Research Article

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Iron Deficiency Anemia Contributes To The Development Of Multiple Cardiovascular Diseases: A Two-Sample Mendelian Randomization Study

Chengjia Li[1], Tianwei Meng[1], Xiaobing Zhang[1], Nan Jiang[2], Jiarui Li[1], Zhiping Liu[3,4]* and Huijun Chen[3]*

1Heilongjiang University of Chinese Medicine, Harbin, 150000, China;
2Geriatric Psychiatry, Second People's Hospital of Zhoushan, Zhoushan, 316000, China
3The second affiliated hospital, Heilongjiang University of Chinese Medicine, Harbin, 150001, China
4Geriatrics, the first affiliated hospital, Heilongjiang University of Chinese Medicine, Harbin, 150000, China

***Corresponding Author:**
Huijun Chen, Zhiping Liu,
4Geriatrics, the first affiliated hospital and The second affiliated hospital, Heilongjiang University of Chinese Medicine, Harbin, 150001, China
Email: liuzhipingtcm@163.com; 13845053131@163.com, hymanli001@163.com

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1.Abstract

1.1. Objectives: Observational studies have shown an association between iron deficiency anemia (IDA) and multiple cardiovascular diseases (CVD), but whether IDA promotes the development of CVD is still controversial.

1.2. Methods: Large-scale genome-wide association study (GWAS) data on unstable angina (UA), stable angina (SA), heart failure (HF), ischemic heart disease (IHD), atrial fibrillation (AF), and myocardial infarction (MI) were obtained from the Open Gwas website. Select strongly correlated single nucleotide polymorphisms (SNPs) in GWAS as instrumental variables (P < 5e-06), and set the threshold of F statistics to 10. A total of five statistical methods, including Inverse variance weighted (IVW), MR-Egger, Weighted median, Simple mode, and Weighted mode, were used to analyze the results, with the IVW method as the main analysis method. and assess the reliability of the results for heterogeneity, pleiotropy, and sensitivity.

1.3. Results: IDA has a promoting effect on the incidence of UA, SA, HF and IHD (UA: OR,1.25, 95% CI, 1.10–1.40, P = 0.0003; SA: OR, 1.14, 95% CI, 1.04–1.26, P = 0.006; HF: OR,1.13, 95% CI, 1.02–1.25, P = 0.0187; IHD: OR, 1.13, 95% CI, 1.02–1.25, P = 0.0238); IDA was not found to have a significant promoting effect on AF and MI (P >0.05). The results passed the tests of heterogeneity, pleiotropy and sensitivity. Conclusions: IDA can increase the risk of UA, SA, HF, and IHD; there is no evidence to support that IDA can increase the risk of MI and AF.

2. Keywords:
Mendelian Randomization; iron deficiency anemia; cardiovascular disease

3. Introduction

With the global control of morbidity and mortality in various infectious diseases, the incidence rate of non-communicable diseases is on the rise. Being the primary cause of death among non-communicable diseases, the impact of CVD has garnered increasing attention. In the last decade, global CVD-related deaths have risen by 12.5%, constituting one-third of all global deaths [1]. IDA is still one of the five main causes of disability, ranking first among women. The relationship between IDA and CVD remains unclear. Iron deficiency anemia may adversely affect CVD through the combined effects of mitochondrial dysfunction and hypoxia. Current evidence from multiple observational studies shows that iron deficiency is associated with the morbidity and mortality of coronary heart disease, heart failure, and acute myocardial infarction[2-5]. Another study demonstrates that iron supplementation can markedly improve outcomes in heart failure patients, even when the patient's iron deficiency is unrelated to anemia[6]. There are also some research data showing that anemia is associated with various CVD outcomes such as heart failure, acute coronary syndrome, atrial fibrillation, heart failure, atherosclerosis, etc[7-10]. Nevertheless, another observational study discovered that the association between anemia and CVD outcomes was not statistically significant after adjusting for covariates[11]. In summary, it is evident that confounding factors in clinical research, such as BMI and smoking, make the results challenging to ascertain. Hence, there is a lack of consistent speculation regarding the relationship between iron deficiency anemia and CVD.

