# Causal Association Between Genetically Determined Iron Status and Lung Cancer: A Two-Sample Mendelian Randomization Study

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## Abstract

**Background:** observational studies have demonstrated that the causal relationship between iron status and lung cancer remains unclear causality. A two-sample mendelian randomization (MR) study was performed to identify the causal association between iron status traits and risk of lung cancer.

**Methods:** Single nucleotide polymorphisms (SNPs) strongly associated ($P < 5 \times 10^{-8}$) with four biomarkers of systemic iron status were obtained from a genome-wide association study containing 48,972 subjects from the Genetics of Iron Status Consortium (GISC). Summary-level data for the genetic associations with lung cancer were extracted from International Lung Cancer Consortium (ILCCO) on 11,348 cases and 15,861 controls. We used a two-sample MR study to obtain causal estimates and assessed by sensitivity analyses.

**Results:** Genetically determined serum iron [OR, 0.86; 95% confidence interval (CI), 0.76-1.00; *P*=0.025)] had a negative effect on lung cancer, while no significant association with lung cancer was found in transferrin saturation (OR, 0.91; 95% CI, 0.81-1.02; *P*=0.110), log10 ferritin (OR, 0.76; 95% CI, 0.52-1.12; *P*=0.170), and transferrin (OR, 1.09; 95% CI, 0.86-1.38; *P*=0.470).

**Conclusion:** In conclusion, our results strongly supported a causal link between genetically determined lower serum iron levels and increased risk of lung cancer. These findings may provide a potential clinical prevention and treatment strategy.


## Introduction

Lung cancer is the leading cause of malignancy-related mortality worldwide, accounting for 18% of the total cancer deaths [1]. Although molecular mechanisms underlying initiation and progression of lung cancer remains unclear, it is already known that the major risk factors for lung cancer include cigarette smoking, air pollution, unhealthy lifestyles, occupational lung carcinogens, and genetic susceptibility [2]. The heritability of lung cancer is estimated at approximately 18%, which suggests that genetic factors play an important role in the etiology of lung cancer [3]. With the development of next generation sequencing and microarray technologies, GWAS has been widely used to identify genetic variations associated with complex diseases. Since the GWAS study of lung cancer was first carried out in 2008 [4], over 40 genetic loci have been identified with susceptibility of lung cancer in European and Asian populations [5, 6, 7]. These susceptibility loci identification will help predict high-risk for lung cancer in population, as well as develop new therapeutic targets. Advances in understanding genetic predisposing factors for lung cancer can improve our diagnostic, prognostic, and therapeutic classification systems.

Previous epidemiological studies have indicated that systemic iron dysregulation is closely associated with the incidence and development of lung cancer. As an essential trace element, iron plays a crucial role in a variety of cellular events including heme biosynthesis, mitochondrial respiration, and DNA repair [8]. Many experimental studies also supported a direct or indirect association between iron and lung cancer [9, 10]. Particularly, iron-dependent ferroptosis has recently been investigated in lung cancer, and may be a promising target for anti-cancer therapy. However, there are still some unsolved questions and controversies for iron status in lung cancer according to previous epidemiological data. Several studies showed that high dietary iron intake significantly increased the risk of lung cancer [11, 12, 13]. On the contrary, Muka and fellows reported that high intake of iron was associated with reduced risk of lung cancer in a prospective study [14]. Furthermore, a meta-analysis result revealed that serum iron levels had no significant association with lung cancer risk [15]. The link between iron status and the risk of lung cancer is therefore under much debate.

Mendelian randomization (MR) analysis is a genetic epidemiological method to investigate the causal association between exposures and disease outcomes by using genetic instrument variants (IVs), such as single-nucleotide polymorphisms (SNPs) [16]. Because of the destined germline genotypes, MR has the possibility of overcoming limitations of confounding and reverse causation bias in observational study [16]. Hence, we conducted a two-sample MR analysis to uncover the genetic association between serum iron status (serum iron, log10 ferritin, transferrin saturation, and transferrin) and lung cancer.

