeliac disease | + |
| | C | G | Self-reported multiple sclerosis | - |
| | C | G | Self-reported nasal polyps | + |
| | C | G | Self-reported polymyalgia rheumatica | + |
| | C | G | Self-reported psoriasis | - |
| | C | G | Self-reported rheumatoid arthritis | + |
| | C | G | Self-reported sjogrens syndrome or sicca syndrome | + |
| | C | G | Self-reported type 1 diabetes | + |
| | C | G | Self-reported ulcerative colitis | - |
| | C | G | Started insulin within one year diagnosis of diabetes | + |
| | C | G | Taking other prescription medications | + |
| | C | G | Thyrotoxicosis | + |
| | C | G | Treatment with becotide 50 inhaler | + |
| | C | G | Treatment with fludrocortisone | + |
| | C | G | Treatment with folic acid product | + |
| | C | G | Treatment with hydroxychloroquine | + |
| | C | G | Treatment with insulin | + |
| | C | G | Treatment with insulin product | + |
| | C | G | Treatment with levothyroxine sodium | + |
| | C | G | Treatment with methotrexate | + |
| | C | G | Treatment with montelukast product | + |
| | C | G | Treatment with plaquenil 200mg tablet | + |
| | C | G | Treatment with prednisolone | + |
| | C | G | Treatment with salbutamol | + |
| | C | G | Treatment with seretide 50 evohaler | + |
| | C | G | Treatment with sulfasalazine | + |
| | C | G | Treatment with symbicort 100 or 6 turbohaler | + |
| | C | G | Treatment with thyroxine product | + |
| | C | G | Treatment with thyroxine sodium | + |
| | C | G | Treatment with ventolin 100micrograms inhaler | + |
| | C | G | Ulcerative colitis | - |
| | C | G | Vitamin and mineral supplements: folic acid or folate | + |
| | C | G | Wheeze or whistling in the chest in last year | + |
| | C | G | Rheumatoid arthritis | + |
| rs7184768 | A | G | Arm fat-free mass left | + |
| | A | G | Arm fat-free mass right | + |
| | A | G | Arm predicted mass left | + |
| | A | G | Arm predicted mass right | + |
| | A | G | Basal metabolic rate | + |
| | A | G | Height | + |
| | A | G | Leg fat-free mass left | + |
| | A | G | Leg fat-free mass right | + |
| | A | G | Leg predicted mass left | + |
| | A | G | Leg predicted mass right | + |
| | A | G | Sitting height | + |
| | A | G | Trunk fat-free mass | + |
| | A | G | Trunk predicted mass | + |
| | A | G | Weight | + |
| | A | G | Whole body fat-free mass | + |

**Table S3** Information on instrumental variables for anemia in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Beta | SE | *P*-val |
|---|---|---|---|---|---|---|---|---|
| rs149614393 | 1 | 112820064 | C | A | 0.124 | 0.099 | 0.020 | 1.09E-06 |
| rs12615303 | 2 | 26917705 | C | T | 0.336 | -0.072 | 0.014 | 4.21E-07 |
| rs6728914 | 2 | 184421981 | T | G | 0.007 | 0.388 | 0.081 | 1.86E-06 |
| rs2169706 | 4 | 19271294 | T | C | 0.312 | 0.070 | 0.014 | 1.12E-06 |
| rs35172598 | 6 | 103952813 | T | C | 0.113 | -0.116 | 0.022 | 8.50E-08 |
| rs847720 | 7 | 71116080 | T | C | 0.727 | 0.069 | 0.015 | 4.32E-06 |
| rs79005670 | 11 | 101545718 | C | G | 0.065 | -0.128 | 0.028 | 4.16E-06 |
| rs80068492 | 15 | 41542957 | A | G | 0.039 | -0.163 | 0.035 | 3.61E-06 |
| rs116940507 | 15 | 75566965 | C | T | 0.030 | 0.181 | 0.039 | 4.08E-06 |
| rs7184768 | 16 | 81630188 | G | A | 0.348 | -0.067 | 0.014 | 2.16E-06 |
| rs117725035 | 17 | 59212300 | A | G | 0.027 | 0.409 | 0.040 | 1.09E-24 |
| rs117372722 | 17 | 61401255 | G | T | 0.025 | 0.311 | 0.042 | 9.22E-14 |
| rs117885142 | 20 | 2464755 | C | T | 0.009 | 0.337 | 0.070 | 1.75E-06 |
| rs145020240 | 20 | 23989840 | C | T | 0.060 | -0.152 | 0.029 | 2.35E-07 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S4** Mendelian randomization analysis of anemia and heart failure in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Anemia | | | Heart failure | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs116940507 | 15 | 75566965 | T | C | 0.03 | 0.181 | 0.039 | 4.08E-06 | 0.045 | 0.032 | 0.167 |
| rs117372722 | 17 | 61401255 | T | G | 0.025 | 0.311 | 0.042 | 9.22E-14 | 0.055 | 0.035 | 0.117 |
| rs117725035 | 17 | 59212300 | G | A | 0.027 | 0.409 | 0.04 | 1.09E-24 | 0.02 | 0.03 | 0.51 |
| rs12615303 | 2 | 26917705 | T | C | 0.336 | -0.072 | 0.014 | 4.21E-07 | -0.023 | 0.008 | 0.005 |
| rs145020240 | 20 | 23989840 | T | C | 0.06 | -0.152 | 0.029 | 2.35E-07 | -0.026 | 0.02 | 0.194 |
| rs149614393 | 1 | 112820064 | A | C | 0.124 | 0.099 | 0.02 | 1.09E-06 | 0.01 | 0.016 | 0.553 |
| rs2169706 | 4 | 19271294 | C | T | 0.312 | 0.07 | 0.014 | 1.12E-06 | 0.011 | 0.008 | 0.188 |
| rs35172598 | 6 | 103952813 | C | T | 0.113 | -0.116 | 0.022 | 8.50E-08 | 0.000 | 0.01 | 0.986 |
| rs7184768 | 16 | 81630188 | A | G | 0.348 | -0.067 | 0.014 | 2.16E-06 | -0.013 | 0.008 | 0.106 |
| rs79005670 | 11 | 101545718 | G | C | 0.065 | -0.128 | 0.028 | 4.16E-06 | -0.016 | 0.018 | 0.377 |
| rs80068492 | 15 | 41542957 | G | A | 0.039 | -0.163 | 0.035 | 3.61E-06 | 0.068 | 0.04 | 0.085 |
| rs847720 | 7 | 71116080 | C | T | 0.727 | 0.069 | 0.015 | 4.32E-06 | -0.002 | 0.008 | 0.779 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S5** Mendelian randomization analysis of anemia and coronary artery disease in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Anemia | | | Coronary artery disease | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs117372722 | 17 | 61401255 | T | G | 0.025 | 0.311 | 0.042 | 9.22E-14 | 0.004 | 0.063 | 0.951 |
| rs117725035 | 17 | 59212300 | G | A | 0.027 | 0.409 | 0.040 | 1.09E-24 | 0.025 | 0.039 | 0.520 |
| rs12615303 | 2 | 26917705 | T | C | 0.336 | -0.072 | 0.014 | 4.21E-07 | -0.025 | 0.011 | 0.017 |
| rs145020240 | 20 | 23989840 | T | C | 0.060 | -0.152 | 0.029 | 2.35E-07 | -0.004 | 0.035 | 0.904 |
| rs149614393 | 1 | 112820064 | A | C | 0.124 | 0.099 | 0.020 | 1.09E-06 | 0.011 | 0.027 | 0.689 |
| rs2169706 | 4 | 19271294 | C | T | 0.312 | 0.070 | 0.014 | 1.12E-06 | 0.014 | 0.011 | 0.196 |
| rs35172598 | 6 | 103952813 | C | T | 0.113 | -0.116 | 0.022 | 8.50E-08 | -0.014 | 0.014 | 0.306 |
| rs6728914 | 2 | 184421981 | G | T | 0.007 | 0.388 | 0.081 | 1.86E-06 | 0.008 | 0.051 | 0.880 |
| rs7184768 | 16 | 81630188 | A | G | 0.348 | -0.067 | 0.014 | 2.16E-06 | -0.007 | 0.011 | 0.501 |
| rs79005670 | 11 | 101545718 | G | C | 0.065 | -0.128 | 0.028 | 4.16E-06 | -0.011 | 0.029 | 0.711 |
| rs80068492 | 15 | 41542957 | G | A | 0.039 | -0.163 | 0.035 | 3.61E-06 | 0.059 | 0.058 | 0.304 |
| rs847720 | 7 | 71116080 | C | T | 0.727 | 0.069 | 0.015 | 4.32E-06 | 0.010 | 0.012 | 0.374 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S6** Mendelian randomization analysis of anemia and atrial fibrillation in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Anemia | | | Atrial fibrillation | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs116940507 | 15 | 75566965 | T | C | 0.030 | 0.181 | 0.039 | 4.08E-06 | 0.106 | 0.105 | 0.313 |
| rs117372722 | 17 | 61401255 | T | G | 0.025 | 0.311 | 0.042 | 9.22E-14 | 0.168 | 0.106 | 0.114 |
| rs117725035 | 17 | 59212300 | G | A | 0.027 | 0.409 | 0.040 | 1.09E-24 | 0.034 | 0.059 | 0.561 |
| rs117885142 | 20 | 2464755 | T | C | 0.009 | 0.337 | 0.070 | 1.75E-06 | -0.027 | 0.186 | 0.886 |
| rs12615303 | 2 | 26917705 | T | C | 0.336 | -0.072 | 0.014 | 4.21E-07 | 0.015 | 0.013 | 0.245 |
| rs145020240 | 20 | 23989840 | T | C | 0.060 | -0.152 | 0.029 | 2.35E-07 | -0.010 | 0.050 | 0.837 |
| rs149614393 | 1 | 112820064 | A | C | 0.124 | 0.099 | 0.020 | 1.09E-06 | 0.040 | 0.034 | 0.235 |
| rs2169706 | 4 | 19271294 | C | T | 0.312 | 0.070 | 0.014 | 1.12E-06 | 0.016 | 0.013 | 0.235 |
| rs35172598 | 6 | 103952813 | C | T | 0.113 | -0.116 | 0.022 | 8.50E-08 | -0.035 | 0.017 | 0.041 |
| rs6728914 | 2 | 184421981 | G | T | 0.007 | 0.388 | 0.081 | 1.86E-06 | -0.066 | 0.061 | 0.276 |
| rs7184768 | 16 | 81630188 | A | G | 0.348 | -0.067 | 0.014 | 2.16E-06 | -0.009 | 0.013 | 0.494 |
| rs80068492 | 15 | 41542957 | G | A | 0.039 | -0.163 | 0.035 | 3.61E-06 | -0.128 | 0.101 | 0.205 |
| rs847720 | 7 | 71116080 | C | T | 0.727 | 0.069 | 0.015 | 4.32E-06 | 0.007 | 0.014 | 0.601 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S7** Mendelian randomization analysis of anemia and any stroke in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Anemia | | | Any stroke | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs116940507 | 15 | 75566965 | T | C | 0.030 | 0.181 | 0.039 | 4.08E-06 | 0.009 | 0.045 | 0.835 |
| rs117372722 | 17 | 61401255 | T | G | 0.025 | 0.311 | 0.042 | 9.22E-14 | -0.036 | 0.051 | 0.483 |
| rs117725035 | 17 | 59212300 | G | A | 0.027 | 0.409 | 0.040 | 1.09E-24 | -0.054 | 0.036 | 0.130 |
| rs117885142 | 20 | 2464755 | T | C | 0.009 | 0.337 | 0.070 | 1.75E-06 | 0.045 | 0.069 | 0.519 |
| rs12615303 | 2 | 26917705 | T | C | 0.336 | -0.072 | 0.014 | 4.21E-07 | -0.011 | 0.010 | 0.252 |
| rs145020240 | 20 | 23989840 | T | C | 0.060 | -0.152 | 0.029 | 2.35E-07 | -0.018 | 0.027 | 0.513 |
| rs149614393 | 1 | 112820064 | A | C | 0.124 | 0.099 | 0.020 | 1.09E-06 | 0.038 | 0.021 | 0.066 |
| rs2169706 | 4 | 19271294 | C | T | 0.312 | 0.070 | 0.014 | 1.12E-06 | -0.005 | 0.010 | 0.616 |
| rs35172598 | 6 | 103952813 | C | T | 0.113 | -0.116 | 0.022 | 8.50E-08 | -0.028 | 0.012 | 0.019 |
| rs6728914 | 2 | 184421981 | G | T | 0.007 | 0.388 | 0.081 | 1.86E-06 | -0.073 | 0.046 | 0.112 |
| rs7184768 | 16 | 81630188 | A | G | 0.348 | -0.067 | 0.014 | 2.16E-06 | -0.016 | 0.010 | 0.101 |
| rs79005670 | 11 | 101545718 | G | C | 0.065 | -0.128 | 0.028 | 4.16E-06 | 0.005 | 0.023 | 0.838 |
| rs80068492 | 15 | 41542957 | G | A | 0.039 | -0.163 | 0.035 | 3.61E-06 | 0.015 | 0.053 | 0.772 |
| rs847720 | 7 | 71116080 | C | T | 0.727 | 0.069 | 0.015 | 4.32E-06 | 0.010 | 0.010 | 0.317 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S8** Mendelian randomization analysis of anemia and any ischemic stroke in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Anemia | | | Any ischemic stroke | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs116940507 | 15 | 75566965 | T | C | 0.030 | 0.181 | 0.039 | 4.08E-06 | 0.011 | 0.048 | 0.828 |
| rs117372722 | 17 | 61401255 | T | G | 0.025 | 0.311 | 0.042 | 9.22E-14 | -0.054 | 0.055 | 0.325 |
| rs117725035 | 17 | 59212300 | G | A | 0.027 | 0.409 | 0.040 | 1.09E-24 | -0.054 | 0.039 | 0.164 |
| rs117885142 | 20 | 2464755 | T | C | 0.009 | 0.337 | 0.070 | 1.75E-06 | 0.040 | 0.075 | 0.590 |
| rs12615303 | 2 | 26917705 | T | C | 0.336 | -0.072 | 0.014 | 4.21E-07 | -0.013 | 0.011 | 0.234 |
| rs145020240 | 20 | 23989840 | T | C | 0.060 | -0.152 | 0.029 | 2.35E-07 | -0.017 | 0.029 | 0.550 |
| rs149614393 | 1 | 112820064 | A | C | 0.124 | 0.099 | 0.020 | 1.09E-06 | 0.042 | 0.023 | 0.059 |
| rs2169706 | 4 | 19271294 | C | T | 0.312 | 0.070 | 0.014 | 1.12E-06 | -0.005 | 0.011 | 0.623 |
| rs35172598 | 6 | 103952813 | C | T | 0.113 | -0.116 | 0.022 | 8.50E-08 | -0.028 | 0.013 | 0.032 |
| rs6728914 | 2 | 184421981 | G | T | 0.007 | 0.388 | 0.081 | 1.86E-06 | -0.053 | 0.050 | 0.295 |
| rs7184768 | 16 | 81630188 | A | G | 0.348 | -0.067 | 0.014 | 2.16E-06 | -0.018 | 0.010 | 0.081 |
| rs79005670 | 11 | 101545718 | G | C | 0.065 | -0.128 | 0.028 | 4.16E-06 | 0.021 | 0.025 | 0.404 |
| rs80068492 | 15 | 41542957 | G | A | 0.039 | -0.163 | 0.035 | 3.61E-06 | 0.010 | 0.057 | 0.868 |
| rs847720 | 7 | 71116080 | C | T | 0.727 | 0.069 | 0.015 | 4.32E-06 | 0.008 | 0.011 | 0.476 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S9** Associations of genetically predicted anemia with risk of cardiovascular disease in the MR-PRESSO analysis in FinnGen study

