

The association between serum iron status and risk of asthma: a 2-sample Mendelian randomization study in descendants of Europeans

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ABSTRACT

Background: Observational studies present conflicting results about a possible association of iron status with asthma risk, pointing to potential modifiable targets for prevention.

Objective: The aim of this study was to use Mendelian randomization (MR) to estimate associations between iron status and asthma risk.

Methods: We used the Genetics of Iron Status consortium to identify genetic variants that could be used as instrumental variables for the effect of systemic iron status. The following sets of instruments were used: a conservative set (instruments restricted to variants with concordant relations to 4 iron status biomarkers) and a liberal set (instruments selected using variants associated with at least 1 of 4 iron status biomarkers). Associations of these genetic variants with asthma risk were estimated in data from the Trans-National Asthma Genetics Consortium (TAGC) and the GABRIEL consortium (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community). Data on the association of genetic variants with iron status and with asthma were combined to assess the influence of iron status on asthma risk.

Results: In the conservative approach, the MR OR of asthma was 1.00 (95% CI: 0.91, 1.10) per SD increase in iron, 0.96 (95% CI: 0.78, 1.18) in log-transformed ferritin, 0.99 (95% CI: 0.93, 1.06) in transferrin saturation, and 1.03 (95% CI: 0.93, 1.14) in transferrin in the TAGC dataset (none of the values were statistically significant). An age at onset-stratified analysis in the GABRIEL dataset suggested no effect of iron status in childhood onset, later onset, or unknown age at onset asthma. Findings from the liberal approach were similar, and the results persisted in sensitivity analyses (all $P > 0.05$).

Conclusions: This MR study does not provide evidence of an effect of iron status on asthma, suggesting that efforts to change iron concentrations will probably not result in decreased risk of asthma. *Am J Clin Nutr* 2019;00:1–10.

Keywords: iron, ferritin, transferrin saturation, transferrin, asthma, Mendelian randomization

Introduction

Oxidative stress and inflammatory responses appear to play major roles in the pathogenesis of asthma (1). Iron, an important trace element that plays key roles in diverse biological processes, such as enzyme activity regulation, oxygen transport, and immune function (2), may influence the development of asthma. In an animal model, iron administration was indicated to inhibit airway hyperreactivity and eosinophilia, suggesting that higher iron status might be protective against asthma (3). In another animal experiment, treating with iron-chelating complex attenuated asthma-associated inflammatory responses (4). In some observational studies, increases in iron status were positively associated with asthma across different ethnicities (5, 6), whereas some other studies suggested that higher iron concentrations were inversely associated with asthma (7, 8). Altogether, these data suggested a controversial association between iron status and

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Supplemental Figures 1–6 and Supplemental Tables 1 and 2 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations used: *ARNTL*, aryl hydrocarbon receptor nuclear translocator like; CEU, Utah residents with Northern and European ancestry; *FADS2*, fatty acid desaturase 2; GABRIEL, Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community; GIANT, Genetic Investigation of Anthropometric Traits; GIS, Genetics of Iron Status; GWAS, genome-wide association study; IV, instrumental variable; IVW, inverse variance weighted; MR, Mendelian randomization; MR-Egger, Mendelian randomization–Egger regression method; *NAT2*, *N*-acetyltransferase 2; SNP, single nucleotide polymorphism; TAGC, Trans-National Asthma Genetics Consortium.

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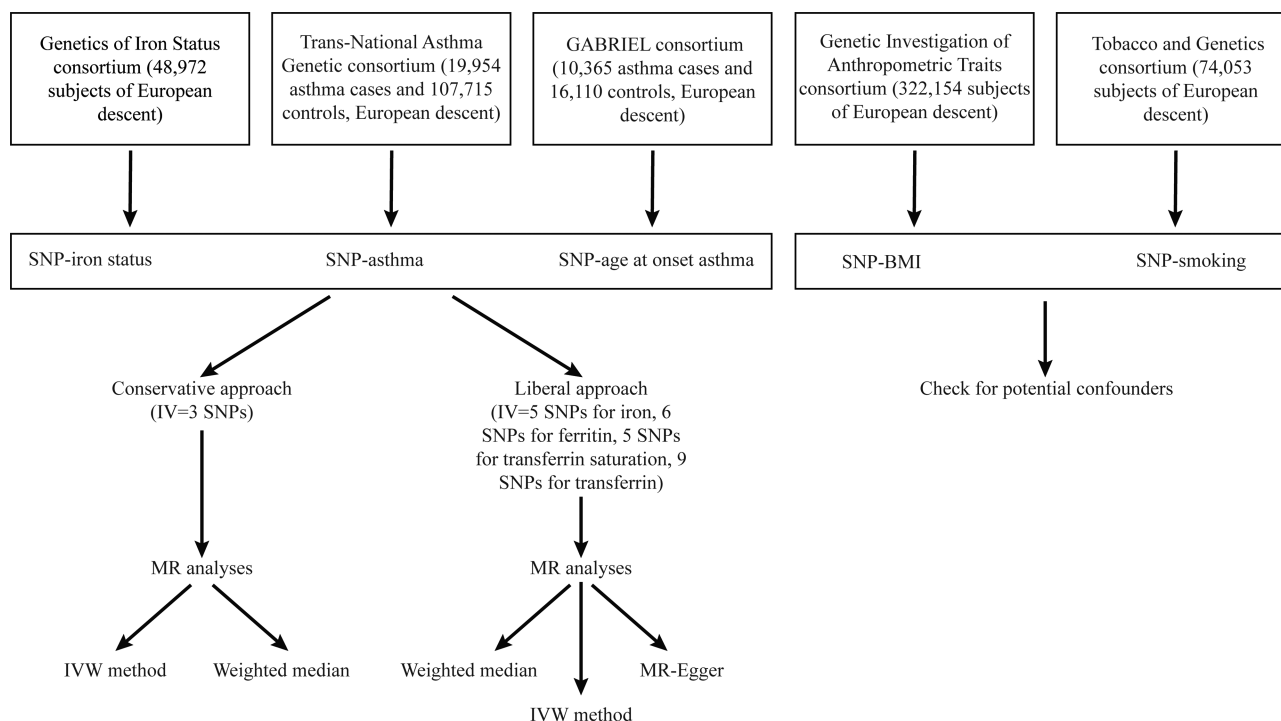


FIGURE 1 Participant flow chart and analysis plan using MR. Summary statistics from SNP phenotypes were obtained from GWAS consortium datasets. The effect of iron status on asthma was estimated using a conservative approach (IVs: only SNPs associated with increased concentrations of serum iron and ferritin, increased transferrin saturation, and decreased concentrations of transferrin) and a liberal approach (instrumental variables: SNPs associated with at least 1 of 4 systemic iron status biomarkers). For the conservative approach, only the IVW and weighted median method were used. For the liberal approach, the IVW, weighted median, and MR-Egger methods were used. GABRIEL, Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community; GWAS, genome-wide association study; IVW, inverse variance weighted; MR, Mendelian randomization; MR-Egger, Mendelian randomization–Egger regression method; SNP, single nucleotide polymorphism.

risk of asthma. If iron status were a risk factor for asthma, it would be useful for public health because disruption of iron status is correctable. Iron deficiency is a treatable disorder that affects up to 2 billion people worldwide (9) and is correctable using iron supplementation. Additionally, some high iron status may be a promising target for asthma prevention and therapy, robust evidence of involvement in the role of asthma is first required.

The limitation of observational epidemiological studies is that causality cannot be confirmed, because the conclusions could have been influenced by confounders or reverse causality leading to overestimation of the risk estimate. These problems can be largely solved in Mendelian randomization (MR) studies by using genetic variants as instrumental variables for an exposure of interest (10). Since genetic variants are expected to be randomly allocated at conception, the MR approach is not as vulnerable to confounding. Moreover, because genetic variants are always allocated preceding the onset of disease, this approach is also free from reverse causality bias. Hence, MR studies can substantially improve causal inference from observational investigations and are conceptually similar to randomized controlled trials. To our knowledge, MR studies of the relationship between iron status and asthma have not been conducted to date.

In the present study, we applied MR design to investigate whether iron status is associated with the development of asthma

by using publicly available summary statistics from large-scale genome-wide association studies (GWAS).

Methods

We performed a 2-sample MR study using summary-level data from GWAS consortia studies (Figure 1). Ethical approval had been obtained previously for our original research. Single nucleotide polymorphisms (SNPs), previously proposed to be associated with systemic iron status, were used as instrumental variables to investigate the effect of iron status on asthma risk. The effect of iron status on asthma risk was estimated by combining the data for the association of SNPs with systemic iron status and asthma. In order to investigate the presence of potential bias or mediation of the effect of iron status on asthma through other asthma risk factors, the associations between the iron status-related SNPs and asthma risk factors were analyzed.

Data sources

Summary data on the association between SNPs and the phenotypes of interest were extracted from the public databases of the following consortia (Figure 1): Genetics of Iron Status (GIS) consortium for iron status (11), Trans-National Asthma Genetics Consortium (TAGC) for asthma (12), GABRIEL consortium (A Multidisciplinary Study to Identify the Genetic and

Environmental Causes of Asthma in the European Community) for age at onset–stratified analysis (asthma onset in childhood or later or unknown age at onset) (13), Genetic Investigation of Anthropometric Traits (GIANT) consortium for BMI (up to 322,154 individuals of European ancestry) (14), and the Tobacco and Genetics consortium for tobacco smoking (up to 74,053 individuals of European ancestry) (15).

