Journal of the American Heart Association

ORIGINAL RESEARCH

Iron Status and Risk of Heart Disease, Stroke, and Diabetes: A Mendelian Randomization Study in European Adults

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BACKGROUND: The relevance of iron status biomarkers for coronary artery disease (CAD), heart failure (HF), ischemic stroke (IS), and type 2 diabetes (T2D) is uncertain. We compared the observational and Mendelian randomization (MR) analyses of iron status biomarkers and hemoglobin with these diseases.

METHODS AND RESULTS: Observational analyses of hemoglobin were compared with genetically predicted hemoglobin with cardiovascular diseases and diabetes in the UK Biobank. Iron biomarkers included transferrin saturation, serum iron, ferritin, and total iron binding capacity. MR analyses assessed associations with CAD (CARDIOGRAMplusC4D [Coronary Artery Disease Genome Wide Replication and Meta-Analysis Plus The Coronary Artery Disease Genetics], n=181 522 cases), HF (HERMES [Heart Failure Molecular Epidemiology for Therapeutic Targets), n=115 150 cases), IS (GIGASTROKE, n=62 100 cases), and T2D (DIAMANTE [Diabetes Meta-Analysis of Trans-Ethnic Association Studies], n=80 154 cases) genome-wide consortia. Observational analyses demonstrated J-shaped associations of hemoglobin with CAD, HF, IS, and T2D. In contrast, MR analyses demonstrated linear positive associations of higher genetically predicted hemoglobin levels with 8% higher risk per 1 SD higher hemoglobin for CAD, 10% to 13% for diabetes, but not with IS or HF in UK Biobank. Bidirectional MR analyses confirmed the causal relevance of iron biomarkers for hemoglobin. Further MR analyses in global consortia demonstrated modest protective effects of iron biomarkers for CAD (7%–14% lower risk for 1 SD higher levels of iron biomarkers), adverse effects for T2D, but no associations with IS or HF.

CONCLUSIONS: Higher levels of iron biomarkers were protective for CAD, had adverse effects on T2D, but had no effects on IS or HF. Randomized trials are now required to assess effects of iron supplements on risk of CAD in high-risk older people.

Key Words: coronary artery disease ■ heart failure ■ hemoglobin ■ iron status ■ ischemic stroke ■ type 2 diabetes

Iron deficiency is a common nutritional deficiency worldwide that preferentially affects women, older people, and low-income populations.[1] Iron overload is a less common problem that chiefly affects men and people of Celtic ancestry in which excess iron is deposited in multiple organs, such as the heart, liver, or pancreas.[2,3] Previous studies reported higher risks of cardiometabolic diseases (CMD) associated with iron status outside the normal range.[4–19] Moreover, iron deficiency is a therapeutic target for the treatment of patients with heart failure with reduced ejection fraction.[20–24] Given the fact that there is no consensus on the optimum biomarkers for assessing iron status and its effects on health outcomes,[25,26] it is important to assess the levels of iron status by multiple biomarkers[27,28] and explore their associations with CMD and other selected diseases.

Most of the body stores of iron are located in hemoglobin within circulating erythrocytes, where their primary role is to transport oxygen to body tissues.[1]

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This article was sent to Jacquelyn Y. Taylor, PhD, PNP-BC, RN, FAHA, FAAN, Guest Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.031732

For Sources of Funding and Disclosures, see page 11.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Higher levels of genetically predicted iron status biomarkers were inversely associated with coronary artery disease (7%–14% lower risk for 1 SD higher iron status biomarkers), positively associated with diabetes, but there was no evidence of any associations with ischemic stroke or heart failure.

What Are the Clinical Implications?

- Mendelian randomization analyses demonstrated modest protective effects of higher iron status biomarkers for coronary artery disease, adverse effects for type 2 diabetes, and no support from Mendelian randomization for heart failure or ischemic stroke.
- The results could guide the design of large-scale randomized trials of iron supplements in high-risk older people for prevention of coronary artery disease.

Nonstandard Abbreviations and Acronyms

CMD	cardiometabolic diseases
GRS	genetic risk score
IS	ischemic stroke
LAGE	localized average causal effects
MR	Mendelian randomization
PheWAS	phenome-wide associations
T2D	type 2 diabetes
TIBC	total iron binding capacity
TSAT	transferrin saturation
UKB	UK Biobank

Previous observational studies have examined associations of hemoglobin levels with CMD and other diseases,[4–12] but their results have been inconsistent, ranging from null to positive, linear, and nonlinear (U-shaped or J-shaped) relationships, in addition to differences by sex. Moreover, few studies have explored the strength of associations of hemoglobin levels and iron biomarkers with CMD,[29] in addition to their causal relevance using linear and nonlinear Mendelian randomization (MR) analyses in the same study.[30,31]

In contrast to other well-established iron status biomarkers such as transferrin saturation (TSAT), serum iron, ferritin, and total iron binding capacity (TIBC), hemoglobin has been more extensively examined in large-scale cohort studies. The UK Biobank (UKB), with detailed data collected by questionnaire and health outcomes in relation to hemoglobin levels and genetic data,[32] affords a unique opportunity to conduct observational and genetic analyses of iron status markers and hemoglobin levels with disease outcomes. Assessing the causal relevance of these associations could guide the design of large-scale randomized trials to assess the effects of iron supplements on the risk of coronary artery disease (CAD), heart failure (HF), ischemic stroke (IS), and type 2 diabetes (T2D).

The aims of this study were to (1) examine the observational associations of hemoglobin with CMD in UKB; (2) assess the shape and strength of associations of hemoglobin with CMD using linear and nonlinear 1-sample MR analyses in UKB; (3) compare the genetic associations of iron status markers (including TSAT, serum iron, ferritin, and TIBC) with hemoglobin; and (4) investigate the associations of iron status markers with CAD, HF, IS, and T2D through 2-sample MR analyses using summary data from published genome-wide consortia. Additional analyses included phenome-wide associations (PheWAS) of genetically predicted differences in hemoglobin with a wide range of disease outcomes in UKB and sex-specific analyses of observational and genetic associations of hemoglobin with CMD in UKB.

METHODS

Study Population

The study design and methods used in UKB have been previously reported.[32–34] In brief, UKB is a prospective study of over 500 000 participants, aged 37 to 73 years when recruited from 22 assessment centers in the United Kingdom between 2006 and 2010 (Table 1; Figure S1; Table S1). Participants provided information on their health and lifestyle using questionnaires supplemented by interviews and had physical measurements recorded and blood samples collected for biochemical and genetic measurements.[32–34] Among the 502 507 participants in the UKB, we excluded individuals who reported current use of iron supplements (n=115 035), or who withdrew from the study (n=99), or had missing data on hemoglobin (n=24 331), leaving 368 406 participants for the present analyses (Table 1). Ethics approval for UKB was provided by the National Information Governance Board for Health and Social Care and the National Health Service North West Multicenter Research Ethics Committee (REC reference: 11/NW/03820). All participants provided written informed consent.

Ascertainment of Exposures and Outcomes

Among samples collected at recruitment, hemoglobin concentrations (Field ID 30020, mg/dL) were

Table 1. Baseline Characteristics of Participants in the Observational Analysis in UK Biobank, by Sex

