Association between iron levels and the risk of type 2 diabetes mellitus: NHANES 2009−2018 and Mendelian randomization study

Keywords
risk factor, type 2 diabetes mellitus, NHANES, Mendelian randomization, iron level

Abstract

Introduction
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Material and methods
In total, 19,483 participants from the National Health and Nutrition Examination Survey (NHANES) 2009–2018 were included in this study. The weighted t-test and chi-squared test were used to compare differences in iron levels and covariates between individuals with and without T2DM. Subsequently, weighted multivariate logistic regression models were constructed to assess the association between iron levels and T2DM risk. Furthermore, Mendelian randomization (MR) analysis was conducted to evaluate the causal effect of iron levels on T2DM. Sensitivity analysis and Steiger's test were employed to validate the dependability and effectiveness of the MR findings.

Results
Weighted multivariate logistic regression analyses revealed a statistically significant inverse association between iron levels and T2DM risk, with odds ratios of 0.955, 0.948, and 0.983 in models 1, 2, and 3, respectively. Receiver operating characteristic curve analysis implied that iron levels accurately identified patients with T2DM. MR analysis demonstrated a marked causal impact of iron levels on T2DM risk, and iron levels represented a protective factor to some extent. Sensitivity analysis confirmed the robustness of the MR findings, and Steiger's test confirmed the plausibility and validity of the causal effect direction of iron levels on T2DM.

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This study supported a negative correlation between iron levels and T2DM risk, which holds potential clinical significance for T2DM prevention and early risk assessment.

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Introduction

Diabetes mellitus (DM) is a serious global public health issue. Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all cases of DM. Individuals with DM can experience life-threatening complications, leading to diminished quality of life, increased mortality, and substantial medical expenses [1, 2]. Consequently, it is imperative to manage the occurrence and progression of DM. In addition to lifestyle modifications, hypoglycemic drugs have demonstrated efficacy in preventing or delaying the onset and progression of T2DM. However, because of the individual heterogeneity among patients with T2DM, effective blood glucose control or delay in the onset of DM-related complications are only achieved in select patients. Consequently, exploring factors associated with T2DM onset and progression is crucial for its treatment and prevention.

Iron plays a crucial role in various metabolic and physiological processes. Iron levels in the body have been linked to several diseases, including cerebral stroke, cardiovascular disease, and thalassemia [3-5]. Furthermore, iron level imbalance is a significant factor contributing to the development of T2DM. However, evidence regarding the relationship between iron levels and the risk of T2DM remains inconsistent. Harrison et al. [6] identified elevated iron levels as a risk factor for T2DM, correlating this finding with decreased insulin secretion, insulin resistance, and increased hepatic gluconeogenesis. Conversely, Zhao et al. [7] reported that serum iron levels were lower in patients with T2DM and macroalbuminuria than in healthy controls. A study from the China Adult Chronic Disease and Nutrition Surveillance indicated that Chinese women of childbearing age with T2DM exhibited higher iron levels than their counterparts without T2DM; however, no significant association was found

between iron levels and the risk of developing T2DM among women within this demographic group [8]. Because of insufficient evidence from randomized controlled trials and conflicting findings from observational studies, establishing a causal link between iron levels and the risk of developing T2DM has proven challenging in previous research.

The National Health and Nutrition Examination Survey (NHANES) is a continuous, stratified, multistage sample study conducted by the US Centers for Disease Control and Prevention. Data are collected through structured interviews with individuals, health screenings, and laboratory sample analysis [9, 10]. Therefore, NHANES might provide high-quality, large-sample data for assessing the correlation between iron levels and T2DM risk. Mendelian randomization (MR) is used to assess causality between a modifiable exposure or risk factor and a clinically relevant outcome. MR combines insights from epidemiologists with statistical theory borrowed from genetics and economics to address problems in epidemiology and human biology. In a MR study, a genetic variation associated with an exposure of interest is utilized as an instrumental variable (IV) to infer whether a risk factor causally affects a health outcome [11, 12]. Recently, MR has been used to integrate genome-wide association study (GWAS) summary data for large numbers of genetic variants. Zhao et al. [13] integrated data from the NHANES with MR methodology to investigate the genetic causal relationships among T2DM, mild cognitive impairment, and their shared biomarkers. Therefore, the causal effect of iron levels on T2DM can be elucidated using the NHANES database in conjunction with MR analysis.

In this study, we analyzed the relationship between iron levels and T2DM risk through both epidemiological analysis and genetic perspectives by utilizing cross-sectional data from the NHANES database spanning 2009–2018 alongside MR analysis. This approach aimed to elucidate the causal effect of iron levels on T2DM risk while addressing limitations posed by confounding factors and

reverse causality inherent in traditional observational studies, thus providing valuable theoretical insights for treatment strategies and prevention measures concerning T2DM.

Material and methods

Participants in NHANES

NHANES is designed to estimate the health status of individuals in the US through comprehensive surveys and physical examinations. All data were available directly from the online database (https://wwwn.cdc.gov/nchs/nhanes/search/). The study protocol was approved by the National Center for Health Statistics Research Ethics Review Committee (#2005-06, #2011-17), and written informed consent was provided by all participants. Participants who participated in the NHANES survey during five consecutive periods (2009–2010, 2011–2012, 2013–2014, 2015–2016, and 2017–2018) were included in this analysis. The exclusion criteria were as follows: lack of demographic data; lack of information on smoking, drinking status, or waist circumference; and lack of information on serum iron levels. Based on these criteria, 19,483 participants were enrolled (Figure 1).

Diagnostic criteria for T2DM and serum iron levels in NHANES

Participants were classified as having T2DM if they met any of the following criteria: prior diagnosis of T2DM; use of antidiabetic medication; fasting plasma glucose (FPG) ≥ 7.0 mmol/L; 2-h oral glucose tolerance test (OGTT) glucose ≥ 11.1 mmol/L; and hemoglobin A1c (HbA1c) ≥ 6.5%. Iron level data were obtained from laboratory testing results in the NHANES database.