| Cardiovascular disease | SNP | Outliers | p_glo | p_dis | OR | 95% CI | *P*-val |
|---|---|---|---|---|---|---|---|
| Heart failure | 12 | 0 | 0.314 | NA | 1.108 | 1.03~1.193 | 0.019 |
| Coronary artery disease | 12 | 0 | 0.885 | NA | 1.112 | 1.041~1.188 | 0.009 |
| Atrial fibrillation | 13 | 0 | 0.365 | NA | 1.109 | 0.983~1.251 | 0.119 |
| Any stroke | 14 | 0 | 0.069 | NA | 1.038 | 0.946~1.139 | 0.441 |
| Any ischemic stroke | 14 | 0 | 0.133 | NA | 1.038 | 0.942~1.143 | 0.463 |

Abbreviation: SNPs, singe nucleotide polymorphisms; p_glo, p value for global test; p_dis, p value for distortion test; OR, odds ratio; CI, confidence interval; NA, not available.

**Table S10** F statistics for the causal effect of cardiovascular disease on anemia in MR analysis in FinnGen study

| Diseases | Data sources | Sample size (cases/controls) | Ancestry | $R^2$(%) for CVDs (Total) | F for CVDs (Total) |
|---|---|---|---|---|---|
| Heart failure | HERMES | 47,309/930,014 | European | 1.3 | 1221.8 |
| Coronary artery disease | CARDIoGRAMplusC4D | 60,801/123,504 | Mixed | 8.1 | 647.1 |
| Atrial fibrillation | AFGen | 65,446/522,744 | European | NA | NA |
| Any stroke | MEGASTROKE | 40,585/406,111 | European | 1.2 | 767.7 |
| Any ischemic stroke | MEGASTROKE | 34,217/406,111 | European | 1.3 | 966.2 |

FinnGen: the FinnGen Consortium; HERMES; Heart Failure Molecular Epidemiology for Therapeutic Targets; CARDIoGRAMplusC4D, Coronary Artery Disease Genome-wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics; AFGen, Atrial Fibrillation Genetics; MEGASTROKE, the MEGASTROKE Consortium.
Note: $R^2$: Variability explained by genetic instruments. The $R^2$ was calculated using the formula[1]: $R^2=\beta^2(1-EAF)*2EAF$. β is the association coefficient between the SNP and phenotype, and EAF is the minor allele frequency. Furthermore, the F statistics were calculated using the formula[2]: $F= R^2(N-K-1)/[K(1-R^2)]$. $R^2$ is the proportion of the variability of CVD explained by each instrument, K is the number of SNP-CVD association, N is the sample size of the GWAS for the SNP-CVD association.