	Men	Women	All
Number of participants	177 535 (48.2)	190 871 (51.8)	368 406
Demographic factors			
Age, y	56.8 (8.2)	56.5 (8.0)	56.6 (8.1)
Race, White, %	167 472 (94.3)	181 554 (95.1)	349 026 (94.7)
Socioeconomic deprivation tertiles			
1 (least deprived)	59 105 (33.3)	63 690 (33.4)	122 795 (33.3)
2	58 231 (32.8)	64 573 (33.8)	122 804 (33.3)
3 (most deprived)	60 199 (33.9)	62 608 (32.8)	122 807 (33.3)
Education, university/college, %	59 133 (33.3)	57 582 (30.2)	116 715 (31.7)
Lifestyle factors			
Current smoker, %	22 444 (12.6)	17 366 (9.1)	39 810 (10.8)
Current drinker, %	166 031 (93.5)	172 515 (90.4)	338 546 (91.9)
Postmenopausal (Yes in women), %	…	116 375 (61.0)	116 375 (31.6)
Physical activity, metabolic equivalent of task, h/week	29.8 (13.2, 61.5)	28.0 (12.9, 55.6)	29.0 (13.0, 58.8)
Family history of diseases, N (%)			
Family history of cardiovascular disease	124 389 (70.1)	145 489 (76.2)	269 878 (73.3)
Family history of diabetes	36 514 (20.6)	42 879 (22.5)	79 393 (21.6)
Medical history, N (%)			
Use of antihypertensive drugs	44 920 (25.3)	35 045 (18.4)	79 965 (21.7)
Use of cholesterol-lowering drugs	41 536 (23.4)	25 334 (13.3)	66 870 (18.2)
Baseline coronary artery disease	15 160 (8.5)	6399 (3.4)	21 559 (5.9)
Baseline ischemic stroke	702 (0.4)	306 (0.2)	1008 (0.3)
Baseline heart failure	1454 (0.8)	465 (0.2)	1919 (0.5)
Baseline type 2 diabetes	11 665 (6.6)	6351 (3.3)	18 016 (4.9)
Clinical measurements			
Body mass index, kg/m^2	27.9 (4.2)	27.2 (5.2)	27.5 (4.8)
Waist circumference, cm	97.1 (11.3)	84.9 (12.6)	90.8 (13.5)
Waist-to-hip ratio	0.93 (0.89, 0.98)	0.81 (0.77, 0.86)	0.88 (0.81, 0.94)
Systolic blood pressure, mm Hg	141.1 (17.5)	135.7 (19.2)	138.3 (18.6)
Diastolic blood pressure, mm Hg	84.2 (10.0)	80.8 (10.0)	82.4 (10.1)
Random glucose, mmol/L	4.95 (4.60, 5.36)	4.91 (4.60, 5.28)	4.93 (4.60, 5.32)
Hemoglobin A1c, %	5.38 (5.15, 5.65)	5.37 (5.15, 5.61)	5.38 (5.15, 5.63)
Hemoglobin, mg/dL	15.0 (14.4, 15.7)	13.5 (12.9, 14.1)	14.2 (13.4, 15.1)
High-density lipoprotein cholesterol, mmol/L	1.28 (0.31)	1.59 (0.38)	1.44 (0.38)
Low-density lipoprotein cholesterol, mmol/L	3.47 (0.87)	3.63 (0.87)	3.56 (0.87)
Triglycerides, mmol/L	1.69 (1.18, 2.44)	1.34 (0.97, 1.90)	1.49 (1.05, 2.16)
Total cholesterol, mmol/L	5.48 (1.14)	5.88 (1.13)	5.69 (1.15)
C-reactive protein, mg/L	1.29 (0.67, 2.56)	1.40 (0.66, 3.01)	1.34 (0.66, 2.78)

Values are shown as numbers (percentages) for categorical variables and means (SD) or median (interquartile range) for continuous variables.

measured in EDTA-vacutainers using the Sysmex XN-1000 hematology analyzer at the laboratory of the UK Biocenter (https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/haematology.pdf).[35,36] The primary outcomes included incident cases of CAD, HF, IS, and T2D, which were identified through linkage to Hospital Episodes Statistics and mortality data, with diagnostic criteria defined by the *International Classification of Diseases, Ninth Revision* (*ICD-9*) and *Tenth Revision* (*ICD-10*) codes (Table S2). Data from UKB are available to bona fide researchers on application to https://www.ukbiobank.ac.uk. Data from other genetic consortia are also available on application to the relevant consortia.

Statistical Analysis

Observational Analyses of Hemoglobin With CMD

Logistic regression was used to estimate the odds ratios (OR) and their 95% CIs for CAD, HF, IS, and T2D

in individuals who were free of cardiovascular disease (CVD) or diabetes at baseline. All analyses were adjusted for age, sex, ethnicity, education, socioeconomic status, family history of CVD/diabetes, smoking status, alcohol intake, physical activity, body mass index, baseline diabetes, medication use, systolic blood pressure, and triglycerides where appropriate. Townsend Deprivation Index was used as an area-based proxy measure for socioeconomic status, with lower levels indicating higher socioeconomic status.[37] Body mass index was calculated by dividing body weight in kilograms by the square of height in meters (kg/m^2).

Cubic spline curves[38] were used to assess the shape of the associations of hemoglobin levels with incident disease outcomes. Nonlinearity was evaluated using a Wald test.[38] All analyses were stratified by sex and any heterogeneity by sex was assessed using a Cochran's *Q* test.[39]

Genetic Analyses

Genetic analyses in UKB were restricted to individuals of White-British descent, and individuals were excluded if they were related to each other (third-degree relatives or closer), had mismatched information between self-reported and genetic sex, had missing values and outliers for hemoglobin levels, or had missing data for any of the covariates, resulting in 331 964 participants for the present analysis (Figure S1; Table S1).

MR Analyses of Hemoglobin With CMD

In linear MR analysis, we generated a weighted genetic risk score (GRS) consisting of 532 single-nucleotide variant (SNVs, instrument strength: *F*-statistics=22335.7, R^2=13%), derived from a recent genome-wide association study (GWAS) of 563 085 European ancestry participants,[40] as an instrumental variable for blood hemoglobin concentrations (Tables S3 and S4). A 2-stage least squares analysis was performed to assess the associations of genetically predicted hemoglobin levels with prevalent and incident CAD, HF, IS, and T2D. Both stages of the least square regression were adjusted for age, sex, assessment centers, top 10 principal components, and genotyping array. To assess the consistency of results, an independent set of instrumental variables (140 SNVs reported by Astle et al.[41] *F*-statistics=12934.7, R^2=7%) and 2-sample MR were conducted using summary data from the most recent publicly available genome-wide consortia: CARDIOGRAMplusC4D (Coronary Artery Disease Genome Wide Replication and Meta-Analysis Plus the Coronary Artery Disease Genetics), HERMES (Heart Failure Molecular Epidemiology for Therapeutic Targets), GIGASTROKE, and DIAMANTE (Diabetes Meta-Analysis of Trans-Ethnic Association Studies).

We used nonlinear MR[30,42] with fractional polynomials to assess the nonlinear associations of genetically determined hemoglobin levels with CMD. First, the study participants were categorized into 4 strata according to the residual levels of hemoglobin after regressing on the GRS of hemoglobin. Second, we calculated the localized average causal effects within each stratum, which are the ratio of coefficients of the GRS-hemoglobin–outcome association to those of the GRS-hemoglobin–hemoglobin association. Third, these the localized average causal effects estimates were meta-regressed against the mean levels of hemoglobin in each stratum by fitting fractional polynomial exposure–outcome models. Maximum likelihood estimates of model parameters were obtained among all possible fractional polynomial models using 1 or 2 degrees of freedom and the likelihood ratio test statistic was used to select the best-fitting models. *P* values from the quadratic test and Cochran's *Q* test were reported for nonlinearity.

For 1-sample and nonlinear MR analyses, we performed sex-stratified analyses in UKB to explore whether the effect of hemoglobin on CMD differed by sex using Cochran's *Q* test for heterogeneity. All statistical analyses were performed in R (version 3.6.3), in addition to Plink (version 2.0) software for the GRS calculation and the "nlmr" package for nonlinear MR analysis.

PheWAS of Hemoglobin With Multiple Diseases

Among the 331 964 participants who passed quality control, we conducted a PheWAS[43] to examine the associations of GRS-hemoglobin with a range of diseases using PHESANT.[44] All disease outcomes were obtained from the first occurrence of each disease (Category 1712) in UKB that were derived from hospital inpatient records, death registers, disease registers, and self-reported health conditions. We restricted analyses to diseases involving more than 1000 cases (377 diseases) and used multivariable logistic regression to assess associations with diseases after adjusting for age, sex, assessment center, top 10 principal components of ancestry, and genotyping array for study participants. Power calculations for PheWAS analysis[45] suggested that a sample size of 1000 cases had 80% power to detect an OR of 1.25 or 0.76 for any disease outcome. We also performed sex-specific PheWAS analyses using a threshold of 500 cases, leaving 319 and 376 diseases in men and women, respectively. All statistical tests were 2-sided and corrected for multiple testing using a 5% false discovery rate.[46]

MR Analyses of Iron Status Biomarkers With Hemoglobin and CMD

We selected the SNVs independently contributing to 4 biomarkers of iron status: TSAT (12 SNVs, N=131 471, *F*-statistics [R^2]=8157.8 [6%]), serum iron (16 SNVs, N=163 511, *F*-statistics [R^2]=6412.2 [4%]), ferritin (42 SNVs, N=246 139, *F*-statistics [R^2]=5981.0 [2%]), and TIBC (16 SNVs, N=135 430, *F*-statistics [R^2]=12989.5 [9%]), based on recent GWAS[47] (Tables S4 and S5). For SNVs associated with iron status biomarkers, we retrieved GWAS summary statistics from outcome data sets obtained from publicly available genetic databases, for CAD using CARDIOGRAMplusC4D (N=181 522 cases),[48] HF using HERMES (N=115 150 cases),[49] IS using GIGASTROKE (N=62 100 cases),[50] and T2D using DIAMANTE (N=80 154 cases),[51] and for cardiovascular risk factors using other publicly genetic databases. Additional details of data sets included in the analysis are provided in Table S6.