Mendelian randomization (MR) analysis method has been widely used in epidemiological causality research in recent years. The fundamental

principle of MR is that parental gametes are randomly assigned to offspring and remain unaffected by environmental confounding factors and reverse causality when investigating disease relationships. Thus, MR can yield more reliable results compared to observational studies. To date, no studies have investigated the relationship between IDA and CVD using MR. Based on large-sample genome-wide association study (GWAS) data of IDA, Unstable angina (UA), Stable angina (SA), Heart Failure (HF), Ischemic heart disease (IHD), Atrial fibrillation (AF), and Myocardial infarction (MI), this study conducted a two-sample MR study to explore the association between IDA and various CVD outcomes.

4. Materials and Methods

4.1. Overview of study design

This study followed Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) when designing experiments and writing the manuscript. All GWAS data used in the MR analysis were obtained from published studies, which had obtained informed consent from patients and approval by the ethics committee before being conducted, so this study did not require additional ethical approval. We selected SNPs strongly correlated with exposure as instrumental variables (IVs) to exposure the association between IDA and CVD. The three assumptions of MR research are as follows: (1) There is a strong correlation between IVs and exposure factors; (2) IVs only affect outcomes through exposure factors and are not directly related to outcomes; (3) IVs cannot affect the association between exposure and outcomes through other confounding factors. (Figure 1)

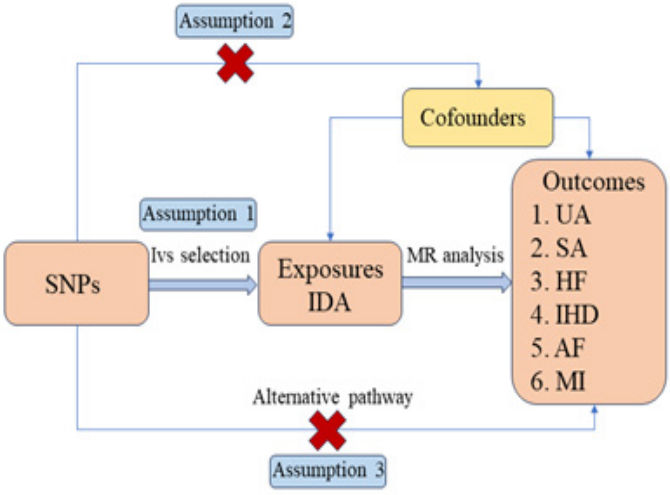


Figure 1: Overview of MR assumptions, design, and procedures. Abbreviations: IDA = iron deficiency anemia; UA = Unstable angina; SA = Stable angina; HF = Heart Failure; IHD = Ischemic heart disease; AF = Atrial fibrillation; MI = Myocardial infarction; MR = Mendelian randomization; IVs = instrumental variables.

4.2. Data sources

The summary data of IDA, UA, SA, HF, IHD, AF, and MI are from the publicly available GWAS in the open gwas database (https://gwas.mrcieu.ac.uk/), and the ethnic data are all from Europe. The ID, sample size, number of SNPs and query links of each data are shown in the table below (Table 1)

Phenotypes	GWAS ID	Sample size	SNP	Link
IDA	ebi-a-GCST90018872	480941	24180477	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018872/
UA	ebi-a-GCST90018932	456468	24179929	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018932/
SA	ebi-a-GCST90018915	343026	19057124	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018915/
HF	finn-b-I9_HEARTFAIL_ALLCAUSE	23397	16380447	https://gwas.mrcieu.ac.uk/datasets/finn-b-I9_HEARTFAIL_ALLCAUSE/
IHD	finn-b-I9_ISCHHEART	30952	16380466	https://gwas.mrcieu.ac.uk/datasets/finn-b-I9_ISCHHEART/
AF	ebi-a-GCST009541	537409	7773021	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST009541/
	ukb-d-I50	1030836	9806537	https://gwas.mrcieu.ac.uk/datasets/ukb-d-I50/
	ukb-d-HEARTFAIL	407746	9858439	https://gwas.mrcieu.ac.uk/datasets/ukb-d-HEARTFAIL/
MI	ebi-a-GCST90038610	484598	9587836	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90038610/
	ebi-a-GCST90018877	461823	24172914	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90018877/
	ebi-a-GCST011364	395795	10290368	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST011364/