**Table S11** Mendelian randomization analysis of heart failure and anemia in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Heart failure | | | Anemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs11745324 | 5 | 137676482 | A | G | 0.228 | -0.053 | 0.010 | 2.35E-08 | -0.017 | 0.016 | 0.285 |
| rs1510226 | 6 | 160395377 | T | C | 0.981 | -0.162 | 0.029 | 1.27E-08 | -0.119 | 0.063 | 0.057 |
| rs17042102 | 4 | 110747470 | A | G | 0.115 | 0.110 | 0.012 | 5.71E-20 | 0.049 | 0.019 | 0.012 |
| rs17617337 | 10 | 119667372 | T | C | 0.221 | -0.056 | 0.010 | 3.65E-09 | -0.022 | 0.016 | 0.174 |
| rs4135240 | 6 | 36679903 | T | C | 0.659 | 0.049 | 0.008 | 6.84E-09 | 0.008 | 0.014 | 0.567 |
| rs4746140 | 10 | 73657491 | C | G | 0.154 | -0.067 | 0.011 | 1.10E-09 | -0.026 | 0.018 | 0.135 |
| rs55730499 | 6 | 160584578 | T | C | 0.069 | 0.106 | 0.016 | 1.83E-11 | 0.091 | 0.032 | 0.004 |
| rs56094641 | 16 | 53772541 | A | G | 0.584 | -0.045 | 0.008 | 1.21E-08 | -0.013 | 0.014 | 0.351 |
| rs600038 | 9 | 133276354 | T | C | 0.791 | -0.057 | 0.010 | 3.68E-09 | -0.066 | 0.016 | 3.84E-05 |
| rs660240 | 1 | 109275216 | T | C | 0.213 | -0.061 | 0.010 | 3.25E-10 | -0.023 | 0.016 | 0.152 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S12** Mendelian randomization analysis of coronary artery disease and anemia in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Coronary artery disease | | | Anemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs10455872 | 6 | 160589086 | G | A | 0.054 | 0.285 | 0.027 | 9.23E-27 | 0.092 | 0.032 | 0.004 |
| rs10947786 | 6 | 39188634 | A | G | 0.208 | -0.072 | 0.013 | 1.77E-08 | -0.022 | 0.015 | 0.138 |
| rs113113862 | 19 | 11072901 | A | G | 0.218 | -0.075 | 0.013 | 2.78E-09 | -0.046 | 0.016 | 0.005 |
| rs11556924 | 7 | 130023656 | T | C | 0.298 | -0.069 | 0.013 | 4.00E-08 | 0.002 | 0.014 | 0.895 |
| rs1332329 | 10 | 89243662 | C | A | 0.362 | 0.079 | 0.011 | 2.68E-13 | 0.001 | 0.014 | 0.939 |
| rs1510226 | 6 | 160395377 | C | T | 0.027 | 0.226 | 0.035 | 6.16E-11 | 0.119 | 0.063 | 0.057 |
| rs1870634 | 10 | 43985363 | G | T | 0.618 | 0.070 | 0.011 | 8.35E-11 | -0.021 | 0.015 | 0.150 |
| rs2019090 | 11 | 103798234 | T | A | 0.642 | -0.065 | 0.011 | 3.65E-09 | -0.001 | 0.016 | 0.952 |
| rs2327426 | 6 | 133881552 | C | T | 0.298 | -0.063 | 0.011 | 1.22E-08 | -0.015 | 0.016 | 0.337 |
| rs2505083 | 10 | 30046193 | C | T | 0.395 | 0.061 | 0.011 | 6.93E-09 | 0.003 | 0.014 | 0.824 |
| rs2681472 | 12 | 89615182 | G | A | 0.194 | 0.073 | 0.013 | 6.11E-09 | -0.028 | 0.025 | 0.278 |
| rs28451064 | 21 | 34221526 | A | G | 0.119 | 0.122 | 0.018 | 6.10E-12 | -0.003 | 0.019 | 0.880 |
| rs35700460 | 1 | 222638065 | G | A | 0.647 | 0.082 | 0.012 | 1.91E-11 | -0.003 | 0.015 | 0.834 |
| rs41290120 | 19 | 44879418 | A | G | 0.032 | -0.186 | 0.031 | 2.62E-09 | -0.035 | 0.040 | 0.376 |
| rs4773141 | 13 | 110302006 | G | C | 0.345 | 0.080 | 0.013 | 4.69E-10 | 0.000 | 0.015 | 0.982 |
| rs4977574 | 9 | 22098575 | G | A | 0.482 | 0.189 | 0.010 | 4.58E-75 | 0.021 | 0.014 | 0.124 |
| rs653178 | 12 | 111569952 | T | C | 0.558 | -0.077 | 0.012 | 2.84E-11 | -0.044 | 0.014 | 0.001 |
| rs7173743 | 15 | 78849442 | C | T | 0.432 | -0.064 | 0.010 | 7.75E-10 | -0.011 | 0.014 | 0.436 |
| rs72689147 | 4 | 155718736 | T | G | 0.180 | -0.074 | 0.013 | 1.65E-08 | -0.020 | 0.016 | 0.212 |
| rs72934535 | 2 | 203104250 | C | T | 0.093 | 0.141 | 0.019 | 3.36E-14 | -0.024 | 0.023 | 0.306 |
| rs7528419 | 1 | 109274570 | G | A | 0.202 | -0.101 | 0.013 | 9.92E-16 | -0.024 | 0.016 | 0.136 |
| rs9349379 | 6 | 12903725 | G | A | 0.411 | 0.131 | 0.011 | 9.37E-35 | 0.026 | 0.013 | 0.053 |
| rs9970807 | 1 | 56499992 | T | C | 0.084 | -0.111 | 0.018 | 1.81E-09 | -0.017 | 0.022 | 0.433 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S13** Mendelian randomization analysis of atrial fibrillation and anemia in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Atrial fibrillation | | | Anemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs11264280 | 1 | 154890476 | T | C | NA | 0.115 | 0.014 | 6.41E-17 | 0.014 | 0.014 | 0.314 |
| rs1152591 | 14 | 64214130 | A | G | NA | 0.082 | 0.013 | 1.04E-10 | -0.017 | 0.014 | 0.217 |
| rs11598047 | 10 | 103582915 | A | G | NA | -0.162 | 0.017 | 1.67E-22 | -0.020 | 0.023 | 0.389 |
| rs11718898 | 3 | 12807323 | T | C | NA | -0.073 | 0.013 | 4.68E-08 | 0.017 | 0.014 | 0.208 |
| rs12664873 | 6 | 122142045 | T | G | NA | 0.078 | 0.014 | 1.19E-08 | -0.006 | 0.016 | 0.704 |
| rs2106261 | 16 | 73017721 | T | C | NA | 0.185 | 0.016 | 8.18E-32 | 0.010 | 0.016 | 0.533 |
| rs2288327 | 2 | 178546938 | A | G | NA | -0.089 | 0.016 | 2.05E-08 | 0.034 | 0.018 | 0.057 |
| rs28681402 | 4 | 110850907 | T | G | NA | 0.138 | 0.013 | 7.90E-26 | -0.011 | 0.014 | 0.444 |
| rs2967791 | 5 | 137677417 | T | C | NA | 0.072 | 0.013 | 2.73E-08 | 0.013 | 0.013 | 0.346 |
| rs337711 | 5 | 114412874 | T | C | NA | 0.070 | 0.013 | 2.93E-08 | -0.004 | 0.014 | 0.775 |
| rs3771537 | 2 | 69811660 | A | C | NA | 0.085 | 0.012 | 7.92E-12 | 0.022 | 0.013 | 0.101 |
| rs4946333 | 6 | 118244502 | A | G | NA | -0.074 | 0.012 | 1.89E-09 | -0.001 | 0.013 | 0.967 |
| rs520525 | 1 | 170669192 | A | G | NA | 0.113 | 0.014 | 6.39E-16 | 0.012 | 0.016 | 0.428 |
| rs6843082 | 4 | 110796911 | A | G | NA | -0.371 | 0.014 | 3.41E-155 | -0.038 | 0.015 | 0.009 |
| rs7026071 | 9 | 94730238 | T | C | NA | 0.091 | 0.013 | 1.31E-12 | 0.019 | 0.014 | 0.180 |
| rs74022964 | 15 | 73384923 | T | C | NA | 0.111 | 0.017 | 2.37E-11 | -0.019 | 0.017 | 0.264 |
| rs7508 | 8 | 18056461 | A | G | NA | 0.088 | 0.014 | 5.16E-10 | 0.014 | 0.015 | 0.353 |
| rs75190942 | 11 | 128894676 | A | C | NA | 0.159 | 0.028 | 1.59E-08 | 0.020 | 0.023 | 0.381 |
| rs7915134 | 10 | 73660422 | T | C | NA | -0.113 | 0.018 | 1.68E-10 | -0.027 | 0.019 | 0.152 |
| rs80056983 | 10 | 103750144 | T | C | NA | 0.115 | 0.018 | 8.41E-11 | 0.016 | 0.021 | 0.436 |
| rs883079 | 12 | 114355435 | T | C | NA | 0.108 | 0.014 | 1.80E-15 | -0.007 | 0.014 | 0.643 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S14** Mendelian randomization analysis of any stroke and anemia in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Any stroke | | | Anemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs10774624* | 12 | 111395984 | A | G | 0.529 | -0.065 | 0.009 | 4.04E-12 | -0.046 | 0.014 | 0.001 |
| rs11242678 | 6 | 1336945 | T | C | 0.255 | 0.064 | 0.011 | 8.71E-10 | -0.016 | 0.015 | 0.273 |
| rs11587860 | 1 | 156187160 | C | G | 0.355 | -0.069 | 0.010 | 2.54E-12 | 0.002 | 0.014 | 0.896 |
| rs1537375 | 9 | 22116072 | T | C | 0.498 | -0.052 | 0.009 | 1.24E-08 | -0.025 | 0.014 | 0.062 |
| rs2107595 | 7 | 19009765 | A | G | 0.167 | 0.080 | 0.012 | 3.59E-11 | 0.028 | 0.017 | 0.099 |
| rs2634074 | 4 | 110755885 | A | T | 0.789 | -0.084 | 0.011 | 6.56E-14 | -0.036 | 0.016 | 0.025 |
| rs475937 | 11 | 102816969 | A | C | 0.132 | 0.076 | 0.014 | 2.92E-08 | -0.017 | 0.016 | 0.285 |
| rs4942561 | 13 | 46635212 | T | G | 0.758 | 0.064 | 0.011 | 2.05E-09 | 0.008 | 0.016 | 0.608 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error; "rs10774624*" was removed as outliers in the MR-PRESSO analysis.