Definition of covariates in NHANES

This study assessed covariates that might affect the relationship between serum iron levels and T2DM, including age, sex (male or female), education (college graduate or above, high school grad, or less than high school grad), race (Mexican-American, non-Hispanic Black, non-Hispanic White, or

other race), marital status (divorced, living with partner, married, never married, separated, or widowed), smoking status (yes or no), drinking status (yes, at least 12 times drinking per year; no, fewer than 12 times drinking per year), poverty income ratio (low, ≤1.3; medium, 1.3–3.5, high, >3.5), hypertension ("The doctor told you that you have hypertension:" yes or no), body mass index (BMI; normal, 25; overweight, 25 ≤ BMI < 30; obese, ≥30), waist circumference (normal, ≤102 cm for males and ≤88 cm for females; obese, >102 cm for males and >88 cm for females), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), alanine transaminase, gamma glutamate transpeptidase, serum creatinine, serum uric acid, and hemoglobin.

Association analysis of serum iron levels with T2DM risk in NHANES

Given the complexity of the sample, weighted analysis was performed according to NHANES recommendations. For baseline characteristics, the weighted *t*-test and weighted chi-squared test were applied to complete the comparison of discrepancies in serum iron levels and covariates between participants with and without T2DM. The baseline table was created using the tableone package (v 0.13.2) [14]. Next, multivariate logistic regression models were adopted to reveal the association between serum iron levels and T2DM, calculating odds ratios (ORs) and 95% confidence intervals (95% CIs). Three models were constructed: model 1 (crude model), featuring only serum iron levels without adjustment for covariates; model 2 (incompletely adjusted model), adjusted for age, race, and sex; and model 3 (fully adjusted model), adjusted for all covariates. To address potential bias arising from HbA1c–based T2DM diagnosis, additional sensitivity analyses were conducted using alternative diagnostic criteria for T2DM. Specifically, T2DM was redefined based solely on FPG (≥ 7.0 mmol/L) or solely on 2-h OGTT glucose (≥ 11.1 mmol/L). Weighted multivariable logistic regression models were then refitted among participants with available FPG or OGTT data, maintaining the same

covariate adjustment strategy as in the primary analysis. In addition, subgroup analysis was performed to examine the consistency of the findings in distinct populations. Finally, the predictive effect of serum iron levels on T2DM risk was assessed by creating receiver operating characteristic (ROC) curves using pROC (v 1.18.0) [15].

Construction and evaluation of the nomogram

To further analyze the ability of the covariates in model 3 to predict the occurrence of T2DM, a nomogram model featuring all participants in the NHANES database was created the "rms" (v 6.8.1). The calibration curve (the slope approaching 1, $p < 0.05$), ROC curve (1 > area under the curve [AUC] > 0.7), and decision curves (net benefit > 0) of the nomogram were drawn using "rms" (v 6.8.1), "pROC" (v 1.18.5) and "rmda" (v 1.6), respectively, to assess the predictive capability of the nomogram model.

Study design and data extraction in the MR study

We used a two-sample MR study to clarify the causality between iron levels in the spleen (exposure) and T2DM (outcome). The groundwork for MR design was laid by three key hypotheses: IVs were significantly linked to the exposure; IVs had no relationship to confounders in the exposure–outcome connection; and IVs influenced the outcome solely as a result of the exposure. GWAS summary data for iron levels in the spleen were retrieved from the Integrated Epidemiology Unit (IEU) Open-GWAS database (https://gwas.mrcieu.ac.uk/). Specifically, ebi-a-GCST90101831 for iron levels in the spleen contained 9,275,407 single-nucleotide polymorphisms (SNPs) from the samples of 35,324 European individuals. In addition, the FinnGen database (https://r10.finngen.fi/pheno/T2D_WIDE) was used to obtain summary data for T2DM (finngen_R10_T2D_WIDE). The databased contained 337,038 SNPs from 379,631 European samples (42,593 T2DM and 337,038 control samples). Statistical power analysis was conducted using the pwr package (v 1.3.0; available at

https://CRAN.R-project.org/package=pwr). The significance level (α) was set at 0.05. The final calculated statistical power was 99.99844%, which exceeded the threshold of 80%, indicating sufficient statistical power.

Selection of IVs in the MR study

Based on these three assumptions, we identified IVs through rigorous quality control. First, SNPs significantly linked to the exposure factor were screened as IVs using the extract_instruments function in TwoSampleMR (v 0.5.6) [16], with the screening condition of $p < 5 \times 10^{-8}$. Next, IVs in linkage disequilibrium were excluded using clump = TRUE, $r^2 = 0.001$, and kb = 1000 to ensure independence between IVs. Subsequently, based on the GWAS data of the outcome and the aforementioned IVs, the IVs that were significantly associated with the outcome were removed, and the effect alleles and effect sizes were harmonized using the harmonize data function, followed by exposure–IV–outcome matching. Finally, IVs with F-statistics < 10 were excluded to reduce potential weak instrumental bias. The F-statistic was calculated using the formula $F = R^2 \times \frac{N-2}{1-R^2}$, $R^2 = 2 \times EAF \times (1-EAF) \times \beta^2$, were R^2 was the cumulative explained variance of the selected IVs for the exposure factor, effect allele frequency (EAF) was the effect allele frequency, β was the estimated effect of the SNPs, and N was the sample size of the GWAS. The F statistic <10 can be used to assess the strength of IVs, ensuring that the selected IVs have sufficient explanatory power to avoid being affected by weak instrumental bias.

MR study and sensitivity analysis

We employed five distinct algorithms to assess the OR and *p*-value regarding the causal impact of iron levels in the spleen on T2DM risk: MR-Egger [17], inverse variance weighted (IVW) [18], weighted median [19], simple mode [16], and weighted mode [20]. Of these, our primary emphasis was

on the results derived from IVW. Scatter and forest plots were used to visualize the MR outcomes, and a funnel plot was used to confirm adherence to Mendel's second law. Scatter plots were used to display the association trend between genetic IVs and outcome variables, and to intuitively present the linear relationship and the strength between exposure factors and outcome variables. Forest plots summarized the effect estimates and their CIs for each independent IV and simultaneously displayed the overall effect size and its significance level. This facilitated systematic evaluation of the consistency among different IVs and the overall effect. Funnel plots were utilized to assess potential publication bias and heterogeneity in the study results. Through symmetry analysis, funnel plots were used to verify the reliability of the data and ensure the robustness of the MR analysis results. Furthermore, we conducted sensitivity analysis to ensure the dependability of the MR estimates. Briefly, heterogeneity and horizontal pleiotropy were assessed using Cochran's Q-test (mr_heterogeneity function) [21] and the MR-Egger intercept test (mr_pleiotropy function) [22], where $p > 0.05$ implied no heterogeneity or horizontal pleiotropy between the IVs. Furthermore, we performed leave-one-out (LOO) analysis, in which individual SNPs were excluded to determine whether the MR estimate was influenced by a specific SNP [23]. Finally, Steiger's directionality test was undertaken by applying the directionality test function to assess the validity and confidence in the direction of the causal effect of iron levels in the spleen on T2DM [24].

