Interaction of dietary and genetic factors influencing body iron status and risk of type 2 diabetes within the EPIC-InterAct Study

Short title: Gene-Haem iron interactions and type 2 diabetes

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Abstract

Objective: Meat intake has been consistently shown to be positively associated with incident type 2 diabetes. Part of that association may be mediated by body iron status, which is influenced by genetic factors. We aimed to test for interactions of genetic and dietary factors influencing body iron status in relation to the risk of incident type 2 diabetes.

Research Design and Methods: The case-cohort comprised 9,347 cases and 12,301 subcohort participants from eight European countries. SNPs were selected from genome-wide association studies on iron status biomarkers and candidate gene studies. A ferritin related gene score was constructed. Multiplicative and additive interactions of haem iron and SNPs as well as the gene score were evaluated using Cox-proportional hazards regression.

Results: Higher haem iron intake (per 1 sd) was associated with higher ferritin levels (beta (95% CI) = 0.113 (0.082, 0.144)), but not with transferrin (-0.019 (-0.043, 0.006) or transferrin saturation (0.016 (-0.006, 0.037)). Five SNPs located in four genes (rs1799945 (*HFE* H63D), rs1800562 (*HFE* C282Y), rs236918 *(PCK7*), rs744653 (*SLC40A1)*, rs855791 (*TMPRSS6* V736A)) were associated with ferritin. We did not detect an interaction of haem iron and the gene score on the risk of diabetes in the overall study population (p_{add}=0.16, p_{mult}=0.21), but a trend towards a negative interaction in men (p_{add}=0.04, p_{mult}=0.03).

Conclusions: We found no convincing evidence that the interplay of dietary and genetic factors related to body iron status associates with type 2 diabetes risk above the level expected from the sum or product of the two individual exposures.

A number of studies have shown a positive association of meat intake and incident type 2 diabetes (1-4). Haem iron from meat has been reported as the strongest dietary determinant of plasma ferritin concentrations (5) and part of the effect of red meat on type 2 diabetes risk seems mediated by ferritin (6). In accordance with this, biomarkers of body iron status i.e. ferritin, transferrin and transferrin saturation (TSAT) have been linked with type 2 diabetes in a number of studies, including the EPIC-Interact study (7-10). Ferritin is the major intracellular iron storage protein and is directly associated with incident type 2 diabetes (7; 11). Transferrin is the iron transport protein in the circulation and its saturation with iron is reflected by TSAT. Transferrin concentrations are inversely and TSAT directly correlated with ferritin. Data on dietary determinants of transferrin and TSAT are scarce (12), but since both markers are related to body iron status, a relation with meat intake, the major source of dietary iron with a high bioavailability, seems plausible.

Genome-wide association studies have identified genetic variants associated with body iron status (13-16). Most of them were located in genes functionally related to iron absorption, transport and storage (13-16). We hypothesize that interactions between dietary and genetic factors influencing body iron status and the risk for type 2 diabetes may exist. An interaction of rs1799945 SNP (*HFE* H63D) and haem iron intake on the risk of type 2 diabetes has previously been described in women (17). However, other studies on SNPs in *TMPRSS6* and *TF* genes as well as a genome-wide interaction analysis did not reveal significant interactions with haem iron intake on the risk of type 2 diabetes (18; 19). Nevertheless, these studies were limited in power by their sample size and it is therefore not possible to exclude interaction effects of moderate size. Therefore, we aim to analyze interactions between genetic factors influencing body iron stores and meat, a major dietary determinant of body iron stores, and risk of type 2 diabetes in the large prospective EPIC-InterAct study.

Research Design and Methods

Study Population

The design and methods of the InterAct Study, nested within the European Prospective Investigation into Cancer (EPIC) cohorts, hereafter called the 'EPIC-InterAct Study' are described in detail elsewhere (20). Briefly, the sampling frame (n=340,234) included participants from 26 centres in 8 out of 10 EPIC participating countries (France, Italy, Spain, the UK, the Netherlands, Germany, Denmark and Sweden). Participants without stored blood (n=109,625) or without information on diabetes (n=5821) were excluded. All ascertained and verified incident type 2 diabetes cases between 1991 and 2007 (3.99 million person-years at risk, n=12,403) comprised the 'case' group. A centre-stratified, representative subcohort of 16,835 individuals was selected as the comparison ('control') group to assess the exposure distribution in the cohort. Prevalent diabetes cases (n=548) and individuals with uncertain diabetes status (n=133) were excluded from the subcohort, leaving 16,154 individuals for analysis. Of the total 12,403 incident type 2 diabetes cases, a random set of 778 cases were part of the subcohort as a result of the random selection of this group.

For the current analysis, we excluded participants with abnormal estimated energy intake (top 1% and bottom 1% of the distribution of the ratio of reported energy intake over basal metabolic rate; $n_{subcohort}$=305; n_{Cases}=339), missing information on dietary intake ($n_{subcohort}$=51; n_{Cases}=70), no genetic data (including samples removed due to relatedness or non-European ethnicity; $n_{subcohort}$=3,142; n_{Cases}=2,389) and missing covariate data ($n_{subcohort}$=821; n_{Cases}=723) leaving a sample of 9,347 cases and 12,301 subcohort participants, including 577 cases in the subcohort (Supplementary Figure 1). Cross-sectional analyses for biomarkers were carried out within the subcohort and additionally excluded samples with

missing biomarker measurement. Sample size varied between 10,657-11,576 individuals between analyses, because ferritin on the one hand and transferrin and iron on the other were measured in slightly different sample size (Supplementary Figure 1).

Case ascertainment

Ascertainment of incident type 2 diabetes involved a review of the existing EPIC datasets at each centre using multiple sources of evidence including self-report, linkage to primary-care registers, secondary-care registers, medication use (drug registers), hospital admissions and mortality data. Information from any follow-up visit or external evidence with a date later than the baseline visit was used. Cases in Sweden and Denmark were not ascertained by self-report, but identified via local and national diabetes and pharmaceutical registers and hence all ascertained cases were considered to be verified. To increase the specificity of the definition for these cases, we sought further evidence including individual medical records review in some centres. Follow-up was censored at the date of diagnosis, 31 December 2007 or the date of death, whichever occurred first.

Dietary assessment

Self- or interviewer-administered country-specific validated dietary questionnaires and/or diet records (Sweden) were used to assess usual food intakes of participants (21; 22). Red meat was calculated as the sum of the daily intake (in g) of unprocessed pork, beef, veal, mutton, lamb, goat and horse as well as minced meat including that in hamburgers and meatballs. Processed meat describes the sum of the daily intake (in g) of bacon-, ham- and liver-containing items and all other processed meats such as black pudding, chorizo, sausages, corned beef. Total meat was derived by summing up intakes of red meat, processed meat, poultry and offal. Energy and nutrient (iron, calcium, vitamin C, fibre, alcohol) intakes were

estimated using the standardized EPIC Nutrient Database (ENDB) (23). The calculation of haem iron was based on the proportion of haem iron on total iron content of the specific meat item (65% beef, 39% pork, 52% remaining red meat and processed meat, 26% poultry and fish, 21% offal) (24; 25).

Covariate assessment

Questionnaires were used to collect information on lifestyle factors and socioeconomic status at baseline (26). For the current analysis, we used a four category physical activity index reflecting occupational and recreational physical activity (27). Educational attainment was categorized as none, primary school, technical school, secondary school and further education including university degree. Smoking status was categorized as never, former, and current smoker. Anthropometric measures including weight, height and waist circumference were collected at baseline by standardized procedures and adjusted for clothing (28).

DNA extraction, genotyping and SNP selection

DNA extraction and genotyping procedures were published previously (29). Briefly, participants were selected across all centres for genome-wide genotyping using the Illumina 660W-Quad BeadChip, Illumina HumanCoreExome-12v1 and the Illumina HumanCoreExome-24v1 BeadArrays (Illumina, San Diego, CA, USA) at different points in time. The number of individuals selected per centre was proportional to the percentage of total cases in that centre. Illumina 660 and Core Exome datasets were separately quality controlled and imputed to the dataset of the Haplotype Reference Consortium (30) using IMPUTE2 (31) at the Wellcome Trust Centre for Human Genetics in Oxford.

We selected candidate SNPs that were associated with iron status biomarkers in genome-wide association studies for ferritin, transferrin, TSAT and soluble transferrin receptor and that were known, by gene function, to be directly involved in iron metabolism (Supplementary Table 1). For loci where several variants were described in different studies, the lead SNP of the largest study (13) was used. Furthermore, we systematically searched Pubmed (32) (Supplementary Table 2) for candidate genes functionally related to body iron metabolism that were associated with type 2 diabetes ($p<0.05$). The search revealed two candidate SNPs (rs3817672, rs17788379) in the Transferrin receptor-1 gene (*TFRC*) (33) and a microsatellite polymorphism of the *HMOX1* gene promoter (34).