## Materials And Methods

## Study Design

We performed a two-sample mendelian randomization (TSMR) analysis based on the publicly available summary-level statistics of genome-wide association (GWAS) studies. The genetic variants used as IVs in this TSMR study are compliant with three basic assumptions (Fig. 1). First, the genetic variants selected as IVs are strongly associated with exposure (relevance assumption). Second, the selected IVs are not associated with any known confounders (independence assumption). Third, the used IVs can only affect the risk of outcome through the exposure, independent from pleiotropy (exclusion assumption).

## Data Sources

In this TSMR study, iron status traits (serum iron, log10 ferritin, transferrin saturation, and transferrin) were used as the exposure. Summary data of iron status were obtained from the Genetics of Iron Status Consortium (GISC), which contained 23,986 subjects of European ancestry from 11 discovery and 24,986 subjects of European ancestry 8 replication cohorts [17]. For the integrated population from 11 cohorts, 55% were women and the average age was 47. Summary statistical data on the association of IVs with lung cancer were extracted from International Lung Cancer Consortium (ILCCO) on

11,348 cases and 15,861 controls [18]. All data used in this study are available in the online public database. Ethical approval and patient consent can be retrieved in the primary GWAS studies.

## Genetic Instrumental Variables

The single nucleotide polymorphisms (SNPs) used as IVs in this study were identified with a strong association ($p < 5 \times 10^{-8}$) and independent inheritance ($R^2 < 0.001$, 10Mb threshold window) without any linkage disequilibrium (LD) based on the 1,000 genomes reference panel. Detailed information for each SNP is presented in Table 1. These SNPs have been used as IVs for iron status (serum iron, log10 ferritin, transferrin saturation, and transferrin) in previous MR studies and were not considered as weak instrumental variables (F statistic > 10).

Table 1
The genetic instrumental variables proxied for serum iron status

| | | | | Iron, µmol/L | | Transferrin Saturation, % | | Log10 Ferritin, µg/L | | Transferrin, g/L | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SNPs | Locus | EA/OA | EAF | Beta (SE) | *P* | Beta (SE) | *P* | Beta (SE) | *P* | Beta (SE) | *P* |
| rs1800562 | HFE | A/G | 0.067 | 0.328 (0.016) | 2.72E-97 | 0.577 (0.016) | 2.19E-270 | 0.204 (0.016) | 1.54E-38 | -0.479 (0.016) | 8.9E-196 |
| rs1799945 | HFE | G/C | 0.150 | 0.189 (0.010) | 1.10E-81 | 0.231 (0.010) | 5.13E-109 | 0.065 (0.010) | 1.71E-10 | -0.114 (0.010) | 9.36E-30 |
| rs855791 | TMPRSS6 | G/A | 0.554 | 0.181 (0.007) | 1.32E-139 | 0.190 (0.008) | 6.41E-137 | 0.055 (0.007) | 1.38E-14 | -0.044 (0.007) | 1.98E-09 |

EA/OA, effect allele/ other allele; EAF, effect allele frequency; SE, standard error.

## Statistical Analyses

We used inverse variance weighted (IVW) to assess the causal associations between iron status and the risk of lung cancer. The effect measures were the odds ratio (OR) of the risk of lung cancer, which was normalized to one SD increment in each iron status biomarkers. Weighted median, MR Egger, simple mode, and weighted mode methods were used for statistical sensitivity analyses to ensure the robustness of selected IVs. All MR analyses were performed by using the "TwoSampleMR (0.5.6)" package for R version 4.1.3.

