MATERIALS AND METHODS

GENETIC ASSOCIATIONS

As markers of iron status, we consider serum iron (μmol/l), transferrin saturation (%), log₁₀-transformed ferritin (μg/l) and transferrin (g/l) (1). The SNP-iron status association estimates are obtained from combined discovery and replication cohorts in a published genome-wide association study (GWAS) meta-analysis of 48,972 subjects of European descent performed by the Genetics of Iron Status (GIS) consortium, where adjustments were made for age, population stratification (ancestry principal components) and other study specific covariates (1).

For the SNP-CAD association estimates, we use publicly available results from the CARDIoGRAMplusC4D 1000 Genomes-based GWAS (referred to here as CARDIoGRAMplusC4D 1000G) and CARDIoGRAMplusC4D Metabochip (2, 3). CARDIoGRAMplusC4D 1000G is a GWAS meta-analysis of 60,801 CAD cases and 123,504 controls that adjusts for population stratification (genomic control method) (2). Participants are of European, east Asian, south Asian, Hispanic and African American ancestry (2). The diagnosis of CAD varies between studies; cases included subjects with documented acute coronary syndrome, coronary artery bypass grafting, percutaneous coronary revascularization, stenosis of greater than 50% in one or more of the coronary vessels, and cardiac angina (2). CARDIoGRAMplusC4D Metabochip is a meta-analysis of 63,746 CAD cases and 130,681 controls genotyped with either the Metabochip array or GWAS data imputed using HapMap (3). Participants are of European and south Asian descent (3). The study uses CAD definitions similar to CARDIoGRAMplusC4D 1000G and corrects for population stratification, age and sex (3). Results for both CARDIoGRAMplusC4D 1000G and CARDIoGRAMplusC4D Metabochip can be downloaded from www.CARDIoGRAMplusC4D.org (2, 3). We obtain SNP-CAD association estimates by meta-analysis of results from CARDIoGRAMplusC4D 1000G and CARDIoGRAMplusC4D Metabochip using a summary data method that accounts for participant overlap between the two studies (34,997 cases and 49,512 controls) (4). The approach ‘decouples’ the results from the two studies by transforming the covariance structure of the data such that meta-analysis methods assuming independence may consequently be applied (4).

INSTRUMENT SELECTION

Increased systemic iron status is associated with increased levels of serum iron, transferrin saturation and ferritin, and decreased levels of transferrin (1, 5-8). Thus, these four correlated markers may be treated as surrogates of systemic iron status, the single
intermediate phenotype of interest in our MR study. Genetic instruments for iron status should therefore be expected to have a concordant association with each of these four markers, and specifically SNPs that are deemed to increase systemic iron status should be associated with increased levels of serum iron, transferrin saturation and ferritin, and decreased levels of transferrin (9-11). The meta-analysis performed by the GIS consortium identified 12 genetic loci that differentially affect the four considered iron status markers (Supplementary Table 1) (1). Three loci (rs1800562 and rs1799945 in the HFE gene, and rs855791 in TMPRSS6) are associated with all four iron status markers at genome-wide significance (p < 5 x 10^{-8}) in a pattern consistent with an effect on systemic iron status (i.e. increased levels of serum iron, transferrin saturation and ferritin, and decreased levels of transferrin) (Supplementary Table 1) (1, 11), and only these are considered as instruments for systemic iron status in our MR analysis. The rs1800562 and rs1799945 SNPs in the HFE gene are not in linkage disequilibrium (LD r^2 < 0.01).

The strength of each instrument is evaluated using its F statistic, and only SNPs with an F statistic greater than 10 are used, thus minimising any weak instrument bias (12, 13).

**Mendelian Randomization Estimates**

To derive MR estimates, a two-sample summary data approach is performed separately for each SNP using the Wald-type estimator, with standard error derived using the Delta method (14). Combined MR estimates are obtained by pooling MR estimates across SNPs using a fixed-effect inverse-variance weighted (IVW) meta-analysis.

The overall study design is demonstrated graphically in Figure 1.

**Pleiotropy**

To explore the possibility that the instruments for iron status may be exerting effects on CAD risk through pleiotropic pathways that are independent of iron status and thus biasing the results of the MR analysis (15, 16), an online database of SNP-phenotype associations (PhenoScanner, http://www.phenoscanner.medschl.cam.ac.uk/phenoscanner) is used to search for secondary phenotypes associated with the three selected instruments at genome-wide significance (p < 5 x 10^{-8}) (17).

All analyses are performed using the statistical programme R (version 3.3.1).

**References**