All identified SNPs were available from genome-wide genotyping or imputation with a confidence threshold >0.90 (Supplementary Table 1). The microsatellite polymorphism in *HMOX1* was not available from the genotyping chips. Therefore, haplotypes covering the chromosomal location of the microsatellite were constructed using data from 55 genetic variants (Supplementary Table 3)within the PHASE software version 2.1.1 (35). We observed eight common haplotypes and used them in statistical analyses (Supplementary Table 3).

A weighted ferritin related gene score was constructed including all SNPs functionally related to iron metabolism and associated with ferritin levels in GWAS (rs1799945, rs1800562, rs744653, rs855791). Weights were based on the betas reported in literature (13).

Biomarker measurement

Samples were stored from collection in liquid nitrogen at −196°C in the coordinating centre at the International Agency for Research into Cancer (IARC) in Lyon, France, or in liquid nitrogen in local biorepositories with the exception of Umeå, where −80°C freezers were

used. Samples from all centers were analyzed centrally at the SHL-Groep, Etten-Leur, Netherlands. Ferritin, iron and transferrin were measured by Cobas® (Roche Diagnostics, Mannheim, Germany) assays on a Roche Hitachi Modular P analyser in serum, except for participants from Umeå where only plasma samples were available and only ferritin was measured (7). TSAT was calculated as follows: [iron(μmol/L) x 100)] / [transferrin(g/L) x 22.75].

Statistical Analysis

Analysis strategy

Based on the hypothesis that dietary and genetic factors are more likely to interact when they are related to a common biomarker, we selected specific dietary and biological factors for interaction instead of testing all possible interactions. Starting from biomarkers of body iron status (ferritin, transferrin and transferrin saturation) previously shown to be associated with type 2 diabetes within the EPIC-InterAct study (7), we selected firstly dietary and secondly genetic factors for our interaction analysis that were individually related to a common biomarkers of body iron status and tested for their interaction in a third step. All analyses were carried out stratified by sex, because of the differences in iron requirements and iron stores between men and women.

Selection of dietary and genetic factors (cross-sectional analysis)

Association analyses of genetic and dietary exposures on concentrations of ferritin, transferrin and TSAT used linear regression analysis, stratified by sex and country. Ferritin concentrations were log10 transformed. All biomarkers were standardized (mean=0, SD=1) based on the distribution in the subcohort.

Dietary exposures (total meat, red meat, processed meat, red and processed meat, iron from meat, haem iron defined as described above) were energy adjusted by the residual method (36) and standardized based on the distribution in the subcohort. Linear regression models with dietary exposures were adjusted for age, study centre, physical activity (4 categories), total energy intake (kcal/day) and the intakes of fibre (g/day), alcohol (g/day), calcium (mg/day), vitamin C (mg/day), tea (g/day) and coffee (g/day) because these dietary factors may influence bioavailability of iron. Analyses of dietary exposures in women were additionally adjusted for menopausal status (premenopausal, postmenopausal, perimenopausal and surgical postmenopausal) and use of hormone replacement therapy (yes/no).

Association analyses of genetic exposures were adjusted for age, study centre, genotyping chip, and eigenvalues of the first 10 coordinates from multidimensional scaling on common and low-frequency variants (MAF>0.01).

Interaction analyses were only carried out for dietary and genetic exposures that showed a significant association ($p<0.05$) in preceding analyses.

Interaction of dietary and genetic factors on biomarker levels

Interaction analyses were carried out by including a multiplicative interaction term of SNP (0,1,2 coded) *dietary factor (continuous) adjusted for all covariates listed above. Subgroup effects were calculated by the use of dummy variables based on cross-tabulation of genetic and dietary exposure. The dietary exposure was split into low and high intake groups by the sex-specific medians. The SNP variable was combined in two groups, with one group of individuals homozygous for the allele associated with lower ferritin concentrations and the second group comprising all carriers of the ferritin increasing allele.

Analyses on type 2 diabetes

Association and interaction analyses of genetic and dietary exposures on the risk of diabetes were carried out by Cox proportional hazard regression with Prentice weighting stratified by sex and country. Age was used as the underlying time scale and the baseline hazard function was stratified by centre and age at recruitment, truncated to full years. Analyses of genetic exposures were adjusted for genotyping chip, eigenvalues of 10 coordinates, and BMI. Interaction analyses of genetic and dietary exposures were carried out by including a multiplicative interaction term of SNP (0,1,2-coded) *dietary factor (continuous) in the model and were additionally adjusted for energy intake, education, smoking, physical activity, alcohol intake and menopausal status (women only). Effect estimates and p-values of the multiplicative interaction term are reported as measures of multiplicative interaction. In addition, the relative excess risk due to interaction (RERI) as a measure of additive interaction was calculated based on the same model by the method described by Li et al (37). Standard errors of RERI were calculated using the delta method (37). Again, we calculated subgroup effects based on the cross-tabulation of genetic and dietary exposure (described above) to characterize the interaction.

Meta-analyses and multiple testing

Sex- and country-specific effect estimates of cross-sectional as well as longitudinal analyses were combined by random-effects meta-analysis. Sex-specific and sex combined estimates are reported. Differences between the sexes were assessed based on Cochran's Q. Selection of genetic and dietary factors for interaction analyses was based on association analyses of iron status biomarkers. These analyses were not corrected for multiple testing in order to include all possible relevant factors in the interaction analysis. In all other analyses, p–values were

corrected for multiple testing using the linear step-up method of the false discovery rate from Benjamini and Hochberg (38). Furthermore, associations of SNPs with diabetes from logistic regression within the EPIC-InterAct study were combined with data from DIAGRAM by meta-analysis. All analyses used the SAS Enterprise Guide 6.1, SAS 9.4 and R Version 3.1.2.

Results

Study population characteristics at baseline are displayed in Table 1. The analytical study population was on average middle aged (median (25^{th}-75^{th}percentile): 52.7 (46.6-59.3) years), comprised 61.6% women and had a median (25^{th}-75^{th}percentile) BMI of 25.5 (23.1-28.3) kg/m^2. Participants were followed up for a median of 12.5 years.

Selection of dietary factors

Intake of all analysed meat and iron items was directly associated with ferritin concentrations (Table 2). The strongest association was observed for haem iron (beta (95%CI)= 0.113 (0.082, 0.144) sd in ferritin/1 sd haem iron, $p=1.3*10^{-12}$, Table 2). No significant associations were detected for any of the analysed dietary factors with transferrin or TSAT (Table 2). Differences in associations between men and women were not observed ($p_{sex\_diff}>0.05$). Based on these results, the selection of SNPs for interaction analyses was restricted to those associated with the biomarker ferritin. Subsequent interaction analyses were carried out with haem iron.

Selection of genetic factors

Among the analysed SNPs, the previously reported association of rs1799945 (*HFE* H63D) and rs1800562 (*HFE* C282Y*)*, rs744653 (*SLC40A1)* and rs855791 (*TMPRSS6*) with ferritin concentrations were replicated (Supplementary Table 4). The associations of rs1799945

(*HFE*) and rs855791 (*TMPRSS6*) differed between sexes (p_{sex_diff} <0.05) and were stronger in men than in women (Supplementary Table 4). Also, rs236918 (*PCSK7),* primarily known for its association with soluble transferrin receptor was associated with ferritin concentrations beta (95%CI)=-0.037(-0.071;-0.003). None of the SNPs located in the transferrin (*TF*), the transferrin receptor (*TFRC*, *TFR2*) and haem oxygenase 1 (*HMOX1*) genes were associated with ferritin concentrations (Supplementary Table 4). The association of the gene score with ferritin concentrations was also stronger in men than in women (men: beta =0.092 (0.068;0.117), women: beta =0.052 (0.031;0.072), p for heterogeneity between sexes = 0.01). Based on the observed associations, further association and interaction analyses were restricted to all SNPs associated with ferritin concentrations (see above), and the gene score.

Main effect of genetic determinants of ferritin on risk of type 2 diabetes

None of the ferritin related variants and gene score were significantly associated with risk of diabetes in the EPIC-InterAct study taking multiple testing into account (Table 3). Meta-analysis of results from EPIC-InterAct with those from DIAGRAM (39) indicated significant positive associations of the ferritin increasing alleles of rs1799945 (*HFE*) with diabetes (OR (95%CI)=1.06 (1.02;1.09), p_{FDR}=0.02) and of rs744653 (*SLC40A1*) (OR (95%CI)=1.05 (1.02;1.09), p_{FDR}=0.02; Table 3).