Table 1: GWAS data sources and details

Abbreviations: IDA = iron deficiency anemia; UA = Unstable angina; SA = Stable angina; HF = Heart Failure; IHD = Ischemic heart disease; AF = Atrial fibrillation; MI = Myocardial infarction; GWAS = genome-wide association study; SNP = single-nucleotide polymorphism.

4.3. Selection and validation of SNPs

IVs were screened according to the core hypothesis. Since the amount of gwas data about IDA is small, P <5e-06 was set to screen out SNPs that are significant to IDA. R studio software was used for clump calculation, and the parameter was set to r2=0.001. kb =10000 to remove linkage disequilibrium[12]. Upload all SNPs to PhennoScanner V2 (http://www.phenoscanner.medschl.cam.ac.uk/) and download relevant phenotypic data to eliminate confounding factors directly related to CVD. Calculate the F value of the remaining SNPs (F=β2/se2), and set the F threshold to 10 to ensure strong correlation. SNPs related to each CVD outcome were extracted, and after integration, the MR-PRESSO algorithm was used to remove outliers.

4.4. MR analysis

The "TwoSampleMR" package in R studio software was used to analyze the causal relationship between IDA and various CVD outcomes. Inverse variance weighted (IVW) was used as the main analysis method, and MR-Egger, Weighted median, Simple mode, and Weighted mode analysis methods are supplemented. The IVW method is the most accurate method for MR analysis. The premise of using this method is that all instrumental variables meet the core assumptions. The intercept term is not considered in the regression and the inverse of the outcome variance is used as the weight to fit the analysis[13,14]. The estimation framework of the MR-Egger method relies on the IVW method, but the existence of the intercept term is considered in the regression analysis, and the testing hypothesis standard is relaxed to only need to meet the InSIDE hypothesis, that is, the estimated value of the causal effect is not related to the pleiotropy of the instrumental variable[15]. The principle of the weighted median method is that if at least 50% of the weights come from valid SNPs, the weighted median will produce an estimate consistent with the causal effect[16]. Simple mode is a median ratio estimate, but if the estimated effect of a single SNP is very different, the estimation power of the Simple mode method is reduced[17,18]; The weighted mode method can obtain a single causal effect estimate from multiple genetic variation instrumental variables. In most cases, it has less bias and a lower type I error rate than other methods. Its estimation efficiency is not as good as the IVW method and the WME method, but better than the MR- Egger method[19]. When only the results of the IVW method are statistically significant in a certain analysis, but other analysis methods are not statistically significant but tend to be in the same direction as the IVW method, the overall analysis result is considered to be statistically significant.

4.5. Reliability evaluation

Heterogeneity, pleiotropy, and sensitivity analyzes were performed on the studies to detect whether there was bias in the studies. First, use the "mr_heterogeneity" function to obtain Cochran Q statistics to evaluate data heterogeneity; secondly, use the "mr_pleiotropy_test" function to evaluate data pleiotropy through the MR-Egger intercept method; Finally, use the "mr_leaveoneout" function to remove SNPs one by one and evaluate whether the remaining SNPs point to the same result to analyze whether the result is affected by a single SNP.

5. Results

5.1 Screened IVs

After correlation analysis and linkage disequilibrium screening, there were a total of 16 SNPs related to IDA, of which 1 SNP was identified as a confounding factor and excluded. The identification results of confounding factors can be viewed in the supplementary material. There were no outliers, and the remaining 15 SNPs were included in the analysis, among which the F statistics was the lowest at 21.19, indicating that all SNPs are strong instrumental variables (Table 2).