Statistical analysis

Unless otherwise stated, all statistical analyses in NHANES were performed using the nhanesR-package (v 1.0) [25] in R (R Foundation for Statistical Computing, Vienna, Austria), and all statistical analyses in MR study were undertaken using the TwoSampleMR-package (v 0.5.6) [16] in R. The significance threshold was $p < 0.05$.

Results

Comparison of baseline characteristics

In total, 19,483 participants enrolled in the NHANES cross-sectional study, including 2657 patients with T2DM and 16,826 individuals without T2DM, were identified. Significant differences in serum iron levels and all covariates were observed between individuals with and without T2DM ($p < 0.05$; Table I). Of note, the mean serum iron levels in individuals with and without T2DM were 13.99 ± 5.59 and 15.55 ± 6.41 μmol/L, respectively ($p < 0.01$). The mean age of patients with T2DM was 42.01 ± 12.75 years, 53.0% of these patients were male, 35.5% of these patients were non-Hispanic White, and 58.8% of these patients were obese. In addition, patients with T2DM exhibited higher TC, gamma-glutamate transpeptidase, serum creatinine, serum uric acid, FPG, 2-h OGTT glucose, and HbA1c levels and lower HDL-C levels, than those without T2DM ($p < 0.01$).

Negative correlation between serum iron levels and T2DM risk

Multivariate logistic regression models revealed a significantly negative correlation between serum iron levels and T2DM risk, as indicated by an OR of 0.955 (95% CI 0.946-0.964, $p < 0.01$) in models 1, 0.948 (95% CI 0.937-0.959, $p < 0.01$) in models 2, and 0.983 (95% CI 0.971-0.995, $p < 0.01$) in models 3. However, the OR values in all 3 models were close to 1, the clinical significance of the models needed to be further verified. To assess whether the observed association might be confounded by HbA1c-related diagnostic bias, we conducted additional sensitivity analyses using FPG- and 2-h OGTT glucose-based definitions of T2DM. Among the 9,409 participants with available FPG data, 1,176 were classified as having T2DM based on the FPG ≥ 7.0 mmol/L. The inverse association between serum iron levels and T2DM risk remained statistically significant across all three models, including the fully adjusted model (OR = 0.893, 95% CI 0.799-0.998, $p < 0.05$; Appendix Table I).

Among the 6,210 participants with available 2-h OGTT glucose data, 446 were classified as having T2DM based on the 2-h OGTT glucose ≥ 11.1 mmol/L. In this analysis, serum iron levels showed no statistically significant association with 2-h OGTT glucose-defined T2DM risk in any model ($p > 0.05$, Appendix Table II). Subgroup analyses further revealed a significant negative association between serum iron levels and T2DM risk in several subgroups within model 3 (Figure 2), including non-Hispanic Black individuals (OR, 0.782, 95% CI 0.632-0.966, $p < 0.05$), non-Hispanic White individuals (OR, 0.510, 95% CI 0.420-0.620, $p < 0.01$), and groups defined by TC (OR, 0.993, 95% CI 0.991-0.995, $p < 0.01$), HDL-C (OR, 0.970, 95% CI 0.964-0.975, $p < 0.01$), hemoglobin (OR, 0.993, 95% CI 0.987-0.999, $p < 0.05$), serum uric acid (OR, 0.983, 95% CI 0.977-0.990, $p < 0.01$), and drinking status (OR, 0.829, 95% CI 0.714-0.963, $p < 0.05$). Additionally, ROC curve analysis revealed AUCs of 0.758 and 0.830 for models 2 and 3, respectively (Figure 3).

Nomogram exhibited excellent performance for T2DM diagnosis

The nomogram was constructed on the basis of the covariates in model 3 (Figure 4a). The probability of having T2DM increased as the nomogram score increased. The calibration curve of the nomogram revealed a *p*-value of 5.22e−05 and a slope near 1, demonstrating the accuracy the nomogram prediction accuracy (Figure 4b). In addition, the ROC curve revealed an AUC of 0.831 for the nomogram (Figure 4c), and the decision curve demonstrated the high clinical benefit of the nomogram (Figure 4d). This nomogram, incorporating the covariates in model 3, provided a reliable tool for predicting T2DM risk.

A causal relationship existed between iron levels and T2DM risk

MR analysis was performed to explore the causal relationship between iron levels in the spleen and the risk of T2DM from a genetic perspective. After rigorous screening, four SNPs were acquired as IVs

(Appendix Table III). The MR results, presented in Table II, revealed a significant causal impact of iron levels in the spleen on T2DM using the IVW method (OR, 0.821, 95% CI 0.732–0.922, $p < 0.01$). The OR suggested that iron levels in the spleen protected against T2DM to some extent. This finding was further supported by the negative slope observed in the scatter plot and an MR effect size smaller than 0 in the forest plot (Figure 5a–b). Additionally, the funnel plot illustrated that there is no deviation in our analysis results (Figure 5c).

MR estimates proved reliable

Cochran's Q-test revealed no significant heterogeneity concerning the impact of iron levels in the spleen on T2DM risk ($p > 0.05$; Table III). The MR-Egger intercept test presented no evidence of horizontal pleiotropy ($p > 0.05$), implying that the IVs must influence the outcome through the exposure. Furthermore, LOO analysis confirmed that the causal effect between iron levels in the spleen and T2DM was not influenced by any single SNP (Figure 6). Finally, the Steiger's test confirmed the plausibility and validity of the causal effect direction from iron levels in the spleen on T2DM ($p < 0.01$).