Interaction of dietary and genetic factors

With regard to the risk of type 2 diabetes, neither multiplicative nor additive interactions between dietary and genetic factors related to body iron status were observed in the overall study population (Table 4), when taking multiple testing into account. A nominally significant negative interaction of haem iron intake and rs855791 was detected in sex-combined analyses on the multiplicative scale (p_{mult_raw}=0.046, Table 4). In women, a statistically significant

positive interaction of rs744653 (*SLC40A1*) and haem iron was detected (p_{mult_raw}=0.002, p_{add_raw}=0.02,Table 4). Further nominally significant interactions were observed for rs236918 (PCK7) and haem iron in women (p_{mult_raw}=0.01, p_{add_raw}=0.04), rs1799945 (HFE) and haem iron in men (p_{mult_raw}=0.01, p_{add_raw}=0.02) and the gene score and haem iron in men (p_{mult_raw}=0.03, p_{add_raw}=0.04, Table 4). Significant differences between sexes were observed for the interaction of rs1799945 (*HFE*) and rs744653 (*SLC40A1*) (p_{sex_diff} <0.05). The observed interaction effects were negative except for rs744653 (Table 4).

Interaction of rs855791 and haem iron

For the negative interaction of rs855791 and haem iron, we observed a HR (95%CI) of 1.11 (0.87; 1.42) in participants carrying one or two ferritin raising alleles with low haem intake, a HR of 1.28 (1.01; 1.61) in participants with high haem intake and no ferritin raising allele and a HRof 1.20 (0.98; 1.46) in participants having both a high haem intake and at least one ferritin raising allele compared to participants having a low haem intake and no ferritin raising allele (Table 4).

Interaction of rs744653 and haem iron in women

With regard to the positive interaction of rs744653 and haem iron in women, we observed no increase in diabetes risk for women carrying the ferritin raising allele with low haem intake (HR (95%CI): 0.97 (0.78;1.21)), a slightly increased risk for women with high haem intake, but no ferritin raising alleles (1.07 (0.93;1.24)) and a moderate increased risk in women with high haem intake carrying ferritin raising alleles (1.21 (0.97;1.52)) compared to women carrying no ferritin raising alleles with low haem intake (Table 4).

Interaction of rs1799945 (HFE H63D) and haem iron in men

Cross-tabulation of diabetes risk by rs1799945 and haem intake in men revealed similar raised diabetes risk for all subgroups: a HR (95%CI) of 1.22 (1.05;1.41) in men carrying the ferritin raising allele with low haem intake, a HR of 1.23 (1.09;1.38) in men with high haem intake, but no ferritin raising allele and a HR of 1.22 (1.01;1.48) in men having both a high haem intake and at least one ferritin raising allele compared to men having a low haem intake and no ferritin raising allele (Table 4).

Interaction of gene score and haem iron in men

The interaction of the gene score and haem iron (Table 4, Suppl. Figure 2) in men can be characterized as follows. The diabetes HR among men with a high haem intake was slightly higher in the group of men with a low gene score (HR (95%CI)=1.33 (1.12;1.59)) than that of men with a high gene score (HR=1.26 (1.10;1.45)). Among men with a low haem iron intake, the HR was higher when the gene score was high (HR =1.20 (0.97;1.47)) compared to when it was low (reference category)..

Based on these results, we tested if we could also observe a tendency for interactions of haem iron and genetic factors on ferritin levels in a cross-sectional analysis. However, we did not find any indication that such interactions exist (Supplementary Table 5).

Conclusions

We studied the interaction of ferritin related genetic variants and haem iron intake on the risk of diabetes. After correction for multiple testing, we did not identify interactions in the entire study population, neither for the ferritin-related gene score nor for single variants. However, we observed a nominally significant interaction of rs855791 and a trend towards a few sex-specific interactions, e.g. for the gene score. In addition, we identified stronger associations in men than in women for rs1799945 (*HFE*), rs855791 (*SLC40A1*) and the gene score with ferritin concentrations .

The EPIC-InterAct study is a large, prospective cohort study and as such it provides major advantages with regard to power and temporality of the observed associations and interactions. Still, we were not able to identify a convincing interaction of haem iron intake and several genetic variants influencing body iron stores. This may imply, that either no such interactions exist or that despite the large sample size, our power was insufficient to detect them. Indeed, main effects on risk of type 2 diabetes for two of the analysed SNPs were only detected when pooling our data with data from large scale meta-analysis. Therefore, it can be assumed that even larger sample size will be required to be able to detect interaction effects.

The EPIC-InterAct study includes participants from eight European countries and therefore provides a high external validity within European populations. Within the different EPIC countries baseline information has been collected in a standardized way, and large efforts have been taken to integrate, in particular, dietary information from the distinct countries (23). Still, measurement error in dietary intake variables derived from self-reports is inevitable and will attenuate interaction effects (40). With our study design we have carefully selected candidates for the interaction analysis with the aim to reduce multiple testing penalties.

However, this procedure will overlook potential interactions of dietary and genetic factors that are not individually associated with biomarkers of body iron status.

The sex-specific effects we observed in our analysis maybe caused by differences in iron requirements and iron stores between men and women. However, they could also originate from residual confounding in women, despite adjustment for menopausal status or from the smaller numbers in the sex-specific analysis that make results generally more prone to chance findings.

We did observe direct associations of some (rs1799945, rs744653), but not all ferritin associated genetic variants and overall no association of the gene score and risk of type 2 diabetes. Both SNPs showed some interaction effects, rs1799945 in men and rs744653 in women, but not in the overall study population.

The detected interaction of rs744653 and haem iron intake on risk of type 2 diabetes in women has not been described before and requires further confirmation. An interaction of *HFE* variants and haem iron intake has been described before in the female participants of the Nurses' Health Study (17) with a linear trend for the association of haem iron intake and type 2 diabetes in carriers of the hemochromatosis associated alleles of the *HFE* variants rs1799935 (H63D) and rs1800562 (C282Y), only. We observed a similar trend in female carriers of the *HFE* D63 allele, but this interaction did not reach statistical significance in our analysis. In contrast, we observe a nominally significant negative interaction of rs1799945 in men. Another study, in contrast to ours, didn't find evidence for an interaction of *TMPRSS6* variant rs855791 and haem iron (18). It can be assumed, that our larger samples size allowed us to identify this nominally significant interaction, which requires further replication.

Besides analysing interaction effects of various single SNPs, we additionally analysed the interaction of a ferritin related gene score and haem iron. We detected no statistically significant interaction within the overall study population, but a tendency towards a negative interaction in men. The analysis shows that individuals genetically predisposed to increased ferritin levels, who have a high haem iron intake, are indeed at a higher risk for type 2 diabetes, but maybe lower than expected from the sum or product of the two individual exposures.

Iron homeostasis is mainly controlled at the level of absorption by a negative feedback mechanism via hepcidin (41). Still normal-range, elevated ferritin concentrations as a consequence of genetic variation might therefore downregulate iron absorption from the diet, thus protecting from additional iron accumulation and from a further increase in diabetes risk by nutritional determinants of body iron stores. This biological mechanism may potentially explain the observed negative interactions. However, we did not detect interactions between haem iron and genetic variants with ferritin concentrations.

In summary, we found no convincing evidence that the interplay of dietary and genetic factors related to body iron status associates with risk of type 2 diabetes above the level expected from the sum or product of the two individual exposures. Large-scale studies of several cohorts will be required to examine the trend observed that the diabetes risk may be even lower than expected from the sum or product of the two individual exposures.

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Authors contribution: KM conceptualized the project, wrote the analysis plan, conducted statistical analysis, and drafted the manuscript. KM had access to all data for this study and take responsibility for the manuscript contents. CP, JK, YTvdS, BB and MBS revised the analysis plan and helped with data interpretation and writing of the manuscript. CA, LA, AB, AJC, CD, KE, GF, PWF, MJG, JMH, PJ, MJ, VAK, TJK, KTK, TK, CK, FRM, OM, PMN, TFO, DP, SP, JRQ, MRB, CS, IS, AMWS, MS, AT, RT, NGF, SJS, CL, ER, NJW contributed to the conception and design of the study, interpretation of the data, critical revision of the article for important intellectual content, All authors approved the final version.

