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Dietary iron, iron homeostatic gene polymorphisms and the risk of advanced colorectal adenoma and cancer

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Dietary iron intake and variation in iron homeostasis genes may affect colorectal neoplasia risk. We conducted two nested case–control studies within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial: one of advanced colorectal adenoma (1205 cases; 1387 controls) and one of colorectal cancer (370 cases; 401 controls). Iron intake was estimated with a food frequency questionnaire and genotyping was performed for 21 genes. Unconditional logistic regression was used to estimate odds ratio (OR) and 95% confidence intervals (95% CIs) for colorectal neoplasia risk within quartiles of intake. Several single nucleotide polymorphisms (SNPs) modified the association between iron intake and the risk of adenoma or cancer. Dietary iron was positively associated with colorectal adenoma among three SNPs of *HEPHL1*, including carriers of the AA genotype at rs7946162 ($OR_{Q4–Q1}$ = 2.22, 95% CI 1.15–4.27, P_{trend} = 0.03; $P_{interaction}$ = 0.10), the TT genotype at rs2460063 ($OR_{Q4–Q1}$ = 2.39, 95% CI 1.26–4.54, P_{trend} = 0.02; $P_{interaction}$ = 0.04) and the GG genotype at rs7127348 ($OR_{Q4–Q1}$ = 2.40, 95% CI 1.23–4.67, P_{trend} = 0.02; $P_{interaction}$ = 0.09). Heme iron was positively associated with colorectal cancer among those with GG genotypes for *ACO1* rs10970985 ($OR_{Q4–Q1}$ = 2.45, 95% CI 3.40–8.06, P_{trend} = 0.004; $P_{interaction}$ = 0.05). However, none of the associations were statistically significant after adjustment for multiple comparisons. Future studies should target the specific genes and SNPs for which the association was significant prior to multiple comparison correction.

Introduction

Substantial data from epidemiologic studies support a dose–response relationship between increased red meat consumption and colorectal adenoma and colorectal cancer risk (1–3), although no adverse association between white meat and colorectal neoplasia has been documented (4–7). Compared with white meat, red meat contains 5-fold higher levels of iron (8). Both total iron and heme iron have potential carcinogenic properties, including the formation of hydroxyl radicals through the Fenton reaction, catalyzation of endogenous *N*-nitrosation and increased cytotoxicity and cell proliferation (9–13). Evidence for an association between biomarkers of iron status and colorectal neoplasia is mixed (14–18). Inter- and intraindividual factors, such as age, sex, inflammation, day-to-day variation and undetected disease impact biomarkers of iron status and the interpretation of a single value from reflecting longer term iron status that could contribute to carcinogenic potential (19). Dietary iron intake is not highly correlated with biomarkers of iron status since estimates of iron absorption in healthy persons range from 1 to 40% (19). Heme iron intake, which has rates of absorption of 15–40%, has been moderately correlated with serum ferritin concentration (18). Nonetheless, unabsorbed iron is relevant as a potential risk factor for colorectal carcinogenesis since unabsorbed iron remains in contact with colorectal tissue and is highly concentrated in feces (12). The epidemiologic evidence linking iron intake to colorectal neoplasia is also inconsistent and graded as 'limited-suggestive' in a 2007 review and meta-analysis commissioned by the World Cancer Research Fund/American Institute for Cancer Research (1), coupled with a Continuous Update Project completed in 2010 (20). Dietary heme iron may have a greater carcinogenic potential than non-heme iron (9,21), which induces production of genotoxic free radicals in the colonic stream and of potentially carcinogenic *N*-nitroso compounds in the gastrointestinal tract (20). The latter seems prominent when nitrate or nitrite-cured meat is consumed. In previous studies, heme iron intake has been assessed via one of two methods, both based on standard proportions of total iron from meat (22,23), and these methods fail to account for individual meat types and cuts, as well as the effect of cooking method on the conversion of heme iron to non-heme iron. Previous work has shown that heat treatment, type of cooking methods and the duration of cooking can influence the conversion of heme to non-heme iron by 10 to 100% (24).

Interindividual variation in iron uptake and metabolism could be explained by polymorphisms in genes governing iron homeostasis. Several studies have examined the hemochromatosis (*HFE*) and transferrin receptor-1 (*TFR1*) genes in relation to colorectal neoplasia, but the results have been largely mixed (25–30). It is hypothesized that possessing variants that result in increased iron uptake and consequential elevation of circulating and/or stored body iron lead to high cellular iron levels and may predispose an individual to colorectal neoplasia via an enhanced oxidative environment within the colonocyte. However, no study has comprehensively investigated variation in a range of genes related to iron uptake and absorption or the interaction of iron intake with variation in multiple genes involved in iron homeostasis with colorectal adenoma or cancer.

In this study, we investigated intake of total iron (from diet and supplements), dietary iron, heme iron from meat using a novel database and variation in 21 genes involved in iron homeostasis in relation to advanced colorectal adenoma and colorectal cancer risk in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire; OR, odds ratio; PLCO, Prostate, Lung, Colorectal and Ovarian; SNP, single nucleotide polymorphism.

Materials and methods

Study population

The PLCO Cancer Screening Trial is a randomized, multicenter clinical trial investigating the efficacy of screening for prostate, lung, colorectal and ovarian cancer (31,32). Participants aged 55–74 years were recruited from 10 centers in the USA from 1993 to 2001 and randomly assigned to the screened or control arm of the trial (31). The study was approved by the Institutional Review Board at the National Cancer Institute and the 10 study centers. All participants provided written informed consent.