SNPs	chr	EA/OA	EAF	β	SE	P	F
rs272534	1	G/A	0.129242	0.0803	0.0172	2.91E-06	21.80
rs11887481	2	A/G	0.315907	0.0755	0.0164	4.02E-06	21.19
rs35603314	2	A/C	0.297615	-0.0622	0.0125	6.38E-07	24.76
rs140689022	3	C/T	0.006895	-0.4042	0.0832	1.20E-06	23.60
rs71542412	6	C/T	0.440642	0.063	0.0133	2.38E-06	22.44
rs9273305	6	C/T	0.467106	0.1072	0.0161	2.40E-11	44.33
rs75686622	6	G/A	0.075593	0.1204	0.0248	1.19E-06	23.57
rs4917016	7	C/T	0.756462	0.0652	0.0139	2.65E-06	22.00
rs75836714	9	T/C	0.146101	0.086	0.0181	2.02E-06	22.58
rs11112625	12	C/T	0.5484	0.0625	0.013	1.57E-06	23.11
rs35851133	14	C/A	0.042116	0.1525	0.0323	2.33E-06	22.29
rs7171366	15	G/T	0.062207	0.1061	0.0229	3.77E-06	21.47
rs75380888	17	C/T	0.04368	0.3271	0.0643	3.64E-07	25.88
rs1292043	17	G/A	0.263458	0.0764	0.013	3.73E-09	34.54
rs6088764	20	A/C	0.235687	-0.0632	0.0134	2.55E-06	22.24

Table 2: Detailed information on IDA-related SNPs

SNPs: Single nucleotide polymorphisms; chr: Chromosome; EA: Effect allele; OA: Other allele; EAF: Effect allele frequency; β: Effect allele value; SE: Standard error; F: Statistics for assessing the impact of weak instrumental variables.

5.2 Two-sample Mendelian randomization results

IVW results show that IDA has a promoting effect on the incidence of UA, SA, HF, and IHD. (UA: OR,1.25, 95% CI, 1.10–1.40, P = 0.0003; SA: OR, 1.14, 95% CI, 1.04–1.26, P = 0.006; HF: OR,1.13, 95% CI, 1.02–1.25, P = 0.0187; IHD: OR, 1.13, 95% CI, 1.02–1.25, P = 0.0238); It was not found that IDA has a significant promoting effect on AF and MI (P>0.05). Different GWAS data sets were selected for negative results and repeated verification twice, the results were also not statistically significant (Figure 2).