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Table 1: Baseline characteristics of EPIC-InterAct study population based on the subcohort (n=12,301)

Characteristic	Missing Data (%)	All (n=12,301)	Men (n=4,726)	Women (n=7,575)
Age (y)	0	52.7 (46.5 - 59.3)	53.2 (47.3 - 59.4)	52.4 (46.1 - 59.2)
BMI (kg/m^2)	0	25.5 (23.1 - 28.3)	26.2 (24.2 - 28.6)	24.9 (22.5 - 28.0)
Smoking	0			
Never		5,683 (46.2)	1,518 (32.1)	4,165 (55.0)
Former		3,354 (27.3)	1,722 (36.4)	1,632 (21.5)
Current		3,264 (26.5)	1,486 (31.4)	1,778 (23.5)
Education	0			
None		873 (7.1)	238 (5.0)	635 (8.4)
Primary		4,008 (32.6)	1,565 (33.1)	2,443 (32.3)
Technical		3,008 (24.4)	1,106 (23.4)	1,902 (25.1)
Secondary		1,814 (14.8)	610 (12.9)	1,204 (15.9)
Further Education		2,598 (21.1)	1,207 (25.5)	1,391 (18.4)
Cambridge Index of Physical Activity	0			
Inactive		2,793 (22.7)	851 (18.0)	1,942 (25.6)
Moderately Inactive		4,139 (33.7)	1,456 (30.8)	2,683 (35.4)
Moderately active		2,835 (23.0)	1,220 (25.8)	1,615 (21.3)
Active		2,534 (20.6)	1,199 (25.4)	1,335 (17.6)
Country	0			
France		302 (2.5)	0 (0.0)	302 (4.0)
Italy		1,448 (11.8)	497 (10.5)	951 (12.6)
Spain		2,487 (20.2)	927 (19.6)	1,560 (20.6)
UK		894 (7.3)	348 (7.4)	546 (7.2)
Netherlands		1,096 (8.9)	184 (3.9)	912 (12.0)
Germany		1,773 (14.4)	729 (15.4)	1,044 (13.8)
Sweden		2,416 (19.7)	1,034 (21.9)	1,382 (18.2)
Denmark		1,885 (15.3)	1,007 (21.3)	878 (11.6)
Biomarker				
Ferritin (pmol/L)	5.9	188.7 (89.9 - 357.3)	330.3 (184.3 - 548.3)	132.6 (65.2 - 242.7)
Transferrin (g/L)	12.3	2.7 (2.5 - 3.0)	2.6 (2.4 - 2.9)	2.8 (2.5 - 3.0)
TSAT (%)	12.3	27.0 (20.8 - 33.7)	28.7 (23.0 - 36.0)	25.8 (19.6 - 32.3)
Dietary Intake				
Total meat (g/day)	0	98.6 (65.6 - 137.6)	123.9 (85.4 - 169.9)	85.6 (57.5 - 117.5)
Red meat (g/day)	0	38.1 (18.6 - 65.6)	49.7 (25.0 - 81.7)	32.9 (16.3 - 55.4)
Processed meat (g/day)	0	28.9 (15.1 - 50.1)	39.3 (21.9 - 64.2)	23.5 (12.6 - 41.2)
Poultry (g/day)	0	15.6 (6.8 - 29.6)	16.4 (7.9 - 32.2)	14.4 (6.1 - 27.3)
Fish (g/day)	0	28.5 (14.8 - 50.9)	33.9 (16.8 - 58.8)	26.2 (13.1 - 46.1)
Haem iron (g/day)	0	1.2 (0.7 - 1.7)	1.5 (1.0 - 2.1)	1.0 (0.7 - 1.4)
Menopausal status				
Premenopausal				2,460 (32.5)
Postmenopausal				3,643 (48.1)
Perimenopausal				1,232 (16.3)
Surgical postmenopausal				240 (3.2)
Use of hormones for menopause				1,120 (15.4)
Prevalent stroke	8.7	98 (0.9)	52 (1.2)	46 (0.7)
Prevalent myocardial infarction	1.7	173 (1.4)	127 (2.8)	46 (0.6)

Given is median (25th - 75th percentile) or n (%)

Table 2: Cross-sectional association of meat and iron intake with ferritin, transferrin and TSAT in the subcohort of the EPIC-Interact study

	Ferritin (n=11,291)			**Transferrin (n=10,657)**			**TSAT (n=10,657)**		
Dietary Intakes (per sd)	**beta (95%CI)**	**p-value**	**I^2 [%]**	**beta (95%CI)**	**p-value**	**I^2 [%]**	**beta (95%CI)**	**p-value**	**I^2 [%]**
Iron from meat	0.099 (0.071, 0.127)	5.1E-12	63.4	-0.011 (-0.031, 0.008)	0.26	0.0	0.012 (-0.009, 0.033)	0.26	0.0
Haem Iron	0.113 (0.082, 0.144)	1.3E-12	70.1	-0.019 (-0.043, 0.006)	0.14	26.9	0.016 (-0.006, 0.037)	0.15	0.0
Red meat	0.080 (0.058, 0.102)	6.4E-13	43.9	-0.001 (-0.027, 0.026)	0.97	48.4	0.012 (-0.008, 0.031)	0.24	0.0
Processed meat	0.066 (0.044, 0.088)	4.7E-09	40.5	-0.005 (-0.024, 0.013)	0.58	0.0	0.007 (-0.013, 0.027)	0.50	0.0
Red & processed meat	0.103 (0.081, 0.126)	3.7E-20	41.8	-0.006 (-0.031, 0.019)	0.64	37.7	0.014 (-0.007, 0.034)	0.19	0.0
Total meat	0.105 (0.087, 0.124)	4.7E-28	23.4	-0.009 (-0.034, 0.017)	0.50	39.9	0.007 (-0.016, 0.030)	0.58	20.7

Effect estimates are given for a change in standardized (and in case of ferritin log transformed) biomarker per standard deviation in dietary intake. Exposures and outcomes were standardized to a mean of 0 and a standard deviation of 1. Ferritin was log-transformed prior to standardization. Effect estimates, p-values and measures of heterogeneity (I^2) were derived from random-effects meta-analysis from country- and sex-specific linear regression models. Linear regression models were adjusted for age, centre, physical activity (4 categories), total energy intake (kcal/day) and the

intakes of fibre (g/day), alcohol (g/day), calcium (mg/day), vitamin C (mg/day), tea (g/day) and coffee (g/day). Analyses in women were additionally adjusted for menopausal status and usage of hormone replacement therapy. No significant ($p<0.05$) heterogeneity between sexes was observed. Therefore, sex-combined estimates were reported, only.

Table 3:Association of ferritin associated genetic variants and type 2 diabetes

SNP	A_2/A_1	EPIC-InterAct (HR (95% CI))* (n_{T2D}=8,794; $n_{controls}$=11,032)	DIAGRAM (OR (95% CI)) (n_{T2D}=26,488; $n_{controls}$=83,964)	Meta-analysis (OR (95% CI)) † (n_{T2D}=35,282; $n_{controls}$=94,996)	p_{RAW}	p_{FDR}
ferritin related SNPs						
HFE rs1799945	G/C	1.06 (0.99, 1.14)	1.06 (1.02; 1.11)	1.06 (1.02; 1.09)	0.002	0.02
HFE rs1800562	A/G	0.90 (0.79, 1.03)	1.02 (0.94; 1.11)	1.01 (0.94; 1.08)	0.812	0.81
PCK7 rs236918	G/C	0.94 (0.85, 1.03)	0.97 (0.94; 1.00)	0.97 (0.95; 1.00)	0.037	0.10
SLC40A1 rs744653	C/T	1.07 (0.98, 1.16)	1.05 (1.01; 1.09)	1.05 (1.02; 1.09)	0.005	0.02
TMPRSS6 rs855791	G/A	1.00 (0.94, 1.06)	0.99 (0.97; 1.02)	0.99 (0.97; 1.02)	0.523	0.70
gene score		1.01 (0.85, 1.20)				

* Cox proportional hazard regression with age as the underlying time scale and adjusted for genotyping chip, eigenvalues of 10 coordinates, and BMI (kg/m^2).