The present investigation is restricted to 77 469 individuals randomized to the screening arm of the trial who completed a self-administered risk factor questionnaire and food frequency questionnaire (FFQ), donated biological samples for use in etiological studies, had no history of cancer (other than non-melanoma skin cancer) or self-reported colon disease (Crohn's disease, ulcerative colitis, familial polyposis or Gardner's syndrome). Individuals with a prior history of colorectal polyps were also excluded from the analyses of adenoma. When suspect lesions were identified, the participant was referred to their health-care provider for further diagnostic work-up. The majority of

subjects with a suspicious lesion underwent a follow-up colonoscopy (33), and results from follow-up visits were abstracted from medical records by trained personnel to identify pathologically confirmed cases of adenoma and cancer, as well as the location, size and morphology of the tumor. All participants were sent an annual questionnaire querying recent cancer diagnoses.

Colorectal adenoma study

A nested case–control study of advanced adenoma was conducted among participants who had a successful sigmoidoscopy examination (defined as identification of a suspicious lesion or insertion to at least 50 cm and >90% of the mucosa visible) at baseline. Cases were participants found to have at least one advanced colorectal adenoma (≥1 cm in size, containing villous/tubulovillous characteristics or had severe dysplasia) of the distal colon or rectum at baseline (n = 1205). A total of 1387 controls, defined as those with a baseline sigmoidoscopy exam that was negative for polyps in the distal colon and rectum, were frequency matched to cases. All controls were matched to the adenoma cases on sex and ethnicity; 46% of the adenoma controls were also matched on age.

Colorectal cancer study

We also conducted a nested case–control study of colorectal cancer with follow-up time through 31 December 2006. Cancer cases with available DNA (n = 370) were identified by colonoscopy conducted as part of the screening trial or by an annual questionnaire asking about recent cancer diagnoses. Medical records were verified for all colorectal cancers reported on the questionnaire or through death certificate. Controls (n = 401) were free of colorectal cancer at the time the case was diagnosed and were frequency matched to the cases on sex, 5-year age category, ethnicity, year of randomization and time since randomization.

Dietary data

Dietary and supplement intake were ascertained from a validated 137-item FFQ administered at baseline that queried usual intake in the previous 12 months (34). Most (89%) participants in the trial completed the FFQ prior to or on the same day as the baseline sigmoidoscopy. The FFQ was administered in conjunction with a meat module in which participants were asked to select the cooking method and degree of doneness that best represented their usual intake of a particular meat. The data from the meat module were linked to a database containing heme iron values quantified by atomic absorption spectrometry for a variety of meats cooked by varying degrees and cooking methods (35). In total, three categories of iron intake were examined: total iron (from diet and supplement sources), dietary iron and heme iron from meat. Participants were excluded from the analyses if they had ≥8 missing responses or invalid responses on the FFQ (n = 63 and n = 7 for the adenoma and cancer study samples, respectively).

Genotyping

A total of 312 single nucleotide polymorphisms (SNPs) from 21 genes directly involved in iron homeostasis (*ACO1*, *B2M*, *CP*, *CYBRD1*, *FTH1*, *FTL*, *HAMP*, *HEPH*, *HEPHL1*, *HFE*, *HFE2*, *HMOX1*, *HMOX2*, *HP/HPR*, *IGFBP3*, *IREB2*, *TF*, *TFR2*, *TFRC*, *SLC11A2*, *SLC40A1*) were selected for genotyping. These genes are specifically involved in iron uptake, transport and absorption (e.g. *CYBRD1*, *HAMP*, *HEPH*, *HEPHL1*, *HFE*, *HFE2*, *TFR2*, *TFRC*), as well as storage (*ACO1*, *IREB2*), and other general roles in maintaining iron homeostasis. Tag SNPs were selected for each gene, including the region 20 kb upstream and 10 kb downstream of the gene, using the HapMap Utah residents with ancestry from northern and western Europe (CEU), Japanese and Han Chinese (JPT + CHB) populations and Carlson method (36), as implemented in Tagzilla with a r^2 threshold of 0.8 and minor allele frequency ≥5%. SNPs with known or putative functional significance (i.e. non-synonymous, promoter, intron–exon splice sites) were also included whenever possible. The SNPs were genotyped on a custom iSelect panel utilizing Illumina's Infinium platform. Eighteen of the 312 SNPs selected failed validation using HapMap samples and were eliminated from analysis.

Whole blood or buffy coat DNA was extracted with QIAamp DNA Blood Midi or Maxi Kits. For quality control purposes, replicate samples from 195 individuals (~6% of the sample) were interspersed randomly within the plates. Genotyping was conducted at the National Cancer Institute Core Genotyping Facility, National Institutes of Health. Participants with genotype rate <90% were excluded from analyses and the overall concordance rate was >99% for replicated samples. After excluding the SNPs with call rate <90%, minor allele frequency <1% or Hardy–Weinberg equilibrium P-value < 1×10^{-6} among Caucasian controls, >87% of the SNPs remained for analysis (277/294 in the adenoma set and 272/294 in the colorectal cancer set).

Statistical analysis

Analyses for the advanced colorectal adenoma and colorectal cancer cases were conducted separately. Descriptive characteristics were assessed using chi-square tests for categorical variables and t-tests for continuous variables.