Before implementing the conventional MR analyses, we estimated genetic correlation (R_g) for iron markers with hemoglobin, CMD, and cardiometabolic risk factors (Figures S2 and S3), to evaluate the shared genetic background among these phenotypes. R_g was estimated by using a range of publicly available GWAS summary statistics through the -rg option of linkage disequilibrium score regression[52–54] (https://github.com/bulik/ldsc), with the values > or <0 indicating positive or negative correlations, respectively.

The inverse-variance weighted method was used to estimate the associations of iron markers with hemoglobin and CMD, respectively, which were accessed by regression of the SNV-outcome (ie, hemoglobin, CAD, and HF) associations on the SNV-exposure (4 iron markers) associations. Sensitivity analyses investigated the possible relevance of directional (unbalanced horizontal) pleiotropic effects: (1) MR-Egger provides a statistical test for the presence of pleiotropic effects due to aggregation of invalid genetic instruments, assuming absence of dose–response confounding of SNVs through pleiotropic pathways[55]; (2) weighted median MR should provide a valid causal effect estimate if more than 50% of the information were derived from the genetic instrumental variables[56]; and (3) the model-based estimate is consistent when the largest subset of individual-instruments which identify the causal effect are valid instruments.[57]

For iron markers–hemoglobin association, bidirectional MR analyses were conducted to check whether genetically predicted differences in iron status biomarkers were associated with hemoglobin concentrations and vice versa. To identify the role of established risk factors that potentially mediate the associations of iron status biomarkers with CMD, we further explored whether genetic predisposition for higher iron status was associated with higher levels of cardiometabolic risk factors (Table S6), including anthropometric measurements (ie, body mass index, waist circumference, waist-to-hip ratio, birth weight, and height), glycemic traits (ie, fasting glucose, fasting insulin, and hemoglobin A1c), blood lipids (ie, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and total cholesterol), blood pressure traits (ie, systolic blood pressure, diastolic blood pressure, and pulse pressure), imaging phenotypes (ie, liver fat, liver iron measurement, and pancreas iron measurement), and other risk factors (ie, smoking status, educational attainment, and C-reactive protein). All statistical analyses were performed in R (version 3.6.3) using the "TwoSampleMR" package.[58]

RESULTS

The Central Illustration outlines the study design and key results of the 4 components of this study: (1) observational analyses of hemoglobin with CMD in UKB; (2) MR analyses of genetically predicted hemoglobin with disease outcomes in UKB; (3) MR analyses of genetically predicted iron with hemoglobin; and (4) MR analyses of genetically predicted iron with CAD, HF, IS, and T2D in worldwide GWAS consortia.

Among the 368 406 participants included in the observational analyses in UKB (Table 1), the mean (SD) age of participants was 56.6 (8.1) years, and 51.8% were women. About 10.8% were current smokers, and 91.9% were alcohol drinkers, respectively. The median (interquartile range) hemoglobin concentration was 15.0 (14.4, 15.7) mg/dL in men and 13.5 (12.9, 14.1) mg/dL in women. The characteristics of individuals in the genetic analyses (Table S1) were comparable with those in the observational analyses in UKB.

Observational and Genetic Associations of Hemoglobin With CMD

The observational analyses in Figure 1 demonstrated U-shaped associations of hemoglobin levels with CAD, wherein both lower and higher levels of hemoglobin were each associated with higher risks of CAD (reference level 14 mg/dL). However, there was no evidence of nonlinearity in the MR analyses (Cochran *Q* *P*=0.853, quadratic test *P*=0.703, Table S7). For the associations of hemoglobin with HF and IS, the associations were L-shaped (leveling off at about 14 mg/dL and increasing slightly after 16 mg/dL) and U-shaped in the observational analyses, but no significant association, in addition to nonlinear associations in the genetic analyses. For hemoglobin–T2D relationship, both conventional and genetic analyses demonstrated positive associations whereby higher levels of hemoglobin were

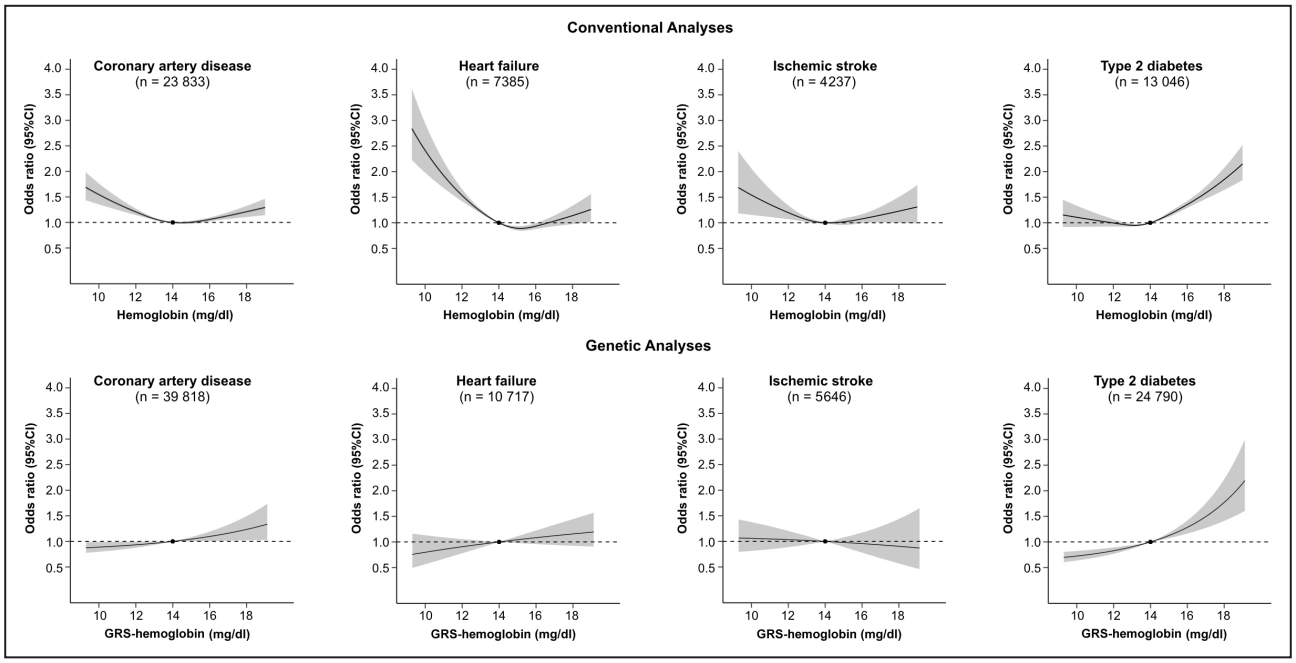

Figure 1. Associations of hemoglobin with coronary artery disease, heart failure, ischemic stroke, and type 2 diabetes in UK Biobank.

Associations of hemoglobin with coronary artery disease, heart failure, ischemic stroke, and type 2 diabetes were obtained from conventional analyses and nonlinear Mendelian randomization analyses in UK Biobank. GRS indicates genetic risk score.

associated with T2D at hemoglobin levels ≥14 mg/dL. No significant linear association was observed with hemoglobin levels <14 mg/dL in the observational analyses, but a modest protective effect was found in the genetic analyses (Cochran Q *P*=0.065, quadratic test *P*=0.177 in MR analysis, Table S7). Overall, there was no evidence of nonlinear associations of hemoglobin with either CAD, HF, IS, or T2D in the overall population, with concordant associations obtained from linear 2-sample MR analysis (Table S8).

The results from sex-stratified analyses were comparable with those obtained for the overall population (Figures S4 and S5; Tables S9 and S10), with the exception of the stronger associations of hemoglobin with CAD in men and the potential nonlinear association of hemoglobin-T2D in women in the MR analyses (Cochran Q *P*=0.030, quadratic test *P*=0.027).