**Table S15** Mendelian randomization analysis of any ischemic stroke and anemia in FinnGen study

| SNP | Chr | Position | A1 | A2 | EAF | Any ischemic stroke | | | Anemia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Beta | SE | *P*-val | Beta | SE | *P*-val |
| rs11242678 | 6 | 1336945 | T | C | 0.255 | 0.072 | 0.011 | 2.70E-10 | -0.016 | 0.015 | 0.273 |
| rs2066864 | 4 | 154604543 | A | G | 0.245 | 0.063 | 0.012 | 3.51E-08 | 0.019 | 0.015 | 0.196 |
| rs2107595 | 7 | 19009765 | A | G | 0.167 | 0.088 | 0.013 | 2.33E-11 | 0.028 | 0.017 | 0.099 |
| rs2634074 | 4 | 110755885 | A | T | 0.788 | -0.094 | 0.012 | 5.91E-15 | -0.036 | 0.016 | 0.025 |
| rs3184504 | 12 | 111446804 | T | C | 0.472 | 0.078 | 0.010 | 1.23E-14 | 0.040 | 0.014 | 0.003 |
| rs473238* | 11 | 102829629 | T | C | 0.133 | 0.083 | 0.015 | 1.65E-08 | -0.018 | 0.016 | 0.263 |
| rs4942561 | 13 | 46635212 | T | G | 0.759 | 0.066 | 0.012 | 1.77E-08 | 0.008 | 0.016 | 0.608 |
| rs635634* | 9 | 133279427 | T | C | 0.192 | 0.077 | 0.013 | 9.18E-09 | 0.071 | 0.017 | 2.55E-05 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error; "rs2634074*" and "rs4942561*" was removed as outliers in the MR-PRESSO analysis.

**Table S16** Associations of genetically predicted anemia with risk of cardiovascular disease in the MR-PRESSO analysis in FinnGen study

| Cardiovascular disease | SNP | Outliers | *P*_glo | *P*_dis | OR | 95%CI | *P*-val |
|---|---|---|---|---|---|---|---|
| Heart failure | 10 | 0 | 0.441 | NA | 1.639 | 1.372~1.958 | 4.10E-04 |
| Coronary artery disease | 23 | 0 | 0.098 | NA | 1.155 | 1.066~1.252 | 0.002 |
| Atrial fibrillation | 21 | 0 | 0.343 | NA | 1.064 | 1.009~1.121 | 0.032 |
| Any stroke | 8 | 1 | 0.02 | 0.175 | 1.223 | 0.961~1.558 | 0.146 |
| Any stroke* | 7 | 0 | 0.093 | NA | 1.133 | 0.905~1.419 | 0.317 |
| Any ischemic stroke | 8 | 2 | 0.001 | 0.988 | 1.307 | 1.014~1.685 | 0.078 |
| Any ischemic stroke* | 6 | 0 | 0.153 | NA | 1.302 | 1.057~1.603 | 0.056 |

Abbreviation: SNPs, singe nucleotide polymorphisms; p_glo, p value for global test; p_dis, p value for distortion test; OR, odds ratio; CI, confidence interval; NA, not available; Any stroke*, "rs10774624" was removed as outliers in the MR-PRESSO analysis; Any ischemic stroke*, "rs2634074" and "rs4942561" was removed as outliers in the MR-PRESSO analysis.

**Table S17** MR-Steiger test in two-sample bidirectional Mendelian randomization analysis in FinnGen study

| Exposure | Outcome | SNP_$r^2$.exposure | SNP_$r^2$.outcome | Correct_causal_direction | Steiger_pval |
|---|---|---|---|---|---|
| Anemia | Heart failure | 0.020 | 1.51E-06 | TRUE | 2.01E-27 |
| Anemia | Coronary artery disease | 0.020 | 2.10E-05 | TRUE | 1.41E-25 |
| Heart failure | Anemia | 0.000 | 1.59E-05 | TRUE | 6.31E-10 |
| Coronary artery disease | Anemia | 0.003 | 5.67E-05 | TRUE | 2.18E-63 |
| Atrial fibrillation | Anemia | 0.002 | 2.95E-05 | TRUE | 1.31E-84 |
| Any ischemic stroke | Anemia | 0.001 | 1.33E-05 | TRUE | 1.07E-17 |

**Table S18** Information on instrumental variables for anemia in UK Biobank study

| SNP | Chr | A1 | A2 | EAF | Beta | SE | *P*-val |
|---|---|---|---|---|---|---|---|
| rs11863726 | 16 | G | A | 0.501 | 0.094 | 0.456 | 1.22E-06 |
| rs1894251 | 22 | T | C | 0.078 | 0.183 | 0.915 | 5.99E-07 |
| rs35239007 | 6 | A | C | 0.118 | 0.143 | 0.666 | 3.24E-06 |
| rs7189975 | 16 | A | G | 0.472 | 0.093 | 0.450 | 1.18E-06 |
| rs8099569 | 18 | A | G | 0.077 | 0.184 | 0.929 | 4.62E-07 |

Abbreviation: SNP, single nucleotide polymorphism; Chr, chromosome; A1, Effect_allele; A2, Other_allele; EAF, effect allele frequency; SE, standard error.

**Table S19** Mendelian randomization analysis of anemia and cardiovascular disease in UK Biobank study

| Cardiovascular disease | Method | SNP | Beta | SE | OR | 95%CI | *P*-val |
|---|---|---|---|---|---|---|---|
| **Heart failure** | IVW (fixed effects) | 5 | 0.046 | 0.056 | 1.047 | 0.939~1.168 | 0.409 |
| | IVW (multiplicative random effects) | 5 | 0.046 | 0.025 | 1.047 | 0.997~1.099 | 0.064 |
| | Maximum likelihood | 5 | 0.100 | 0.293 | 1.105 | 0.623~1.961 | 0.733 |
| | MR Egger | 5 | 0.021 | 0.225 | 1.021 | 0.656~1.589 | 0.932 |
| | Weighted median | 5 | 0.012 | 2.709 | 1.012 | 0.005~204.651 | 0.996 |
| | RAPS | 5 | 0.100 | 0.310 | 1.105 | 0.602~2.028 | 0.748 |
| | MR-PRESSO | 5 | 0.046 | 0.025 | 1.047 | 0.997~1.099 | 0.137 |
| **Coronary artery disease** | IVW (fixed effects) | 5 | -0.004 | 0.068 | 0.996 | 0.872~1.138 | 0.955 |
| | IVW (multiplicative random effects) | 5 | -0.004 | 0.067 | 0.996 | 0.874~1.135 | 0.955 |
| | Maximum likelihood | 5 | -7.753 | 89.250 | 0.000 | 0~4.02E+72 | 0.931 |
| | MR Egger | 5 | -0.078 | 0.296 | 0.925 | 0.518~1.653 | 0.810 |
| | Weighted median | 5 | 0.014 | 0.240 | 1.014 | 0.633~1.625 | 0.952 |
| | RAPS | 5 | 0.563 | 3.973 | 1.756 | 0.001~4230.364 | 0.887 |
| | MR-PRESSO | 5 | -0.004 | 0.067 | 0.996 | 0.874~1.135 | 0.957 |
| **Atrial fibrillation** | IVW (fixed effects) | 5 | -0.024 | 0.089 | 0.976 | 0.819~1.163 | 0.786 |
| | IVW (multiplicative random effects) | 5 | -0.024 | 0.109 | 0.976 | 0.788~1.209 | 0.824 |
| | Maximum likelihood | 5 | -1.598 | 11.678 | 0.202 | 0~1763400900 | 0.891 |
| | MR Egger | 5 | 0.001 | 0.509 | 1.001 | 0.369~2.715 | 0.998 |
| | Weighted median | 5 | -0.142 | 0.141 | 0.868 | 0.658~1.145 | 0.315 |
| | RAPS | 5 | -2.339 | 49.644 | 0.096 | 0~1.75E+41 | 0.962 |
| | MR-PRESSO | 5 | -0.024 | 0.109 | 0.976 | 0.788~1.209 | 0.835 |
| **Any stroke** | IVW (fixed effects) | 5 | -0.054 | 0.071 | 0.947 | 0.824~1.088 | 0.442 |
| | IVW (multiplicative random effects) | 5 | -0.054 | 0.080 | 0.947 | 0.809~1.108 | 0.498 |
| | Maximum likelihood | 5 | -1.433 | 11.683 | 0.239 | 0~2100028637 | 0.902 |
| | MR Egger | 5 | 0.171 | 0.341 | 1.186 | 0.608~2.312 | 0.651 |
| | Weighted median | 5 | -0.028 | 0.604 | 0.972 | 0.298~3.174 | 0.963 |
| | RAPS | 5 | -2.116 | 49.380 | 0.120 | 0~1.3E+41 | 0.966 |
| | MR-PRESSO | 5 | -0.054 | 0.080 | 0.947 | 0.809~1.108 | 0.535 |
| **Any ischemic stroke** | IVW (fixed effects) | 5 | -0.052 | 0.075 | 0.949 | 0.819~1.1 | 0.486 |
| | IVW (multiplicative random effects) | 5 | -0.052 | 0.086 | 0.949 | 0.802~1.123 | 0.541 |
| | Maximum likelihood | 5 | -1.356 | 7.803 | 0.258 | 0~1130136.753 | 0.862 |
| | MR Egger | 5 | 0.203 | 0.366 | 1.226 | 0.598~2.51 | 0.617 |
| | Weighted median | 5 | -0.048 | 0.786 | 0.954 | 0.204~4.452 | 0.952 |
| | RAPS | 5 | -2.450 | 59.290 | 0.086 | 0~2.5E+49 | 0.967 |
| | MR-PRESSO | 5 | -0.052 | 0.086 | 0.949 | 0.802~1.123 | 0.574 |

Abbreviation: SNPs, Single nucleotide polymorphisms; SE, standard error; OR, Odds ratio; CI, Confidence interval; IVW, inverse-variance weighted; IVW (fixed), fixed-effects inverse-variance weighted; MR-RAPS, MR-robust adjusted profile score; MR-PRESSO, MR-pleiotropy residual sum and outlier.