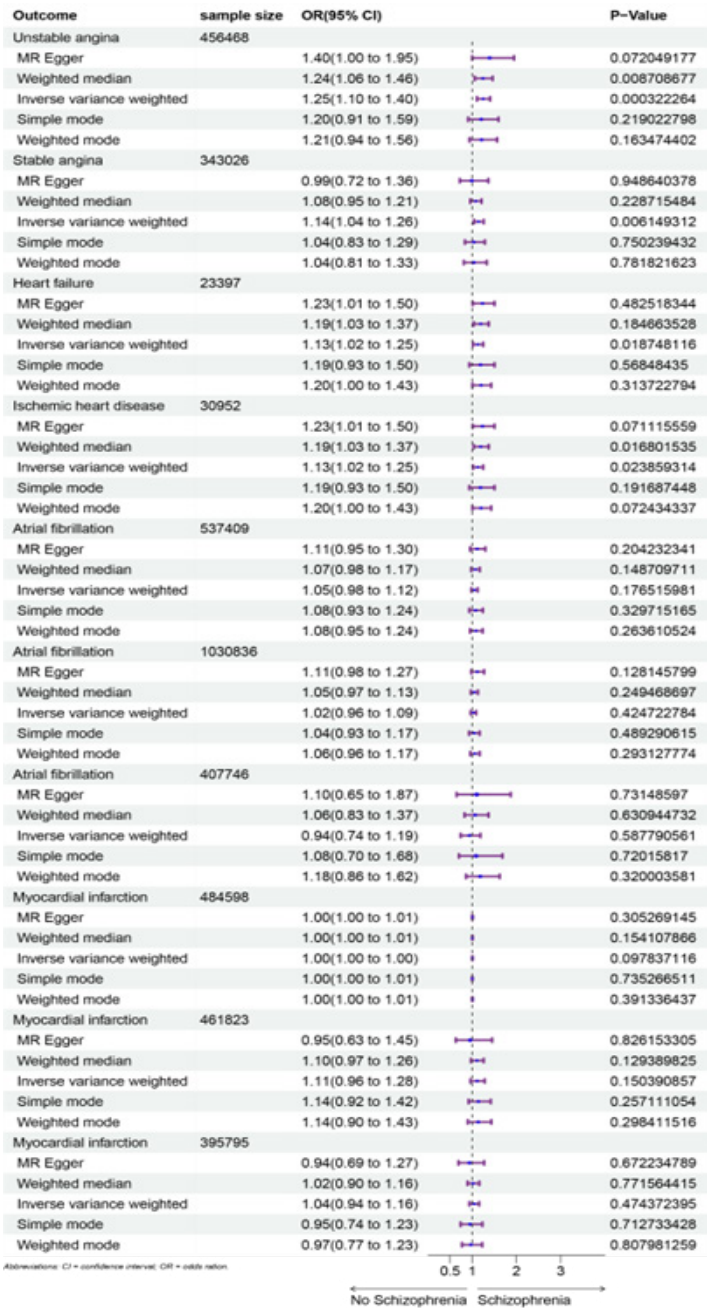
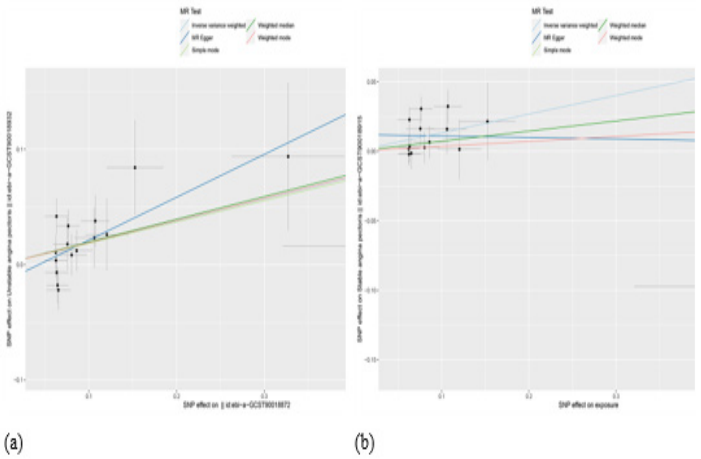


Figure 2: Two-sample Mendelian randomization analysis results.

After Cochran Q test and MR-Egger test, no heterogeneity and pleiotropy were found in each study (Table 3). After using the Leave-one-out method to remove SNP one by one, the statistical results were not affected, proving that the results are reliable. All MR analysis and Reliability evaluation results can be viewed in the supplementary material. Show the scatter plot and forest plot of the positive results as Figure 3, Figure 4.

Out-comes	Method	Pleiot-ropy		Hetero geneity			
		egger_ intercept	se	pval	Q	Q_df	Q_ pval
UA	MR Egger	-0.016	0.015	0.297	16. 680	14	0.274
	Inverse variance weighted				18. 077	15	0.259
SA	MR Egger	0.010	0.013	0.479	17. 891	12	0.119
	Inverse variance weighted				18. 687	13	0.133
HF	MR Egger	0.007	0.013	0.627	15. 790	9	0.071
	Inverse variance weighted				16. 234	10	0.093
IHD	MR Egger	-0.010	0.010	0.331	6.5 46	9	0.684
	Inverse variance weighted				7.6 02	10	0.668

Table 3: Heterogeneity and pleiotropy detection of positive results
Abbreviations: UA = Unstable angina; SA = Stable angina; HF = Heart Failure; IHD = Ischemic heart disease.