Genetic Associations of Hemoglobin With Multiple Diseases

In PheWAS analyses (Figure 2), we investigated the associations of GRS-hemoglobin with 377 diseases in UKB after correction for multiple testing. Strong associations of genetically predicted higher hemoglobin levels were associated with iron deficiency anemia, other causes of anemia, and disorders of mineral metabolism, with ORs of 0.90 (95% CI, 0.89–0.92), 0.88 (95% CI, 0.86–0.89), and 1.23 (95% CI, 1.18–1.27), respectively. Moreover, 1 SD higher levels of hemoglobin were associated with 3% to 12% higher risks of acute myocardial infarction, hypertension, diabetes, disorders of porphyrin and bilirubin metabolism, fibrosis and cirrhosis of liver, duodenal ulcer, and psoriasis. Sex-specific PheWAS results are shown separately in Figures S6 and S7 for men and women, respectively (>500 cases), with 11 out of 319 diseases and 8 out of 376 diseases exceeding thresholds for multiple-testing correction.

MR Analyses of Iron Status Biomarkers With Hemoglobin

Table 2 shows that using all iron biomarkers, genetically predicted higher iron status was associated with higher levels of hemoglobin, with an increase of 0.37 (95% CI, 0.20–0.54) mg/dL for TSAT, 0.49 (95% CI, 0.30–0.67) mg/dL for serum iron, 0.51 (95% CI, 0.26–0.76) mg/dL for ferritin, and −0.16 (95% CI, −0.34 to 0.02) mg/dL for TIBC (reflecting lower systemic iron) obtained from an inverse-variance weighted MR method. Table S11 presents the results of additional bi-directional MR analyses for genetically predicted hemoglobin levels with each iron biomarker.

MR Analyses of Iron Status Biomarkers With CMD

Figure 3 shows the results of MR analysis for iron markers with CMD, which are reported as ORs per 1

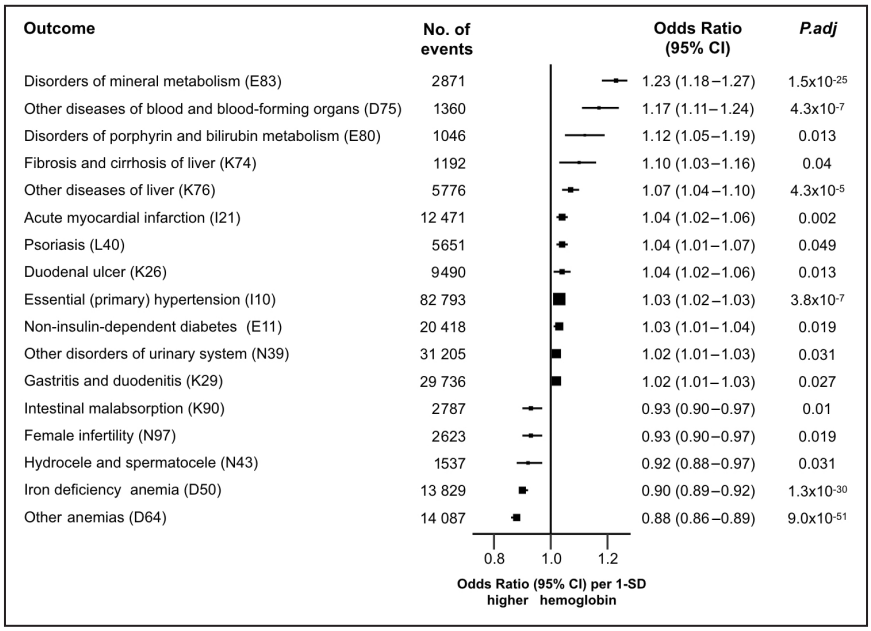

Figure 2. Associations of a genetic risk score for hemoglobin with different disease outcomes in UK Biobank.
Odds ratios obtained from PheWAS represent the estimates for the effect of 1-unit higher genetic risk scores for hemoglobin (GRS-hemoglobin), after adjusting for age, sex, center, genotyping array, and genetic principal components (the first 10 principal components). Analyses were restricted to outcomes with greater than 1000 cases (377 diseases) and showed the associations that survived from the multiple testing. *International Classification of Diseases, Tenth Revision* codes were also shown in the parentheses for each outcome. Error bars represent 95% CIs. All statistical tests were 2-sided, following a correction for multiple testing using a strategy controlling for the false discovery rate. GRS indicates genetic risk score; and PheWAS, phenome-wide associations.

SD higher level of each iron status biomarker. There were modest inverse associations of genetically predicted higher iron status biomarkers with CAD, with ORs of 0.93 (95% CI, 0.88–0.98) for TSAT, 0.91 (95% CI, 0.83–0.99) for serum iron, 0.86 (95% CI, 0.77–0.96) for (log-transformed) ferritin, and 1.04 (95% CI, 0.96–1.12) for TIBC (reflecting lower systemic iron). In contrast, we found adverse effects of higher levels of iron status with 7% higher risks of T2D per 1 SD higher level of TSAT but not for other iron status biomarkers. There was no evidence of associations of iron status markers with HF or IS or with other CVD outcomes (Table S12). Sensitivity analyses using different analytical methods or different sets of SNVs demonstrated consistent results with the overall analyses (Tables S13 through S15).

For the associations of iron markers with cardiometabolic risk factors, Tables S16 through S18 show that a 1 SD higher level of iron status biomarkers was associated with higher levels of waist circumference, height, triglycerides, diastolic blood pressure, estimated glomerular filtration rate, and C-reactive protein and lower levels of hemoglobin A1c, LDL, and total cholesterol, respectively.

Sex-Specific Associations of Hemoglobin With CMD

Figure 4 shows that higher levels of hemoglobin were associated with lower risks of CAD in women in the observational analyses, but genetic analyses from 1-sample MR analyses did not provide support for the causal relevance of such associations. In addition, a 1 SD higher genetically predicted hemoglobin was associated with 8% higher risks of CAD in men (OR, 1.08 [95% CI, 1.04–1.13]). There was evidence of heterogeneity by sex for hemoglobin-CAD associations in the genetic analyses (*P* for heterogeneity 0.003). For the hemoglobin-HF relationship, strong inverse associations were observed in both men and women in the observational analyses but not in the genetic analyses. The findings from both observational and genetic analyses were consistent with hemoglobin associations with T2D, showing 10% to 12% and 10% to 13% higher

Table 2. Causal Effects of a 1 SD Higher Plasma Level of Iron Biomarkers on Hemoglobin Concentrations

	Transferrin saturation (n=131 471)			Serum iron (n=163 511)			Serum ferritin (n=246 139)			Total iron binding capacity (n=135 430)		
MR method	SNP	Beta (95% CI)	*P*	SNP	Beta (95% CI)	*P*	SNP	Beta (95% CI)	*P*	SNP	Beta (95% CI)	*P*
Inverse variance weighted	10	0.37 (0.20 to 0.54)	1.5×10^{-5}	14	0.49 (0.30 to 0.67)	2.1×10^{-7}	37	0.51 (0.26 to 0.76)	7.9×10^{-5}	15	−0.16 (−0.34 to 0.02)	0.077
Weighted median	10	0.41 (0.36 to 0.45)	1.2×10^{-73}	14	0.60 (0.56 to 0.64)	2.7×10^{-200}	37	0.16 (0.09 to 0.24)	9.0×10^{-6}	15	−0.08 (−0.14 to −0.02)	0.007
Weighted mode	10	0.44 (0.41 to 0.47)	3.0×10^{-10}	14	0.59 (0.56 to 0.63)	8.4×10^{-14}	37	0.13 (0.06 to 0.20)	8.3×10^{-4}	15	−0.07 (−0.10 to −0.03)	2.6×10^{-3}
MR Egger	10	0.48 (0.25 to 0.71)	3.5×10^{-3}	14	0.66 (0.41 to 0.90)	2.0×10^{-4}	37	0.72 (0.26 to 1.18)	4.4×10^{-3}	15	−0.16 (−0.40 to 0.08)	0.214
Egger intercept		−0.02 (−0.05 to 0.01)	0.223		−0.02 (−0.04 to 0.00)	0.088		−0.01 (−0.03 to 0.01)	0.294		−0.00 (−0.04 to 0.03)	0.948

Estimates represent the effect of a 1-SD increase in levels of transferrin saturation, serum iron, log-transformed serum ferritin, and total iron binding capacity on hemoglobin. MR was implemented through the "TwoSampleMR" package in R-version 3.6.3. The *F*-statistics (R2) of iron markers were 8157.8 (0.06) for transferrin saturation (12 SNVs), 6412.2 (0.04) for serum iron (16 SNVs), 5981.0 (0.02) for serum ferritin (42 SNVs), and 12989.5 (0.09) for total iron binding capacity (16 SNVs), respectively. MR indicates Mendelian randomization; and SNV single-nucleotide variant.

risks per 1 SD higher hemoglobin, respectively, in both men and women. There were no significant associations for hemoglobin with IS in the observational analyses. However, we found a potential protective effect of hemoglobin on IS in women only (OR, 0.86 [95% CI, 0.74–1.01]), with *P* for heterogeneity by sex of 0.039 in the genetic analyses.

