Interaction of Dietary and Genetic Factors Influencing Body Iron Status and Risk of Type 2 Diabetes Within the EPIC-InterAct Study

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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 case subjects and 12,301 subcohort participants from eight European countries. Single nucleotide polymorphisms (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 heme iron and SNPs as well as the gene score were evaluated using Cox proportional hazards regression.

RESULTS

Higher heme iron intake (per 1 SD) was associated with higher ferritin levels (β = 0.113 [95% CI 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*], and rs855791 [*TMPRSS6* V736A]) were associated with ferritin. We did not detect an interaction of heme iron and the gene score on the risk of diabetes in the overall study population (P_{add} = 0.16, P_{mult} = 0.21) but did detect a trend toward 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). Heme 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, including ferritin, transferrin, and transferrin saturation (TSAT), have been linked with type 2 diabetes in a number of studies, including the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct study (7–10).

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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 because 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 single nucleotide polymorphism (SNP) (*HFE* H63D) and heme 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 and a genome-wide interaction analysis did not reveal significant interactions with heme iron intake on the risk of type 2 diabetes (18,19). Nevertheless, these studies were limited in power by their sample size, and excluding interaction effects of moderate size is therefore not possible. We therefore aim to analyze interactions between genetic factors influencing body iron stores and meat, a major dietary determinant of body iron stores, and the 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 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 centers in 8 of 10 countries participating in EPIC (France, Italy, Spain, the U.K., the Netherlands, Germany, Denmark, and Sweden). Participants without stored blood (n = 109,625) or without information on diabetes (n = 5,821) were excluded. All individuals ascertained and verified with incident type 2 diabetes between 1991 and 2007 (3.99 million person-years at risk, n = 12,403) comprised the case group. A center-stratified, representative subcohort of 16,835 individuals was selected as the comparison (control) group to assess the exposure distribution in the cohort. Case subjects with prevalent diabetes (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 case subjects with incident type 2 diabetes, a random set of 778 case subjects was 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; $n_{\text{subcohort}}$ = 305; $n_{\text{case subjects}}$ = 339), missing information on dietary intake ($n_{\text{subcohort}}$ = 51; $n_{\text{case subjects}}$ = 70), no genetic data (including samples removed because of relatedness or non-European ethnicity; $n_{\text{subcohort}}$ = 3,142; $n_{\text{case subjects}}$ = 2,389), and missing covariate data ($n_{\text{subcohort}}$ = 821; $n_{\text{case subjects}}$ = 723), leaving a sample of 9,347 case subjects and 12,301 subcohort participants, including 577 case subjects in the subcohort (Supplementary Fig. 1). Cross-sectional analyses for biomarkers were performed within the subcohort and additionally excluded samples with missing biomarker measurement. Sample size varied between 10,657 and 11,576 individuals between analyses, because ferritin on the one hand and transferrin and iron on the other were measured in a slightly different sample size (Supplementary Fig. 1).

Case Ascertainment

Ascertainment of incident type 2 diabetes involved a review of the existing EPIC data sets at each center 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 were 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 individuals with type 2 diabetes, we sought further evidence, including individual medical records review in some centers. 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

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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 items containing bacon, ham, and liver and all other processed meats such as black pudding, chorizo, sausages, and corned beef. Total meat was derived by summing intakes of red meat, processed meat, poultry, and offal. Energy and nutrient (iron, calcium, vitamin C, fiber, alcohol) intakes were estimated using the standardized EPIC Nutrient Database (23). The calculation of heme iron was based on the proportion of heme iron on the total iron content of the specific meat item (65% beef, 39% pork, 52% remaining red meat and processed meat, 26% poultry and fish, and 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 centers for genome-wide genotyping using the Illumina 660W-Quad BeadChip and the Illumina HumanCoreExome-12v1 and -24v1 BeadArrays (Illumina, San Diego, CA) at different times. The number of individuals selected per center was proportional to the percentage of the total case subjects in that center. Illumina 660 and CoreExome data sets were separately quality controlled and imputed to the data set 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 2.1.1 software (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 genome-wide association studies (rs1799945, rs1800562, rs744653, and rs855791). Weights were based on the betas reported in the literature (13).