The GIS consortium performed the largest GWAS meta-analysis on serum iron, ferritin, transferrin saturation, and transferrin phenotypes to date, which comprised 48,972 subjects of European descent from 19 cohorts (11). Adjustments were made for age, principal component scores, and other study-specific covariates. The cohorts included in the GIS consortium had a mean $\pm$ SD age ranging from 14.8 ± 2.2 to 68.2 ± 15.5 y. The proportion of male subjects was 46.9%. Details of the cohort information have been described previously (11), and are available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4215164/bin/NIHMS619770-supplement-1.pdf>.

The TAGC conducted a GWAS meta-analysis of asthma with ethnically diverse populations using 23,948 cases and 118,538 controls from 66 studies (12). In the present MR study, we drew on the GWAS data of the subsample of data from participants with European ancestry, which consisted of 19,954 asthma cases and 107,715 controls from 56 studies. Descriptions of the participants are available online at https://static-content.springer.com/esm/art%3A10.1038%2F41588-017-0014-7/MediaObjects/41588_2017_14_MOESM1_ESM.pdf. The definition of asthma was based on doctors' diagnoses and/or standardized questionnaires.

Specifically, TAGC includes data from the GABRIEL consortium. The meta-analysis on asthma from the GABRIEL consortium consisted of 10,365 cases and 16,110 controls from 23 different studies of populations of European descent (13). Asthma was considered present if diagnosed by a physician. Childhood-onset asthma was defined as asthma onset before age 16 y, and later-onset asthma was defined as disease that started at age 16 y or older. Other subgroups were made up of cases of unknown age of onset, occupational asthma, or severe asthma. The proportion of male subjects was 54.0%. Descriptions of the participants are available online at https://www.nejm.org/doi/suppl/10.1056/NEJMoa0906312/suppl_file/nejmoa0906312_appendix.pdf. The available summary statistics allowed us to further conduct an age at onset–stratified analysis in the GABRIEL consortium, with separate analyses for childhood onset (6783 cases and 7720 controls), later onset (1947 cases and 3669 controls), and unknown age at onset asthma (1635 cases and 4721 controls).

Instrumental variables selection

The SNPs for instrumental variables analyses were obtained from the SNPs strongly associated with iron status in a GWAS meta-analysis from GIS ($P < 5 \times 10^{-8}$) (Table 1) (11). All of the SNPs were in linkage equilibrium (all pairwise $r^2 \leq 0.01$). The SNP rs1799945 could not be found in the TAGC and GABRIEL asthma dataset and was replaced by a proxy SNP in high linkage disequilibrium [rs129128 with $r^2 = 0.91$ in the Utah residents with Northern and Western European ancestry (CEU) population]. Five SNPs (rs8177240, rs411988, rs651007, rs4921915, and rs174577) could not be found in the GABRIEL asthma dataset and were replaced by proxy SNPs (rs3811647

with $r^2 = 0.96$, rs368243 with $r^2 = 0.98$, rs495828 with $r^2 = 1.00$, rs1495741 with $r^2 = 1.00$, and rs174576 with $r^2 = 1.00$ in the CEU population, respectively). Specifically, rs7385804 was not selected for stratified analysis, since genetic association estimates for the rs7385804 were not available for the GABRIEL asthma dataset, and neither was an appropriate proxy with high-linkage disequilibrium available.

We used the following 2 sets of instruments (Table 1): 1) Conservative instrumental variable analyses, in which only SNPs associated with increased concentrations of serum iron and ferritin, increased transferrin saturation, and decreased concentrations of transferrin ($P < 5 \times 10^{-8}$) were considered eligible ($n = 3$ SNPs). Because increased systemic iron status was associated with increased concentrations of serum iron and ferritin, increased transferrin saturation, and decreased concentrations of transferrin (16), genetic instrumental variables for iron status should therefore be expected to have a concordant association with each of these 4 markers. 2) Liberal instrumental variable analyses, in which SNPs associated with at least 1 of 4 systemic iron status biomarkers (serum iron, ferritin, transferrin saturation, and transferrin) at genome-wide significance were included ($P < 5 \times 10^{-8}$). This identified 5 SNPs, 6 SNPs, 5 SNPs, and 9 SNPs for iron, ferritin, transferrin saturation, and transferrin for use as instruments, respectively.

Validation of instrumental variables

To assess the validity of SNPs as instrumental variables, the following core instrumental variable assumptions were tested (17): strong association with the exposure (iron status), independent of confounding of the exposure–outcome association, and affects the outcome (asthma) via only the exposure (iron status) and not through any other pathway (Figure 2).

To limit the influence of possible weak instrumental variable bias, an F statistic above 10 was expected to be of sufficient strength for the main study (18). To minimize the possibility of bias owing to population stratification, the analyses were restricted to individuals of European descent. We addressed the issues of pleiotropy by using the following different strategies: 1) The associations of SNPs with known asthma risk factors were assessed. 2) The conservative and the liberal approaches were compared. The estimate using the conservative approach was regarded as the primary analysis, and that using the liberal approach as the secondary analysis. 3) The Mendelian randomization–Egger regression method (MR-Egger), which accounts for unknown directional pleiotropy, was used for MR analyses.

MR estimates

For both liberal and conservative approaches, the main MR estimates for the influence of each measure of iron status on asthma risk were conducted using the inverse variance–weighted (IVW) method for the data in the TAGC consortium and GABRIEL consortium, respectively (19). The MR analyses were further conducted on an age at onset–stratified sample in the GABRIEL consortium, which was performed separately for asthma of childhood onset, later onset, and unknown age at onset.

TABLE 1 Characteristics of SNPs selected for each analytical approach¹

Association with iron status (<i>n</i> = 48,972)																								
SNP	Chr	Closest gene	EA	EAF	C3	Iron (μmol/L)			Log10 ferritin (μg/L)			Transferrin saturation (%)			Transferrin (g/L)									
						β	SE	P	β	SE	P	β	SE	P	β	SE	P							
rs1800562	6	HFE	A	0.07	✓	0.328	0.016	2.72 × 10 ⁻⁹⁷	0.204	0.016	1.54 × 10 ⁻³⁸	0.577	0.016	2.19 × 10 ⁻²⁷⁰	-0.479	0.016	8.90 × 10 ⁻¹⁹⁶							
rs1799945	6	HFE	G	0.15	✓	0.189	0.010	1.10 × 10 ⁻⁸¹	0.065	0.010	1.71 × 10 ⁻¹⁰	0.231	0.010	5.13 × 10 ⁻¹⁰⁹	-0.114	0.010	9.36 × 10 ⁻³⁰							
rs855791	22	TMPRSS6	G	0.55	✓	0.181	0.007	1.32 × 10 ⁻¹³⁹	0.055	0.007	1.38 × 10 ⁻¹⁴	0.190	0.008	6.41 × 10 ⁻¹³⁷	-0.044	0.007	1.98 × 10 ⁻⁹							
rs8177240	3	TF	G/T ²	0.33/0.67		0.066	0.007	6.65 × 10 ⁻²⁰				0.100	0.008	7.24 × 10 ⁻³⁸	-0.380	0.007	8.43 × 10 ⁻⁶¹⁰							
rs7385804	7	TFR2	A	0.62		0.064	0.007	1.36 × 10 ⁻¹⁸				0.054	0.008	6.07 × 10 ⁻¹²										
rs411988	17	TEX14	G	0.44					0.044	0.007	1.59 × 10 ⁻¹⁰						1.35 × 10 ⁻¹¹							
rs744653	2	WDR75- SLC40A1	C	0.15					0.089	0.010	8.37 × 10 ⁻¹⁹				-0.068	0.010								
rs651007	9	ABO	C	0.80					0.050	0.009	1.31 × 10 ⁻⁸													
rs990333	3	TFR2	T	0.46											-0.051	0.007	1.95 × 10 ⁻¹³							
rs4921915	8	NAT2	G	0.22											-0.079	0.009	7.05 × 10 ⁻¹⁹							
rs6486121	11	ARNTL	T	0.63											-0.046	0.007	3.89 × 10 ⁻¹⁰							
rs174577	11	FADS2	C	0.67											-0.062	0.007	2.28 × 10 ⁻¹⁷							

¹β indicates the per-allele effect on the SD units of the iron marker. P values indicate genome-wide significance in the joint analysis; ABO, blood group system ABO; ARNTL, aryl hydrocarbon receptor nuclear translocator like; C3, the 3 SNPs used in the conservative analyses; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; FADS2, fatty acid desaturase 2; HFE, homeostatic iron regulator; NAT2, N-acetyltransferase 2; SNP, single nucleotide polymorphism; TEX14, testis expressed 14; TF, transferrin; TFR2, transferrin receptor; TFR2, transferrin receptor 2; TMPRSS6, transmembrane serine protease 6; TEWDR75-SLC40A1, WD repeat domain 75, and solute carrier family 40 member; ✓, the SNP selected for C3.

²G is the effect allele for iron (associated with higher iron status) and T is the effect allele for transferrin saturation and transferrin (associated with higher transferrin saturation status and lower transferrin status).