Unconditional multivariable logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (95% CIs) for the association between total, dietary and heme iron and advanced colorectal adenoma and cancer. Iron intake was categorized into quartiles based on the combined intake distribution of the adenoma and cancer controls. The P-trend values for the dietary variables were calculated using the median intake value for each category. We present a minimally adjusted model (adjusted for age, sex and ethnicity) and a multivariable model, adjusted for age at selection (continuous); sex; ethnicity (non-Hispanic white, non-Hispanic black, other); body mass index, kg/m^2; education (<high school diploma, at least some college); smoking status (never, former, current); vigorous physical activity (none, <1, 1, 2, 3, 4+ h/week); total energy intake (kcal/day); dietary fiber (g/day); dietary calcium (mg/day); alcohol intake (g/day); study center; regular use of aspirin or ibuprofen (yes/ no) and first-degree family history of colorectal cancer (yes/no).

Initial genetic analyses to examine the SNP main effects were conducted using PLINK, a whole-genome association analysis toolset (37). Unconditional logistic regression models assuming an additive model were calculated to identify SNPs associated with the outcomes of advanced colorectal adenoma or colorectal cancer. Results were adjusted for sex, age and ethnicity and corrected for multiple testing using the false discovery rate (38), separately for adenoma and cancer.

A two-step approach to identify the SNPs involved in significant gene–environment (G–E) interactions (39) was employed. First, we examined the association of each SNP with total, dietary or heme iron in linear regression models among cases and controls, combined. Due to a skewed intake distribution, we transformed the exposure variables using the inverse normal rank transformation for total and dietary iron and the $\log_e$ transformation for heme iron. Linear regressions were calculated using PLINK. The subset of SNPs that exceeded the significance threshold of $P < 0.10$ was taken forward to Step 2 to test the G–E interaction. In Step 2, an unconditional logistic regression model was calculated with a multiplicative interaction term of the SNP coded 0, 1, 2 for number of minor alleles with total, dietary or heme iron coded by median intake value for quartile category. We corrected for multiple comparisons by the false discovery rate and by multiplying the P-interaction by the total of number of SNPs taken forward to Step 2 (i.e. those associated with iron intake at $P \leq 0.10$), separately. All interactions that met the significance threshold of $P \leq 0.10$ prior to multiple comparison adjustment were further evaluated using logistic regression models to examine the association between total, dietary or heme iron and colorectal adenoma or cancer, stratified by genotype assuming a dominant model. The P-trend values were calculated using the median intake value for each category. Models for Step 2 as well as the models for the association between the iron variables and colorectal adenoma or cancer were analyzed using STATA version 9.0.

Results

Analyses included a total of 1205 advanced colorectal adenoma cases (776 males and 429 females) and 370 colorectal cancer cases (218 males and 152 females) (Table I). Study participants were predominately white (>90%). Colorectal adenoma and cancer cases tended to be less educated but consumed more alcohol than their respective controls. In addition, adenoma cases were slightly older, more likely to have a family history of colorectal cancer, more likely to be current smokers, less likely to use aspirin or ibuprofen regularly, less physically active and consumed less fiber, calcium and dietary iron but more red meat relative to controls.

Iron intake

The median daily intake of total iron, dietary iron and heme iron was 24.8 mg, 17.0 mg and 365.1 µg, respectively, in the colorectal adenoma study and 25.1 mg, 16.7 mg and 344.2 µg, respectively, in the colorectal cancer study. Total iron and dietary iron were inversely associated with advanced colorectal adenoma in the minimally adjusted model among individuals in the highest versus the lowest quartile of intakes (OR = 0.66, 95% CI 0.53–0.82; P_{trend} = 0.0001 and OR = 0.79, 95% CI 0.63–0.99; P_{trend} = 0.01, respectively) (Table II); however, these associations were not statistically significant in the fully adjusted model. Heme iron intake was positively associated with adenoma risk in the minimally adjusted model (OR = 1.28, 95% CI 1.01–1.62; P_{trend} = 0.06), but this association did not persist in the fully adjusted model. Neither total iron, dietary iron, nor heme iron intake were associated with colorectal cancer risk in either the minimally or fully adjusted models (Table II).

Table I. Baseline characteristics of subjects in the nested case–control studies of advanced colorectal adenoma and incident colorectal cancer in the PLCO Cancer Screening Trial