(a) (b)

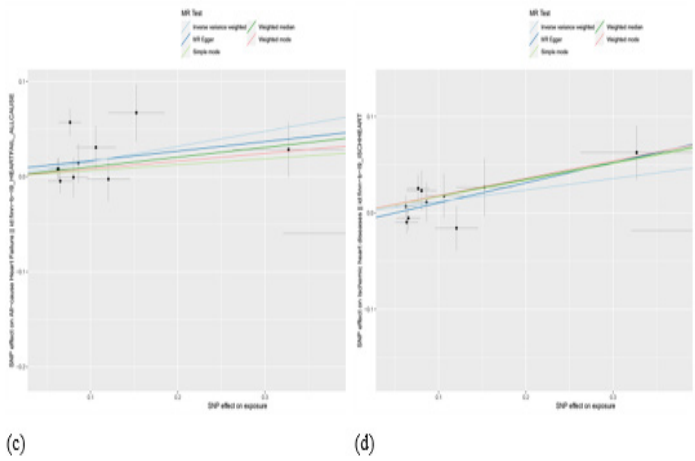


Figure 3: Scatter plots for Two-sample Mendelian randomization analysis. Scatter plot represents the estimated effect of using 5 statistical methods to analyze the causal association of IDA (x-axis) on each outcome (y-axis). The slope of each line represents the OR value of this method. (a) IDA and UA; (b) IDA and SA; (c) IDA and HF; (d) IDA and IHD.

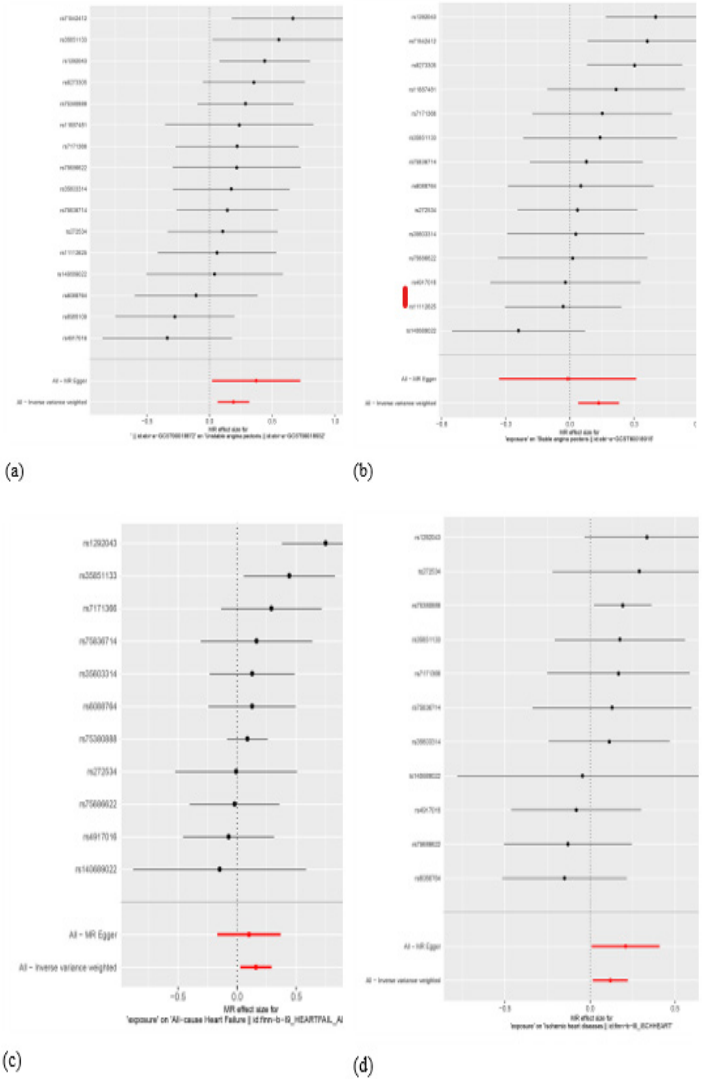


Figure 4: Forest plots for Two-sample Mendelian randomization analysis. Each black point in the Forest plot represents the risk effect value estimate of a single SNP of IDA on the outcome, and the black line represents the 95% CI. The two red dots represent the estimates of the overall effect size between IDA and outcome using MR-Egger and IVW respectively, and the red line represents the 95% CI. (a) IDA and UA; (b) IDA and SA; (c) IDA and HF; (d) IDA and IHD.