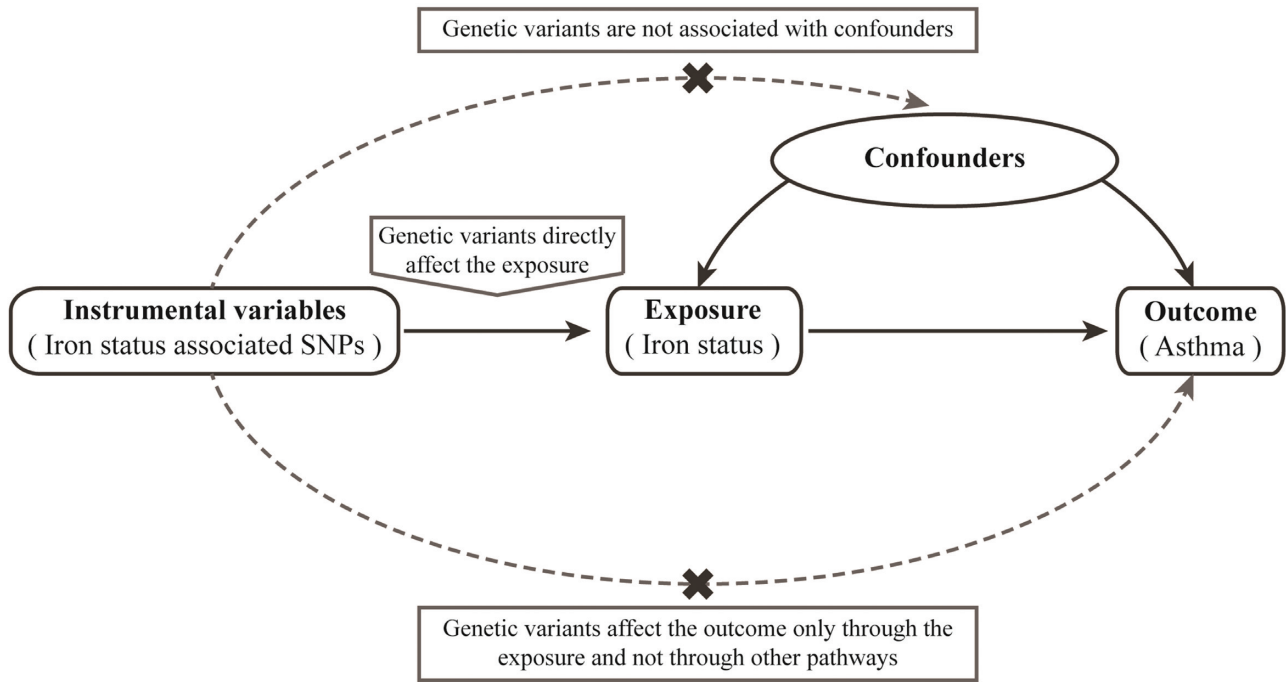


FIGURE 2 Diagram of the instrumental variable assumptions for the Mendelian randomization study. SNP, single nucleotide polymorphism

For the liberal approach, we also used the IVW method to evaluate the combined effect of iron status on asthma risk factors (obesity and smoking), using the publicly available GWAS data of the GIANT consortium and the Tobacco and Genetics consortium (Figure 1). Given the limited publicly available summarized data, the associations between instrumental variables, and other potential confounders such as drinking and exercise, were difficult to assess. An online database of Ensembl (http://grch37.ensembl.org/Homo_sapiens/Info/Index) was further used to search for secondary phenotypes associated with the selected instrument SNP and to exclude possible pleiotropic effects by manually removing these SNPs from the MR analyses.

Sensitivity analyses

Meta-analytic methods including forest plot of per SNP ratio estimate, Cochran's Q statistic, and I^2 values were applied to evaluate heterogeneity in IVW estimates (20–22). Moreover, in order to identify overly influential SNPs, leave-1-out analyses were conducted by removing 1 SNP in turn and recalculating the overall instrumental variable estimates. In addition, we conducted weighted median and MR-Egger statistical sensitivity analyses, which were more robust to the inclusion of pleiotropic instruments. The weighted median method provides accurate estimates if more than half of the instrumental variables are valid (20). The MR-Egger regression allows the intercept to be freely estimated as an indicator of average pleiotropic bias (23). The MR-Egger method was not applied to conservative sets, since it is not sensible to make inferences based on a straight line fitted to only 3 points (3 SNPs) (24).

Analyses were conducted using R, version 3.4.3.

Results

Association of the genetic instrument with iron status and asthma risk

Table 1 presents the associations with iron status of SNPs used as instrumental variables in the conservative (3 SNPs) and liberal analyses (5 SNPs for iron, 6 SNPs for ferritin, 5 SNPs for transferrin saturation, and 9 SNPs for transferrin). F -statistics of the individual genetic variants ranged from 39 (rs651007, *ABO* gene) to 3340 (rs8177240, *TF* gene), making introducing weak instruments bias unlikely. Table 2 presents the SNP asthma estimates from the meta-analysis of the TAGC and GABRIEL data, respectively.

Association of the genetic instruments with asthma risk factors

None of the individual SNPs was associated with asthma risk factors (BMI and smoking). In general, iron-increasing variants were not associated with asthma risk factors in the conservative and liberal approaches (all $P > 0.002$, Table 3).

Effect of iron status on asthma risk

Figure 3 presents the results of all MR analyses estimating the association of genetically predicted iron status with asthma risk. The results were presented as ORs for asthma per SD increase in each iron status biomarker. Using the conservative approach, we observed no association between the 4 genetically predicted markers of iron status and asthma in the TAGC dataset (for iron, IVW OR: 1.00, 95% CI: 0.91, 1.10, $P = 0.97$; for log-transformed ferritin, OR: 0.96, 95% CI: 0.78, 1.18, $P = 0.68$; for

TABLE 2 Characteristics of included SNPs associated with asthma¹

SNP	EA	TAGC ²			GABRIEL ³											
		β	SE	P	Total sample (n = 10,365 cases and 16,110 controls)			Childhood onset (n = 6783 cases and 7720 controls)			Later onset (n = 1947 cases and 3669 controls)			Unknown age at onset (n = 1635 cases and 4721 controls)		
					β	SE	P	β	SE	P	β	SE	P	β	SE	P
rs1800562	A	-0.033	0.027	0.21	-0.012	0.044	0.78	-0.028	0.060	0.64	-0.002	0.099	0.99	0.010	0.083	0.90
rs1799945 ⁴	G	0.034	0.018	0.06	0.044	0.028	0.12	0.090	0.038	0.02	-0.062	0.059	0.30	0.038	0.061	0.54
rs855791	G	-0.003	0.013	0.79	0.004	0.020	0.84	0.017	0.027	0.54	0.000	0.042	1.00	-0.023	0.043	0.59
rs8177240 ⁵	G	0.001	0.014	0.95	0.002	0.021	0.93	-0.003	0.028	0.91	-0.014	0.044	0.76	0.030	0.045	0.50
rs7385804 ⁶	A	-0.015	0.014	0.26												
rs411988 ⁷	G	0.003	0.017	0.84	0.003	0.020	0.87	0.005	0.027	0.84	0.023	0.042	0.58	-0.011	0.042	0.80
rs744653	C	-0.027	0.018	0.14	-0.036	0.029	0.21	-0.054	0.039	0.17	-0.025	0.061	0.69	-0.004	0.061	0.95
rs651007 ⁸	C	-0.047	0.016	0.00	-0.026	0.023	0.26	-0.060	0.031	0.05	-0.016	0.047	0.73	0.056	0.052	0.28
rs9990333	T	-0.007	0.013	0.60	-0.025	0.020	0.21	-0.037	0.027	0.17	-0.005	0.042	0.91	-0.018	0.043	0.67
rs4921915 ⁹	G	-0.001	0.016	0.96	-0.017	0.024	0.48	-0.015	0.032	0.64	-0.047	0.050	0.34	0.020	0.085	0.82
rs6486121	T	-0.007	0.013	0.57	-0.010	0.021	0.63	-0.005	0.028	0.85	-0.077	0.042	0.07	0.052	0.044	0.24
rs174577 ¹⁰	C	0.060	0.014	1.15×10^{-5}	0.043	0.021	0.04	0.020	0.029	0.48	0.050	0.029	0.08	0.074	0.046	0.10

¹ β indicates log odds ratio of asthma for each additional iron-increasing allele; *P* values indicate genome-wide significance in the joint analysis. EA, effect allele; GABRIEL, Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community; SNP, single nucleotide polymorphism; TAGC, Trans-National Asthma Genetics Consortium.

²Results are derived from the TAGC asthma consortium.

³Results are derived from the GABRIEL asthma consortium.

⁴Proxy (rs129128; $r^2 = 0.91$ and $D' = 1.00$ in European descent individuals) was used in the TAGC and GABRIEL asthma data.

⁵Proxy (rs3811647; $r^2 = 0.96$ and $D' = 1.00$ in European descent individuals) was used in the GABRIEL asthma data.

⁶Genetic association estimates for the rs7385804 were not available for the GABRIEL asthma data, and neither was a suitable proxy available.

⁷Proxy (rs368243; $r^2 = 0.98$ and $D' = 1.00$ in European descent individuals) was used in the GABRIEL asthma data.

⁸Proxy (rs495828; $r^2 = 1.00$ and $D' = 1.00$ in European descent individuals) was used in the GABRIEL asthma data.