DISCUSSION

In this large study of UKB and worldwide GWAS consortia, we observed linear positive associations of higher genetically predicted hemoglobin with risk for CAD and T2D but found no evidence of any associations with HF or IS. However, MR analyses of iron status biomarkers demonstrated that 1 SD higher genetically instrumented levels of iron status were associated with 10% lower risks of CAD but higher risks of T2D.

This is the first study to assess both the shape and strength of associations of hemoglobin with CMD, in addition to assessing possible heterogeneity by sex using both observational and genetic analyses. Previous observational studies of hemoglobin concentrations with disease outcomes in diverse populations have reported conflicting associations with CVD,[4–8,10,11] dementia,[12] and all-cause mortality.[5,9] The Korean Heart Study involving 407 858 participants,[8] reported that men with lower or higher hemoglobin levels had higher risks of total CVD, coronary heart disease, and stroke, and higher risk of total CVD related to higher hemoglobin only in women. The REGARDS (Reasons for Geographical and Racial Differences in Stroke) study of 16 332 participants[4] reported higher risks of coronary heart disease in both the lowest and highest quintiles of hemoglobin without any differential effects by sex, whereas in a separate report by Panwar et al,[6] there was a U-shaped association of hemoglobin with incident stroke in women only. Among the few studies that explored possible causality, Wang et al[29] reported that higher genetically determined hemoglobin levels were associated with higher risks of CAD, but they did not assess effects on other diseases. In the present study, higher genetically determined hemoglobin levels were associated with higher risks of CAD and T2D but not with HF and IS. Moreover, the present study reported heterogeneity in hemoglobin-CAD associations, by sex ($P_{interaction}$ <0.05, male only). The genetic associations of hemoglobin with CVD outcomes were more likely to be linear than the observational analyses, perhaps reflecting incomplete control of confounding or reverse causality due to smoking, kidney function, chronic obstructive pulmonary disease, and other clinical or subclinical diseases in the observational analyses.[59–64] Consistent with previous studies, the present study suggested additional potential targets for higher genetically predicted hemoglobin, including hypertension[59–62] and liver disease,[63,64] but these associations will require further investigation.

In addition to hemoglobin concentrations, the present study also evaluated the causal relevance and strength of associations of iron status biomarkers with CAD, stroke, T2D, and HF. A meta-analysis of 17 studies[19] conducted before 2014, involving 9236 cases of coronary heart disease and 156 427 participants, demonstrated an inverse association of TSAT and coronary heart disease, and no such associations were found for serum iron, ferritin, and TIBC. Moreover, previous MR studies reported that higher genetically predicted iron status, instrumented by 3 loci obtained from the Genetics of Iron Status consortium involving 48 972 participants,[65] were associated with lower risk for CAD[66,67] and higher

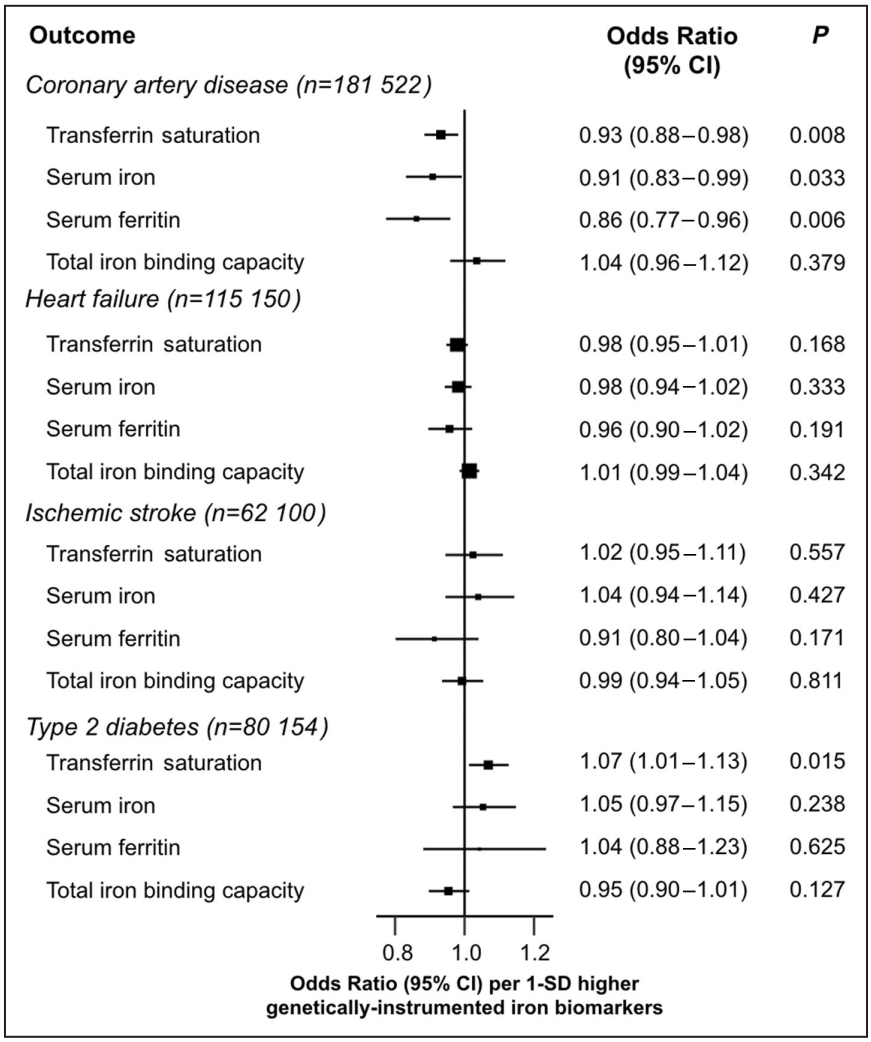

Figure 3. Associations of genetically determined iron markers with coronary artery disease, heart failure, ischemic stroke, and type 2 diabetes.
Odds ratios represent the estimates for the effect of a 1-SD increase in levels of transferrin saturation, serum iron, log-transformed serum ferritin, and total iron binding capacity, respectively, on disease outcomes.

risk for stroke[68] and T2D.[69] Using the latest summary results of genetic studies that yielded 62 independent variants associated with iron status biomarkers at 56 loci with sample sizes ranging from 131 471 to 246 139 participants,[47] the present study demonstrated modest protective effects of higher iron status for CAD, possible hazards for T2D, but no evidence of any associations with HF or IS.

Strengths and Limitations

This MR study assessed a broad panel of laboratory biomarkers of iron status (because no single biomarker provides all relevant information[27,28]) to derive optimum instrumental variables for MR analyses. Bidirectional MR analyses provided support for the causal relevance of higher levels of hemoglobin with iron status biomarkers (TSAT, serum iron, ferritin, and TIBC). The study demonstrated the protective effects of higher iron biomarkers for CAD but adverse effects of higher hemoglobin on CAD. The reasons for these discrepant results are not fully understood but may reflect the effects of iron status markers on established CVD risk factors or direct effects of iron independent of hemoglobin. The analyses used linear and nonlinear MR methods[30] to assess the shape and strength of the causal associations of hemoglobin with CAD and T2D in addition to PheWAS analyses[43] to identify other possible targets of altered hemoglobin levels. The use of 2-sample MR analyses involving worldwide genetic consortia enhanced the statistical power of the present study when compared with previous MR studies.[70–78] However, the present study also had several