**Table S20** Associations of genetically predicted anemia with risk of cardiovascular disease in sensitivity analysis in UK Biobank study

| Cardiovascular disease | Heterogeneity test MR-Egger | | | Heterogeneity test IVW | | | Pleiotropy test | | |
|---|---|---|---|---|---|---|---|---|---|
| | Q | Q_df | Q_pval | Q | Q_df | Q_pval | egger_ intercept | SE | *P*-val |
| Heart failure | 0.779 | 3 | 0.854 | 0.792 | 4 | 0.939 | 0.003 | 0.029 | 0.916 |
| Coronary artery disease | 3.778 | 3 | 0.287 | 3.862 | 4 | 0.425 | 0.011 | 0.041 | 0.813 |
| Atrial fibrillation | 5.990 | 3 | 0.112 | 5.995 | 4 | 0.199 | -0.003 | 0.066 | 0.962 |
| Any stroke | 4.456 | 3 | 0.216 | 5.149 | 4 | 0.272 | -0.029 | 0.043 | 0.544 |
| Any ischemic stroke | 4.429 | 3 | 0.219 | 5.200 | 4 | 0.267 | -0.034 | 0.046 | 0.522 |

Abbreviation: MR, Mendelian randomization; Q, heterogeneity statistic Q; Q_df, degree of freedom.

**Table S21** Mendelian randomization analysis of cardiovascular disease and anemia in UK Biobank study

| Cardiovascular disease | Method | SNP | Beta | SE | OR | 95%CI | *P*-val |
|---|---|---|---|---|---|---|---|
| **Heart failure*** | IVW (fixed effects) | 5 | 0.003 | 0.000 | 1.003 | 1.002~1.004 | 1.58E-10 |
| | IVW (multiplicative random effects) | 5 | 0.003 | 0.000 | 1.003 | 1.002~1.004 | 1.94E-10 |
| | Maximum likelihood | 5 | 0.003 | 0.001 | 1.003 | 1.002~1.004 | 1.66E-08 |
| | MR Egger | 5 | 0.002 | 0.001 | 1.002 | 1~1.004 | 0.201 |
| | Weighted median | 5 | 0.003 | 0.001 | 1.003 | 1.002~1.004 | 3.01E-06 |
| | RAPS | 5 | 0.003 | 0.001 | 1.003 | 1.002~1.004 | 9.95E-08 |
| | MR-PRESSO | 5 | 0.003 | 0.000 | 1.003 | 1.002~1.004 | 0.003 |
| **Coronary artery disease*** | IVW (fixed effects) | 10 | -0.001 | 0.001 | 0.999 | 0.998~1 | 0.231 |
| | IVW (multiplicative random effects) | 10 | -0.001 | 0.001 | 0.999 | 0.998~1.001 | 0.381 |
| | Maximum likelihood | 10 | -0.001 | 0.001 | 0.999 | 0.998~1 | 0.206 |
| | MR Egger | 10 | 0.001 | 0.003 | 1.001 | 0.995~1.008 | 0.726 |
| | Weighted median | 10 | -0.002 | 0.001 | 0.998 | 0.996~1 | 0.031 |
| | RAPS | 10 | 0.000 | 0.001 | 1.000 | 0.998~1.002 | 0.903 |
| | MR-PRESSO | 10 | -0.001 | 0.001 | 0.999 | 0.998~1.001 | 0.404 |
| **Atrial fibrillation*** | IVW (fixed effects) | 10 | 0.001 | 0.000 | 1.001 | 1~1.001 | 0.094 |
| | IVW (multiplicative random effects) | 10 | 0.001 | 0.000 | 1.001 | 1~1.002 | 0.248 |
| | Maximum likelihood | 10 | 0.001 | 0.000 | 1.001 | 1~1.001 | 0.092 |
| | MR Egger | 10 | -0.001 | 0.001 | 0.999 | 0.997~1 | 0.089 |
| | Weighted median | 10 | 0.001 | 0.001 | 1.001 | 1~1.002 | 0.196 |
| | RAPS | 10 | 0.000 | 0.001 | 1.000 | 0.999~1.001 | 0.888 |
| | MR-PRESSO | 10 | 0.001 | 0.000 | 1.001 | 1~1.002 | 0.278 |
| **Any stroke** | IVW (fixed effects) | 4 | 0.003 | 0.000 | 1.003 | 1.003~1.003 | 7.50E-120 |
| | IVW (multiplicative random effects) | 4 | 0.003 | 0.001 | 1.003 | 1.001~1.005 | 0.002 |
| | Maximum likelihood | 4 | 0.003 | 0.001 | 1.003 | 1.002~1.004 | 1.49E-07 |
| | MR Egger | 4 | -0.026 | 0.069 | 0.974 | 0.851~1.114 | 0.739 |
| | Weighted median | 4 | 0.003 | 0.001 | 1.003 | 1.001~1.005 | 0.006 |
| | RAPS | 4 | 0.001 | 0.002 | 1.001 | 0.998~1.005 | 0.498 |
| | MR-PRESSO | 4 | 0.003 | 0.001 | 1.003 | 1.001~1.005 | 0.052 |
| **Any ischemic stroke** | IVW (fixed effects) | 5 | 0.002 | 0.000 | 1.002 | 1.002~1.003 | 1.17E-110 |
| | IVW (multiplicative random effects) | 5 | 0.002 | 0.001 | 1.002 | 1.001~1.004 | 0.003 |
| | Maximum likelihood | 5 | 0.003 | 0.001 | 1.003 | 1.001~1.004 | 0.000 |
| | MR Egger | 5 | -0.048 | 0.019 | 0.953 | 0.919~0.989 | 0.084 |
| | Weighted median | 5 | 0.002 | 0.001 | 1.002 | 1~1.005 | 0.055 |
| | RAPS | 5 | 0.001 | 0.001 | 1.001 | 0.999~1.004 | 0.296 |
| | MR-PRESSO | 5 | 0.002 | 0.001 | 1.002 | 1.001~1.004 | 0.039 |

Abbreviation: SNPs, Single nucleotide polymorphisms; SE, standard error; OR, Odds ratio; CI, Confidence interval; IVW, inverse-variance weighted; IVW (fixed), fixed-effects inverse-variance weighted; MR-RAPS, MR-robust adjusted profile score; MR-PRESSO, MR-pleiotropy residual sum and outlier; Heart failure*, “rs11745324” was removed as outliers in the MR-PRESSO analysis; Coronary artery disease*, “rs1870634” and “rs653178” were removed as outliers in the MR-PRESSO analysis; Atrial fibrillation*, “rs12664873” and “rs6843082” were removed as outliers in the MR-PRESSO analysis.

**Table S22** Associations of genetically predicted cardiovascular disease with risk of anemia in sensitivity analysis in UK Biobank study

| Cardiovascular disease | Heterogeneity test MR-Egger | | | Heterogeneity test IVW | | | Pleiotropy test | | |
|---|---|---|---|---|---|---|---|---|---|
| | Q | Q_ df | Q_pval | Q | Q_ df | Q_pval | egger_ intercept | SE | *P*-val |
| Heart failure | 23.473 | 4 | 1.02E-04 | 23.484 | 5 | 2.73E-04 | -8.38E-06 | 1.87E-04 | 0.966 |
| Heart failure* | 2.900 | 3 | 0.407 | 4.040 | 4 | 0.401 | 8.53E-05 | 7.99E-05 | 0.364 |
| Coronary artery disease | 29.873 | 10 | 8.99E-04 | 29.933 | 11 | 0.002 | -4.44E-05 | 3.11E-04 | 0.889 |
| Coronary artery disease* | 16.129 | 8 | 0.041 | 16.816 | 9 | 0.052 | -0.00016 | 2.74E-04 | 0.576 |
| Atrial fibrillation | 22.148 | 9 | 0.008 | 26.538 | 10 | 0.003 | 1.50E-04 | 1.12E-04 | 0.214 |
| Atrial fibrillation* | 7.982 | 8 | 0.435 | 18.876 | 9 | 0.026 | 2.53E-04 | 7.67E-05 | 0.011 |
| Any stroke | 152.197 | 2 | 8.93E-34 | 165.843 | 3 | 1.00E-35 | 0.002 | 0.004 | 0.713 |
| Any ischemic stroke | 65.275 | 3 | 4.38E-14 | 220.099 | 4 | 1.79E-46 | 0.004 | 0.001 | 0.076 |

Abbreviation: MR, Mendelian randomization; Q, heterogeneity statistic Q; Q_df, degree of freedom. Heart failure*, “rs11745324” was removed as outliers in the MR-PRESSO analysis; Coronary artery disease*, “rs1870634” and “rs653178” were removed as outliers in the MR-PRESSO analysis; Atrial fibrillation*, “rs12664873” and “rs6843082” were removed as outliers in the MR-PRESSO analysis.