Biomarker Measurement

Samples were stored from collection in liquid nitrogen at −196°C in the coordinating center at the International Agency for Research on Cancer 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 SHL-Groep, Etten-Leur, the Netherlands. Ferritin, iron, and transferrin were measured by Cobas (Roche Diagnostics, Mannheim, Germany) assays on a Roche Hitachi Modular P analyzer 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) × 100)]/[transferrin (g/L) × 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 TSAT) 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 common biomarkers of body iron status and tested for their interaction in a third step. All analyses were 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 $\log_{10}$ 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, and heme 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 center, physical activity (four categories), total energy intake (kcal/day), and the intakes of fiber (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 center, genotyping chip, and eigenvalues of the first 10 coordinates from multidimensional scaling on common and low-frequency variants (minor allele frequency >0.01).

Interaction analyses were only calculated for dietary and genetic exposures that showed a significant association ($P < 0.05$) in the preceding analyses.

Interaction of Dietary and Genetic Factors on Biomarker Levels

Interaction analyses were done 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 performed 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 center 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 done 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 and Chambless (37). The delta method was used to calculate SEs of the RERI (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 and 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 the Cochran *Q* test. 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 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 (FDR) from Benjamini and Hochberg (38). Furthermore, associations of SNPs with diabetes from logistic regression within the EPIC-InterAct study were combined with data from DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) by meta-analysis. All analyses used SAS Enterprise Guide 6.1, SAS 9.4, and R 3.1.2 software.

RESULTS

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

Selection of Dietary Factors

Intake of all analyzed meat and iron items was directly associated with ferritin concentrations (Table 2). The strongest association was observed for heme iron (β = 0.113 [95% CI 0.082; 0.144] SD in ferritin/1 SD heme iron, $P = 1.3 \times 10^{-12}$) (Table 2). No significant associations were detected for any of the analyzed dietary factors with transferrin or TSAT (Table 2). Differences in associations between men and women were not observed ($P_{sex\_diff} > 0.05$). These results were used to restrict the selection of SNPs for interaction analyses to those associated with the biomarker ferritin. Subsequent interaction analyses were done with heme iron.

Selection of Genetic Factors

Among the analyzed 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 (β = –0.037 [95% CI −0.071; –0.003]). None of the SNPs located in the transferrin (*TF*), the transferrin receptor (*TFRC*, *TFR2*), and heme 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: β = 0.092 [0.068; 0.117], women: β = 0.052 [0.031; 0.072], P = 0.01 for heterogeneity between the sexes). The observed associations resulted in further association and interaction analyses being restricted to all SNPs associated with ferritin concentrations (see above) and the gene score.

Main Effect of Genetic Determinants of Ferritin on the Risk of Type 2 Diabetes

The ferritin-related variants and gene scores were not significantly associated with the 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*) (odds ratio [OR] 1.06 [95% CI 1.02; 1.09], P_{FDR} = 0.02) and of rs744653 (*SLC40A1*) (OR 1.05 [1.02; 1.09], P_{FDR} = 0.02) with diabetes (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 heme 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 heme 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 heme iron in women (P_{mult_raw} = 0.01, P_{add_raw} = 0.04), rs1799945 (*HFE*) and heme iron in men (P_{mult_raw} = 0.01, P_{add_raw} = 0.02), and the gene score and heme iron in men

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 (years)	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)
U.K.		894 (7.3)	348 (7.4)	546 (7.2)
The 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 (g/day)				
Total meat	0	98.6 (65.6–137.6)	123.9 (85.4–169.9)	85.6 (57.5–117.5)
Red meat	0	38.1 (18.6–65.6)	49.7 (25.0–81.7)	32.9 (16.3–55.4)
Processed meat	0	28.9 (15.1–50.1)	39.3 (21.9–64.2)	23.5 (12.6–41.2)
Poultry	0	15.6 (6.8–29.6)	16.4 (7.9–32.2)	14.4 (6.1–27.3)
Fish	0	28.5 (14.8–50.9)	33.9 (16.8–58.8)	26.2 (13.1–46.1)
Heme iron	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)

Data are given as median (25th–75th percentile) or *n* (%).

(P_{mult_raw} = 0.03, P_{add_raw} = 0.04) (Table 4). Significant differences between the 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 Heme Iron

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

Interaction of rs744653 and Heme Iron in Women

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

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)	β (95% CI)	P value	I^2 (%)	β (95% CI)	P value	I^2 (%)	β (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
Heme 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 and 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 the standardized (and in the case of ferritin, log-transformed) biomarker per SD in dietary intake. Exposures and outcomes were standardized to a mean of 0 and an SD of 1. Ferritin was log-transformed before 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, center, physical activity (four categories), total energy intake (kcal/day), and the intakes of fiber (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 use of hormone replacement therapy. No significant ($P < 0.05$) heterogeneity between sexes was observed; therefore, sex-combined estimates were reported only.