Discussion

T2DM represents a major global health challenge, with iron level closely associated with its development. Our analysis of the NHANES database revealed a significant negative correlation between iron levels and T2DM risk. Furthermore, a two-sample MR analysis of GWAS data demonstrated a reverse causal relationship between iron levels and T2DM, suggesting that iron deficiency might constitute a modifiable risk factor for T2DM development. Consistent with this, Kim et al. [26] reported that iron deficiency in women was associated with significantly higher odds of HbA1c elevation (≥5.5%). Mukasa et al. [27] found a high prevalence of iron deficiency among

individuals with established T2DM. Notably, Chen et al. [28] reported that lower serum iron levels might increase the risk of T2DM. These findings provide epidemiological evidence supporting an association between low iron levels and heightened T2DM susceptibility. However, recent studies have indicated that the relationship between iron levels and T2DM risk is not linear. Analyses of data from 7308 participants in the China Health and Nutrition Survey revealed a J-shaped association between iron levels with T2DM risk [29]. These findings suggest a potential dose–response relationship between iron levels and the risk of T2DM. This nonlinear pattern implies that both iron deficiency and pathological iron overload, as well as deviations within the conventional "normal" range, might independently elevate T2DM risk. The biological basis of this non-linear relationship lies in the dual effects of iron on pancreatic β-cells [6]. Within β-cells, iron serves as a critical component of iron–sulfur cluster proteins, which are indispensable for proper insulin synthesis, posttranslational processing, and secretory function. Notably, even physiological fluctuations in intracellular iron levels can modulate glucose-stimulated insulin secretion, systemic insulin sensitivity, and hepatic gluconeogenesis [30]. In iron deficiency, the activity of mitochondrial iron-dependent enzymes (e.g., cytochrome c oxidase) is diminished, leading to impaired ATP generation and consequently attenuated glucose-stimulated insulin secretion [6]. Importantly, mitochondrial energy metabolism may be compromised even in individuals with iron deficiency but normal hemoglobin levels, leading to functional impairments in cardiac and skeletal muscle [31]. Conversely, iron overload is cytotoxic and induces oxidative stress, cellular damage, and apoptosis [32]. Specifically, excess iron catalyzes the formation of reactive oxygen species through the Fenton reaction, promoting lipid peroxidation. When the glutathione peroxidase 4 (GPX4) system becomes dysfunctional, ferroptosis is triggered, culminating in plasma membrane rupture and irreversible cell death [33]. The dual effect of iron levels

on pancreatic β-cells exists under pathological conditions and occurs within the normal physiological range. Thus, the observed negative correlation between iron levels and T2DM risk in this study suggests that even subclinical iron deficiency within the physiological range may perturb systemic glucose homeostasis.

HbA1c is a standard diagnostic criterion for DM. In patients with T2DM and iron deficiency anemia (IDA), HbA1c levels may be spuriously elevated. Urrechaga et al. [34] reported a positive correlation between iron deficiency and elevated HbA1c concentrations in DM patients. Similarly, Katwal et al. examined the impact of anemia on HbA1c interpretation and the feasibility of achieving optimal HbA1c targets in both DM and non-DM individuals, based on available evidence. Notably, HbA1c levels were consistently elevated in patients with IDA. Importantly, oral iron supplementation had been shown to reduce HbA1c in anemic patients with concomitant IDA and T2DM [35]. In the study, we conducted additional sensitivity analyses using FPG- and 2-h OGTT-based definitions of T2DM. Our results demonstrated a statistically significant inverse association between serum iron levels and the risk of FPG-defined T2DM. This finding supports the robustness of the observed association, indicating that it is unlikely to be attributable to HbA1c-related diagnostic bias.

Our study revealed that individuals with T2DM exhibited significantly lower serum iron levels alongside higher BMI, body weight, blood pressure, and serum creatinine concentrations. Obesity is frequently associated with chronic low-grade inflammation. Pro-inflammatory cytokines, including interleukin-1 (IL-1) and IL-6, increase hepcidin expression, thereby impairing intestinal iron absorption and promoting iron sequestration within macrophages, ultimately leading to reduced circulating iron levels [36]. Iron deficiency has been strongly linked to inflammatory factor levels in obese patients, and increased levels of obesity-related inflammatory factors are associated with an elevated risk of

T2DM [37]. Kalra et al. [38] emphasized that anemia and iron deficiency were highly prevalent among patients with metabolic syndrome, including those with hypertension. Notably, tissue hypoxia resulting from anemia appears to be a key mechanism underlying the exacerbation of both microvascular and macrovascular complications in T2DM attributable to iron deficiency. In addition, a cross-sectional analysis of 8118 patients with DM from the NHANES database demonstrated that dietary iron intake ≥12.59 mg/day was associated with a significantly lower risk of diabetic nephropathy. Stratified analyses further indicated that this protective association was more pronounced among men, individuals aged <60 years, those with hypertension, and those with higher body weight [39]. Collectively, these findings indicate that serum iron levels are significantly lower in patients with T2DM than in those without DM, concomitant with adverse clinical parameters, including elevated BMI, body weight, blood pressure, and serum creatinine concentration, suggesting that diminished iron level may facilitate both the occurrence and progression of T2DM.

NHANES is a nationally representative survey with a large sample size, thereby ensuring data reliability. MR analysis effectively addresses potential effects of residual confounding and reverse causation. In this study, we conducted an observational analysis utilizing data from the NHANES database from 2009 to 2018, alongside MR analysis, to investigate the causal relationship between iron levels and the risk of T2DM. This integrated approach both provides an unbiased estimate of the causal effect of iron levels on T2DM and deepened our understanding of this association. However, certain limitations inherent in this study. First, the observed population was predominantly concentrated in Europe, which might restrict the generalizability of findings to other ethnic groups. Second, MR analysis mainly assesses the linear exposure effects. If the relationship between iron levels and T2DM is nonlinear, then MR analysis might only be able to capture the slope near the mean. Notably, in this

study, serum iron levels served as the assessment indicator in the NHANES observational analysis, whereas spleen iron levels were used as the exposure in the MR analysis. These two measures differ substantially in anatomical origin, physiological relevance, and biological interpretation. Third, serum iron levels only represent one aspect of iron metabolism, as it does not consider ferritin or transferrin saturation. Moreover, the NHANES dataset provided only a single measurement of serum iron level per participant, precluding adequate assessment of physiological fluctuations, such as those associated with the menstrual cycle in premenopausal women or hormonal shifts during menopause. Lastly, the mechanisms underlying the influence of iron levels on T2DM risk remain inadequately understood.

In conclusion, our study identified a reverse causality relationship between iron levels and T2DM risk based on a large observational data derived from the NHANES database combined with MR analysis. These findings suggest that maintaining serum iron levels within an appropriate range might protect against the risk of T2DM, thereby offering a novel perspective for T2DM prevention. Future research should verify these findings in different populations, assess the impact of iron supplementation on the risk of T2DM through randomized controlled trials, and clarify the underlying mechanisms.