## Results

Three SNPs (rs1800562, rs1799945 and rs855791) were conservative genetic variables strongly associated with serum iron, transferrin saturation, ferritin, and transferrin, which were selected as genetic IVs proxied for systemic iron status in this TSMR study (Table 1). The SNP-lung cancer associations were obtained from GWAS data of the ILCCO Consortium (Table 2). The pooled MR analyses for the effect of the four iron status markers on the risk of lung cancer were shown in Table 3. The causal estimates demonstrated a protective effect on lung cancer risk for serum iron (OR, 0.86; 95% CI, 0.76-1.00; $P = 0.025$) by the inverse variance weighted method, whereas no significance was found in transferrin saturation (OR, 0.91; 95% CI, 0.81–1.02; $P = 0.110$), log10 ferritin (OR, 0.76; 95% CI, 0.52–1.12; $P = 0.170$), and transferrin (OR, 1.09; 95% CI, 0.86–1.38; $P = 0.470$). Furthermore, the association with lung cancer across all three SNPs was estimated by sensitivity analyses including the weighted median, single mode-based, weighted mode-based and MR-Egger methods (Fig. 2), and no heterogeneity and pleiotropy were observed indicating the above results were consistent and robust. Cochran's Q tests also confirmed that no heterogeneity was detected in inverse variance weighted method and MR-Egger regression for serum iron, transferrin saturation, ferritin, and transferrin. Individual MR estimates showed that SNP rs855791 (TMPRSS6) associated with a significant effect of serum iron, transferrin saturation and ferritin on the reduced risk of lung cancer, but exerted an opposite effect of transferrin on lung cancer (Fig. 3). These results indicated that SNP rs855791 could drive a significant effect of serum iron on lung cancer.

Table 2
SNP-lung cancer associations obtained from GWAS data of the ILCCO Consortium

| | | | | Lung cancer | |
|---|---|---|---|---|---|
| SNPs | Locus | EA/OA | EAF | Beta (SE) | *P* |
| rs1800562 | HFE | A/G | 0.067 | -0.021 (0.038) | 0.600 |
| rs1799945 | HFE | G/C | 0.150 | -0.018 (0.025) | 0.490 |
| rs855791 | TMPRSS6 | G/A | 0.554 | -0.046 (0.019) | 0.013 |

Chr, chromosome; EA/OA, effect allele/ other allele; EAF, effect allele frequency; SE, standard error.

Table 3
Mendelian randomization estimates for the causal effect of iron status on lung cancer risk.

| Methods | Serum iron | Transferrin Saturation | Ferritin | Transferrin |
|---|---|---|---|---|
| Inverse variance weighted | | | | |
| OR (95% CI) | 0.86 (0.76, 0.98) | 0.91 (0.81, 1.02) | 0.76 (0.52, 1.12) | 1.09 (0.86, 1.38) |
| Q statistic (*P*-value) | 1.69 (0.43) | 2.95 (0.23) | 3.46 (0.18) | 5.33 (0.07) |
| MR-Egger | | | | |
| OR (95% CI) | 1.12 (0.64, 1.96) | 1.05 (0.85, 1.30) | 1.13 (0.65, 1.96) | 0.95 (0.78, 1.15) |
| Q statistic (*P*-value) | 0.77 (0.38) | 0.71 (0.40) | 0.75 (0.39) | 0.65 (0.42) |
| Intercept (*P*-value) | -0.06 (0.51) | -0.05 (0.38) | -0.043 (0.35) | 0.040 (0.28) |
| Weighted median | | | | |
| OR (95% CI) | 0.89 (0.77, 1.04) | 0.94 (0.84, 1.05) | 0.84 (0.60, 1.18) | 1.06 (0.91, 1.24) |
| Simple mode | | | | |
| OR (95% CI) | 0.92 (0.75, 1.14) | 0.94 (0.80, 1.11) | 0.83 (0.47, 1.45) | 1.11 (0.86, 1.43) |
| Weighted mode | | | | |
| OR (95% CI) | 0.93 (0.74, 1.16) | 0.96 (0.84, 1.08) | 0.88 (0.62, 1.25) | 1.06 (0.90, 1.24) |
| OR, odds ratio; CI, confidence interval | | | | |

## Discussion

In this TSMR study, we estimated the causal link between serum iron status and the risk of lung cancer based on genetic summary data from two previously GWASs. According to the estimates of MR analyses, we revealed that genetic predisposition to lower levels of serum iron was causally associated with a higher risk of lung cancer, while no significant causal effect was observed in transferrin saturation, ferritin, and transferrin. The association was driven by rs855791, which had the largest impact on iron status among the IVs. These findings might provide novel insights into the association of serum iron status with lung tumorigenesis.