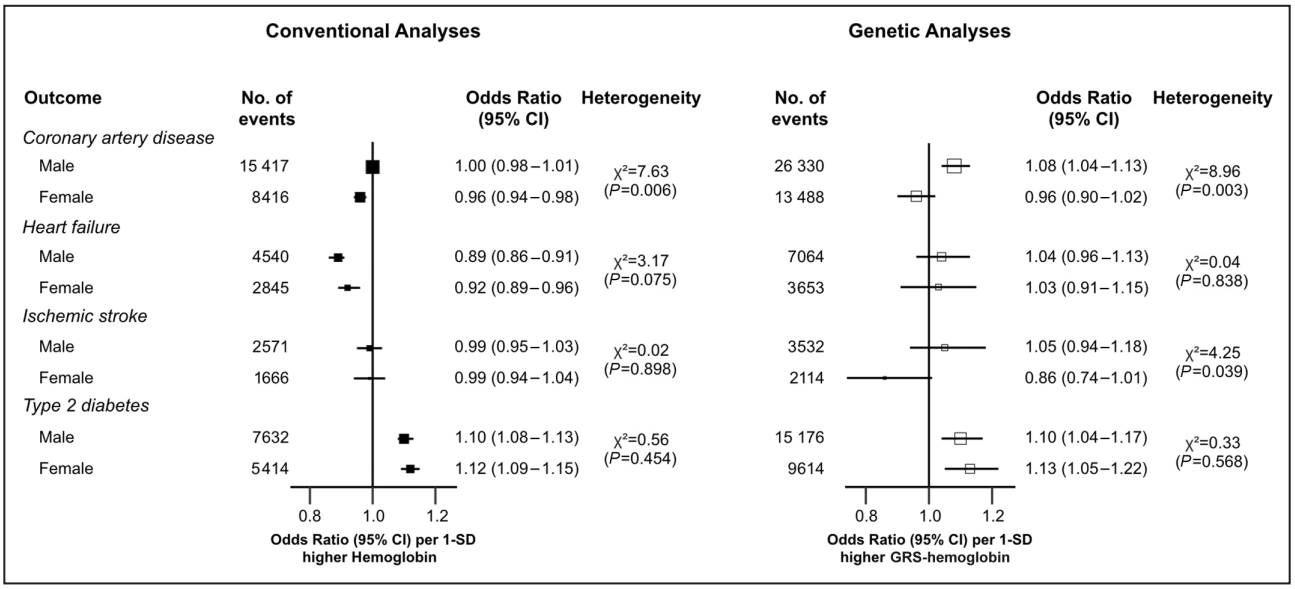

Figure 4. Sex-specific associations of a 1 SD higher level of hemoglobin on risk of cardiometabolic diseases in UK Biobank. We used multivariable logistic regression in observational analysis to estimate the risk of incident coronary artery disease, ischemic stroke, heart failure, and type 2 diabetes among individuals by 1-SD increase in levels of hemoglobin after adjusting sociodemographic factors (age, sex, ethnicity, and education), socioeconomic status (Townsend deprivation index), family history of cardiovascular diseases or diabetes, smoking status, alcohol intake, physical activity, body mass index, baseline diabetes, medication use (antihypertensive or cholesterol-lowering drugs), systolic blood pressure, and triglycerides where appropriate. Odds ratios from Mendelian randomization represent the estimates for the effect of a 1-unit higher genetic risk score for hemoglobin (GRS-hemoglobin). GRS indicates genetic risk score.

limitations. The use of iron supplements was evaluated by self-reported questionnaires, which may have resulted in some misclassification of iron use and possible bias in the results. Randomized trials are required to assess the effects of iron supplements on CVD in high-risk individuals (with stratification for use of multivitamins with or without added minerals). The detailed phenotyping data available in UKB enabled the assessment of associations of genetically predicted hemoglobin with multiple diseases in PheWAS analyses, but this could have introduced some misclassification bias due to the differential case ascertainment derived from multiple sources. However, access to the large sample size in UKB, limiting PheWAS to a minimum of 200 cases,[45] and correction for multiple testing should have minimized the effects of chance findings. MR analyses could also be biased if there was pleiotropy where variants influenced disease outcomes through pathways other than those mediated by iron biomarkers.[79] However, results of sensitivity analyses using other statistical methods and different genetic instruments yielded concordant results with the main analyses. Hence, it is unlikely that pleiotropy could have influenced our results. MR studies using genetic instruments approximate the average effects over the life course, so the strength and shape of the associations may not be comparable with the short-term effects of iron supplements on disease outcomes.[79] To minimize the risk of possible bias due to population stratification, the present genetic analyses were restricted to participants of White-British descent. Although this limits the application of the findings for other ancestry populations, it does not affect the internal validity of the present study findings.

CONCLUSIONS

This MR study demonstrated modest protective effects of higher levels of iron status biomarkers for CAD, possible hazards for T2D, but no evidence for any associations with HF or IS. The discrepant results of protective effects of higher iron status biomarkers for CAD, but adverse effects of higher hemoglobin, are unexplained, raising questions about the likely effects of iron supplements for the prevention of CAD. Large-scale randomized trials are required to assess the efficacy and safety of iron supplements for the prevention of CAD in high-risk older adults, but trials of treatments with support from MR studies are much more likely to have a successful outcome.

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Received July 10, 2023; accepted February 28, 2024.

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Sources of Funding

R.C. and D.A.B. were supported by the British Heart Foundation and the Medical Research Council (United Kingdom), and W.G. was supported by Novo Nordisk.

Disclosures

None.