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All authors would like to acknowledge the use of NHANES database as a data source in this study.

Data availability

The datasets analysed during the current study are available in the [NHANES] repository, [https://wwwn.cdc.gov/nchs/nhanes/search/]; [IEU Open-GWAS] repository, [https://gwas.mrcieu.ac.uk/] and [FinnGen] repository, [https://r10.finngen.fi/pheno/T2D_WIDE].

Ethical approval

The study protocol was previously vetted and ethically approved by the National Center for Health Statistics Research Ethics Review Committee (#2005-06, #2011-17). Consequently, our study did not necessitate additional ethical clearance.

Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Exclusion criteria for participants participating in the NHANES survey.

Figure 2. Weighted logistic regression analysis of iron levels and T2DM risk.

Figure 3. ROC curves for model 1, model 2 and model 3.

Figure 4. Construction and Validation of the Nomogram. (a) Nomogram constructed based on model 3. (b) Calibration curve validation of the nomogram. (c) ROC curve validation of the nomogram. (d) DCA curve validation of the nomogram.

Figure 5. Exploring the causal relationship between iron levels and T2DM. (a) Scatterplot of causal relationship between iron levels and T2DM. (b) Forest plot of causal relationship between iron levels and T2DM. (c) Funnel plot of causal relationship between iron levels and T2DM.

Figure 6. Leave-one-out forest plots between iron levels and T2DM.

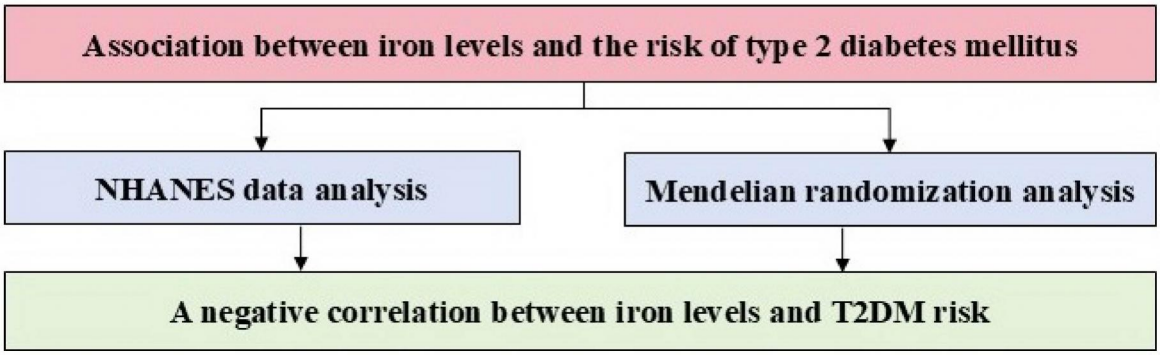

Association between iron levels and the risk of type 2 diabetes mellitus
NHANES data analysis
Mendelian randomization analysis
A negative correlation between iron levels and T2DM risk

Table I. Baseline statistical characteristics

Characteristics	T2DM (n = 2657)	Non-T2DM (n = 16826)	*p* value
Gender (n, %)			＜0.001
Female	1249 (47.0)	8582 (51.0)	
Male	1408 (53.0)	8244 (49.0)	
Age (years, %)	42.01 (12.75)	27.81 (17.32)	＜0.001
BMI (n, %)			＜0.001
Normal	334 (12.6)	5212 (31.0)	
Overweight	760 (28.6)	5603 (33.3)	
Obesity	1563 (58.8)	6011 (35.7)	
Waist circumference (n, %)			＜0.001
Normal	545 (20.5)	7806 (46.4)	
Obesity	2112 (79.5)	9020 (53.6)	
Race (n, %)			＜0.001
Mexican American	479 (18.0)	2323 (13.8)	
Non-hispanic black	640 (24.1)	3295 (19.6)	
Non-hispanic white	944 (35.5)	7339 (43.6)	
Other race	594 (22.4)	3869 (23.0)	
Marital status (n, %)			
Divorced	364 (13.7)	1798 (10.7)	＜0.001
Living with partner	104 (3.9)	1551 (9.2)	

Married	1508 (56.8)	8485 (50.4)	
Never married	234 (8.8)	3455 (20.5)	
Separated	99 (3.7)	528 (3.1)	
Widowed	348 (13.1)	1009 (6.0)	
Economic (n, %)			＜0.001
Low income	939 (35.3)	5209 (31.0)	
Medium income	1049 (39.5)	6229 (37.0)	
High income	669 (25.2)	5388 (32.0)	
Education (n, %)			＜0.001
College graduate or above	1240 (46.7)	9757 (58.0)	
High School Grad	593 (22.3)	3780 (22.5)	
Less Than High School Grad	824 (31.0)	3289 (19.5)	
Smoke status (n, %)			＜0.001
Smoker	1323 (49.8)	7213 (42.9)	
Non-smoker	1334 (50.2)	9613 (57.1)	
Drink status (n, %)			＜0.001
Drinker	1457 (54.8)	10621 (63.1)	
Non-drinker	1200 (45.2)	6205 (36.9)	
Hypertension (n, %)	1839 (69.2)	5029 (29.9)	＜0.001
Hemoglobin (g/L))	72.30 (15.89)	75.85 (15.02)	＜0.001

TC (mmol/L)	103.59 (44.63)	116.54 (40.04)	＜0.001
HDL-C (mmol/L)	37.91 (14.07)	43.89 (16.18)	＜0.001
Alanine transaminanse (U/L)	22.89 (16.58)	21.77 (16.78)	0.001
Glutamate transpeptidase (U/L)	32.75 (35.73)	25.09 (28.45)	＜0.001
Serum creatinine (μmol/L)	69.41 (41.14)	58.23 (24.45)	＜0.001
Serum uric acid (μmol/L)	45.11 (15.36)	42.08 (14.13)	＜0.001
FPG (mmol/L)	8.74 (3.46)	5.64 (1.06)	＜0.001
2-h OGTT glucose (mmol/L)	12.04 (5.45)	6.55 (2.73)	＜0.001
HbA1c (%)	7.45 (1.80)	5.50 (0.58)	＜0.001
Iron level (μmol/L)	13.99 (5.59)	15.44 (6.41)	＜0.001

T2DM: type 2 diabetes mellitus; BMI: body mass index; TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; FPG: fasting plasma glucose; OGTT: oral glucose tolerance test.