**Table S23** Associations of genetic liability to anemia with risk of cardiovascular disease in UK Biobank and FinnGen studies

| Cardiovascular disease | Studies | SNP | OR | LCI | UCI | *P*-val |
|---|---|---|---|---|---|---|
| **Heart failure** | UK Biobank | 5 | 1.047 | 0.939 | 1.168 | 0.409 |
| | FinnGen | 12 | 1.108 | 1.037 | 1.184 | 0.002 |
| | Combined | | 1.092 | 1.032 | 1.155 | 0.002 |
| **Coronary artery disease** | UK Biobank | 5 | 0.996 | 0.872 | 1.138 | 0.955 |
| | FinnGen | 12 | 1.112 | 1.017 | 1.216 | 0.020 |
| | Combined | | 1.075 | 0.998 | 1.158 | 0.056 |
| **Atrial fibrillation** | UK Biobank | 5 | 0.947 | 0.824 | 1.088 | 0.442 |
| | FinnGen | 13 | 1.038 | 0.963 | 1.120 | 0.330 |
| | Combined | | 1.017 | 0.951 | 1.087 | 0.627 |
| **Any stroke** | UK Biobank | 5 | 0.947 | 0.824 | 1.088 | 0.442 |
| | FinnGen | 14 | 1.038 | 0.963 | 1.120 | 0.330 |
| | Combined | | 1.017 | 0.951 | 1.087 | 0.627 |
| **Any ischemic stroke** | UK Biobank | 5 | 0.949 | 0.819 | 1.100 | 0.486 |
| | FinnGen | 14 | 1.038 | 0.956 | 1.127 | 0.374 |
| | Combined | | 1.016 | 0.946 | 1.092 | 0.662 |

Abbreviation: SNPs, Single nucleotide polymorphisms; OR, Odds ratio; LCI, Lower confidence interval; UCI, Upper confidence interval.

**Table S24** Associations of genetic liability to cardiovascular disease with risk of anemia in UK Biobank and FinnGen studies

| Cardiovascular disease | Studies | SNP | OR | LCI | UCI | *P*-val |
|---|---|---|---|---|---|---|
| **Heart failure** | UK Biobank | 6 | 1.002 | 1.002 | 1.003 | 1.01E-07 |
| | FinnGen | 10 | 1.639 | 1.386 | 1.938 | 7.60E-09 |
| | Combined | | 1.272 | 0.786 | 2.060 | 0.328 |
| **Coronary artery disease** | UK Biobank | 10 | 0.999 | 0.998 | 1.000 | 0.231 |
| | FinnGen | 23 | 1.155 | 1.080 | 1.235 | 2.32E-05 |
| | Combined | | 1.070 | 0.928 | 1.233 | 0.351 |
| **Atrial fibrillation** | UK Biobank | 10 | 1.001 | 1.000 | 1.002 | 0.248 |
| | FinnGen | 21 | 1.064 | 1.012 | 1.118 | 0.015 |
| | Combined | | 1.001 | 1.000 | 1.002 | 0.045 |
| **Any stroke** | UK Biobank | 4 | 1.003 | 1.001 | 1.005 | 0.002 |
| | FinnGen | 7 | 1.133 | 0.964 | 1.332 | 0.13 |
| | Combined | | 1.003 | 1.001 | 1.005 | 0.003 |
| **Any ischemic stroke** | UK Biobank | 5 | 1.002 | 1.001 | 1.004 | 0.003 |
| | FinnGen | 6 | 1.302 | 1.112 | 1.524 | 0.001 |
| | Combined | | 1.128 | 0.874 | 1.457 | 0.355 |

Abbreviation: SNPs, Single nucleotide polymorphisms; OR, Odds ratio; LCI, Lower confidence interval; UCI, Upper confidence interval.

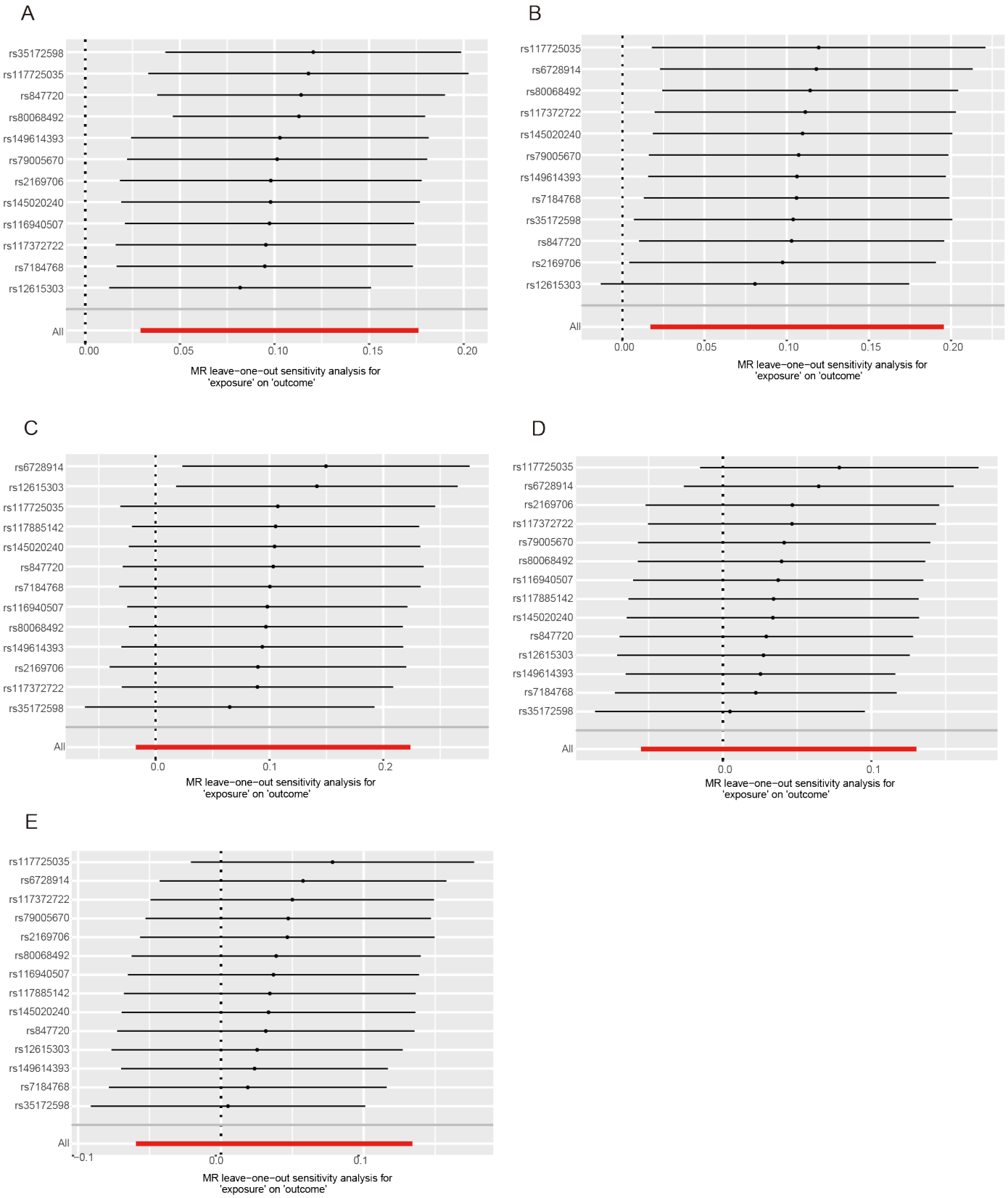


**Figure S1** Leave-one-out sensitivity analysis in the Mendelian randomization analysis of anemia and cardiovascular disease in FinnGen study. (A) Anemia and Heart failure; (B) Anemia and Coronary artery disease; (C) Anemia and Atrial fibrillation; (D) Anemia and Any stroke; (E) Anemia and Any ischemic stroke.