⁹Proxy (rs1495741; $r^2 = 1.00$ and $D' = 1.00$ in European descent individuals) was used in the GABRIEL asthma data.

¹⁰Proxy (rs174576; $r^2 = 1.00$ and $D' = 1.00$ in European descent individuals) was used in the GABRIEL asthma data.

transferrin saturation, OR: 0.99, 95% CI: 0.93, 1.06, $P = 0.82$; for transferrin, OR: 1.03, 95% CI: 0.93, 1.14, $P = 0.55$) or in the GABRIEL dataset (for iron, IVW OR: 1.06, 95% CI: 0.91, 1.22, $P = 0.46$; for log-transformed ferritin, OR: 1.08, 95% CI: 0.78, 1.51, $P = 0.64$; for transferrin saturation, OR: 1.03, 95% CI: 0.93, 1.15, $P = 0.56$; for transferrin, OR: 0.98, 95% CI: 0.83, 1.15, $P = 0.77$). The age at onset-stratified MR analysis in the GABRIEL dataset suggested no effect of iron status on asthma with childhood onset, later onset, or unknown age at onset (all $P > 0.05$). Using the liberal approach, the results were similar (all $P > 0.05$).

Sensitivity analyses

Results for weighted median estimators used with the liberal instrument were consistent with the results for the IVW method in the TAGC and GABRIEL datasets (all $P > 0.05$). With the use of the MR-Egger method with the liberal instrument, no evidence of directional pleiotropy was observed in the TAGC dataset (intercept -0.003 , 0.000 , -0.001 , and -0.009 , respectively; all $P > 0.05$) and in the GABRIEL dataset (intercept 0.007 , 0.005 , 0.009 , and 0.001 , respectively; all $P > 0.05$) (**Supplementary Figure 1**). The MR-Egger analyses also did not provide evidence of a significant association (all $P > 0.05$) (**Figure 3**). The results of age at onset-stratified analysis in the GABRIEL dataset were similar to results from the total sample models (all $P > 0.05$) (**Figure 3**).

There was heterogeneity in IVW estimates among the SNPs from the liberal approach in the TAGC dataset for log-transformed ferritin ($I^2 = 61.4\%$; $P = 0.024$) and transferrin

($I^2 = 70.7\%$; $P = 0.001$) (**Supplementary Figure 2**). However, the leave-1-out analyses did not materially change the MR estimate, although the direction of estimates varied somewhat ($P > 0.05$) (**Supplementary Figures 3, 4, 5** and **6**). In the Ensembl database, we found that rs1800562 was also associated with LDL cholesterol and total cholesterol, rs4921915 was also associated with triglyceride, and rs174577 was also associated with LDL and HDL cholesterol and triglycerides at GWAS significance (**Supplementary Table 1**). Moreover, 3 genes [aryl hydrocarbon receptor nuclear translocator like (*ARNTL*), fatty acid desaturase 2 (*FADS2*), and *N*-acetyltransferase 2 (*NAT2*)] were proposed to be associated with triglycerides. However, removing the 4 SNPs (rs1800562, rs4921915, rs6486121, rs174577) did not change the pattern of results (**Supplementary Figure 3, 4, 5** and **6**).

Discussion

Using summary level data for asthma and iron status from large populations of European descent, the present MR estimates for serum iron, ferritin, transferrin saturation, and transferrin all suggest that there is no association between genetically determined iron status and the risk of asthma. These results provide no rationale for using iron status as a promising target for asthma prevention.

Because MR studies may carry the risk of pleiotropy (25), we investigated this possibility by searching online for secondary effects of the SNPs. Our online search identified 4 SNPs [rs1800562 on homeostatic iron regulator (*HFE*), rs4921915 on *NAT2*, rs6486121 on *ARNTL*, and rs174577 on *FADS2*] for

TABLE 3 *P* values for associations of asthma risk factors with all genetic variants¹

SNP	BMI (kg/m ²) ² <i>P</i>	Cigarettes per day ³ <i>P</i>
rs1800562	0.228	0.444
rs1799945	0.529	0.838
rs855791	0.396	0.799
rs8177240	0.745	0.860
rs7385804	0.240	0.452
rs411988	0.402	0.343
rs744653	0.544	0.602
rs651007	0.775	0.103
rs9990333	0.079	0.453
rs4921915	0.016	0.085
rs6486121	0.661	0.426
rs174577	0.386	0.712
Conservative variants for iron	0.819	0.863
Conservative variants for ferritin	0.764	0.671
Conservative variants for transferrin saturation	0.893	0.728
Conservative variants for transferrin	0.460	0.552
Liberal variants for iron	0.945	0.968
Liberal variants for ferritin	0.843	0.960
Liberal variants for transferrin saturation	0.739	0.852
Liberal variants for transferrin	0.455	0.855

¹After Bonferroni correction, only *P* values lower than 0.002 (0.05 ÷ 11 SNPs ÷ 2 phenotypes) were considered statistically significant. *P* values lower than 0.05 were considered statistically significant for conservative variants and liberal variants. *P* values for the combined effect of iron status on asthma risk factors were conducted by inverse-variance weighted method. GIANT, Genetic Investigation of Anthropometric Traits; SNP, single nucleotide polymorphism; TAGC, Trans-National Asthma Genetics Consortium.

²*P* value for association between SNP and BMI obtained from the GIANT consortium (*n* = 322,154).

³*P* value for association between SNP and smoking quantity obtained from the Tobacco and Genetics Consortium (*n* = 74,053).

iron status nominally associated with LDL cholesterol, total cholesterol, and/or triglyceride, which are potentially related to asthma risk, as reported by previous studies (26, 27). However, removing these SNPs produced no substantive change in MR estimate results, suggesting that the MR estimates in the present study were not likely expected to be biased by lipid profile.

We further tested the robustness of our findings to potential pleiotropy by relaxing IV selection criteria to increase the number of SNPs available for analysis (liberal instrumental variables). We observed a consistent nonsignificant association between iron status and asthma in consequent sensitivity analyses. The small differences in estimates and CI widths for the effects of the different MR analysis methods might be explained by chance and, possibly, differential measurement errors, rather than indicating distinct estimates. Furthermore, MR-Egger results suggested no bias owing to pleiotropy and did not detect an association. The public GWAS data used for our analyses were restricted to those for subjects of European descent, reducing possible bias owing to population stratification. Moreover, the leave-1-out MR estimates were similar to the main MR estimates. Thus, the overall conclusions of the present study were unlikely to be severely influenced by bias. Additionally, we estimated our hypotheses in 2 datasets (TAGC and GABRIEL) and checked the

consistency of our findings by conducting age of onset-stratified MR analysis in the GABRIEL dataset. This analysis suggested no effect of iron status in asthma of childhood onset, later onset, or unknown age at onset, strengthening the conclusions.

To the best of our knowledge, no previous study has conducted MR analysis to investigate the effect of iron status on asthma. A recent investigation used MR to study maternal iron concentrations during pregnancy and respiratory and atopic outcomes in the offspring (28). These investigators did not find evidence for a causal role of iron status during pregnancy in asthma. However, the observational studies exploring the association between iron and asthma have yielded inconsistent results. For instance, a meta-analysis showed that iron concentration might be a biomarker of asthma risk in specific populations (29). Some studies reported that high concentrations of iron were positively associated with the increased risk of asthma (5, 6). It was postulated that higher iron concentrations might increase reactive oxygen species production and reduce total antioxidant capacity production, further resulting in oxidative/antioxidative imbalance, which might increase the risk of asthma (5). On the other hand, the results of some studies have suggested that higher iron concentrations were inversely associated with asthma (7, 8). The potential biological basis might be that elevated iron concentrations reduce airway hyperreactivity and eosinophilia, which play key roles in the inflammation that occurs with asthma (3). Another study, however, did not confirm significant differences in iron concentrations between patients with asthma and controls (30). The lack of a genetically determined association of asthma with iron status suggests that the previously observed associations might be explained by differences in ethnicity, as well as in sample size. In addition, reverse causality and residual confounding cannot be excluded. Unmeasured or unknown risk factors not taken into account as confounders in these observational studies might have influenced the observed associations between iron and asthma.

It has been suggested that obesity and cigarette smoking might increase asthma risk (31). In addition, iron has been shown to be correlated with obesity and smoking (32, 33). These factors might be strong confounders in observational studies. Several mechanisms have been proposed to support the observed associations between iron status and asthma risk. Evidence from our study provided no support for a role for iron status in the risk of asthma. The findings in observational studies might be attributable to reverse causation, namely, increased or decreased iron status might be a consequence of asthma rather than the cause of asthma (34). Moreover, steroid therapies used in asthma might lead to low iron status. In addition, heterogeneity between the observational studies with regard to the considered confounders was common. Even though most studies adjusted for potential confounders such as age, gender, BMI, drinking habits, smoking habits, and exercise, these might not be sufficient to get unconfounded results. Recognized determinants of asthma (e.g., prenatal and perinatal factors, air pollution, anemias, and stress) were hard to be adjusted for completely (34). Lastly, both iron status and elevated risk of asthma may be the result of a common exposure. For instance, inflammation impacts iron homeostasis with increases in serum ferritin while serum iron decreases (35, 36). Previous research suggested that serum ferritin increased in response to inflammation (34). Respiratory inflammation has been considered to impact the development of asthma (37, 38).