† Results from the EPIC-InterAct study and the DIAGRAM consortium (looked up using the PhenoScanner webpage: http://www.phenoscanner.medschl.cam.ac.uk/) were combined by fixed effects meta-analysis after alignment of the reference alleles on the odds ratio scale. Odds ratios in the InterAct study were calculated in a case/noncase design using logistic regression within the EPIC-InterAct study. Logistic regression models were adjusted for age, sex, BMI, genotyping source, principal components and study centre. Country-specific estimates were combined by random-effects meta-analysis within InterAct. Participants from the EPIC centre Norfolk were excluded from the analysis within InterAct, since EPIC-Norfolk was part of the DIAGRAM consortium

Table 4: Prospective analysis of interactions of ferritin related SNPs and haem iron intake on the risk of type 2 diabetes in the EPIC-Interact study

SNP	Sex	G=0|E=0*		G=1|E=0*		G=0|E=1*		G=1|E=1*		p_{mult_raw} †	$RERI_{HR}$ ‡	p_{add_raw} ‡
Gene		N	HR	N	HR (95% CI)	N	HR (95% CI)	N	HR (95% CI)			
weighted	all	1693/4302	1	2385/5669	1.08 (0.90; 1.29)	2244/4746	1.19 (1.01; 1.41)	3025/6354	1.17 (1.00; 1.36)	0.21	-0.09 (-0.22; 0.03)	0.16
gene	♂	823/1835	1	1222/2441	1.20 (0.97; 1.47)	1114/2070	1.33 (1.12; 1.59)	1521/2779	1.26 (1.10; 1.45)	0.03	-0.23 (-0.46; -0.01)	0.04
score	♀	870/2467	1	1163/3228	0.98 (0.74; 1.30)	1130/2676	1.09 (0.84; 1.40)	1504/3575	1.12 (0.85; 1.48)	0.68	0.04 (-0.20; 0.27)	0.66
rs1799945§	all	2923/7186	1	1155/2785	1.11 (0.97; 1.26)	3732/7903	1.15 (1.03; 1.27)	1537/3197	1.26 (1.13; 1.40)	0.32	-0.06 (-0.17; 0.06)	0.29
HFE	♂	1460/3102	1	585/1174	1.22 (1.05; 1.41)	1865/3447	1.23 (1.09; 1.38)	770/1402	1.22 (1.01; 1.48)	0.01	-0.16 (-0.29; -0.02)	0.02
(G/C)	♀	1463/4084	1	570/1611	1.02 (0.82; 1.28)	1867/4456	1.07 (0.92; 1.25)	767/1795	1.27 (1.10; 1.46)	0.49	0.08 (-0.05; 0.20)	0.40
rs1800562	all	3702/9028	1	376/943	0.86 (0.73; 1.01)	4762/10048	1.13 (1.04; 1.24)	507/1052	1.03 (0.84; 1.27)	0.91	-0.03 (-0.10; 0.05)	0.50
HFE	♂	1854/3867	1	191/409	0.83 (0.63; 1.08)	2364/4370	1.15 (1.01; 1.32)	271/479	1.09 (0.72; 1.64)	0.99	-0.04 (-0.19; 0.11)	0.60
(A/G)	♀	1848/5161	1	185/534	0.89 (0.71; 1.11)	2398/5678	1.12 (0.99; 1.26)	236/573	0.98 (0.80; 1.21)	0.99	-0.04 (-0.20; 0.11)	0.52
rs236918‖	all	809/2032	1	3269/7939	1.00 (0.87; 1.15)	1069/2163	1.33 (1.07; 1.65)	4200/8937	1.10 (0.94; 1.28)	0.09	-0.08 (-0.23; 0.07)	0.34
PCK7	♂	404/875	1	1641/3401	0.94 (0.71; 1.24)	523/951	1.26 (0.83; 1.91)	2112/3898	1.06 (0.79; 1.42)	0.79	-0.01 (-0.21; 0.19)	0.91
(G/C)	♀	405/1157	1	1628/4538	1.05 (0.89; 1.24)	546/1212	1.36 (1.11; 1.67)	2088/5039	1.10 (0.93; 1.31)	0.01	-0.21 (-0.39; -0.02)	0.04
rs744653§¶	all	2991/7360	1	1087/2611	1.09 (0.94; 1.25)	3909/8257	1.12 (1.03; 1.23)	1360/2843	1.28 (1.10; 1.48)	0.26	0.02 (-0.04; 0.09)	0.33
SLC40A1	♂	1481/3135	1	564/1141	1.22 (1.05; 1.42)	1977/3643	1.18 (1.06; 1.32)	658/1206	1.36 (1.13; 1.64)	0.44	-0.02 (-0.11; 0.06)	0.56
(C/T)	♀	1510/4225	1	523/1470	0.97 (0.78; 1.21)	1932/4614	1.07 (0.93; 1.24)	702/1637	1.21 (0.97; 1.52)	0.002	0.12 (0.00; 0.23)	0.02
rs855791	all	735/1866	1	3343/8105	1.11 (0.87; 1.42)	986/2061	1.28 (1.01; 1.61)	4283/9039	1.20 (0.98; 1.46)	0.046	-0.04 (-0.09; 0.00)	0.08
TMPRSS6	♂	354/799	1	1691/3477	1.33 (0.96; 1.86)	500/897	1.53 (1.19; 1.97)	2135/3952	1.38 (1.08; 1.77)	0.17	-0.03 (-0.09; 0.02)	0.24
(G/A)	♀	381/1067	1	1652/4628	0.94 (0.67; 1.33)	486/1164	1.07 (0.76; 1.52)	2148/5087	1.04 (0.77; 1.41)	0.19	-0.07 (-0.15; 0.02)	0.14

*G=0 refers to the homozygotes with the lowest ferritin concentrations (reference category); G=1 refers to all heterozygote and homozygote carriers of the ferritin increasing allele; E=0 refers to the group with intake values below the sex-specific median, E=1 refers to the group with intake values above the sex-specific median; Cox models with dummy variables as exposures were stratified by centre and age and adjusted for genotyping chip, BMI (kg/m^2), energy intake, education, smoking, physical activity (4 categories) and alcohol intake (g/day). Country and sex-specific estimates were combined by random effects meta-analysis.

[†]p-value of the multiplicative interaction term; Cox models were stratified by centre and age and adjusted for genotyping chip, BMI (kg/m^2), energy intake (kcal/day), education (5 categories), smoking (3 categories), physical activity (4 categories), alcohol intake (g/day) and menopausal status (4 categories). Country- and sex specific effect estimates were combined by random-effects meta-analysis.

[‡]RERI (relative excess risk due to interaction) = exp ($beta_{GxE}$ + $beta_G$ + $beta_E$)-exp($beta_G$) –exp($beta_E$)+1 ; country- and sex specific effect estimates were combined by random-effects meta-analysis

[§]p for heterogeneity between sexes < 0.05.

[‖]Due to allele frequency distribution of rs236918, G=0 refers to the homozygotes with the lowest ferritin levels and heterozygotes; G=1 refers to homozygote carriers of the ferritin raising allele of rs236918.

[¶] p-value corrected for multiple testing < 0.05 (linear step-up method of Benjamini and Hochberg (38) was used to correct for multiple testing)

Supplementary Tables

Supplementary Table 1: SNP characteristics in the EPIC-InterAct study

gene	variant	function	coded/ non-coded allele	allele frequency	p_{HWE}	confidence threshold of imputed variants[1]	reference
HMOX1	haplotype 1		1/-	0.37			[1]
HMOX1	haplotype 24		1/-	0.05			
HMOX1	haplotype 52		1/-	0.05			
HMOX1	haplotype 84		1/-	0.05			
HMOX1	haplotype 129		1/-	0.05			
HMOX1	haplotype 139		1/-	0.05			
HMOX1	haplotype 207		1/-	0.10			
HMOX1	haplotype 237		1/-	0.19			
HFE	rs1799945	H63D	G/C	0.15	0.16	0.997/-	[2-4]
HFE	rs1800562	C282Y	A/G	0.05	0.56	-	[2-8]
PCSK7	rs236918		C/G	0.10	0.43	0.997/-	[8]
SLC40A1	rs744653		T/C	0.86	0.05	-/0.990	[4]
TF	rs1799852		T/C	0.11	0.47	-	[2,4,6]
TF	rs3811647		A/G	0.32	0.04	-	[2,3,5,7]
TFR2	rs7385804		A/C	0.63	0.13	0.994/-	[3,4]
TFRC	rs17788379		T/C	0.10	1.00	0.988 / 0.937	[9]
TFRC	rs3817672	S142G	T/C	0.55	0.02	-	[9]
TFRC	rs9990333		T/C	0.46	0.99	-/0.985	[4]
TMPRSS6	rs855791	A736V	G/A	0.57	0.68	-	[2,4,5,8,10]

[1] '-' indicates no imputation, number before slash indicates imputation score in individuals genotyped with Illumina Human Quad 660 chip, number after slash indicates imputation score in individuals genotyped with Illumina HumanCoreExome chip

Supplementary Table 2: Search strategy for identification of candidate genes[1]