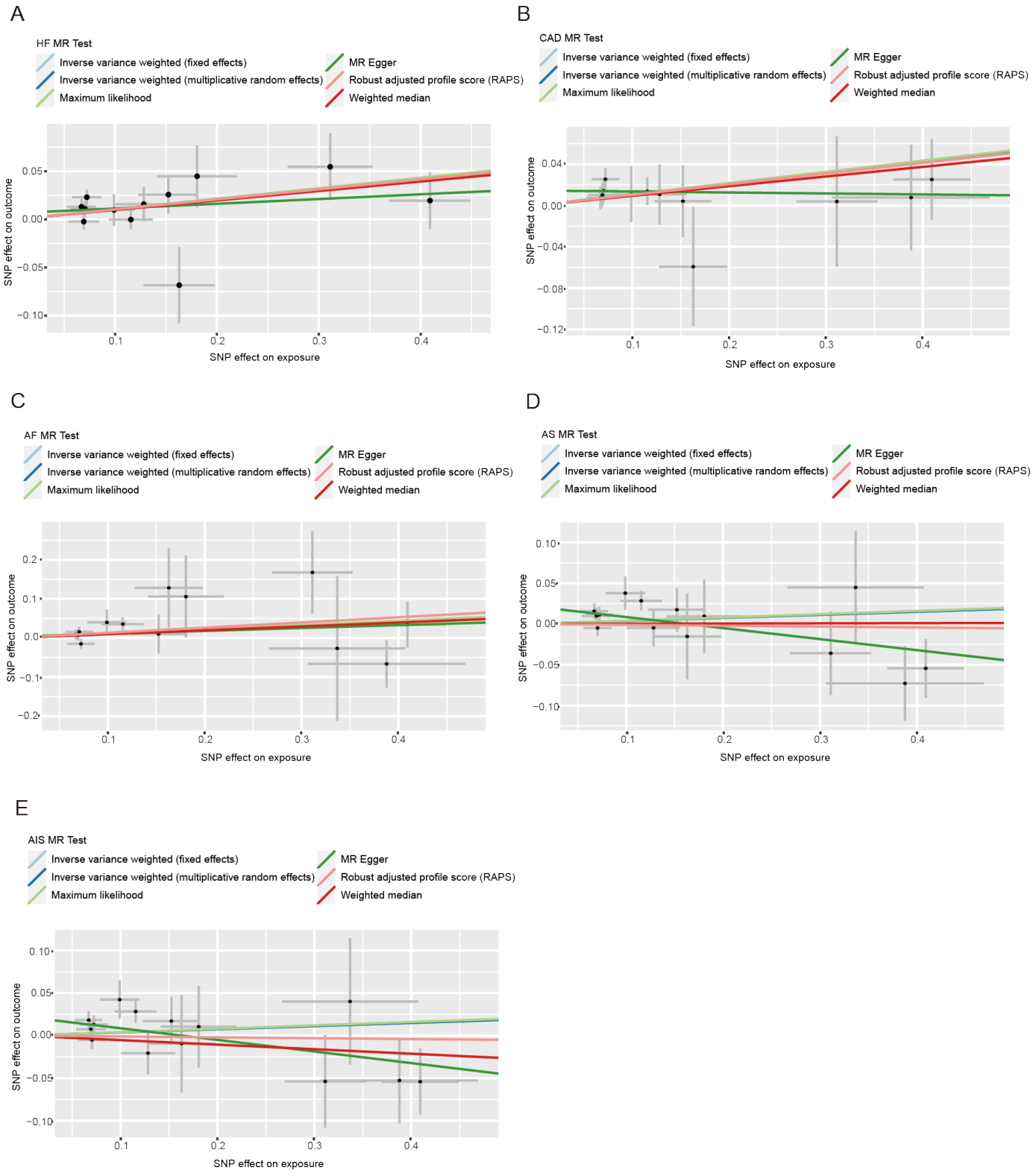


**Figure S2** Scatter_plot in the Mendelian randomization analysis of anemia and cardiovascular disease in FinnGen study. (A) Anemia and Heart failure; (B) Anemia and Coronary artery disease; (C) Anemia and Atrial fibrillation; (D) Anemia and Any stroke; (E) Anemia and Any ischemic stroke.

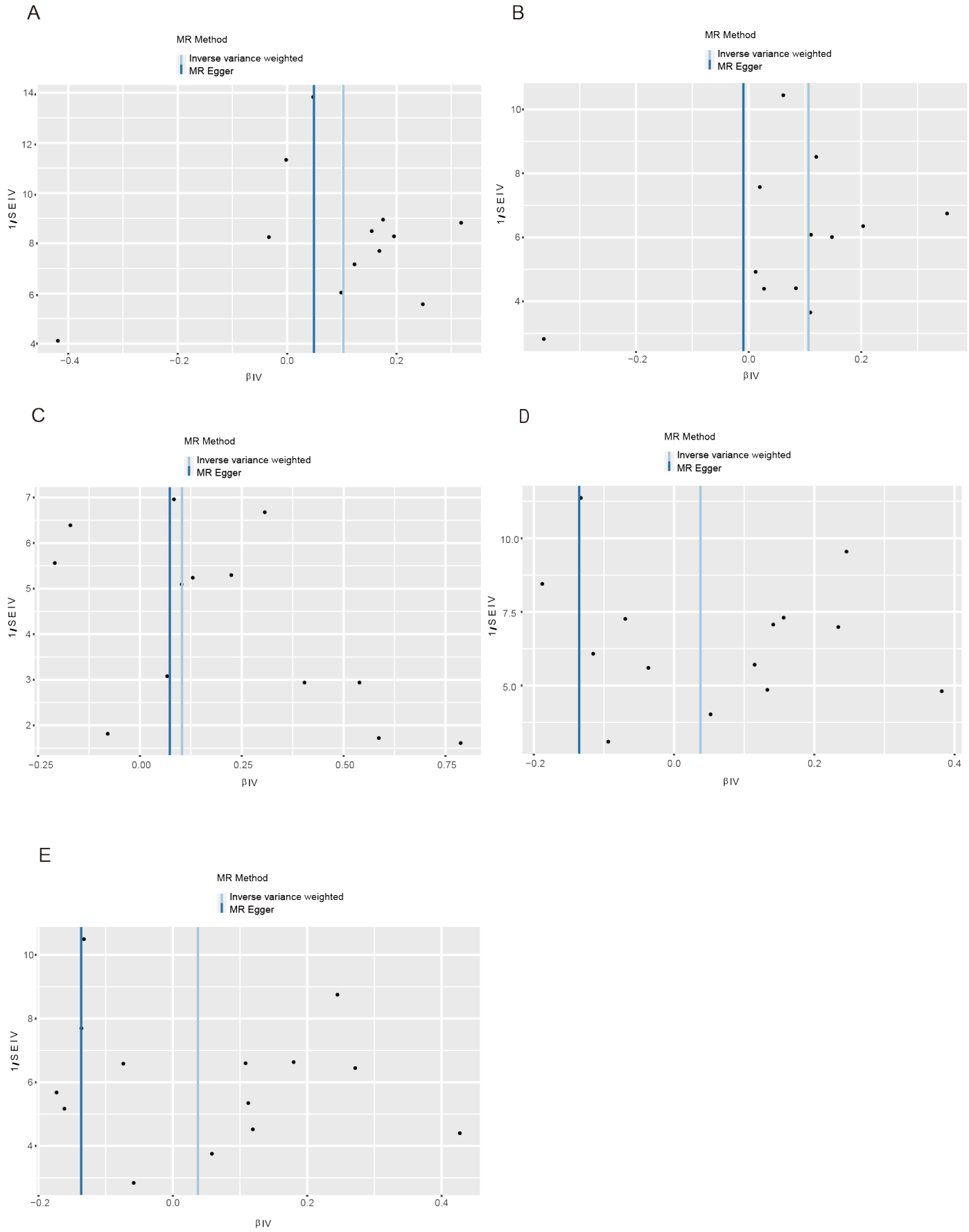


**Figure S3** Funnel_plot in the Mendelian randomization analysis of anemia and cardiovascular disease in FinnGen study. (A) Anemia and Heart failure; (B) Anemia and Coronary artery disease; (C) Anemia and Atrial fibrillation; (D) Anemia and Any stroke; (E) Anemia and Any ischemic stroke.

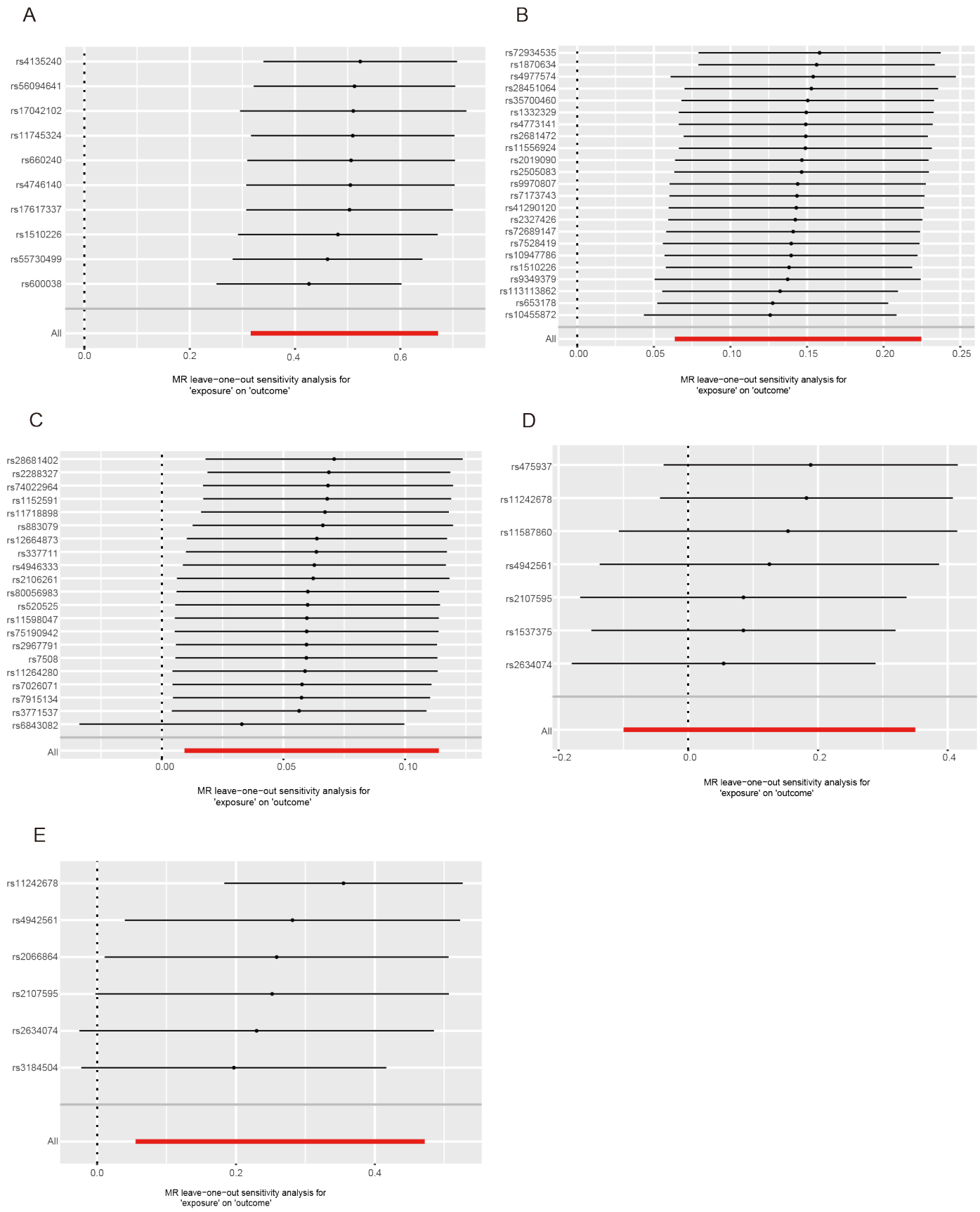


**Figure S4** Leave-one-out sensitivity analysis in the Mendelian randomization analysis of cardiovascular disease and anemia in FinnGen study. (A) Heart failure and Anemia; (B) Coronary artery disease and Anemia; (C) Atrial fibrillation and Anemia; (D) Any stroke and Anemia, "rs10774624" was removed as outliers in the MR-PRESSO analysis; AIS*, any ischemic stroke; (E) Any ischemic stroke and Anemia, "rs2634074" and "rs4942561" was removed as outliers in the MR-PRESSO analysis.