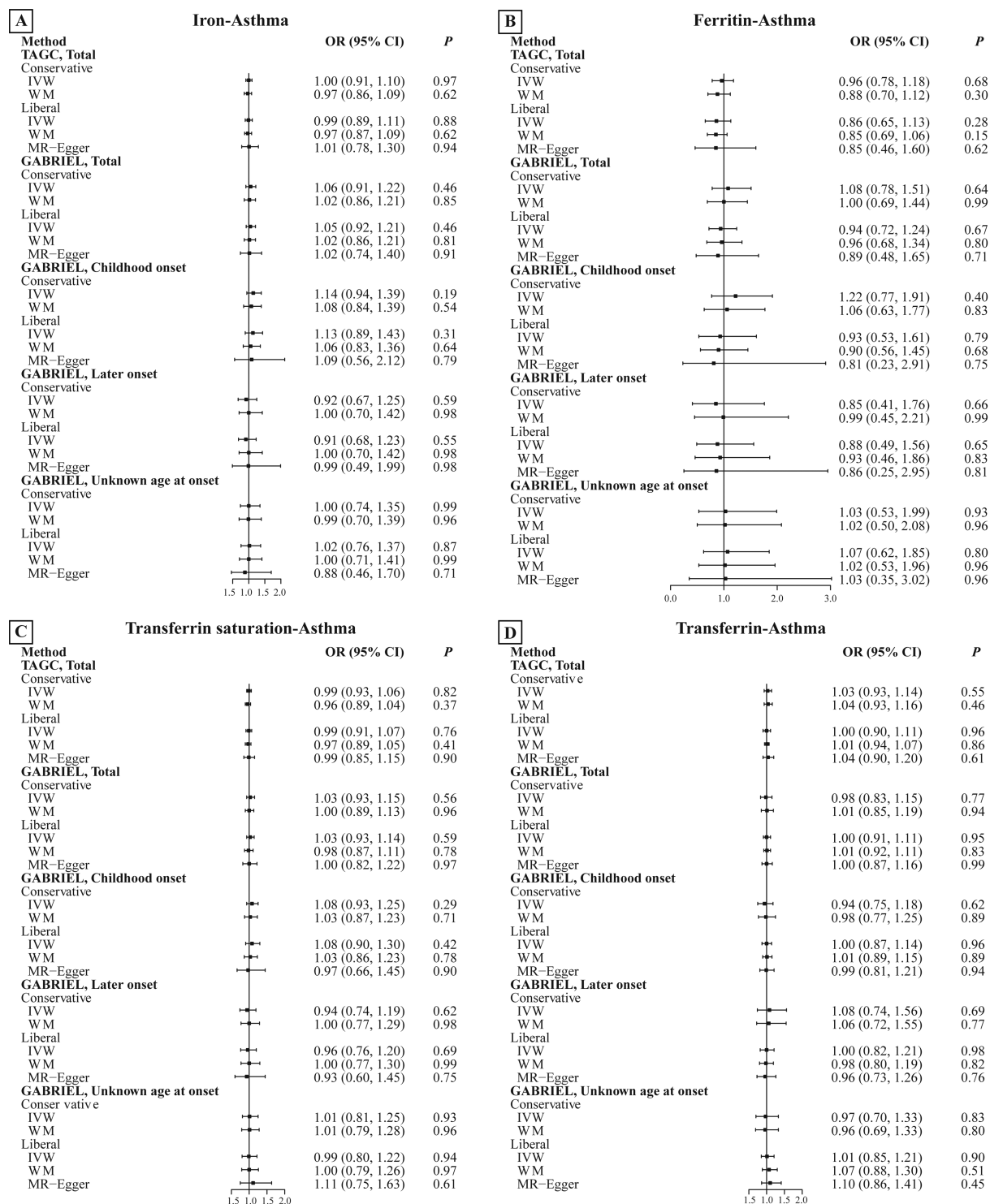


FIGURE 3 The MR estimates of ORs of asthma risk per SD increase in genetically determined iron status markers, (A) for iron; (B) for log-transformed ferritin; (C) for transferrin saturation; and (D) for transferrin using data from the TAGC consortium asthma cases ($n = 19,954$) and controls ($n = 107,715$) and the GABRIEL consortium asthma cases ($n = 10,365$) and controls ($n = 16,110$). The samples for age at onset-stratified analysis in the GABRIEL consortium were 6783 cases and 7720 controls for childhood onset, 1947 cases and 3669 controls for later onset, and 1635 cases and 4721 controls for unknown age at onset asthma. IVW, inverse variance weighted; MR, Mendelian randomization; MR-Egger, Mendelian randomization-Egger regression method; TAGC, Trans-National Asthma Genetics Consortium; WM, weighted median method.

Therefore, inflammation may drive both elevation in iron status and asthma risk. However, this should be elaborated further in future research.

A limitation of using publicly summarized statistics is that it is hard to perform stratified analyses by gender, age, or other categories. Also, we could only evaluate the association between SNPs and key potential confounders (BMI and smoking) in the GIANT and Tobacco and Genetics Consortium datasets due to the limited publicly available GWAS databases. The associations between instruments and other potential confounders such as drinking and exercise were difficult to assess. Nevertheless, several approaches were undertaken to further assess potential confounding in our MR study. Another limitation was the inclusion of liberal instruments, because even though the identification of liberal instruments could offer more power, it might make the study vulnerable to pleiotropy. Although we tried to control for pleiotropy, residual bias may exist because the exact biological function of the SNPs associated with iron status is unknown. Sample size was another possible limitation. Although our study used the large sample size from the GWAS consortium, which enabled us to estimate the associations more precisely than using individual-level data from small studies, the sample size might still be too low to detect the effect of iron status and age at onset-specific asthma (**Supplementary Table 2**). In addition, we could not fully rule out the possibility that age differences between the GIS and TAGC/GABRIEL datasets influenced the null findings. Nevertheless, as the genetically determined effect of iron status on asthma was comparatively lower than that in the observational studies, a possible true effect of iron status on asthma was nearly null and would have little clinical meaning. The null result could also be explained by possible compensatory feedback mechanisms, known as canalization (39). Last, there might be heterogeneity in the asthma diagnosis, since in some GWAS used for the present MR study the asthma was self-reported, while in others the asthma was physician diagnosed.

In conclusion, the results of our MR study provide no support for a role of iron status in the risk of asthma. It is likely that the findings to the contrary of observational studies might be attributable to unmeasured confounding or reverse causality. Efforts to change iron concentrations will probably not result in decreased risk of asthma.

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The authors' responsibilities were as follows—XY and ZM: designed the research; all authors: analyzed the data; LH and LL: wrote the paper; XY: had primary responsibility for final content; and all authors: read and approved the final manuscript.

None of the authors report a conflict of interest.