6. Discussion

This study is the first to use MR to evaluate the relationship between IDA and various CVDs. We included up to 10 GWAS data sets, adopted a two-sample MR design, and hypothesized that IDA is associated with various CVDs. The results showed that IDA does have a causal relationship with the incidence of some CVDs, such as UA, SA, HF, and IHD; at the same time, it is not associated with the incidence of MI and AF. These results are based on the same ethnic data and are reliable, but the underlying mechanisms still need to be further explored. Anemia denotes a relative or absolute deficiency in the body's uptake, transport, and release of oxygen in the blood. According to the cause, it can be divided into reduced red blood cell production, excessive red blood cell destruction, or blood loss. The persistent hypoxic state resulting from anemia can cause severe damage to myocardial cells, with iron deficiency being the primary cause of anemia. In a retrospective study based on 1,061 cases of heart failure patients, researchers evaluated the impact of anemia on CVD. The results showed that for every 1 g/dL decrease in hemoglobin, the risk of death in heart failure patients increased by 13% [20]. Another study showed that even in the presence of moderate anemia (hemoglobin between 10-12 g/dL), mortality in patients with acute coronary syndrome is still increased [8]. HF and acute coronary syndrome, as end-stage diseases, generally have been combined with more basic coronary artery disease (CAD) or preload and postload abnormalities for a long time during the progression of the disease. Although the healthy human heart can withstand acute and severe anemia without causing sustained MI, which CAD may impair [21]. Possible reasons for the association between anemia and heart failure include the following: On the one hand, the oxygen-carrying capacity of the blood is reduced during anemia, which requires the secretion of extra adrenaline to stimulate myocardial pumping to compensate [22]; On the other hand, these anemia patients without subgroup stratification may be combined with other chronic inflammation, and inflammatory cytokines also cause damage to endothelial function [20]. It can be seen that anemia is a relatively stable independent risk factor in various CVDs.

IDA is a type of anemia where the production of red blood cells is diminished due to insufficient hematopoietic substances. While anemia, irrespective of its cause, has been deemed a cardiovascular disease risk factor, iron deficiency might intensify this adverse impact. Iron, a crucial component of hemoglobin, plays a vital role in the formation and oxygenation process of red blood cells. Malnutrition, chronic blood loss, chronic inflammation, and increased hepcidin levels due to chronic kidney disease are common causes of iron deficiency [23]. Additionally, as an essential inorganic element, iron plays a crucial role

in mitochondrial function. Mitochondria serve as the primary subcellular site for intracellular iron accumulation. Mitochondria prompt hemoglobin to adopt an iron-regulatory protein conformation for assembly with Fe/S clusters or heme production, preventing damage by sequestering excess iron in an inactive form. Iron deficiency causes intracellular hemoglobin to adopt an iron-responsive element binding conformation. Mitochondria can only uptake iron but cannot utilize it for assembly and export with Fe/S clusters or heme. The ultimately deposited iron impairs mitochondrial function [24], and studies have indicated that mitochondrial dysfunction is a significant cause of CVD [25]. It is worth noting that a recent MR study exploring the impact of anemia on CVD concluded that anemia was only significantly related to HF[26], which is inconsistent with the results of this study. The reason for this difference may be that the large sample size of unstratified anemia patient data dilutes the possible CVD consequences of iron deficiency anemia, thus masking the possible synergistic negative effects of iron deficiency and anemia on CVD. At the same time, this also shows that for some heart diseases, such as HF, the treatment of anemia should be carefully differentiated between iron supplementation and blood transfusion, which treatment is more appropriate.