	("Diabetes Mellitus, Type 2"[Mesh] OR "type 2 diabetes mellitus" OR T2D OR diabetes)
AND	(polymorphism* OR SNPs OR SNP)
AND	iron

[1]assessed at the 23.11.2015

Supplementary Table 3: HMOX1 haplotypes

haplotype	rs34945111	rs112973136	rs5755709	rs114316959	rs735267	rs735266	rs9306299	rs5755710	rs5995096	rs4821395	rs8135345	rs8136058	rs77069690	rs75689278	rs9610289	rs9619536	rs66481421	rs3761439	rs2071746	rs56161244	rs2071748	rs17885522	rs17882565	rs4820192	rs17883018	rs73172715	rs9306300	rs56033205	rs11913209	rs9619538	rs17885925	rs8139532	rs9607267	rs7285877	rs17879606	rs2071749	rs11912889	rs8142390	rs149362862	rs113611204	rs17879082	rs17878790	rs2269533	rs2269534	rs5999815	rs5755718	rs5755719	rs5755720	rs17886055	rs5995098	rs5995099	rs2413346	rs17879027	rs2003038	rs2285112
1	G	A	A	A	A	A	T	G	A	C	A	C	A	G	A	A	A	G	T	C	A	A	A	C	A	C	G	T	A	G	C	A	C	T	T	G	A	T	G	A	C	A	G	C	G	C	C	G	C	G	T	A	A	T	G
24	G	A	A	A	A	A	T	G	A	C	A	C	A	G	A	A	A	G	T	C	A	A	G	C	A	C	G	T	A	G	C	A	C	T	T	G	A	T	G	A	C	A	G	C	G	C	C	G	C	G	T	A	A	T	G
52	G	A	A	A	A	A	T	G	A	C	A	C	G	G	A	A	A	A	T	C	A	A	A	C	A	C	G	T	A	G	C	A	C	T	T	A	A	T	G	A	C	A	G	C	G	C	C	G	C	G	T	A	A	T	G
84	G	A	A	A	A	A	T	G	G	C	A	C	A	G	A	A	A	A	A	C	A	A	A	C	A	C	G	T	A	G	C	A	C	T	T	A	A	T	G	A	C	A	A	C	G	C	C	G	C	G	T	G	A	T	G
129	G	A	G	T	G	T	G	G	A	T	A	C	A	A	A	G	G	A	A	T	G	C	A	C	C	T	A	C	T	C	T	G	C	C	C	A	G	T	A	G	T	G	A	C	G	T	C	G	T	G	T	A	G	T	A
139	G	C	A	A	A	A	T	G	A	C	A	C	A	G	A	A	A	A	G	T	C	A	A	A	C	A	C	G	T	A	G	C	A	C	T	T	G	A	T	G	A	C	A	G	C	G	C	C	G	C	G	T	A	A	T	G
207	A	A	G	A	G	T	G	C	A	T	G	C	A	G	G	A	A	A	A	C	G	A	A	A	A	A	C	G	T	A	G	C	A	T	T	T	A	A	C	G	A	C	A	A	T	A	T	T	A	C	C	C	A	A	C	A
237	A	A	G	A	G	T	G	C	A	T	G	T	A	G	G	A	A	A	A	C	G	A	A	A	A	A	C	G	T	A	G	C	A	T	T	T	A	A	C	G	A	C	A	A	T	A	T	T	A	C	C	C	A	A	C	A

The minor allele of each SNP is indicated by a black background.

Supplementary Table 4: Adjusted Means and regression coefficients for cross-sectional association of SNPs and ferritin in the EPIC-InterAct study

SNP	Sex	genotype A_1/A_1		genotype A_1/A_2		genotype A_2/A_2		$beta_{std}$ (95% CI)	p
		n	Mean (95% CI) [pmol/l]	n	Mean (95% CI) [pmol/l]	n	Mean (95% CI) [pmol/l]		
HFE rs1799945*	men	3186	296.7 (288.0, 305.7)	1110	323.3 (307.4, 340.0)	110	372.4 (317.3, 437.0)	0.097 (0.050, 0.145)	0.0001
(G/C)	women	5123	120.4 (117.5, 123.3)	1864	119.6 (114.9, 124.6)	183	126.1 (110.9, 143.4)	0.029 (-0.010, 0.068)	0.1455
	overall							0.056 (0.026, 0.085)	0.0003
HFE rs1800562	men	3983	301.0 (293.2, 309.1)	415	336.1 (309.5, 365.0)	8	1,183.7 (654.8, 2,139.7)	0.188 (0.110, 0.266)	2.08E-06
(A/G)	women	6487	118.8 (116.3, 121.4)	666	133.7 (125.0, 142.9)	17	223.6 (146.9, 340.3)	0.149 (0.087, 0.212)	3.08E-06
	overall							0.165 (0.116, 0.213)	3.81E-11
PCSK7 rs236918	men	3514	305.5 (297.0, 314.3)	838	302.6 (285.5, 320.7)	54	302.3 (240.5, 379.9)	-0.024 (-0.079, 0.030)	0.3802
(C/G)	women	5781	121.3 (118.5, 124.1)	1311	116.5 (111.1, 122.2)	78	115.2 (94.7, 140.2)	-0.047 (-0.092, -0.002)	0.0423
	overall							-0.037 (-0.072, -0.003)	0.0337
SLC40A1 rs744653	men	82	424.6 (352.8, 511.0)	1031	312.8 (296.9, 329.6)	3293	300.0 (291.4, 308.9)	-0.069 (-0.146, 0.007)	0.0764
(T/C)	women	166	124.1 (108.5, 141.9)	1706	127.2 (122.0, 132.6)	5298	118.1 (115.3, 120.9)	-0.047 (-0.091, -0.002)	0.0387
	overall							-0.055 (-0.095, -0.015)	0.0070
TMPRSS6 rs855791*	men	840	285.9 (269.8, 303.0)	2132	302.2 (291.4, 313.4)	1434	320.9 (307.0, 335.5)	0.057 (0.023, 0.091)	0.0010
(G/A)	women	1341	120.3 (114.7, 126.1)	3526	119.0 (115.5, 122.5)	2303	122.5 (118.1, 127.0)	0.013 (-0.014, 0.041)	0.3452
	overall							0.031 (0.009, 0.052)	0.0058
weighted gene score*	men	1424	286.2 (273.8, 299.2)	1402	301.2 (288.0, 315.0)	1580	326.4 (312.9, 340.4)	0.092 (0.068, 0.117)	1.03E-13
(thirds)	women	2317	115.4 (111.3, 119.7)	2219	118.4 (114.1, 122.8)	2634	126.5 (122.3, 130.9)	0.052 (0.031, 0.072)	9.57E-07
	overall							0.067 (0.049, 0.086)	1.96E-12
HMOX1 ht 1	men	1682	312.9 (300.3, 325.9)	2128	297.1 (286.5, 308.2)	596	311.1 (290.4, 333.3)	-0.014 (-0.050, 0.021)	0.43
HMOX1 ht 1	women	2899	120.0 (116.2, 123.9)	3211	121.8 (118.1, 125.6)	1060	116.7 (110.7, 123.1)	0.005 (-0.028, 0.039)	0.75
	overall							-0.003 (-0.025, 0.020)	0.80
HMOX1 ht 129	men	3972	305.2 (297.2, 313.4)	422	302.1 (278.4, 327.9)	12	315.8 (194.4, 512.9)	-0.013 (-0.091, 0.065)	0.74
HMOX1 ht 129	women	6419	120.0 (117.5, 122.7)	723	123.0 (115.4, 131.2)	28	115.9 (83.5, 160.8)	0.052 (-0.009, 0.112)	0.09
	overall							0.028 (-0.020, 0.075)	0.25
HMOX1 ht 139	men	3953	305.1 (297.1, 313.4)	445	303.6 (280.4, 328.8)	8	274.7 (151.7, 497.6)	-0.014 (-0.137, 0.110)	0.83
HMOX1 ht 139	women	6397	120.8 (118.3, 123.5)	748	116.5 (109.4, 124.2)	25	104.3 (73.7, 147.5)	-0.040 (-0.117, 0.036)	0.30
	overall							-0.026 (-0.092, 0.041)	0.45
HMOX1 ht 207	men	3579	304.6 (296.2, 313.3)	792	307.7 (289.8, 326.6)	35	278.9 (209.9, 370.5)	0.014 (-0.051, 0.079)	0.67
HMOX1 ht 207	women	5775	120.0 (117.3, 122.8)	1323	121.2 (115.6, 127.1)	72	126.8 (103.3, 155.5)	-0.012 (-0.060, 0.037)	0.63
	overall							-0.002 (-0.040, 0.036)	0.93
HMOX1 ht 237	men	2862	302.3 (292.9, 311.9)	1368	308.1 (294.4, 322.4)	176	324.7 (286.1, 368.5)	0.023 (-0.020, 0.065)	0.29
HMOX1 ht 237	women	4725	121.5 (118.5, 124.6)	2189	116.9 (112.6, 121.3)	256	129.5 (116.2, 144.3)	-0.011 (-0.046, 0.023)	0.52
	overall							0.002 (-0.024, 0.029)	0.86
HMOX1 ht 24	men	3932	305.3 (297.2, 313.6)	462	301.0 (278.3, 325.5)	12	353.0 (217.3, 573.3)	-0.002 (-0.075, 0.072)	0.96