To figure out the genetic association between systemic iron status and lung cancer, we used only the three SNPs proxied for all four iron status markers at genome-wide significance. These SNPs have a concordant direction of effect on serum iron, transferrin saturation, ferritin, and transferrin, thus to be valid instruments. Several sensitivity analyses were performed to support the direct causal relationship between serum iron and lung cancer. However, the causal estimates for transferrin saturation, ferritin, and transferrin showed an insignificant trend on the risk of lung cancer. This inconsistence might be attributed to the selected genetic IVs and the different populations derived from GWAS summary data. Future studies based on more robust GWASs are warranted to address the inconsistence.

Iron is an essential trace element required for cellular activities. Both iron overload and iron deficiency are related to significant abnormalities in cellular function [19]. Given that, experimental studies have demonstrated a dual role of iron in tumor growth and metastasis [19]. Epidemiological studies have suggested an association between excess iron and increased cancer incidence and risk [21]. However, there are still controversies for iron status in cancer initiation and progression. Particularly, epidemiological studies on the role of iron status in lung cancer are sparse and inconsistent. Moreover, a recent TSMR study reported the associations of iron status with overall cancer and 22 site-specific cancers, and provide no evidence in support of a causal association between iron status and lung cancer [22]. To be noted, the summary data for lung cancer used in the study contained only 2,838 individuals from UK Biobank, which might have limited potency to obtain meaningful results. Herein, we took advantage of the summary data from ILCCO composed of 11,348 cases and 15,861 controls, and indicated the causal association of serum iron with lung cancer.

However, there are several limitations in our study. First, our results were based on GWAS summary data from European populations, which impeded the generalization of findings to other populations. Second, linear association of iron status biomarkers with lung cancer was obtained by MR analyses, but U-shaped relationship or threshold effect may be more fitting and proper in practice. Third, this study did not explore the causal effect of iron status on different histologic subtypes of lung cancer including adenocarcinoma, squamous carcinoma, and non-small cell lung cancer.

In summary, our work suggested the causal association of reduced serum iron with an increased risk of lung cancer, while the levels of transferrin saturation, ferritin, and transferrin were not significantly associated with lung cancer. Future studies are required to explore the exact mechanism and relationship of iron status with lung cancer, which may provide a clinical value for prevention and treatment strategy.

## Declarations

### DATA AVAILABILITY

All data generated or analyzed during this study are included in this article.


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We are extremely grateful to the GIS consortium and the ILCCO consortium for providing relevant publicly available public data, which have been mentioned in the data sources section in this study.


### AUTHOR CONTRIBUTIONS

All authors contributed to research design, data analyses and writing of the paper.