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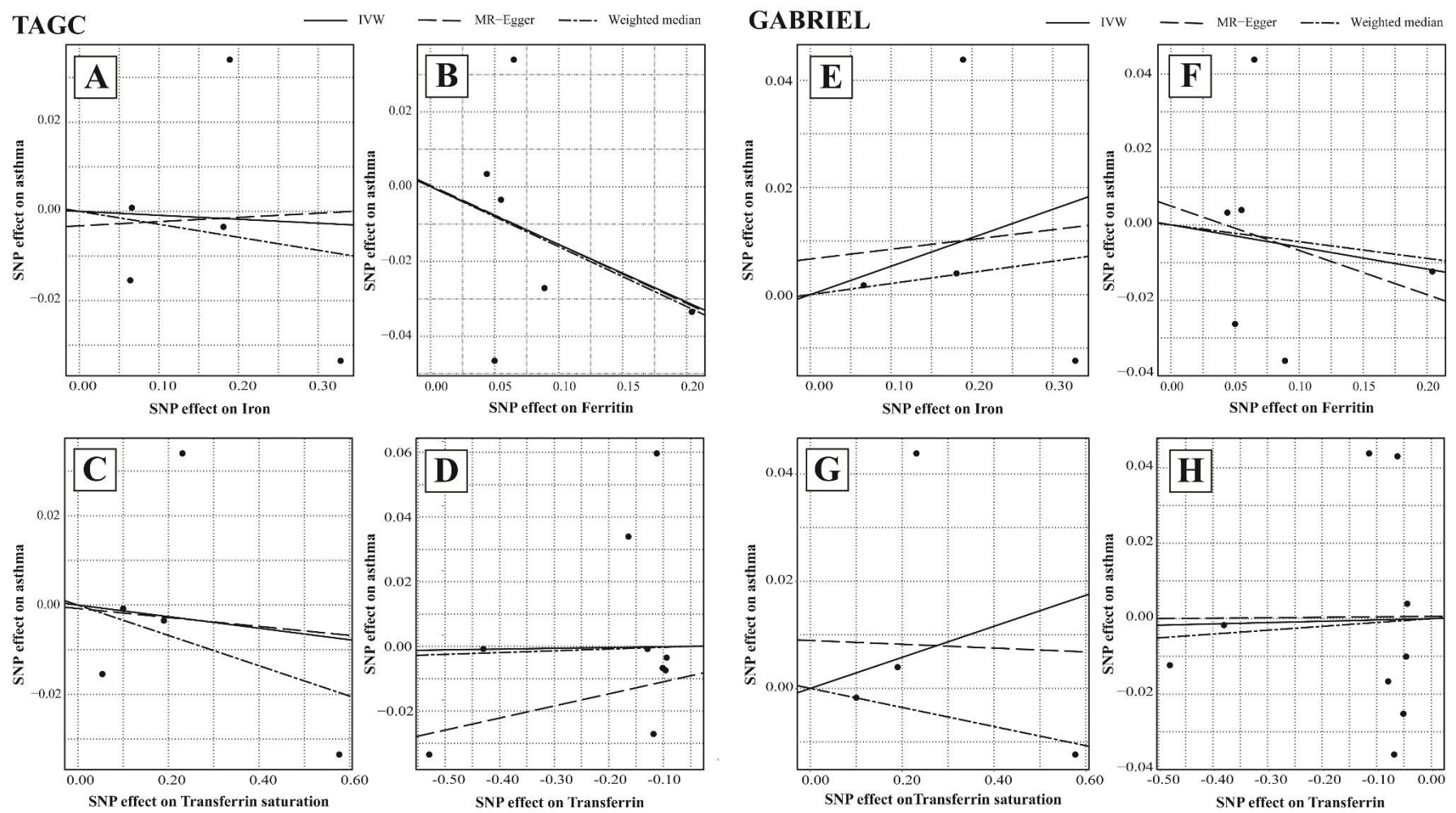
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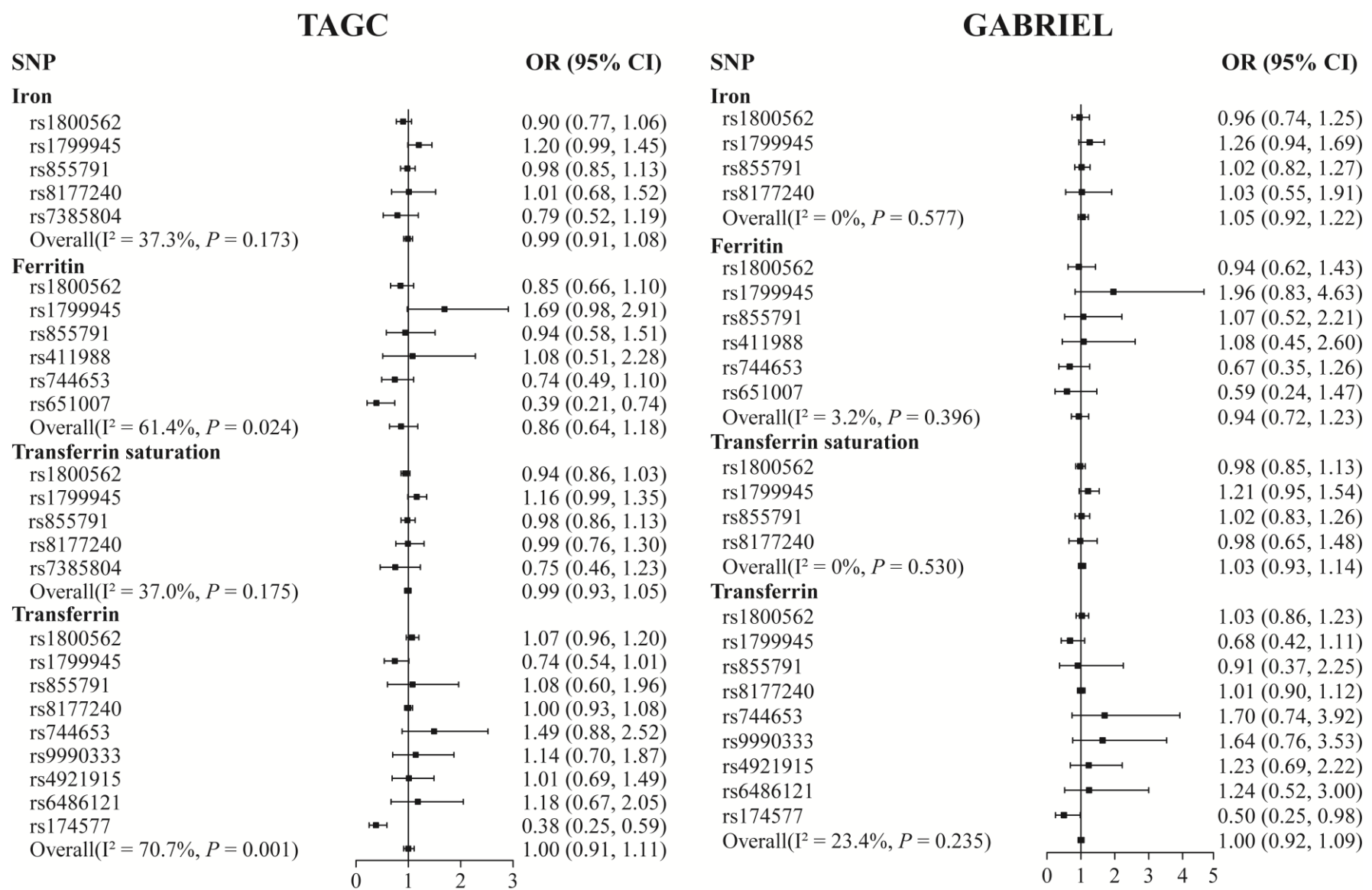