Table II. MR analysis results between iron level and the risk of T2DM

Outcome	Exposure	Method	SNP (n)	*p* value	OR
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	MR Egger	4	0.531449 814	0.893426 826
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	Weighted median	4	0.011138 216	0.841016 112
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	Inverse variance weighted	4	0.000860 341	0.821396 117
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	Simple mode	4	0.121490 22	0.840578 876
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	Weighted mode	4	0.130372 828	0.843749 663

MR: Mendelian Randomization; T2DM: type 2 diabetes mellitus; SNP, single nucleotide polymorphism; OR, odds ratio.

Table III. Heterogeneity test results

Outcome	Exposure	Method	Q	Q df	Q *p* value
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	MR Egger	0.787378514	2	0.6745636 4
Finngen R10 T2DM WIDE	Iron Content: GCST90101831	Inverse variance weighted	1.157565849	3	0.7631987 08

T2DM: type 2 diabetes mellitus; MR: Mendelian Randomization.

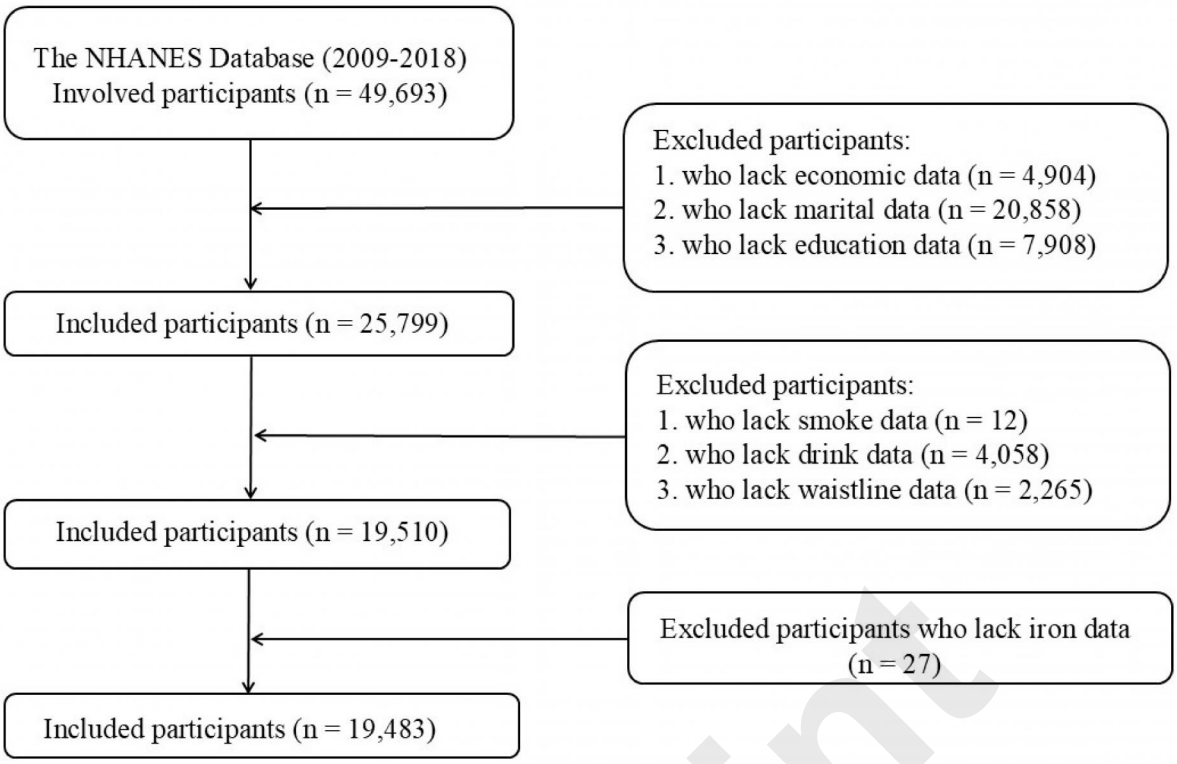


Figure 1. Exclusion criteria for participants participating in the NHANES survey.