In recent years, the relationship between iron storage levels and CVD has become the focus of research, with various studies questioning each other and the results being highly controversial. In 1981, Sullivan published research results stating that higher iron storage levels can lead to cardiac arrhythmias and myocardial fibrosis. This difference causes the incidence of heart disease in premenopausal women to be lower than that in men and postmenopausal women [27]. Based on this hypothesis, Randall recommends that adult men exercise vigorously or donate 1,000 to 1,500 mL of blood every year to correct the high storage of iron in the blood and prevent CVD [28]. Some researchers believe that high storage levels of iron may promote atherosclerosis by inducing the formation of highly reactive oxygen species and lipid peroxidation [29]. However, these results are questionable. A systematic review and meta-analysis published in 2015 included 156,427 patients with CAD and MI. The results show that serum ferritin, total iron binding capacity, serum iron are negatively correlated with CAD and MI, that is, high iron storage can have a protective effect on the heart [30]. Another study found that men who donate more blood are more likely to suffer from MI [31]. According to the results of this study and other MR studies of the same type, iron deficiency anemia is more harmful to the heart than patients with simple anemia, and it can promote the onset of diseases such as UA, SA, HF, and IDA. Although ferroptosis has become a hot topic in CVD research, low storage levels of iron should not be considered to have a protective effect on the myocardium.

After determining the cardioprotective effect of iron supplementation in patients with iron deficiency anemia, it is also worth exploring which iron supplement preparation should be chosen. Clinical treatments for IDA are generally divided into two types: oral iron and intravenous iron supplementation. Each treatment has a variety of preparations to choose from. Oral iron compounds, including ferrous and ferric iron compounds, have been used clinically for many years. However, up to 30% of patients will experience gastrointestinal discomfort, such as constipation, diarrhea or nausea [32,33]. Oral iron supplements can also cause toxic mucosal effects through iron deposition, thereby exacerbating the damage to the gastrointestinal mucosa of patients [34]. Therefore, oral iron therapy for the treatment of IDA has gradually withdrawn from the frontline in the field of CVD. The European Society of Cardiology pointed out in its guidelines that patients with iron deficiency should be treated with intravenous iron supplementation, and specifically recommended ferric carboxymaltose (FCM)[35], and the American Heart Association/ American College of Cardiology also gives similar recommendations and believes that this measure can improve symptoms and quality of life[36]. In a European multicenter, double-blind, two-arm prospective study, researchers enrolled 304 patients with heart failure accompanied by iron deficiency symptoms and randomly assigned them to FCM or placebo treatment groups. The results showed that at the sixth week, the 6-minute walking test results of the FCM treatment group were significantly better than those of the control group, with a difference of 33±11m (P = 0.02). At the same time, FCM treatment could also significantly reduce the risk of hospitalization due to worsening of heart failure (HR 0.39, 95% CI, 0.19–0.82, P = 0.009)[37].

7. Conclusions

We found that IDA can increase the risk of UA, SA, HF, and IHD. There is no evidence to support that IDA can increase the risk of MI and AF. IDA should be considered as a risk factor in patients with UA, SA, HF, and IHD in the future. In addition, patients with IDA during the course of heart disease should actively supplement iron to prevent further aggravation of the condition.Supplementary Materials: All supplementary documents have been uploaded to the review system along with the article. Author Contributions: Conceptualization, Chengjia Li and Huijun Chen; Data curation, Chengjia Li; Formal analysis, Xiaobing Zhang and Jiarui Li; Funding acquisition, Huijun Chen; Investigation, Zhiping Liu; Methodology, Chengjia Li and Tianwei Meng; Project administration, Huijun Chen; Resources, Nan Jiang; Software, Chengjia Li; Supervision, Zhiping Liu; Validation, Tianwei Meng, Xiaobing Zhang and Nan Jiang; Visualization, Tianwei Meng; Writing – original draft, Chengjia Li; Writing – review & editing, Huijun Chen.

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9. Informed Consent Statement:
Everything involved in this article comes from GWAS studies that have received ethical review. Specific approval documents can be obtained from the original data of relevant studies.

10. Data Availability Statement:
Some of the data are presented in the text, and the full raw data are available in the supplementary file.

11. Acknowledgments:

The authors would like to express their sincere gratitude to FinnGen for providing raw data for our Mendelian randomization analysis by making their GWAS summary-level statistics publicly available.

12. Declaration of Interest:

The authors declare no conflict of in-terest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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