**Supplement to “The effect of serum iron status on asthma risk: a 2-sample
Mendelian randomization study”**

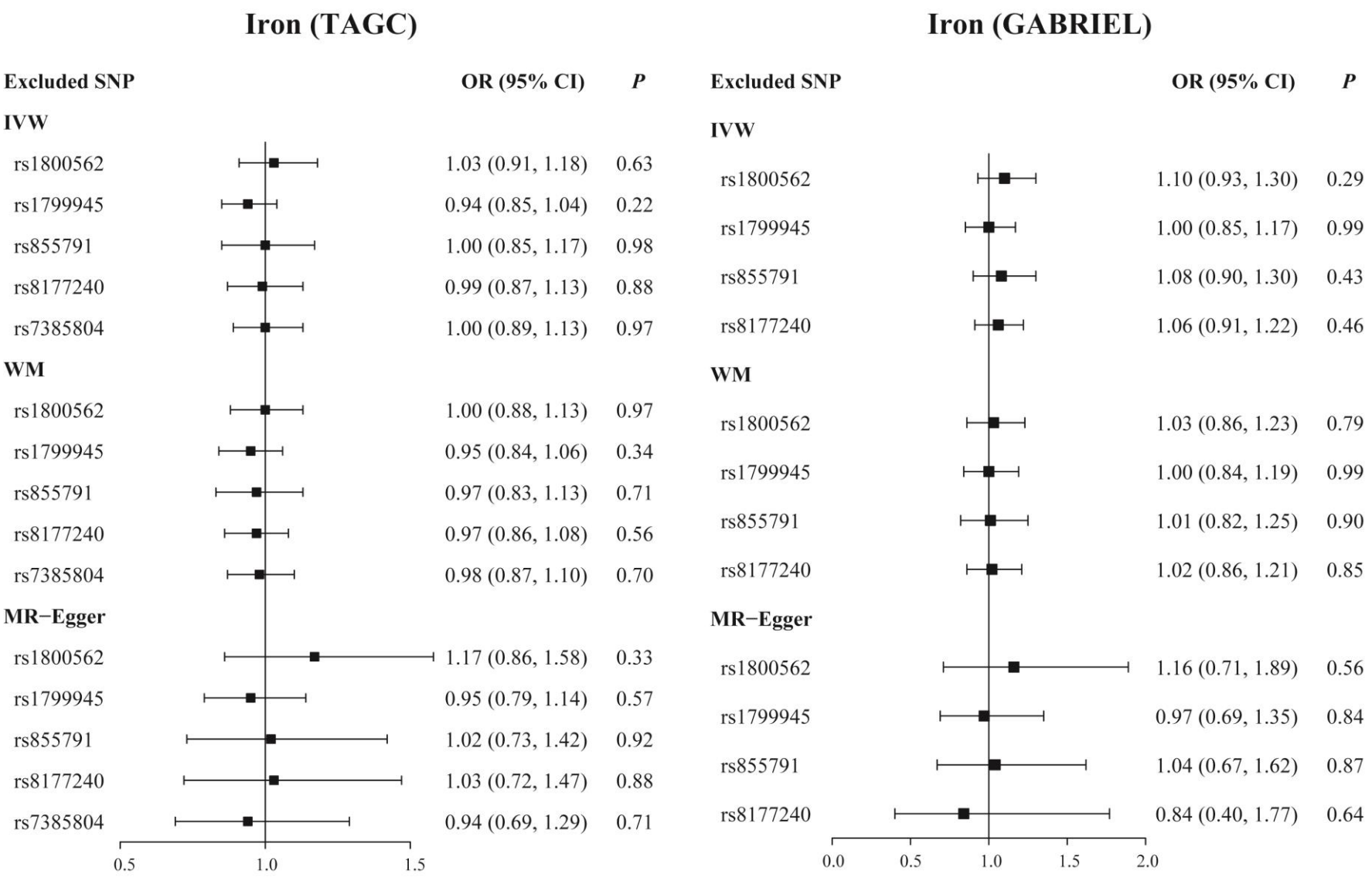
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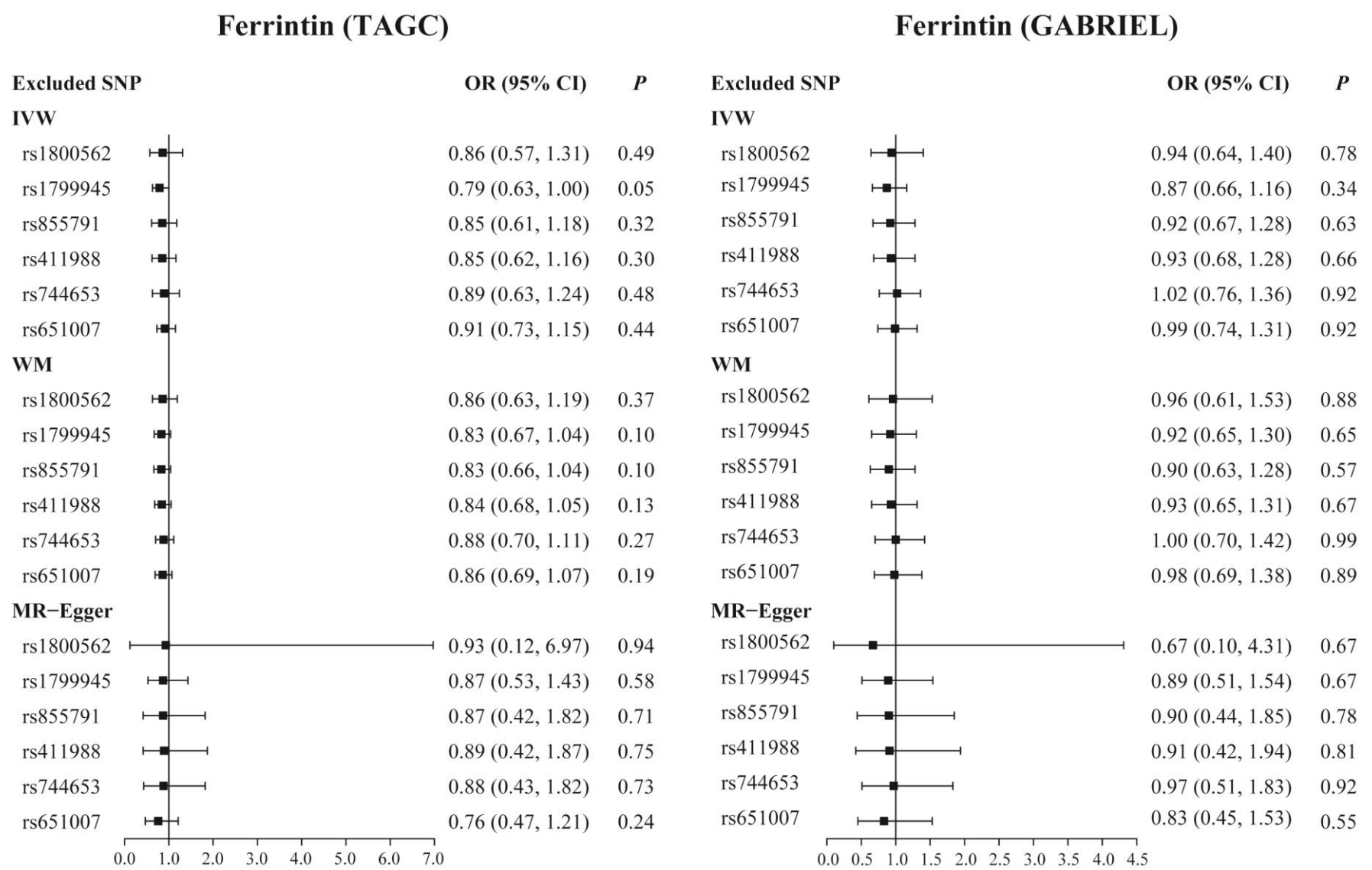