Characteristic	Advanced colorectal adenoma			Colorectal cancer		
	Cases (n = 1205)[a]	Controls (n = 1387)[a]	P-value[b]	Cases (n = 370)[a]	Controls (n = 401)[a]	P-value[b]
Age, years	63.1 ± 5.3	62.6 ± 5.3	0.02	67.6 ± 6.5	67.5 ± 6.3	0.78
Gender, *n* (%)						
Male	776 (64.4)	888 (64.0)	0.84	218 (58.9)	242 (60.4)	0.69
Female	429 (35.6)	499 (36.0)		152 (41.1)	159 (40.0)	
Ethnicity, *n* (%)						
Non-Hispanic white	1136 (94.3)	1284 (92.6)	0.20	337 (91.2)	363 (90.5)	0.96
Non-Hispanic black	29 (2.4)	47 (3.4)		18 (4.9)	21 (5.2)	
Other	40 (3.3)	56 (4.0)		15 (4.1)	17 (4.2)	
First-degree family history of colorectal cancer, *n* (%)	157 (13.1)	136 (9.9)	0.03*	58 (15.8)	45 (11.4)	0.20
Education, *n* (%)						
12 years or less	416 (34.5)	399 (28.7)	<0.01*	139 (37.7)	113 (28.3)	<0.01*
At least some college	789 (65.5)	988 (71.3)		230 (62.3)	286 (71.9)	
Body mass index (kg/m^2)	27.9 ± 4.7	27.4 ± 4.6	0.01*	27.6 ± 4.6	27.5 ± 4.9	0.78
Hours spent in vigorous physical activity, *n* (%)						
None	222 (18.5)	182 (13.2)	<0.001*	46 (12.4)	58 (14.5)	0.88
<1 h/week	216 (18.0)	229 (16.6)		73 (19.7)	72 (18.1)	
1 h/week	153 (12.7)	179 (13.0)		49 (13.2)	48 (12.0)	
2 h/week	184 (15.3)	215 (15.6)		62 (16.8)	68 (17.0)	
3 h/week	184 (15.3)	212 (15.3)		54 (14.6)	66 (16.5)	
4+ h/week	242 (20.2)	365 (26.4)		86 (23.2)	87 (21.8)	
Regular use of aspirin or ibuprofen, *n* (%)	700 (58.1)	867 (62.6)	0.02*	216 (58.4)	253 (63.3)	0.17
Smoking status, *n* (%)						
Never	462 (38.4)	657 (47.4)	<0.0001*	161 (43.5)	169 (42.1)	0.93
Former cigarette smoker	577 (47.9)	635 (45.8)		169 (45.9)	188 (46.9)	
Current cigarette smoker	165 (13.7)	95 (6.9)		40 (10.8)	44 (11.0)	
Alcohol (g/day)	15.5 ± 29.0	12.1 ± 22.9	<0.01*	14.9 ± 30.7	11.9 ± 20.8	0.12
Total caloric intake (kcal/day)	2074 ± 805	2127 ± 823	0.10	2087 ± 807	2078 ± 768	0.87
Dietary fiber (g/1000 kcal)	11.0 ± 3.4	11.7 ± 3.7	<0.0001*	11.4 ± 3.6	11.6 ± 3.3	0.46
Dietary calcium (mg/1000 kcal)	457 ± 163	471 ± 164	0.03*	456 ± 163	463 ± 157	0.61
Red meat (g/1000 kcal)	39.9 ± 22.8	37.7 ± 23.2	0.02*	37.1 ± 21.0	35.8 ± 20.0	0.36
Total iron (mg/day)[c]	25.4 ± 12.9	27.2 ± 13.6	<0.001*	26.1 ± 13.7	26.6 ± 14.1	0.57
Dietary iron (mg/day)	18.0 ± 8.5	19.0 ± 8.7	<0.01*	18.3 ± 8.0	18.8 ± 9.0	0.35
Heme iron (μg/day)	500 ± 429	477 ± 408	0.15	469 ± 394	441 ± 368	0.31

Data are means ± SDs unless otherwise indicated.
[a]Numbers may not sum to total due to missing values.
[b]*P*-values derived from *t* test or chi-square test.
[c]Includes iron from diet and dietary supplements.
*Statistically significant at $P < 0.05$.

SNP main effects

A total of 12 SNPs from eight genes (*HEPHL1*, *CYBRD1*, *CP*, *HFE*, *ACO1*, *HMOX2*, *SLC11A2* and *FTL*) were associated with colorectal adenoma ($P < 0.05$), and 18 SNPs from eight genes (*ACO1*, *HFE2*, *TF*, *HFE*, *FTL*, *IGFBP3*, *B2M* and *SLC40A1*) were associated with colorectal cancer ($P < 0.05$) (Table III). However, none of these associations remained statistically significant after adjustment for multiple comparison testing.

Iron intake and SNP interactions

Analyses of interactions between iron intake and SNPs for colorectal adenoma were detected for dietary iron and four SNPs (located in either the *HEPHL1* or *TFR2* genes) and heme iron and four SNPs from four distinct genes (*CYBRD1*, *ACO1*, *HFE* and *IREB2*), all $P \leq 0.10$ (Table IV). Investigation of potential interactions between total iron and the SNPs in relation to colorectal adenoma was not statistically significant at $P \leq 0.10$. For colorectal cancer, there were several G–E interactions ($P \leq 0.10$) including: total iron and one SNP from the *IGFBP3* gene, dietary iron and four SNPs from four genes (*CYBRD1*, *HAMP*, *TFRC* and *FTL*) and heme iron and two SNPs from two genes (*ACO1* and *HMOX1*) (Table IV).

We further explored the risk pattern of iron exposures stratified by genotype only among interactions that met the unadjusted significance threshold of $P \leq 0.10$ (Table IV). These models are presented minimally adjusted (age, sex and ethnicity only) as well as fully adjusted (age, sex, ethnicity, body mass index, education, smoking status, vigorous physical activity, total energy intake, dietary fiber, dietary calcium, alcohol intake, study center, use of aspirin or ibuprofen and first-degree family history of colorectal cancer). In the fully adjusted model, an increased risk of advanced colorectal adenoma was observed for individuals in the highest quartile of dietary iron, relative to those in the lowest, among individuals possessing two A alleles at *HEPHL1* rs7946162 (OR = 2.22, 95% CI 1.15–4.27; $P_{trend} = 0.03$, $P_{interaction} = 0.10$). Similar results were observed with *HEPHL1* rs2460063 and *HEPHL1* rs7127348; both of which are highly correlated with rs7946162 ($r^2 > 0.94$). Also in the fully adjusted models, an elevated risk of colorectal adenoma was observed for individuals in the highest quartile of heme iron intake, relative to those in the lowest, among those carrying the C allele at *ACO1* rs1041320, but the small number of C allele carriers make interpretation of the results difficult.