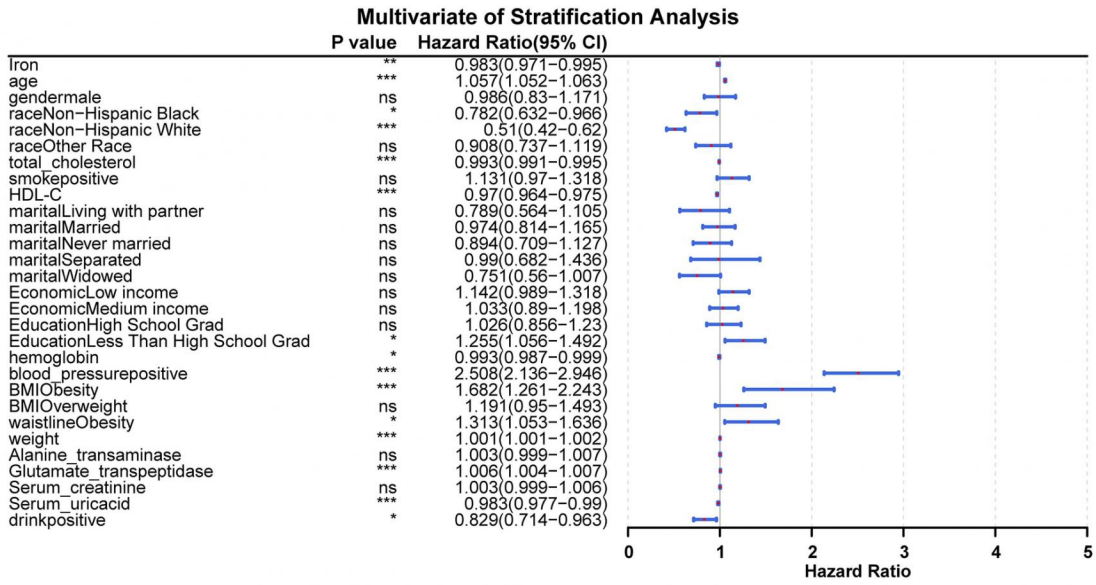
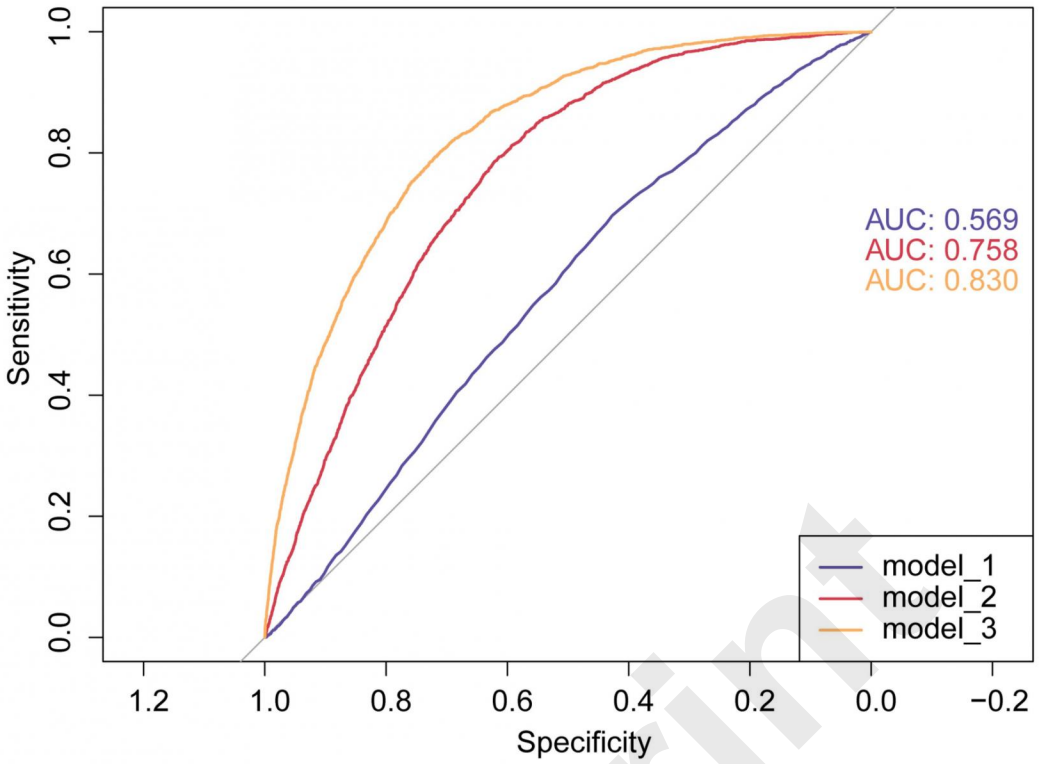
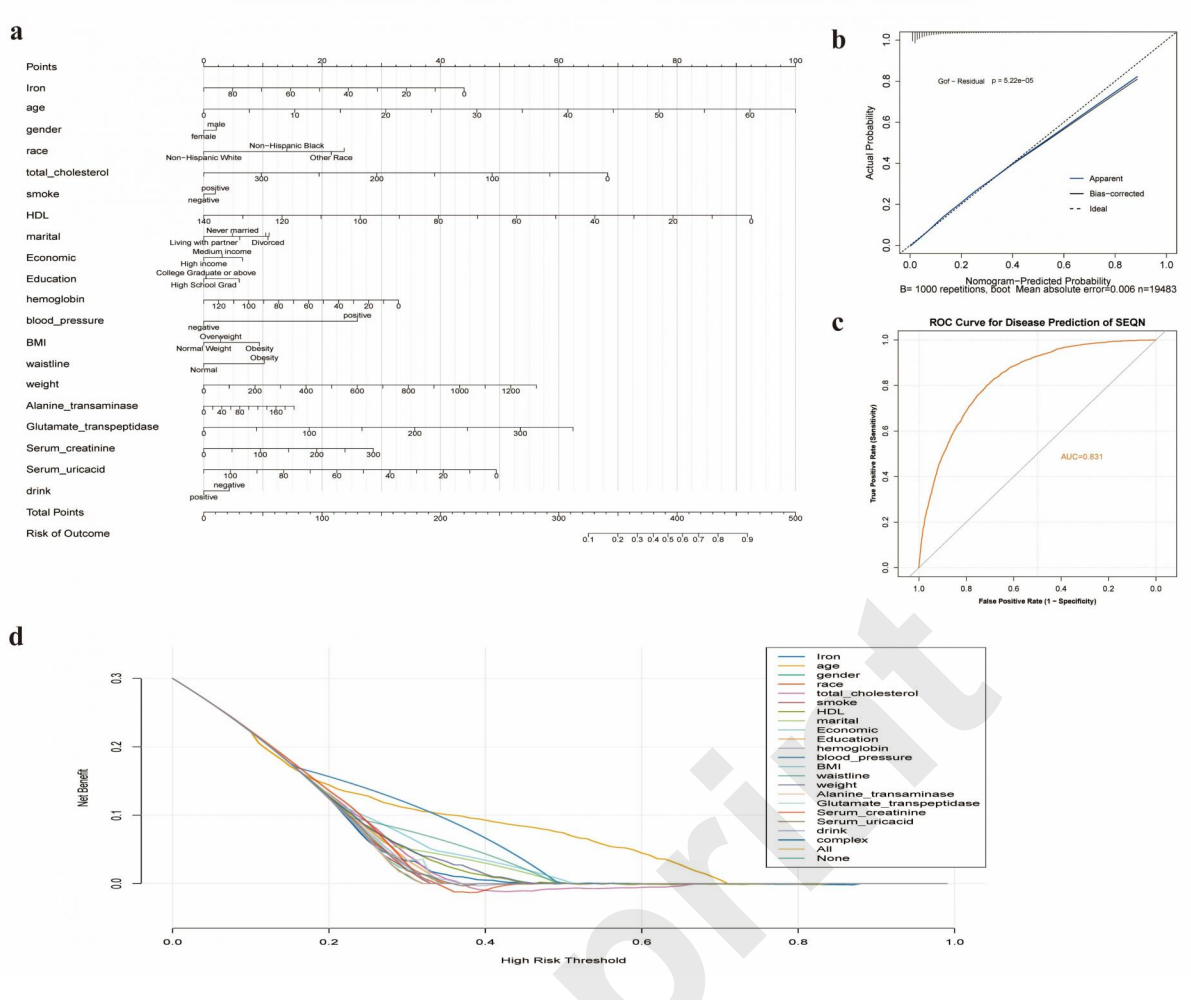
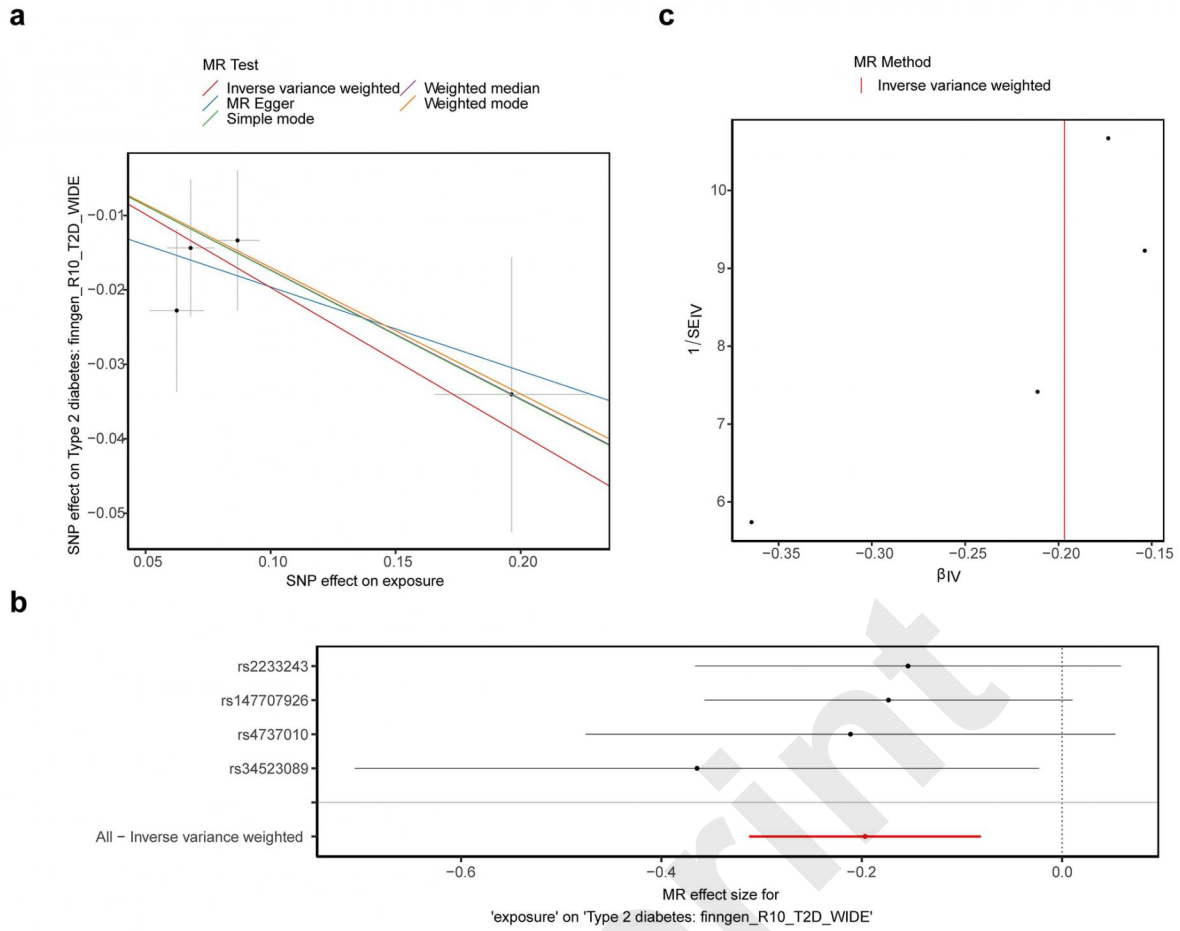