Interaction of rs1799945 (*HFE* H63D) and Heme Iron in Men

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

Interaction of Gene Score and Heme Iron in Men

The interaction of the gene score and heme iron (Table 4 and Supplementary Fig. 2) in men can be characterized as follows: the diabetes HR among men with a high heme intake was slightly higher in the men with a low gene score (HR 1.33 [95% CI 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 heme iron intake, the HR was higher when the gene score was high (HR 1.20 [0.97; 1.47]) compared with when it was low (reference category).

Based on these results, we tested whether we could also observe a tendency for interactions of heme iron and genetic factors on ferritin levels in a cross-sectional analysis. However, we found no indication that such interactions exist (Supplementary Table 5).

CONCLUSIONS

We studied the interaction of ferritin-related genetic variants and heme 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 toward 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 thus 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 heme iron intake and several genetic variants influencing body iron stores. This may imply that no such interactions exist or that our power was insufficient to detect them

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

		EPIC-InterAct	DIAGRAM	Meta-analysis		
		n_{T2D} = 8,794; $n_{control\ subjects}$ = 11,032	n_{T2D} = 26,488; $n_{control\ subjects}$ = 83,964	n_{T2D} = 35,282; $n_{control\ subjects}$ = 94,996		
SNP	A_2/A_1	HR (95% CI)*	OR (95% CI)	OR (95% CI)†	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)				

T2D, type 2 diabetes. *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 OR scale. ORs in the EPIC-InterAct study were calculated in a case/noncase design using logistic regression. Logistic regression models were adjusted for age, sex, BMI, genotyping source, principal components, and study center. Country-specific estimates were combined by random-effects meta-analysis within EPIC-InterAct. Participants from the EPIC center Norfolk were excluded from the analysis within EPIC-InterAct, because EPIC-Norfolk was part of the DIAGRAM consortium.

Table 4—Prospective analysis of interactions of ferritin-related SNPs and heme iron intake on the risk of type 2 diabetes in the EPIC-InterAct study