In the fully adjusted models of colorectal cancer, there was an increased risk among those homozygous for the G allele at *ACO1* rs10970985 among individuals in the highest quartile of heme iron intake relative to those in the lowest (OR = 3.40, 95% CI 1.43–8.06; $P_{trend} = 0.004$, $P_{interaction} = 0.05$). Increased risk of colorectal cancer was also seen among individuals carrying at least one T allele at *HMOX1* rs737777 in the highest quartile of heme iron intake relative to those in the lowest (OR = 14.91, 95% CI 2.13–104.69). Following adjustment

Table II. ORs and 95% CIs for the main effect of iron intake and risk of advanced colorectal adenoma and colorectal cancer

Characteristic	Advanced colorectal adenoma				Colorectal cancer			
	Cases	Controls	OR[a] (95% CI)	OR[b] (95% CI)	Cases	Controls	OR[a] (95% CI)	OR[b] (95% CI)
Total iron								
Quartile 1	361	331	1.00	1.00	107	117	1.00	1.00
Quartile 2	314	366	0.77 (0.62, 0.95)	0.86 (0.68, 1.09)	94	80	1.31 (0.88, 1.96)	1.36 (0.87, 2.12)
Quartile 3	272	335	0.74 (0.59, 0.92)	0.85 (0.67, 1.08)	84	112	0.83 (0.56, 1.21)	0.82 (0.54, 1.25)
Quartile 4	258	355	0.66 (0.53, 0.82)	0.86 (0.66, 1.13)	85	92	1.03 (0.69, 1.53)	1.13 (0.70, 1.83)
P-trend			0.0001*	0.24			0.82	0.96
Dietary iron								
Quartile 1	336	334	1.00	1.00	95	114	1.00	1.00
Quartile 2	330	348	0.94 (0.76, 1.16)	1.09 (0.86, 1.38)	102	98	1.26 (0.85, 1.86)	1.37 (0.89, 2.11)
Quartile 3	257	360	0.69 (0.56, 0.87)	0.94 (0.71, 1.25)	86	87	1.20 (0.80, 1.80)	1.38 (0.83, 2.27)
Quartile 4	282	345	0.79 (0.63, 0.99)	1.31 (0.91, 1.90)	87	102	1.04 (0.69, 1.58)	1.26 (0.66, 2.43)
P-trend			0.01*	0.16			0.99	0.62
Heme iron								
Quartile 1	266	352	1.00	1.00	87	95	1.00	1.00
Quartile 2	282	334	1.13 (0.90, 1.42)	1.07 (0.85, 1.36)	102	113	1.00 (0.67, 1.50)	1.39 (0.79, 2.43)
Quartile 3	322	337	1.31 (1.04, 1.64)	1.19 (0.93, 1.53)	87	110	0.89 (0.59, 1.35)	1.00 (0.64, 1.57)
Quartile 4	335	364	1.28 (1.01, 1.62)	1.18 (0.88, 1.58)	94	83	1.34 (0.86, 2.11)	1.02 (0.67, 1.56)
P-trend			0.06	0.31			0.17	0.23

[a]Adjusted for age at selection, sex, ethnicity.
[b]Adjusted for age at selection, sex, ethnicity, study center, body mass index, level of education, smoking status, physical activity, total energy intake, alcohol intake, fiber intake, dietary calcium, regular use of aspirin or ibuprofen and first-degree family history of colorectal cancer.
*Statistically significant at $P < 0.05$.

Table III. OR and 95% CI for the main effect of SNPs in genes involved in iron homeostasis with $P < 0.05$ on advanced colorectal adenoma and colorectal cancer in the PLCO Cancer Screening Trial