HMOX1 ht 24	women	6487	120.1 (117.5, 122.7)	662	123.0 (114.9, 131.5)	21	107.2 (73.4, 156.5)	0.002 (-0.060, 0.064)	0.95
	overall							0.000 (-0.047, 0.048)	0.99
HMOX1 ht 52	men	3962	303.5 (295.5, 311.7)	430	315.6 (291.1, 342.2)	14	421.2 (268.9, 659.7)	0.020 (-0.064, 0.105)	0.64
HMOX1 ht 52	women	6458	120.2 (117.7, 122.9)	697	120.5 (112.8, 128.7)	15	153.4 (98.0, 239.9)	0.002 (-0.060, 0.064)	0.96
	overall							0.006 (-0.042, 0.054)	0.81
HMOX1 ht 84	men	4029	304.7 (296.8, 312.9)	364	305.7 (280.0, 333.9)	13	353.2 (221.7, 562.8)	0.015 (-0.066, 0.095)	0.72
HMOX1 ht 84	women	6489	119.5 (117.0, 122.1)	668	127.4 (119.1, 136.2)	13	170.7 (105.5, 276.0)	0.061 (-0.007, 0.128)	0.08
	overall							0.044 (-0.006, 0.094)	0.08
TF rs1799852	men	3476	304.1 (295.6, 312.9)	868	307.1 (290.1, 325.2)	62	321.4 (259.6, 397.9)	0.007 (-0.047, 0.060)	0.81
(T/C)	women	5642	120.3 (117.6, 123.1)	1438	121.2 (115.8, 126.9)	90	107.9 (89.9, 129.5)	0.006 (-0.036, 0.049)	0.77
	overall							0.007 (-0.027, 0.040)	0.70
TF rs3811647	men	2030	298.4 (287.4, 309.7)	1915	310.2 (298.5, 322.3)	461	312.7 (289.2, 338.2)	0.024 (-0.032, 0.080)	0.40
(A/G)	women	3340	120.7 (117.2, 124.4)	3049	120.0 (116.3, 123.9)	781	119.7 (112.5, 127.4)	-0.011 (-0.047, 0.026)	0.56
	overall							0.004 (-0.028, 0.035)	0.83
TFRC rs3817672	men	953	302.8 (286.8, 319.7)	2134	309.6 (298.6, 321.1)	1319	299.0 (285.5, 313.1)	-0.010 (-0.066, 0.045)	0.71
(T/C)	women	1467	120.9 (115.5, 126.5)	3491	121.3 (117.8, 124.9)	2212	118.5 (114.2, 122.9)	-0.014 (-0.048, 0.019)	0.40
	overall							-0.012 (-0.041, 0.018)	0.44
TFRC rs9990333	men	1270	314.0 (299.5, 329.1)	2224	302.1 (291.5, 313.0)	912	299.6 (283.4, 316.7)	-0.030 (-0.065, 0.004)	0.08
(T/C)	women	2097	122.1 (117.6, 126.8)	3540	119.0 (115.6, 122.5)	1533	121.0 (115.8, 126.5)	-0.008 (-0.035, 0.020)	0.59
	overall							-0.016 (-0.038, 0.005)	0.13
TFR2 rs7385804	men	605	304.6 (284.4, 326.1)	2037	302.3 (291.2, 313.7)	1764	308.2 (296.1, 320.8)	0.001 (-0.036, 0.039)	0.95
(A/C)	women	999	117.8 (111.5, 124.4)	3257	119.3 (115.8, 123.0)	2914	122.3 (118.5, 126.3)	0.027 (-0.002, 0.055)	0.06
	overall							0.016 (-0.006, 0.038)	0.15

Means were adjusted for age, centre and genotyping chip. Effect estimates and p-values were derived from random-effects meta-analysis from country- and sex-specific linear regression models. Linear regression models were adjusted for age, centre, genotyping chip and principal components. Effect estimates are given for a change in standardized log transformed ferritin per allele;

* p for heterogeneity between sexes < 0.05

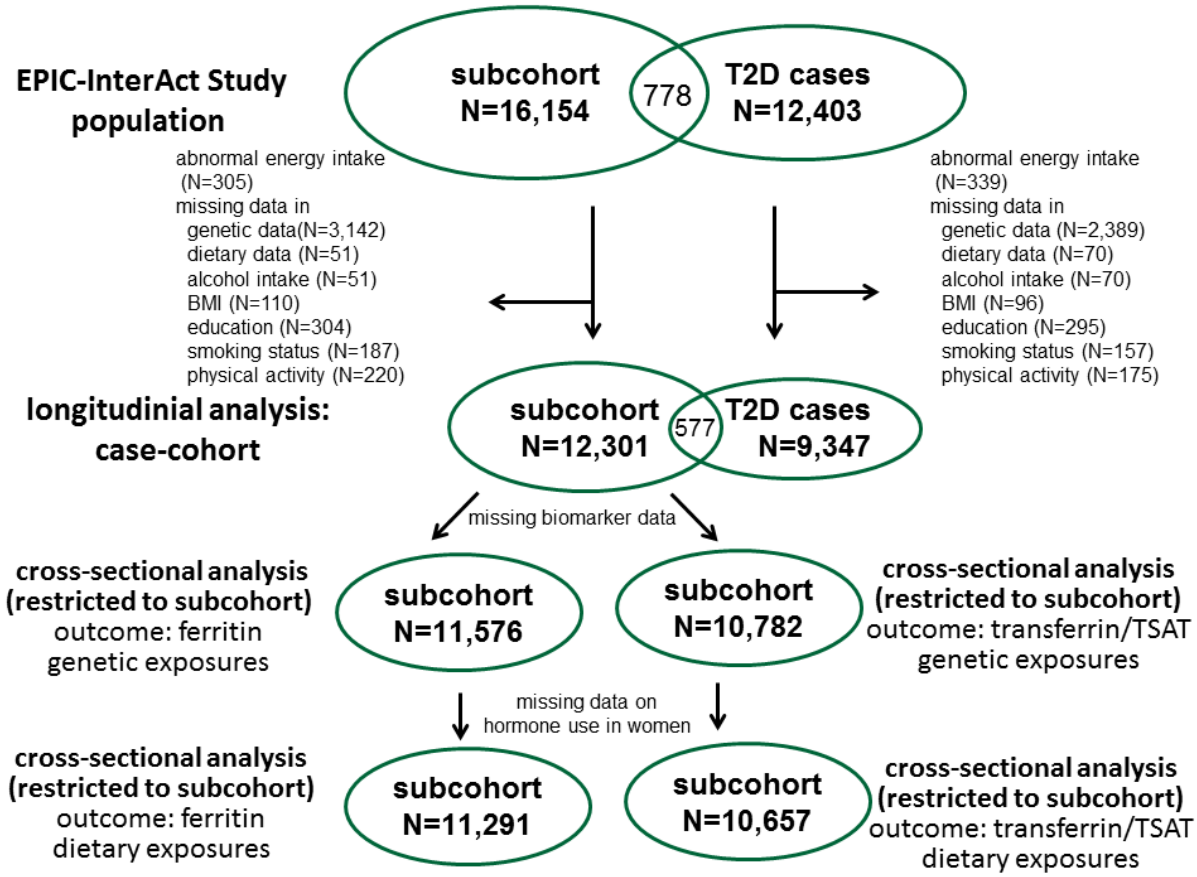
Supplementary Table 5: Cross-sectional analysis on interaction of ferritin related SNPs and haem iron on ferritin levels in the EPIC-InterAct study