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### CONFLICTS OF INTEREST

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

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## Figures

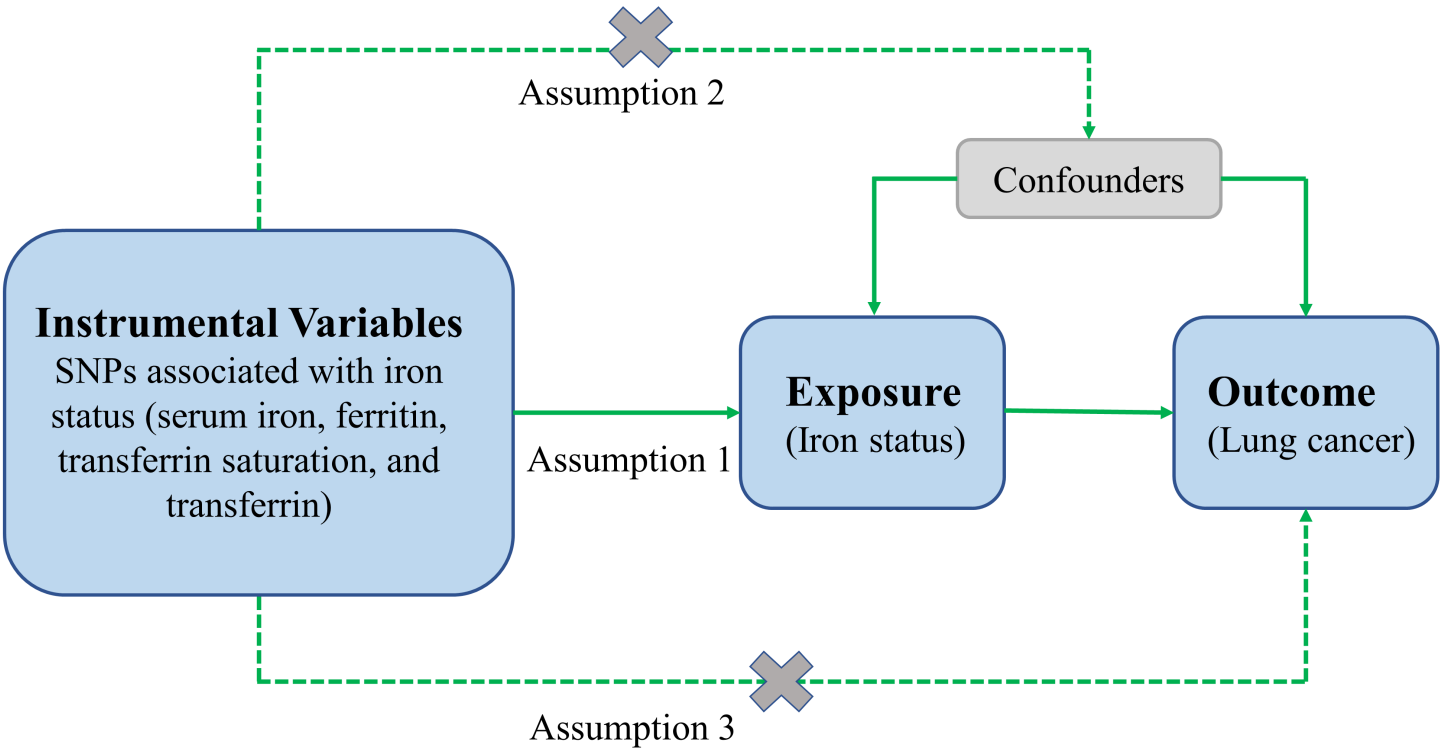


Figure 1

Diagram of three assumptions in Mendelian randomization study.