SNP	Gene	Effect allele	EAF	OR[a]	95% CI	*P*-value[b]
Advanced colorectal adenoma						
rs12236816	*ACO1*	G	0.05	1.32	1.03, 1.68	0.03
rs16861634	*CP*	T	0.11	0.80	0.66, 0.96	0.02
rs701754	*CP*	T	0.17	0.85	0.73, 0.98	0.03
rs10935744	*CP*	A	0.11	0.82	0.69, 0.99	0.04
rs7580094	*CYBRD1*	G	0.26	0.84	0.74, 0.95	0.01
rs1042265	*FTL*	A	0.12	0.83	0.70, 0.99	0.04
rs7116300	*HEPHL1*	G	0.43	1.18	1.06, 1.31	0.00328
rs1401186	*HEPHL1*	C	0.45	1.18	1.06, 1.31	0.00345
rs16919942	*HEPHL1*	T	0.10	1.23	1.03, 1.46	0.02
rs1800702	*HFE*	G	0.39	1.14	1.02, 1.27	0.02
rs2270367	*HMOX2*	A	0.05	1.31	1.02, 1.68	0.03
rs706803	*SLC11A2*	A	0.06	0.76	0.59, 0.98	0.04
Colorectal cancer						
rs7855483	*ACO1*	C	0.17	1.56	1.21, 2.01	0.0006
rs10970971	*ACO1*	G	0.45	0.71	0.57, 0.88	0.0018
rs1023087	*ACO1*	G	0.20	1.41	1.10, 1.79	0.006
rs17288067	*ACO1*	A	0.17	1.42	1.10, 1.83	0.007
rs11793098	*ACO1*	G	0.28	1.29	1.03, 1.61	0.02
rs7866419	*ACO1*	A	0.38	1.27	1.03, 1.56	0.03
rs10758138	*ACO1*	T	0.46	0.80	0.65, 0.98	0.03
rs8043138	*B2M*	T	0.05	1.56	1.02, 2.40	0.04
rs4645900	*FTL*	T	0.04	0.46	0.24, 0.89	0.02
rs905238	*FTL*	G	0.48	0.80	0.65, 0.98	0.03
rs1045537	*HFE*	C	0.08	1.49	1.00, 2.07	0.02
rs2071303	*HFE*	C	0.29	1.28	1.03, 1.59	0.03
rs4970862	*HFE2*	T	0.32	1.34	1.09, 1.66	0.006
rs10282088	*IGFBP3*	A	0.16	1.35	1.04, 1.76	0.02
rs10255707	*IGFBP3*	T	0.20	1.29	1.01, 1.66	0.04
rs12702181	*IGFBP3*	G	0.37	0.81	0.65, 1.00	0.05
rs12638146	*TF*	T	0.01	3.11	1.29, 7.50	0.01
rs1370593	*SLC40A1*	A	0.38	1.24	1.01, 1.52	0.04

EAF, effect allele frequency among Caucasian controls.
[a]OR per risk allele assuming a log-additive model, adjusted for age, sex and ethnicity.
[b]No associations were significant at $P < 0.05$ after adjustment for multiple comparison testing.

Table IV. Association between iron intake and advanced colorectal adenoma and colorectal cancer risk stratified by genotypes possessing iron–SNP interactions $P \leq 0.10$