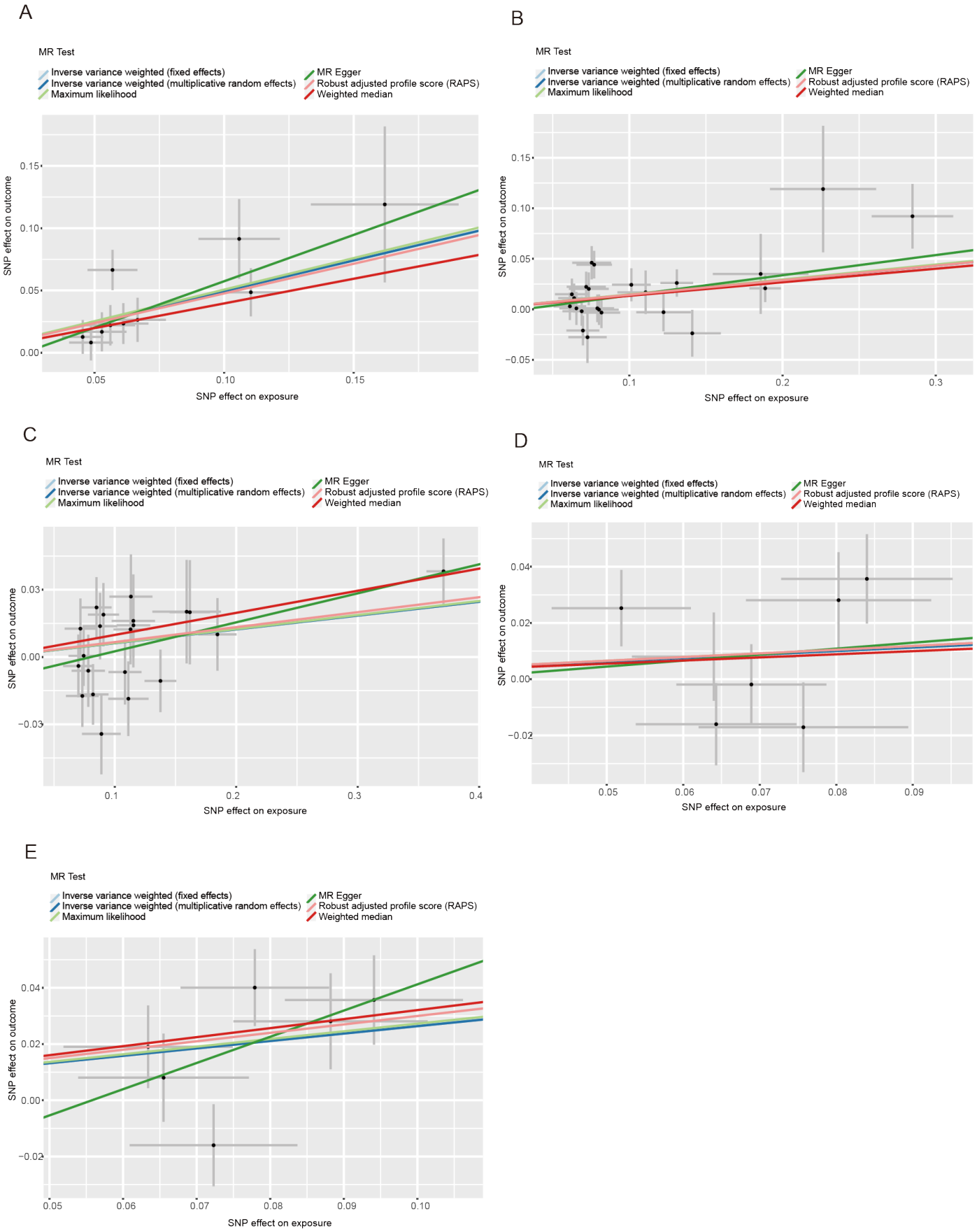


**Figure S5** Scatter_plot in the Mendelian randomization analysis of cardiovascular disease and anemia in FinnGen study. (A) Heart failure and Anemia; (B) Coronary artery disease and Anemia; (C) Atrial fibrillation and Anemia; (D) Any stroke and Anemia, "rs10774624" was removed as outliers in the MR-PRESSO analysis; AIS*, any ischemic stroke; (E) Any ischemic stroke and Anemia, "rs2634074" and "rs4942561" was removed as outliers in the MR-PRESSO analysis.

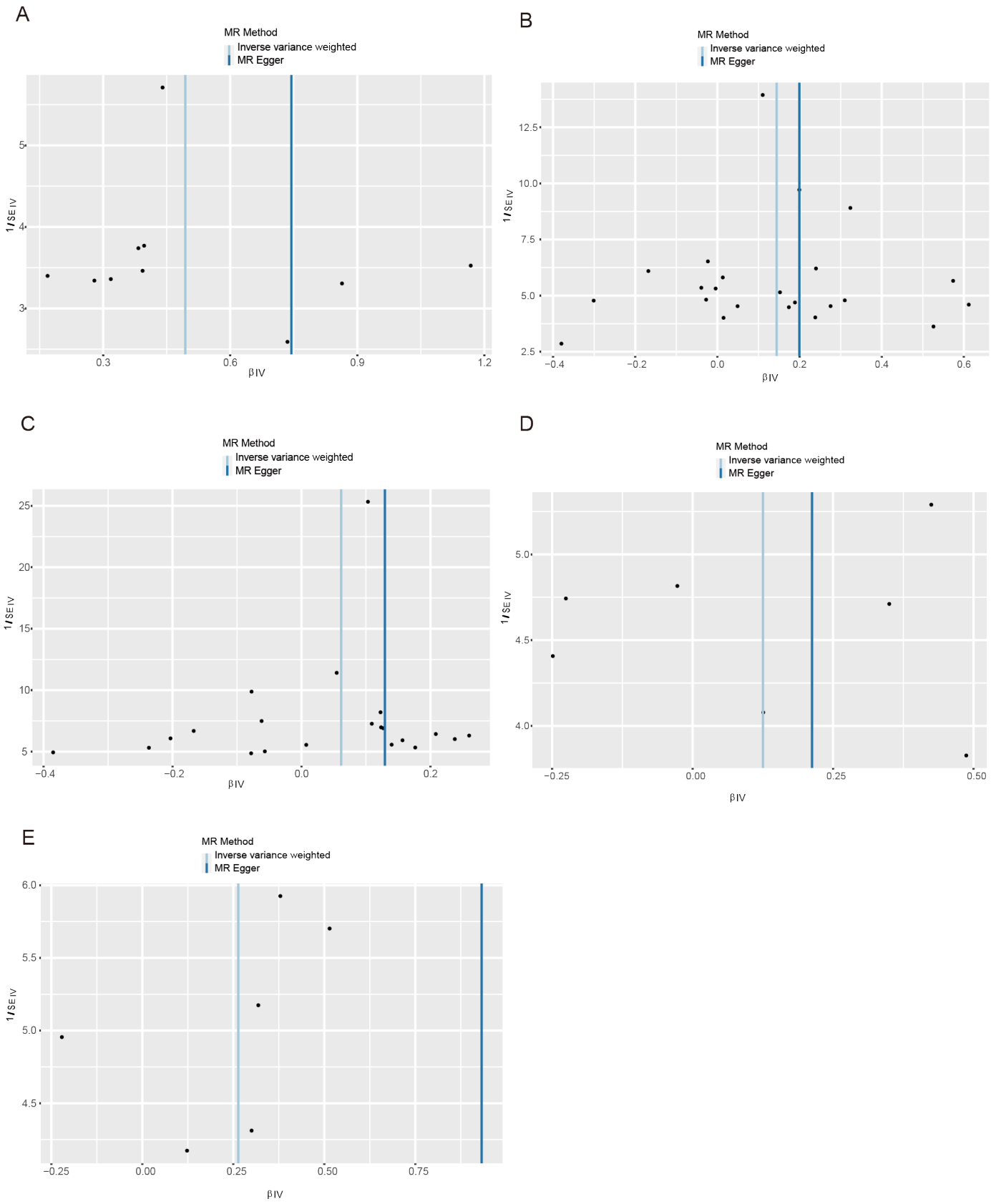


**Figure S6** Funnel_plot in the Mendelian randomization analysis of cardiovascular disease and anemia in FinnGen study. (A) Heart failure and Anemia; (B) Coronary artery disease and Anemia; (C) Atrial fibrillation and Anemia; (D) Any stroke and Anemia, "rs10774624" was removed as outliers in the MR-PRESSO analysis; AIS*, any ischemic stroke; (E) Any ischemic stroke and Anemia, "rs2634074" and "rs4942561" was removed as outliers in the MR-PRESSO analysis.