Supplementary Figure 1 Scatterplot of SNP effects on iron vs. their effects on asthma (A, E), log-transformed ferritin vs. asthma (B, F), transferrin saturation vs. asthma (C, G), transferrin vs. asthma (D, H), with the slope of each line corresponding to estimated Mendelian randomization effect per method, using the TAGC consortium asthma case (n = 19,954) - control (n = 107,715) and GABRIEL consortium asthma case (n = 10,365) - control (n = 16,110) study. SNP, single-nucleotide polymorphism; IVW: inverse-variance weighted method; MR-Egger: Mendelian randomization-Egger regression method.

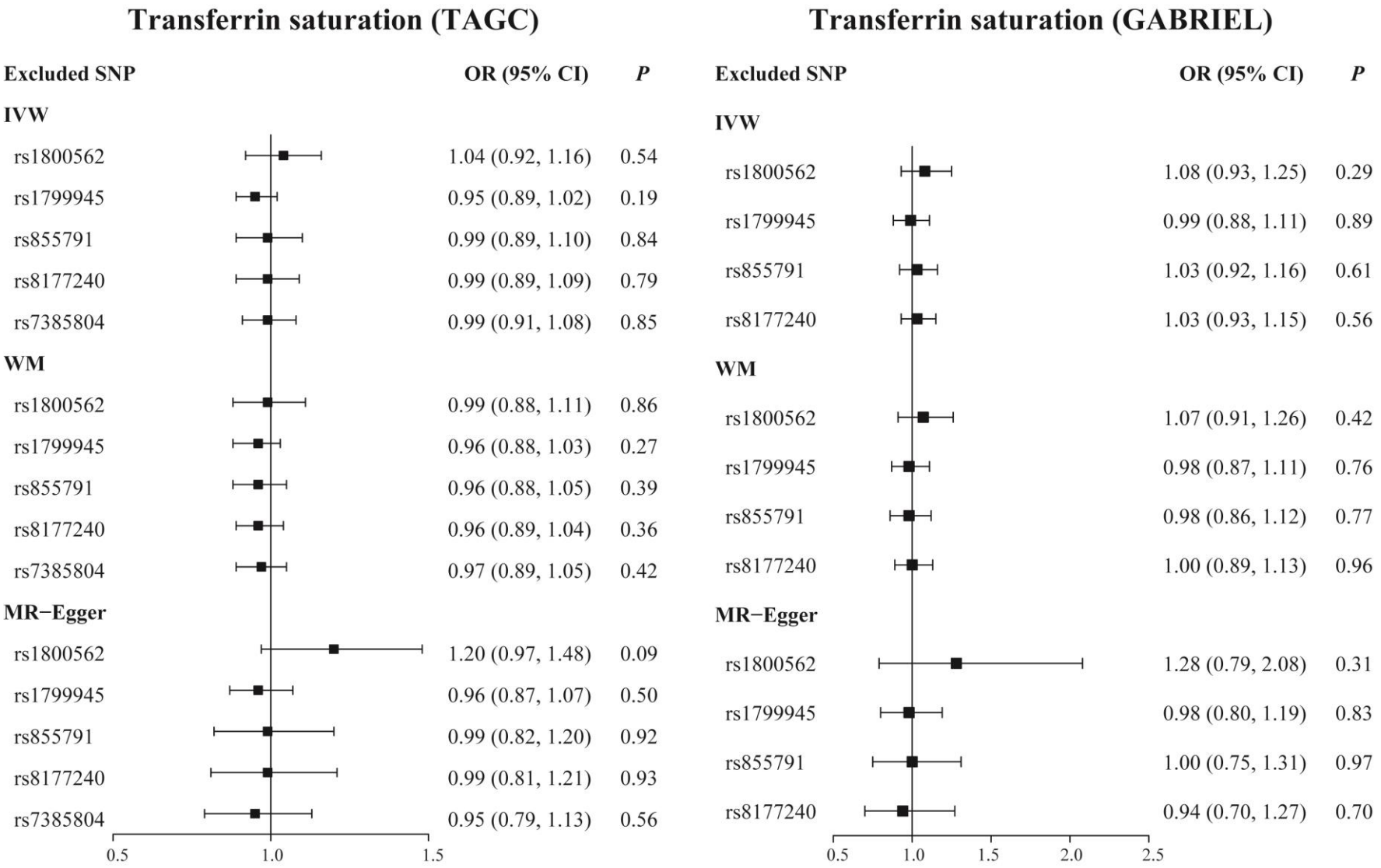




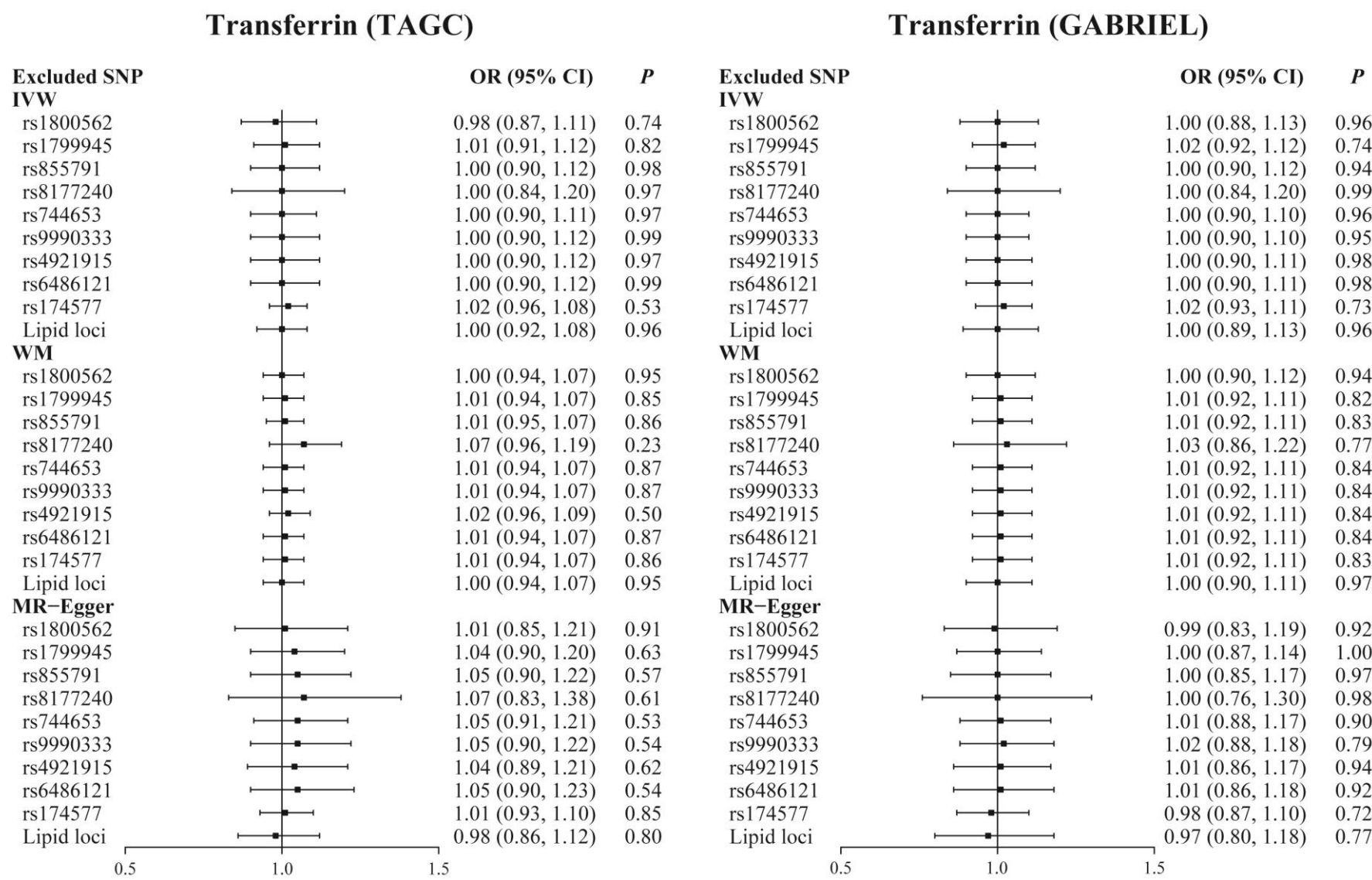
Supplementary Figure 3 Odds ratio of asthma risk per standard deviation increase in iron removing 1 SNP at a time estimated by the IVW, weighted median and MR-Egger methods using the TAGC consortium asthma case (n = 19,954) - control (n = 107,715) and GABRIEL consortium asthma case (n =10,365) - control (n = 16,110) study. SNP: single nucleotide polymorphism; OR: odds ratio; 95% CI, 95% confidence interval; IVW: inverse-variance weighted method; WM: weighted median method; MR-Egger: Mendelian randomization-Egger regression method.



Supplementary Figure 4 Odds ratio of asthma risk per standard deviation increase in log-transformed ferritin removing 1 SNP at a time estimated by the IVW, weighted median and MR-Egger methods using the TAGC consortium asthma case (n = 19,954) - control (n = 107,715) and GABRIEL consortium asthma case (n =10,365) - control (n = 16,110) study. SNP: single nucleotide polymorphism; OR: odds ratio; 95% CI, 95% confidence interval; IVW: inverse-variance weighted method; WM: weighted median method; MR-Egger: Mendelian randomization-Egger regression method.



Supplementary Figure 5 Odds ratio of asthma risk per standard deviation increase in transferrin saturation removing 1 SNP at a time estimated by the IVW, weighted median and MR-Egger methods using the TAGC consortium asthma case (n = 19,954) - control (n = 107,715) and GABRIEL consortium asthma case (n = 10,365) - control (n = 16,110) study. SNP: single nucleotide polymorphism; OR: odds ratio; 95% CI, 95% confidence interval; IVW: inverse-variance weighted method; WM: weighted median method; MR-Egger: Mendelian randomization-Egger regression method.



Supplementary Figure 6 Odds ratio of asthma risk per standard deviation increase in transferrin removing 1 SNP at a time estimated by the IVW, weighted median and MR-Egger methods using the TAGC consortium asthma case (n = 19,954) - control (n = 107,715) and GABRIEL consortium asthma case (n = 10,365) - control (n = 16,110) study. SNP: single nucleotide polymorphism; OR: odds ratio; 95% CI, 95% confidence interval; IVW: inverse-variance weighted method; WM: weighted median method; MR-Egger: Mendelian randomization-Egger regression method.

Supplementary Table 1 Instrument SNPs in the Ensembl database to assess any associated secondary phenotype at genome-wide significance ($P < 1 \times 10^{-8}$) with potential confounding traits

SNP	Significant association
rs1800562	red blood cell traits, hemoglobin, low density lipoprotein cholesterol, total cholesterol, glycatel hemoglobin, transferrin glycosylation
rs1799945	systolic and diastolic blood pressures, hypertension
rs855791	red blood cell traits, hemoglobin, glycatel hemoglobin
rs8177240	-
rs7385804	hemoglobin, mean corpuscular hemoglobin, erythrocyte count, leukocyte count, platelet count, hematocrit
rs411988	-
rs744653	-
rs651007	hematocrit, coagulation factor, e-selectin, blood protein levels, alkaline phosphatase
rs9990333	-
rs4921915	triglyceride
rs6486121	-
rs174577	fasting blood glucose, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride, arachidonic acid measurement, omega-6 polyunsaturated fatty acid measurement

Supplementary Table 2 Power simulations for the Mendelian randomization analyses

Data source	Sample size	Proportion of cases	Type-I error rate (α)	OR	Iron		Ferritin		Transferrin saturation		Transferrin	
					R ²	Power	R ²	Power	R ²	Power	R ²	Power
TAGC	127,669	15.6%	0.05	0.8	4.32%	100%	1.18%	83%	8.06%	100%	10.53%	100%
TAGC	127,669	15.6%	0.05	0.9	4.32%	78%	1.18%	30%	8.06%	96%	10.53%	99%
TAGC	127,669	15.6%	0.05	1.1	4.32%	76%	1.18%	28%	8.06%	95%	10.53%	99%
TAGC	127,669	15.6%	0.05	1.2	4.32%	100%	1.18%	78%	8.06%	100%	10.53%	100%
GABRIEL	26,475	39.2%	0.05	0.8	4.32%	95%	1.18%	47%	8.06%	100%	10.53%	100%
GABRIEL	26,475	39.2%	0.05	0.9	4.32%	41%	1.18%	15%	8.06%	65%	10.53%	77%
GABRIEL	26,475	39.2%	0.05	1.1	4.32%	36%	1.18%	13%	8.06%	58%	10.53%	70%
GABRIEL	26,475	39.2%	0.05	1.2	4.32%	87%	1.18%	36%	8.06%	99%	10.53%	100%
GABRIEL childhood onset	14,503	46.8%	0.05	0.8	4.32%	79%	1.18%	31%	8.06%	97%	10.53%	99%
GABRIEL childhood onset	14,503	46.8%	0.05	0.9	4.32%	26%	1.18%	11%	8.06%	43%	10.53%	54%
GABRIEL childhood onset	14,503	46.8%	0.05	1.1	4.32%	22%	1.18%	10%	8.06%	37%	10.53%	46%
GABRIEL childhood onset	14,503	46.8%	0.05	1.2	4.32%	63%	1.18%	22%	8.06%	88%	10.53%	95%
GABRIEL later onset	5,616	34.7%	0.05	0.8	4.32%	36%	1.18%	13%	8.06%	59%	10.53%	71%
GABRIEL later onset	5,616	34.7%	0.05	0.9	4.32%	12%	1.18%	7%	8.06%	18%	10.53%	22%
GABRIEL later onset	5,616	34.7%	0.05	1.1	4.32%	11%	1.18%	7%	8.06%	17%	10.53%	20%
GABRIEL later onset	5,616	34.7%	0.05	1.2	4.32%	29%	1.18%	11%	8.06%	48%	10.53%	58%
GABRIEL unknown age at onset	6,356	25.7%	0.05	0.8	4.32%	34%	1.18%	13%	8.06%	55%	10.53%	67%
GABRIEL unknown age at onset	6,356	25.7%	0.05	0.9	4.32%	12%	1.18%	7%	8.06%	17%	10.53%	21%
GABRIEL unknown age at onset	6,356	25.7%	0.05	1.1	4.32%	11%	1.18%	7%	8.06%	16%	10.53%	20%
GABRIEL unknown age at onset	6,356	25.7%	0.05	1.2	4.32%	28%	1.18%	11%	8.06%	47%	10.53%	58%

OR: Assumed true odds ratio of asthma risk per standard deviation of the exposure variable.
R²: proportion of variance explained for the association between the genetic instrument and iron status.
The power calculations were performed using the online calculator for Mendelian randomization studies (<http://cnsgenomics.com/shiny/mRnd/>).