Gene	SNP	Genotype	Q1		Q2			Q3			Q4			P_{trend}[c]	P_{trend}[b,c]	$P_{interaction}$[c]
			Cases/ controls	REF	Cases/ controls	OR (95% CI)[a]	OR (95% CI)[b]	Cases/ controls	OR(95% CI)[a]	OR (95% CI)[b]	Cases/ controls	OR(95% CI)[a]	OR (95% CI)[b]			
Advanced colorectal adenoma																
			Dietary iron													
HEPHL1	rs7946162	AA	108/111	1.00	120/103	1.20 (0.82, 1.74)	1.44 (0.94, 2.19)	86/111	0.80 (0.54, 1.18)	1.19 (0.73, 1.94)	113/110	1.06 (0.72, 1.55)	2.22 (1.15, 4.27)	0.87	0.03	0.10
		AG+GG	227/214	1.00	199/235	0.78 (0.60, 1.02)	0.88 (0.66, 1.18)	169/245	0.63 (0.48, 0.83)	0.81 (0.57, 1.15)	166/228	0.64 (0.48, 0.85)	0.98 (0.62, 1.55)	0.002	0.93	
HEPHL1	rs2460063	TT	111/124	1.00	129/114	1.27 (0.89, 0.82)	1.51 (1.01, 2.26)	90/118	0.85 (0.58, 1.25)	1.26 (0.78, 2.03)	116/113	1.15 (0.79, 1.68)	2.39 (1.26, 4.54)	0.82	0.02	0.04
		TC+CC	225/211	1.00	197/230	0.79 (0.60, 1.03)	0.89 (0.66, 1.19)	169/244	0.63 (0.48, 0.83)	0.15 (0.57, 1.15)	163/229	0.63 (0.47, 0.84)	0.95 (0.60, 1.50)	0.001	0.94	
HEPHL1	rs7127348	GG	105/108	1.00	113/105	1.11 (0.76, 1.63)	1.37 (0.90, 2.10)	83/108	0.79 (0.53, 1.18)	1.26 (0.76, 2.07)	109/108	1.05 (0.71, 1.57)	2.40 (1.23, 4.67)	0.96	0.02	0.09
		GA+GG	232/228	1.00	214/239	0.86 (0.66, 1.12)	0.97 (0.73, 1.29)	176/255	0.66 (0.50, 0.86)	0.83 (0.59, 1.17)	173/237	0.68 (0.51, 0.90)	0.83 (0.64, 1.58)	0.03	0.95	
TFR2	rs4729598	CC	269/283	1.00	255/277	0.95 (0.75, 1.21)	1.05 (0.81, 1.37)	199/289	0.70 (0.54, 0.89)	0 .91 (0.66, 1.24)	221/255	0.86 (0.66, 1.10)	1.31 (0.86, 1.98)	0.11	0.21	0.07
		CT+TT	68/53	1.00	72/68	0.84 (0.51, 1.37)	1.08 (0.62, 1.89)	60/74	0.68 (0.41, 1.13)	1.09 (0.57, 2.08)	61/88	0.58 (0.35, 0.96)	1.37 (0.59, 3.20)	0.03	0.45	
			Heme iron													
ACO1	rs1041320	AA	262/338	1.00	286/328	1.12 (0.89, 1.41)	1.05 (0.83, 1.34)	312/331	1.30 (1.03, 1.64)	1.20 (0.93, 1.54)	328/358	1.24 (0.98, 1.57)	1.14 (0.85, 1.54)	0.10	0.42	0.03
		AC+CC	4/14	1.00	4/9	2.21 (0.34, 14.5)	7.25 (0.30, 175.87)	4/7	3.67 (0.50, 14.52)	82.20 (0.60, 2492.20)	5/2	19.27 (1.66, 223.07)	118 440.40 (29.66, 4.73e+08)	0.02	0.004	
CYBRD1	rs16859443	GG	243/332	1.00	274/324	1.15 (0.91, 1.46)	1.09 (0.86, 1.40)	302/327	1.35 (1.07, 1.71)	1.25 (0.97, 1.62)	324/345	1.36 (1.07, 1.73)	1.26 (0.93, 1.70)	0.02	0.16	0.08
		GA+AA	23/20	1.00	15/13	0.80 (0.29, 2.22)	0.66 (0.20, 2.19)	14/11	0.98 (0.33, 2.86)	0.49 (0.20, 2.19)	9/15	0.36 (0.11, 1.19)	0.26 (0.04, 1.56)	0.10	0.15	
HFE	rs1799945	CC	203/277	1.00	214/266	1.06 (0.82. 1.23)	1.01 (0.77, 1.32)	228/262	1.25 (0.96, 1.63)	1.17 (0.88, 1.56)	248/258	1.33 (1.02, 1.76)	1.33 (0.95, 1.86)	0.03	0.07	0.09
		CG+GG	63/75	1.00	76/71	1.36 (0.85, 2.19)	1.30 (0.80, 2.13)	88/76	1.53 (0.95, 2.45)	1.36 (0.81, 2.28)	85/102	1.13 (0.70, 1.84)	0.80 (0.43, 1.48)	0.93	0.19	
IREB2	rs7183034	CC	72/125	1.00	78/90	1.46 (0.95, 2.24)	1.41 (0.90, 2.21)	95/101	1.75 (1.16, 2.65)	1.58 (1.01, 2.47)	98/94	1.86 (1.22, 2.86)	1.55 (0.90, 2.66)	0.009	0.200	0.03
		CT+TT	193/227	1.00	212/247	1.01 (0.77, 1.32)	0.95 (0.72, 1.26)	220/236	1.16 (0.88, 1.53)	1.10 (0.81, 1.48)	235/266	1.09 (0.82, 1.45)	1.06 (0.74, 1.51)	0.52	0.66	
Colorectal cancer																
			Total iron													
IGFBP3	rs2453839	TT	66/77	1.00	57/52	1.34 (0.81, 2.22)	1.53 (0.87, 2.68)	57/58	1.14 (0.70, 1.87)	1.24 (0.72, 2.12)	51/46	1.29 (0.76, 2.17)	1.62 (0.86, 3.05)	0.47	0.28	0.08
		TC+CC	41/40	1.00	36/28	1.24 (0.63, 2.42)	1.13 (0.53, 2.41)	27/53	0.47 (0.24, 0.90)	0.42 (0.21, 0.83)	35/46	0.68 (0.36, 1.29)	0.62 (0.28, 1.35)	0.05	0.03	
			Dietary iron													
CYBRD1	rs12052537	CC	49/64	1.00	62/74	0.98 (0.60, 1.61)	1.18 (0.67, 2.08)	48/71	1.54 (0.91, 2.59)	2.17 (1.10, 4.29)	65/45	1.14 (0.67. 1.93)	2.00 (0.80, 5.04)	0.43	0.10	0.04
		CA+AA	37/30	1.00	40/38	1.41 (0.70, 2.83)	1.46 (0.69, 3.12)	36/35	0.75 (0.37, 1.50)	0.64 (0.28, 1.47)	28/34	0.74 (0.37, 1.49)	0.53 (0.19, 1.50)	0.19	0.12	
FTL	rs8104760	TT	79/87	1.00	96/109	1.24 (0.83, 1.86)	1.26 (0.81, 1.97)	76/97	1.14 (0.75, 1.75)	1.14 (0.67, 1.93)	84/76	0.94 (0.61, 1.45)	0.92 (0.46, 1.84)	0.63	0.66	0.06
		TG+GG	8/8	1.00	8/6	1.17 (0.23, 5.99)	1.49 (0.19, 11.84)	9/11	2.69 (0.53, 13.74)	6.77 (0.72, 63.93)	10/17	2.34 (0.48, 11.42)	11.81 (0.61, 228.94)	0.21	0.06	
HAMP	rs916145	GG	67/76	1.00	79/88	1.31 (0.84, 20.4)	1.51 (0.92, 2.47)	60/94	1.50 (0.95, 2.38)	1.85 (1.03, 3.31)	67/65	1.25 (0.77, 2.03)	1.74 (0.81, 3.77)	0.36	0.20	0.10
		GC+CC	20/19	1.00	25/27	1.02 (0.42, 2.45)	0.77 (0.29, 2.06)	25/14	0.75 (0.37, 1.50)	0.38 (0.13, 1.17)	27/18	0.74 (0.37, 1.49)	0.26 (0.07, 1.05)	0.06	0.04	
TFRC	rs7645812	CC	74/83	1.00	87/99	1.27 (0.83, 0.95)	1.38 (0.86, 2.19)	74/93	1.35 (0.86, 2.11)	1.55 (0.90, 2.68)	85/71	1.28 (0.82, 2.01)	1.57 (0.77, 3.19)	0.34	0.27	0.02
		CT+TT	13/12	1.00	17/16	1.37 (0.48, 3.96)	1.58 (0.43, 5.78)	11/15	0.65 (0.22, 1.994)	0.70 (0.17, 2.88)	9/12	0.22 (0.06, 0.79)	0.30 (0.04, 2.23)	0.02	0.16	
			Heme iron													
ACO1	rs10970985	GG	27/44	1.00	43/50	1.32 (0.70, 2.50)	1.51 (0.75, 3.02)	42/43	1.84 (0.96, 3.56)	2.50 (1.21, 5.13)	50/40	2.45 (1.23, 4.85)	3.40 (1.43, 8.06)	0.009	0.004	0.05
		GC+CC	60/51	1.00	60/63	0.82 (0.49, 1.40)	0.74 (0.42, 1.29)	43/64	0.56 (0.32, 0.97)	0.50 (0.27, 0 .92)	43/42	0.85 (0.45, 1.58)	0.56 (0.25, 1.25)	0.58	0.15	
HMOX1	rs737777	GG	81/84	1.00	88/93	0.95(0.62, 1.46)	0.94 (0.60, 1.47)	73/88	0.92 (0.59, 1.43)	0.97 (0.60, 1.56)	74/71	1.17 (0.72, 1.90)	1.08 (0.59, 1.96)	0.46	0.74	0.10
		GT+TT	5/11	1.00	16/22	1.85 (0.53, 6.57)	2.66 (0.60, 11.85)	12/20	1.41 (0.38, 5.20)	2.29 (0.49, 10.59)	19/12	4.39 (1.09, 17.65)	14.91 (2.13, 104.69)	0.03	0.008	