Figure 2. Weighted logistic regression analysis of iron levels and T2DM risk.


Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
Specificity
AUC: 0.569
AUC: 0.758
AUC: 0.830
model_1
model_2
model_3


a
Points
Iron
age
gender
race
total_cholesterol
smoke
HDL
marital
Economic
Education
hemoglobin
blood_pressure
BMI
waistline
weight
Alanine_transaminase
Glutamate_transpeptidase
Serum_creatinine
Serum_uricacid
drink
Total Points
Risk of Outcome
male
female
Non−Hispanic Black
Non−Hispanic White
Other Race
positive
negative
Never married
Living with partner
Divorced
Medium income
High income
College Graduate or above
High School Grad
Overweight
Normal Weight
Obesity
Normal
negative
positive
b
Gof − Residual p = 5.22e−05
Apparent
Bias−corrected
Ideal
Actual Probability
Nomogram−Predicted Probability
B= 1000 repetitions, boot Mean absolute error=0.006 n=19483
c
ROC Curve for Disease Prediction of SEQN
AUC=0.831
True Positive Rate (Sensitivity)
False Positive Rate (1 − Specificity)
d
Net Benefit
High Risk Threshold
Iron
age
gender
race
total_cholesterol
smoke
HDL
marital
Economic
Education
hemoglobin
blood_pressure
BMI
waistline
weight
Alanine_transaminase
Glutamate_transpeptidase
Serum_creatinine
Serum_uricacid
drink
complex
All
None



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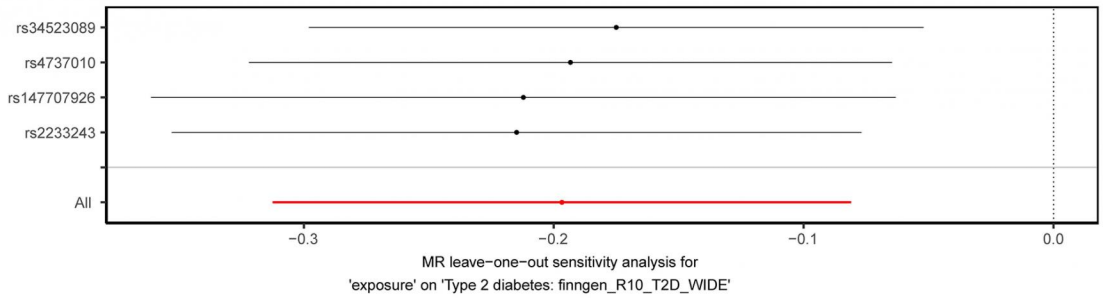

Figure 6. Leave-one-out forest plots between iron levels and T2DM.