Supplemental Material

Tables S1–S8
Figures S1–S7

REFERENCES

1. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. *Lancet*. 2021;397:233–248. doi: 10.1016/S0140-6736(20)32594-0
2. Bulaj ZJ, Ajioka RS, Phillips JD, LaSalle BA, Jorde LB, Griffen LM, Edwards CQ, Kushner JP. Disease-related conditions in relatives of patients with hemochromatosis. *N Engl J Med*. 2000;343:1529–1535. doi: 10.1056/NEJM200011233432104
3. Fleming RE, Ponka P. Iron overload in human disease. *N Engl J Med*. 2012;366:348–359. doi: 10.1056/NEJMra1004967
4. Houghton DE, Koh I, Ellis A, Key NS, Douce DR, Howard G, Cushman M, Safford M, Zakai NA. Hemoglobin levels and coronary heart disease risk by age, race, and sex in the reasons for geographic and racial differences in stroke study (REGARDS). *Am J Hematol*. 2020;95:258–266. doi: 10.1002/ajh.25703
5. Lee G, Choi S, Kim K, Yun JM, Son JS, Jeong SM, Kim SM, Park SM. Association of hemoglobin concentration and its change with cardiovascular and all-cause mortality. *J Am Heart Assoc*. 2018;7:007723. doi: 10.1161/JAHA.117.007723
6. Panwar B, Judd SE, Warnock DG, McClellan WM, Booth JN III, Muntner P, Gutierrez OM. Hemoglobin concentration and risk of incident stroke in community-living adults. *Stroke*. 2016;47:2017–2024. doi: 10.1161/STROKEAHA.116.013077
7. Gotoh S, Hata J, Ninomiya T, Hirakawa Y, Nagata M, Mukai N, Fukuhara M, Ikeda F, Ago T, Kitazono T, et al. Hematocrit and the risk of cardiovascular disease in a Japanese community: the Hisayama study. *Atherosclerosis*. 2015;242:199–204. doi: 10.1016/j.atherosclerosis.2015.07.014
8. Kim MY, Jee SH, Yun JE, Baek SJ, Lee DC. Hemoglobin concentration and risk of cardiovascular disease in Korean men and women - the Korean heart study. *J Korean Med Sci*. 2013;28:1316–1322. doi: 10.3346/jkms.2013.28.9.1316
9. Zakai NA, Katz R, Hirsch C, Shlipak MG, Chaves PH, Newman AB, Cushman M. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the cardiovascular health study. *Arch Intern Med*. 2005;165:2214–2220. doi: 10.1001/archinte.165.19.2214
10. Sarnak MJ, Tighiouart H, Manjunath G, MacLeod B, Griffith J, Salem D, Levey AS. Anemia as a risk factor for cardiovascular disease in the atherosclerosis risk in communities (ARIC) study. *J Am Coll Cardiol*. 2002;40:27–33. doi: 10.1016/S0735-1097(02)01938-1
11. Gagnon DR, Zhang TJ, Brand FN, Kannel WB. Hematocrit and the risk of cardiovascular disease the Framingham study: a 34-year follow-up. *Am Heart J*. 1994;127:674–682. doi: 10.1016/0002-8703(94)90679-3
12. Wolters FJ, Zonneveld HI, Licher S, Cremers LGM; Heart Brain Connection Collaborative Research G, Ikram MK, Koudstaal PJ, Vernooij MW, Ikram MA. Hemoglobin and anemia in relation to dementia risk and accompanying changes on brain MRI. *Neurology*. 2019;93:e917–e926. doi: 10.1212/WNL.0000000000008003
13. Liu J, Li Q, Yang Y, Ma L. Iron metabolism and type 2 diabetes mellitus: a meta-analysis and systematic review. *J Diabetes Investig*. 2020;11:946–955. doi: 10.1111/jdi.13216
14. Diaz-Lopez A, Iglesias-Vazquez L, Palleja-Millan M, Rey Renones C, Flores Mateo G, Arija V. Association between iron status and incident type 2 diabetes: a population-based cohort study. *Nutrients*. 2020;12:3249. doi: 10.3390/nu12113249
15. Jiang L, Wang K, Lo K, Zhong Y, Yang A, Fang X, Akezhuoli H, Song Z, Chen L, An P, et al. Sex-specific association of circulating ferritin level and risk of type 2 diabetes: a dose-response meta-analysis of prospective studies. *J Clin Endocrinol Metab*. 2019;104:4539–4551. doi: 10.1210/jc.2019-00495
16. Silvestre OM, Goncalves A, Nadruz W Jr, Claggett B, Couper D, Eckfeldt JH, Pankow JS, Anker SD, Solomon SD. Ferritin levels and risk of heart failure-the atherosclerosis risk in communities study. *Eur J Heart Fail*. 2017;19:340–347. doi: 10.1002/ejhf.701
17. Klip IT, Voors AA, Swinkels DW, Bakker SJ, Kootstra-Ros JE, Lam CS, van der Harst P, van Veldhuisen DJ, van der Meer P. Serum ferritin and risk for new-onset heart failure and cardiovascular events in the community. *Eur J Heart Fail*. 2017;19:348–356. doi: 10.1002/ejhf.622
18. Podmore C, Meidtner K, Schulze MB, Scott RA, Ramond A, Butterworth AS, Di Angelantonio E, Danesh J, Arriola L, Barricarte A, et al. Association of multiple biomarkers of iron metabolism and type 2 diabetes: the EPIC-InterAct study. *Diabetes Care*. 2016;39:572–581. doi: 10.2337/dc15-0257
19. Das De S, Krishna S, Jethwa A. Iron status and its association with coronary heart disease: systematic review and meta-analysis of prospective studies. *Atherosclerosis*. 2015;238:296–303. doi: 10.1016/j.atherosclerosis.2014.12.018
20. Savarese G, von Haehling S, Butler J, Cleland JGF, Ponikowski P, Anker SD. Iron deficiency and cardiovascular disease. *Eur Heart J*. 2023;44:14–27. doi: 10.1093/eurheartj/ehac569
21. Kalra PR, Cleland JGF, Petrie MC, Thomson EA, Kalra PA, Squire IB, Ahmed FZ, Al-Mohammad A, Cowburn PJ, Foley PWX, et al. Intravenous ferric derisomaltose in patients with heart failure and iron deficiency in the UK (IRONMAN): an investigator-initiated, prospective, randomised, open-label, blinded-endpoint trial. *Lancet*. 2022;400:2199–2209. doi: 10.1016/S0140-6736(22)02083-9
22. Docherty KF, Welsh P, Verma S, De Boer RA, O'Meara E, Bengtsson O, Kober L, Kosiborod MN, Hammarstedt A, Langkilde AM, et al. Investigators D-H and committees. Iron deficiency in heart failure and effect of dapagliflozin: findings from DAPA-HF. *Circulation*. 2022;146:980–994. doi: 10.1161/CIRCULATIONAHA.122.060511
23. Butler J, Khan MS, Friede T, Jankowska EA, Fabien V, Goehring UM, Dorigotti F, Metra M, Pina IL, Coats AJS, et al. Health status improvement with ferric carboxymaltose in heart failure with reduced ejection fraction and iron deficiency. *Eur J Heart Fail*. 2022;24:821–832. doi: 10.1002/ejhf.2478
24. Anker SD, Ponikowski P, Khan MS, Friede T, Jankowska EA, Fabien V, Goehring UM, Metra M, Pina IL, Coats AJS, et al. Responder analysis for improvement in 6-min walk test with ferric carboxymaltose in patients with heart failure with reduced ejection fraction and iron deficiency. *Eur J Heart Fail*. 2022;24:833–842. doi: 10.1002/ejhf.2491
25. Camaschella C. Iron-deficiency anemia. *N Engl J Med*. 2015;372:1832–1843. doi: 10.1056/NEJMra1401038
26. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. *Lancet*. 2016;387:907–916. doi: 10.1016/S0140-6736(15)60865-0
27. Restrepo-Gallego M, Diaz LE, Rondo PHC. Classic and emergent indicators for the assessment of human iron status. *Crit Rev Food Sci Nutr*. 2021;61:2827–2840. doi: 10.1080/10408398.2020.1787326
28. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol*. 2006;1(suppl 1):S4–S8. doi: 10.2215/CJN.01490506
29. Wang K, Shi X, Zhu Z, Hao X, Chen L, Cheng S, Foo RSY, Wang C. Mendelian randomization analysis of 37 clinical factors and coronary artery disease in east Asian and European populations. *Genome Med*. 2022;14:63. doi: 10.1186/s13073-022-01067-1
30. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol*. 2017;41:341–352. doi: 10.1002/gepi.22041
31. Burgess S, Butterworth A, Malarstig A, Thompson SG. Use of Mendelian randomisation to assess potential benefit of clinical intervention. *BMJ*. 2012;345:e7325. doi: 10.1136/bmj.e7325
32. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12:e1001779. doi: 10.1371/journal.pmed.1001779

33. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. The UK biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203–209. doi: 10.1038/s41586-018-0579-z
34. Littlejohns TJ, Sudlow C, Allen NE, Collins R. UK biobank: opportunities for cardiovascular research. *Eur Heart J*. 2019;40:1158–1166. doi: 10.1093/eurheartj/ehx254
35. Oskarsson GR, Oddsson A, Magnusson MK, Kristjansson RP, Halldorsson GH, Ferkingstad E, Zink F, Helgadottir A, Ivarsdottir EV, Arnadottir GA, et al. Predicted loss and gain of function mutations in ACO1 are associated with erythropoiesis. *Commun Biol*. 2020;3:189. doi: 10.1038/s42003-020-0921-5
36. Takeuchi H, Kawashima R. A prospective study on the relationship between iron supplement intake, hemoglobin concentration, and risk of parkinsonism. *Nutrients*. 2022;14:4671–4684. doi: 10.3390/nu14214671
37. Tyrrell J, Jones SE, Beaumont R, Astley CM, Lovell R, Yaghootkar H, Tuke M, Ruth KS, Freathy RM, Hirschhorn JN, et al. Height, body mass index, and socioeconomic status: mendelian randomisation study in UK biobank. *BMJ*. 2016;352:i582. doi: 10.1136/bmj.i582
38. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med*. 2010;29:1037–1057. doi: 10.1002/sim.3841
39. Gan W, Bragg F, Walters RG, Millwood IY, Lin K, Chen Y, Guo Y, Vaucher J, Bian Z, Bennett D, et al. Genetic predisposition to type 2 diabetes and risk of subclinical atherosclerosis and cardiovascular diseases among 160,000 Chinese adults. *Diabetes*. 2019;68:2155–2164. doi: 10.2337/db19-0224
40. Vuckovic D, Bao EL, Akbari P, Lareau CA, Mousas A, Jiang T, Chen MH, Raffield LM, Tardaguila M, Huffman JE, et al. The polygenic and monogenic basis of blood traits and diseases. *Cell*. 2020;182(1214–1231):e11.
41. Astle WJ, Elding H, Jiang T, Allen D, Ruklisa D, Mann AL, Mead D, Bouman H, Riveros-Mckay F, Kostadima MA, et al. The allelic landscape of human blood cell trait variation and links to common complex disease. *Cell*. 2016;167:1415–1429.e19. doi: 10.1016/j.cell.2016.10.042
42. Burgess S, Davies NM, Thompson SG and Consortium EP-I. Instrumental variable analysis with a nonlinear exposure-outcome relationship. *Epidemiology*. 2014;25:877–885. doi: 10.1097/EDE.0000000000000161
43. Denny JC, Ritchie MD, Basford MA, Pulley JM, Bastarache L, Brown-Gentry K, Wang D, Masys DR, Roden DM, Crawford DC. PheWAS: demonstrating the feasibility of a phenome-wide scan to discover gene-disease associations. *Bioinformatics*. 2010;26:1205–1210. doi: 10.1093/bioinformatics/btq126
44. Millard LAC, Davies NM, Gaunt TR, Davey Smith G, Tilling K. Software application profile: PHESANT: a tool for performing automated phenome scans in UK biobank. *Int J Epidemiol*. 2018;47:29–35. doi: 10.1093/ije/dyx204
45. Verma A, Bradford Y, Dudek S, Lucas AM, Verma SS, Pendergrass SA, Ritchie MD. A simulation study investigating power estimates in phenome-wide association studies. *BMC Bioinformatics*. 2018;19:120. doi: 10.1186/s12859-018-2135-0
46. Benjamini Y, Hochberg Y. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *J R Stat Soc B*. 1995;57:289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x
47. Bell S, Rigas AS, Magnusson MK, Ferkingstad E, Allara E, Bjornsdottir G, Ramond A, Sorensen E, Halldorsson GH, Paul DS, et al. A genome-wide meta-analysis yields 46 new loci associating with biomarkers of iron homeostasis. *Commun Biol*. 2021;4:156. doi: 10.1038/s42003-020-01575-z
48. Nelson CP, Goel A, Butterworth AS, Kanoni S, Webb TR, Marouli E, Zeng L, Ntalla I, Lai FY, Hopewell JC, et al. Association analyses based on false discovery rate implicate new loci for coronary artery disease. *Nat Genet*. 2017;49:1385–1391. doi: 10.1038/ng.3913
49. Shah S, Henry A, Roselli C, Lin H, Sveinbjornsson G, Fatemifar G, Hedman AK, Wilk JB, Morley MP, Chaffin MD, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun*. 2020;11:163. doi: 10.1038/s41467-019-13690-5
50. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, Rutten-Jacobs L, Giese AK, van der Laan SW, Gretarsdottir S, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524–537. doi: 10.1038/s41588-018-0058-3
51. Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, Payne AJ, Steinthorsdottir V, Scott RA, Grarup N, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat Genet*. 2018;50:1505–1513. doi: 10.1038/s41588-018-0241-6
52. Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh PR, ReproGen C, Psychiatric Genomics C; Genetic Consortium for Anorexia Nervosa of the Wellcome Trust Case Control C, Duncan L, et al. An atlas of genetic correlations across human diseases and traits. *Nat Genet*. 2015;47:1236–1241. doi: 10.1038/ng.3406
53. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J; Schizophrenia Working Group of the Psychiatric Genomics C, Patterson N, Daly MJ, Price AL, Neale BM. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. 2015;47:291–295. doi: 10.1038/ng.3211
54. Finucane HK, Bulik-Sullivan B, Gusev A, Trynka G, Reshef Y, Loh PR, Anttila V, Xu H, Zang C, Farh K, et al. Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat Genet*. 2015;47:1228–1235. doi: 10.1038/ng.3404
55. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression. *Int J Epidemiol*. 2015;44:512–525. doi: 10.1093/ije/dyv080
56. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304–314. doi: 10.1002/gepi.21965
57. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol*. 2017;46:1985–1998. doi: 10.1093/ije/dyx102
58. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, et al. The MR-base platform supports systematic causal inference across the human phenome. *Elife*. 2018;7:e34408. doi: 10.7554/eLife.34408
59. Timoteo VJ, Chiang KM, Pan WH. Positive or U-shaped association of elevated hemoglobin concentration levels with metabolic syndrome and metabolic components: findings from Taiwan biobank and UK biobank. *Nutrients*. 2022;14:4007. doi: 10.3390/nu14194007
60. Tapio J, Vahanikkila H, Kesaniemi YA, Ukkola O, Koivunen P. Higher hemoglobin levels are an independent risk factor for adverse metabolism and higher mortality in a 20-year follow-up. *Sci Rep*. 2021;11:19936. doi: 10.1038/s41598-021-99217-9
61. Lim WH, Choi EK, Han KD, Lee SR, Cha MJ, Oh S. Impact of hemoglobin levels and their dynamic changes on the risk of atrial fibrillation: a nationwide population-based study. *Sci Rep*. 2020;10:6762. doi: 10.1038/s41598-020-63878-9
62. Zhou J, Zhao R, Wang D, Gao Q, Zhao D, Ouyang B, Hao L, Peng X. Sex-specific association between iron status and the predicted 10-year risk for atherosclerotic cardiovascular disease in hypertensive patients. *Biol Trace Elem Res*. 2022;200:4594–4607. doi: 10.1007/s12011-021-03060-y
63. Scheiner B, Semmler G, Maurer F, Schwabl P, Bucsics TA, Paternostro R, Bauer D, Simbrunner B, Trauner M, Mandorfer M, et al. Prevalence of and risk factors for anaemia in patients with advanced chronic liver disease. *Liver Int*. 2020;40:194–204. doi: 10.1111/liv.14229
64. Singh S, Manrai M, Parvathi VS, Kumar D, Srivastava S, Pathak B. Association of liver cirrhosis severity with anemia: does it matter? *Ann Gastroenterol*. 2020;33:272–276. doi: 10.20524/aog.2020.0478
65. Benyamin B, Esko T, Ried JS, Radhakrishnan A, Vermeulen SH, Traglia M, Gogele M, Anderson D, Broer L, Podmore C, et al. Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. *Nat Commun*. 2014;5:4926. doi: 10.1038/ncomms5926
66. Grote Beverborg N, Said MA, van der Wal HH, Verweij N, van der Meer P, van der Harst P. Genetically determined high levels of iron parameters are protective for coronary artery disease. *Circ Genom Precis Med*. 2020;13:e002544. doi: 10.1161/CIRCGEN.119.002544
67. Gill D, Del Greco MF, Walker AP, Srai SKS, Laffan MA, Minelli C. The effect of iron status on risk of coronary artery disease: a Mendelian randomization study-brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:1788–1792. doi: 10.1161/ATVBAHA.117.309757
68. Gill D, Monori G, Tzoulaki I, Dehghan A. Iron status and risk of stroke. *Stroke*. 2018;49:2815–2821. doi: 10.1161/STROKEAHA.118.022701
69. Wang X, Fang X, Zheng W, Zhou J, Song Z, Xu M, Min J, Wang F. Genetic support of a causal relationship between iron status and type 2 diabetes: a Mendelian randomization study. *J Clin Endocrinol Metab*. 2021;106:e4641–e4651. doi: 10.1210/clinem/dgab454

70. Grammer TB, Kleber ME, Silbernagel G, Pilz S, Scharnagl H, Tomaschitz A, Konig W, Marz W. Hemoglobin, iron metabolism and angiographic coronary artery disease (the Ludwigshafen Risk and Cardiovascular Health study). *Atherosclerosis*. 2014;236:292–300. doi: 10.1016/j.atherosclerosis.2014.07.002
71. Grammer TB, Scharnagl H, Dressel A, Kleber ME, Silbernagel G, Pilz S, Tomaschitz A, Koenig W, Mueller-Myhsok B, Marz W, et al. Iron metabolism, hepcidin, and mortality (the Ludwigshafen risk and cardiovascular health study). *Clin Chem*. 2019;65:849–861. doi: 10.1373/clinchem.2018.297242
72. Liu D, Zhang Y, Wang CC, Xiao-Hong E, Zuo H. Markers of iron metabolism and stroke risk: cross-sectional and longitudinal findings from the China Health and Nutrition Survey (CHNS). *Iran J Public Health*. 2022;51:115–123. doi: 10.18502/ijph.v51i1.8302
73. Del Greco MF, Foco L, Pichler I, Eller P, Eller K, Benyamin B, Whitfield JB; Genetics of Iron Status C, Consortium CK, Pramstaller PP, Thompson JR. Serum iron level and kidney function: a Mendelian randomization study. *Nephrol Dial Transplant*. 2017;32:273–278.
74. Gill D, Benyamin B, Moore LSP, Monori G, Zhou A, Koskeridis F, Evangelou E, Laffan M, Walker AP, Tsilidis KK, et al. Associations of genetically determined iron status across the phenome: a mendelian randomization study. *PLoS Med*. 2019;16:e1002833. doi: 10.1371/journal.pmed.1002833
75. Gill D, Brewer CF, Monori G, Tregouet DA, Franceschini N, Giambartolomei C, Consortium I, Tzoulaki I, Dehghan A. Effects of genetically determined iron status on risk of venous thromboembolism and carotid atherosclerotic disease: a Mendelian randomization study. *J Am Heart Assoc*. 2019;8:e012994. doi: 10.1161/JAHA.119.012994
76. Yang F, Bao Q, Wang Z, Ma M, Shen J, Ye F, Xie X. Sex-specific genetically predicted iron status in relation to 12 vascular diseases: a Mendelian randomization study in the UK biobank. *Biomed Res Int*. 2020;2020:6246041. doi: 10.1155/2020/6246041
77. Zhou J, Liu C, Francis M, Sun Y, Ryu MS, Grider A, Ye K. The causal effects of blood iron and copper on lipid metabolism diseases: evidence from phenome-wide Mendelian randomization study. *Nutrients*. 2020;12:3174. doi: 10.3390/nu12103174
78. Wang T, Cheng J, Wang Y. Genetic support of a causal relationship between iron status and atrial fibrillation: a Mendelian randomization study. *Genes Nutr*. 2022;17:8. doi: 10.1186/s12263-022-00708-9
79. Davies NM, Holmes MV, Davey SG. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi: 10.1136/bmj.k601