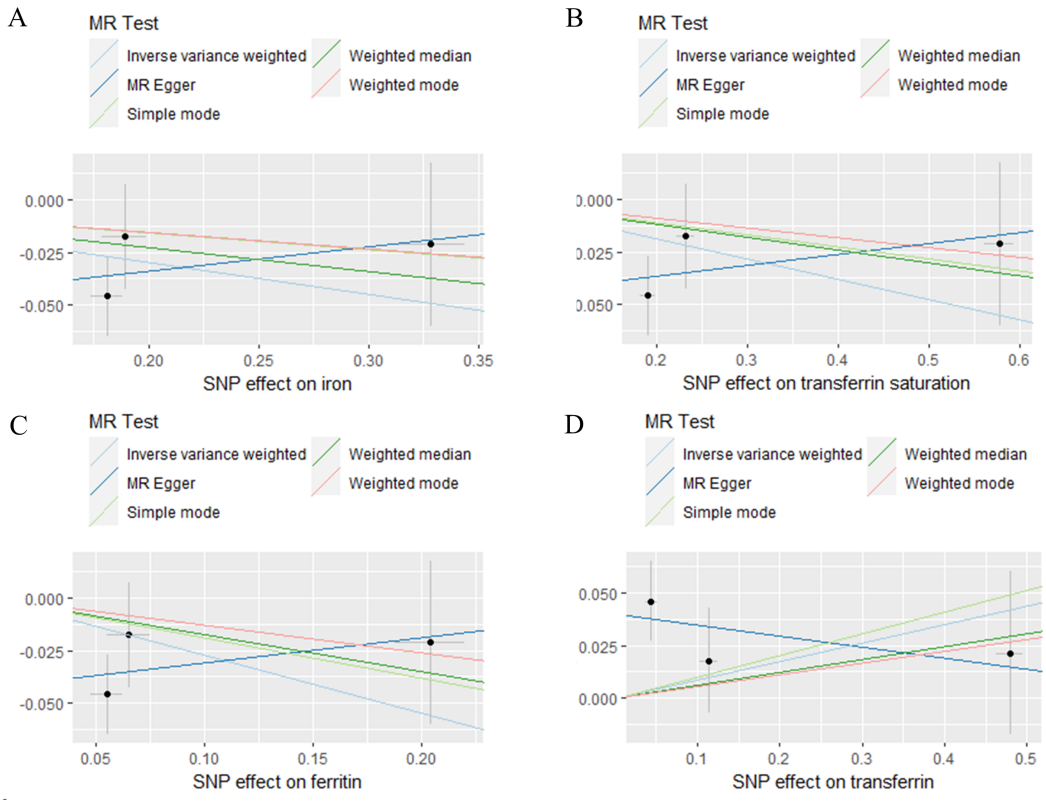

Figure 2

Scatter plots for MR analyses of the causal effect of serum iron status on lung cancer. (A) iron; (B) transferrin saturation; (C) ferritin; (D) transferrin. The slope of each line corresponds to the estimated MR effect per method.

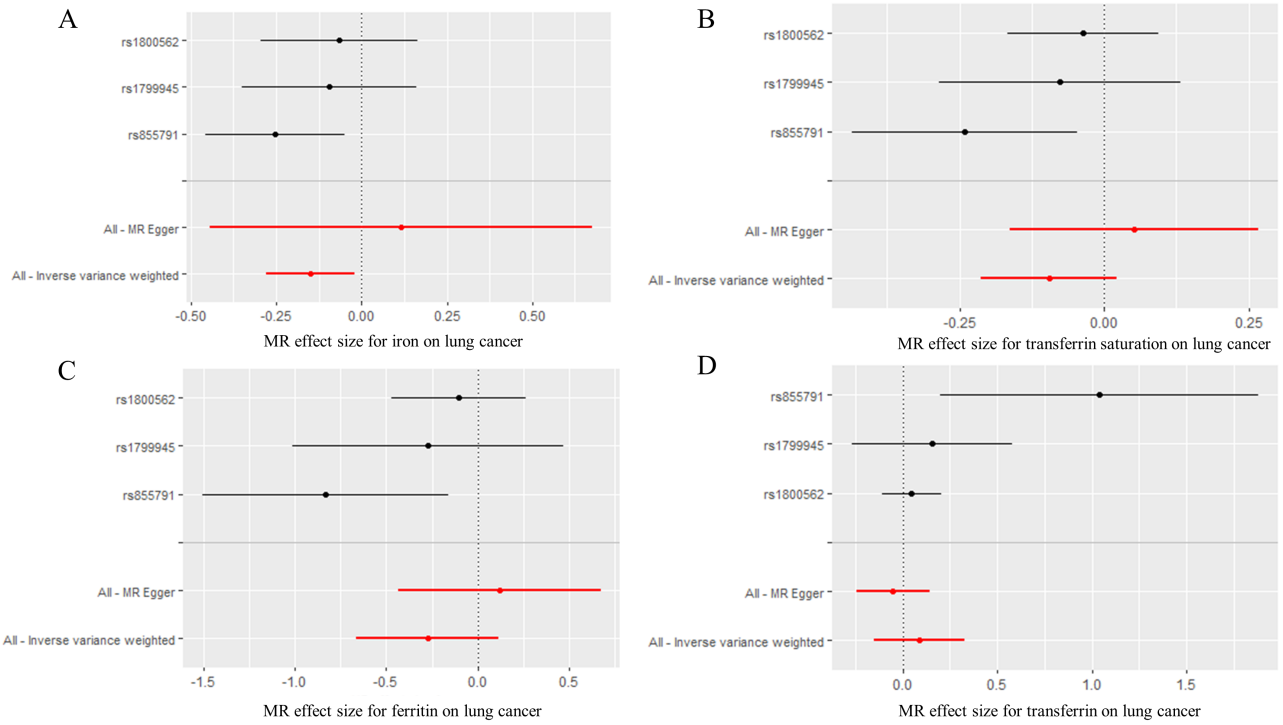


Figure 3

Forest plot of the SNP-specific and pooled MR estimates for the causal effect of each iron status marker on lung cancer risk. (A) iron; (B) transferrin saturation; (C) ferritin; (D) transferrin.