SNP	Sex	G=0|E=0[1]		G=1|E=0[1]		G=0|E=1[1]		G=1|E=1[1]		Interaction				
		N	beta	N	beta (95% CI)	N	beta (95% CI)	N	beta (95% CI)	$beta_{std}$ (95% CI)	p	p_{fdr}	I^2	$p_{sex\ diff}$
weighted gene score	all	2150	0 (Ref)	3489	0.06 (0.01; 0.11)	2196	0.14 (0.09; 0.18)	3456	0.20 (0.16; 0.25)	-0.001 (-0.016; 0.014)	0.88	0.94	10%	0.15
	men	826	0 (Ref)	1379	0.11 (0.04; 0.18)	864	0.13 (0.05; 0.20)	1337	0.25 (0.17; 0.32)	0.006 (-0.010; 0.021)	0.47	0.94		
	women	1324	0 (Ref)	2110	0.03 (-0.04; 0.09)	1332	0.14 (0.08; 0.20)	2119	0.18 (0.12; 0.23)	-0.016 (-0.041; 0.009)	0.22	0.94		
HFE rs1799945	all	4076	0 (Ref)	1578	0.05 (0.00; 0.11)	4024	0.14 (0.10; 0.18)	1613	0.19 (0.15; 0.24)	-0.003 (-0.032; 0.025)	0.81	0.94	0%	0.47
(G/C)	men	1605	0 (Ref)	593	0.13 (0.05; 0.20)	1581	0.16 (0.08; 0.23)	627	0.23 (0.13; 0.34)	-0.012 (-0.048; 0.024)	0.52	0.94		
	women	2471	0 (Ref)	985	0.00 (-0.06; 0.06)	2443	0.13 (0.09; 0.18)	986	0.18 (0.12; 0.24)	0.010 (-0.037; 0.057)	0.67	0.94		
HFE rs1800562	all	5112	0 (Ref)	542	0.16 (0.09; 0.23)	5106	0.15 (0.11; 0.18)	531	0.27 (0.20; 0.35)	-0.004 (-0.054; 0.047)	0.89	0.94	12%	0.14
(A/G)	men	1989	0 (Ref)	209	0.16 (0.03; 0.28)	1994	0.14 (0.07; 0.22)	214	0.30 (0.19; 0.41)	0.019 (-0.031; 0.070)	0.45	0.94		
	women	3123	0 (Ref)	333	0.16 (0.07; 0.25)	3112	0.15 (0.11; 0.19)	317	0.25 (0.13; 0.36)	-0.054 (-0.138; 0.030)	0.21	0.94		
PCK7 rs236918[2]	all	1166	0 (Ref)	4488	0.03 (-0.02; 0.08)	1065	0.13 (0.07; 0.20)	4572	0.17 (0.12; 0.22)	0.014 (-0.021; 0.050)	0.42	0.94	0%	0.67
(G/C)	men	458	0 (Ref)	1740	0.03 (-0.07; 0.13)	434	0.16 (0.03; 0.29)	1774	0.16 (0.08; 0.24)	0.007 (-0.045; 0.059)	0.78	0.94		
	women	708	0 (Ref)	2748	0.03 (-0.04; 0.10)	631	0.13 (0.04; 0.21)	2798	0.18 (0.11; 0.25)	0.024 (-0.030; 0.078)	0.39	0.94		
SLC40A1 rs744653	all	4174	0 (Ref)	1480	0.01 (-0.05; 0.08)	4194	0.12 (0.08; 0.16)	1443	0.22 (0.15; 0.29)	0.006 (-0.042; 0.054)	0.81	0.94	52%	0.75
(C/T)	men	1629	0 (Ref)	569	0.04 (-0.07; 0.14)	1664	0.13 (0.05; 0.21)	544	0.22 (0.08; 0.37)	0.017 (-0.041; 0.075)	0.57	0.94		
	women	2545	0 (Ref)	911	0.00 (-0.08; 0.08)	2530	0.12 (0.08; 0.17)	899	0.22 (0.15; 0.28)	0.001 (-0.079; 0.081)	0.98	0.98		
TMPRSS6 rs855791	all	1080	0 (Ref)	4574	0.05 (-0.01; 0.10)	1050	0.14 (0.07; 0.21)	4587	0.18 (0.13; 0.24)	-0.008 (-0.029; 0.013)	0.48	0.94	0%	0.17
(G/A)	men	437	0 (Ref)	1761	0.08 (-0.01; 0.16)	403	0.13 (0.00; 0.26)	1805	0.21 (0.12; 0.29)	0.005 (-0.023; 0.032)	0.73	0.94		
	women	643	0 (Ref)	2813	0.03 (-0.05; 0.10)	647	0.15 (0.07; 0.24)	2782	0.17 (0.09; 0.25)	-0.025 (-0.058; 0.008)	0.13	0.94		

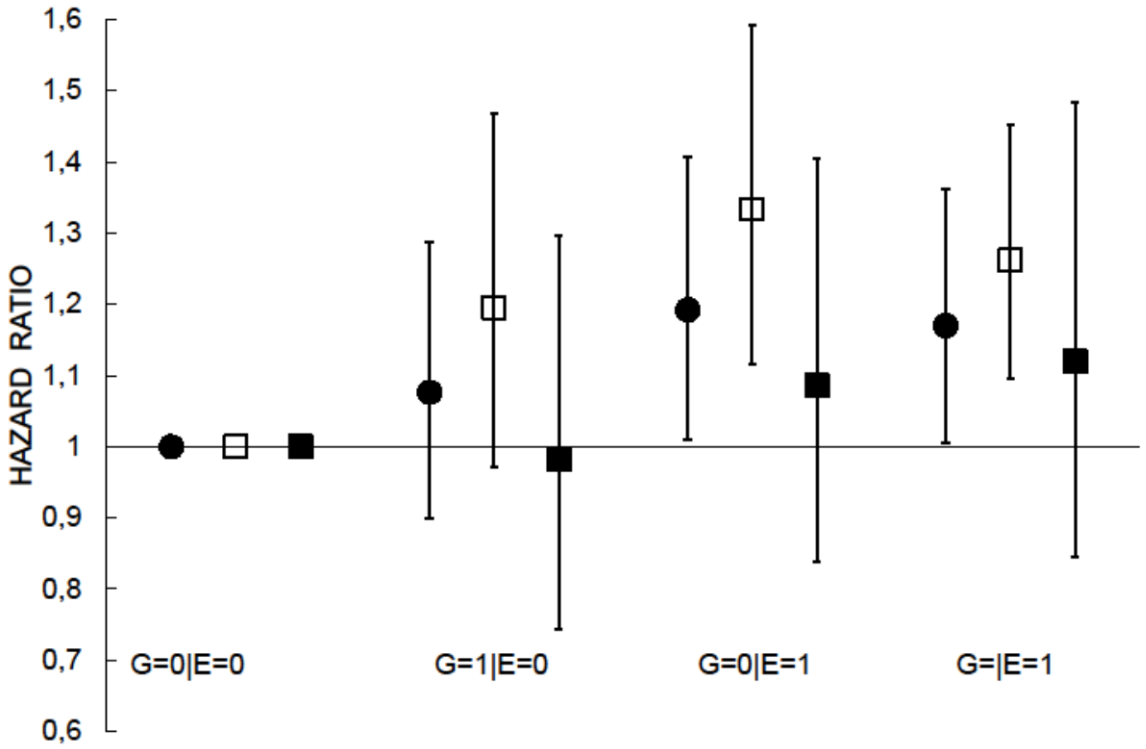
[1]G=0 refers to the homozygotes with the lowest ferritin levels; G=1 refers to all heterozyte and homozygote carriers of the ferritin raising allele; E=0 refers to the group with intake values below the sex-specific median, E=1 refers to the group with intake values above the sex-specific median; country and sex-specific estimates were combinded by random effects meta-analysis, beta is given per standard deviation of log transformed ferritin and refers to estimated ferritin levels of the named subgroup compared to the G=0|E=0 group.

[2] Due to allele frequency distribution of rs236918, G=0 refers to the homozygotes with the lowest ferritin levels and heterozygotes; G=1 refers to homozygote carriers of the ferritin raising allele of rs236918.

Supplementary Figures



Supplementary Figure 1: Deviation of the analytical study population



Supplementary Figure 2: Hazard ratios of incident type 2 diabetes according to haem iron intake and GRS levels

G=0 refers to GRS below median; G=1 refers to GRS equal or above median; E=0 refers to the group with intake values below the sex-specific median, E=1 refers to the group with intake values above the sex-specific median; Cox models with dummy variables as exposures were stratified by centre and age and adjusted for genotyping chip, BMI (kg/m^2), energy intake, education, smoking, physical activity (4 categories) and alcohol intake (g/day). Country and sex-specific estimates were combined by random effects meta-analysis. ● refers to HR in the overall study population, □ refers to HR in men and ■ to HR in women

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