SNP			G = 0 | E = 0*			G = 1 | E = 0*		G = 0 | E = 1*		G = 1 | E = 1*			
Gene	Sex	*N*	HR	*N*	HR (95% CI)	*N*	HR (95% CI)	*N*	HR (95% CI)	P_{mult_raw}†	RERI‡ HR (95% CI)	P_{add_raw}‡
Weighted gene score	All	1,693/4,302	1	2,385/5,669	1.08 (0.90; 1.29)	2,244/4,746	1.19 (1.01; 1.41)	3,025/6,354	1.17 (1.00; 1.36)	0.21	−0.09 (−0.22; 0.03)	0.16
	♂	823/1,835	1	1,222/2,441	1.20 (0.97; 1.47)	1,114/2,070	1.33 (1.12; 1.59)	1,521/2,779	1.26 (1.10; 1.45)	0.03	−0.23 (−0.46; −0.01)	0.04
	♀	870/2,467	1	1,163/3,228	0.98 (0.74; 1.30)	1,130/2,676	1.09 (0.84; 1.40)	1,504/3,575	1.12 (0.85; 1.48)	0.68	0.04 (−0.20; 0.27)	0.66
rs1799945§ *HFE* (G/C)	All	2,923/7,186	1	1,155/2,785	1.11 (0.97; 1.26)	3,732/7,903	1.15 (1.03; 1.27)	1,537/3,197	1.26 (1.13; 1.40)	0.32	−0.06 (−0.17; 0.06)	0.29
	♂	1,460/3,102	1	585/1,174	1.22 (1.05; 1.41)	1,865/3,447	1.23 (1.09; 1.38)	770/1,402	1.22 (1.01; 1.48)	0.01	−0.16 (−0.29; −0.02)	0.02
	♀	1,463/4,084	1	570/1,611	1.02 (0.82; 1.28)	1,867/4,456	1.07 (0.92; 1.25)	767/1,795	1.27 (1.10; 1.46)	0.49	0.08 (−0.05; 0.20)	0.40
rs1800562 *HFE* (A/G)	All	3,702/9,028	1	376/943	0.86 (0.73; 1.01)	4,762/10,048	1.13 (1.04; 1.24)	507/1,052	1.03 (0.84; 1.27)	0.91	−0.03 (−0.10; 0.05)	0.50
	♂	1,854/3,867	1	191/409	0.83 (0.63; 1.08)	2,364/4,370	1.15 (1.01; 1.32)	271/479	1.09 (0.72; 1.64)	0.99	−0.04 (−0.19; 0.11)	0.60
	♀	1,848/5,161	1	185/534	0.89 (0.71; 1.11)	2,398/5,678	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‖ *PCK7* (G/C)	All	809/2,032	1	3,269/7,939	1.00 (0.87; 1.15)	1,069/2,163	1.33 (1.07; 1.65)	4,200/8,937	1.10 (0.94; 1.28)	0.09	−0.08 (−0.23; 0.07)	0.34
	♂	404/875	1	1,641/3,401	0.94 (0.71; 1.24)	523/951	1.26 (0.83; 1.91)	2,112/3,898	1.06 (0.79; 1.42)	0.79	−0.01 (−0.21; 0.19)	0.91
	♀	405/1,157	1	1,628/4,538	1.05 (0.89; 1.24)	546/1,212	1.36 (1.11; 1.67)	2,088/5,039	1.10 (0.93; 1.31)	0.01	−0.21 (−0.39; −0.02)	0.04
rs7446536¶ *SLC40A1* (C/T)	All	2,991/7,360	1	1,087/2,611	1.09 (0.94; 1.25)	3,909/8,257	1.12 (1.03; 1.23)	1,360/2,843	1.28 (1.10; 1.48)	0.26	0.02 (−0.04; 0.09)	0.33
	♂	1,481/3,135	1	564/1,141	1.22 (1.05; 1.42)	1,977/3,643	1.18 (1.06; 1.32)	658/1,206	1.36 (1.13; 1.64)	0.44	−0.02 (−0.11; 0.06)	0.56
	♀	1,510/4,225	1	523/1,470	0.97 (0.78; 1.21)	1,932/4,614	1.07 (0.93; 1.24)	702/1,637	1.21 (0.97; 1.52)	0.002	0.12 (0.00; 0.23)	0.02
rs855791 *TMPRSS6* (G/A)	All	735/1,866	1	3,343/8,105	1.11 (0.87; 1.42)	986/2,061	1.28 (1.01; 1.61)	4,283/9,039	1.20 (0.98; 1.46)	0.046	−0.04 (−0.09; 0.00)	0.08
	♂	354/799	1	1,691/3,477	1.33 (0.96; 1.86)	500/897	1.53 (1.19; 1.97)	2,135/3,952	1.38 (1.08; 1.77)	0.17	−0.03 (−0.09; 0.02)	0.24
	♀	381/1,067	1	1,652/4,628	0.94 (0.67; 1.33)	486/1,164	1.07 (0.76; 1.52)	2,148/5,087	1.04 (0.77; 1.41)	0.19	−0.07 (−0.15; 0.02)	0.14

♀, female; ♂, male. *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 center and age and adjusted for genotyping chip, BMI (kg/m^2), energy intake, education, smoking, physical activity (four categories), and alcohol intake (g/day). Country- and sex-specific effect estimates were combined by random effects meta-analysis. †*P* value of the multiplicative interaction term. Cox models were stratified by center and age and adjusted for genotyping chip, BMI (kg/m^2), energy intake (kcal/day), education (five categories), smoking (three categories), physical activity (four categories), alcohol intake (g/day), and menopausal status (four categories). Country- and sex-specific effect estimates were combined by random-effects meta-analysis. ‡RERI = $\exp(\beta_{G\times E} + \beta_G + \beta_E) - \exp(\beta_G) - \exp(\beta_E) + 1$; country- and sex-specific effect estimates were combined by random-effects meta-analysis. §$P < 0.05$ for heterogeneity between sexes. ‖Owing 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).

despite the large sample size. Indeed, main effects on the risk of type 2 diabetes for two of the analyzed SNPs were only detected when our data were pooled with data from large-scale meta-analysis. Therefore, we assume 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. Baseline information has been collected in a standardized way within the different EPIC countries, 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 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 may be 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 heme 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 heme iron intake has been described before in the female participants of the Nurses' Health Study (17), with a linear trend for the association of heme 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, did not find evidence for an interaction of *TMPRSS6* variant rs855791 and heme iron (18). We assume that our larger samples size allowed us to identify this nominally significant interaction, which requires further replication. Besides analyzing interaction effects of various single SNPs, we additionally analyzed the interaction of a ferritin-related gene score and heme iron. We detected no statistically significant interaction within the overall study population but a tendency toward a negative interaction in men. The analysis shows that individuals genetically predisposed to increased ferritin levels and who have a high heme iron intake are indeed at a higher risk for type 2 diabetes, but the risk may be 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 heme 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 the 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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