[a]Assuming a dominant model, adjusted for age, race and ethnicity.

[b]Assuming a dominant model, adjusted for age, race, ethnicity, education, body mass index, cigarette smoking, use of aspirin and ibuprofen, physical activity, study center, total calories, dietary fiber, dietary calcium, alcohol and family history of colorectal cancer.

[c]No associations were significant after adjustment for multiple comparison testing.

for multiple testing, none of the G–E interactions remained significant at $P \leq 0.05$ for colorectal adenoma or colorectal cancer.

Discussion

In this large study of iron exposure and advanced colorectal adenoma and colorectal cancer, we examined potential interactions between SNPs in 21 genes related to homeostasis and iron intake, in a comprehensive manner. Our study quantified intakes of total iron (diet and supplements), dietary iron and heme iron from meat via a validated FFQ linked to a quantitative heme iron database. Although there was limited evidence to support an independent role of total, dietary or heme iron intake with risk of colorectal neoplasia in these two particular nested case–control studies, we observed multiple instances where intake of dietary or heme iron was significantly associated with advanced colorectal adenoma or colorectal cancer risk among individuals with variants in genes governing iron homeostasis. These results suggest that genetic factors may be important in understanding the role of iron in colorectal neoplasia and could help to explain the mixed findings reported in other observational studies. However, our results did not withstand adjustment for multiple comparisons testing, and thus these signals should be interpreted with caution.

An earlier investigation from the PLCO Cancer Screening Trial reported a positive, though not statistically significant, association between heme iron and risk of colorectal adenoma (18). Our results for heme iron and advanced colorectal adenoma in this subset are similar, but we found no such suggestion for colorectal cancer. Prior studies examining the relationship between heme iron and risk of colorectal neoplasia are limited in number, with some studies indicating a positive association(22,40–43), and others suggesting a null relationship (22,44–46). Furthermore, our null results for total iron are in agreement with other published cohort studies of colorectal adenoma (18,41) and colorectal cancer (15,47,48), although two cohort studies have reported a statistically significant positive association (42,49). Intakes of total iron, dietary iron and heme were comparable with those of other studies, including the large National Institutes of Health-American Association of Retired Persons (NIH-AARP) Diet and Health Study (n = 300 948). In that investigation, the median daily intake of total iron, dietary iron and heme iron were 21.5 mg, 8.5 mg/1000 kcal and 150.3 µg/1000 kcal, respectively (42). The differences in the association of heme iron versus total and dietary iron with the outcome of colorectal cancer highlight the importance of evaluating heme iron, which comes from meat, separately from total or dietary iron, which are comprised largely of non-heme iron sources such as fortified cereals and bread, fruit juice and vegetables. The present study did not evaluate serum indices of iron, but an earlier investigation in the PLCO Cancer Screening Trial reported an inverse association between increased total iron binding capacity and unsaturated iron binding capacity (indicative of decreased overall iron load), and colorectal adenoma (18). Although the utility of a single blood collection as a marker representing longer term iron status is debatable (16), future investigations should consider including biomarkers of iron status at baseline together with genetic analyses. This would also eliminate confounding by iron depletion secondary to increased iron requirements of an undiagnosed malignancy or occult tumor bleeding.

A small number of studies have investigated variation in iron homeostasis genes in relation to colorectal neoplasia. Limited genotyping of eight key genes involved in iron homeostasis were investigated in an earlier study within the PLCO Cancer Screening Trial (18). Variants of *ACO1* (rs4297112, rs1041321, rs4878497, rs10970975) and *TF* (rs1358024) were significantly associated with serum indices of iron, but none of the variants from the eight genes under investigation were associated with risk of colorectal adenoma. Furthermore, the interaction of serum indices of iron with genetic variation on the outcome of colorectal adenoma was not investigated, nor was the interaction of serum values and intake of total, dietary or heme iron with genetic variation on the outcome of colorectal adenoma (18). In addition to the SNPs included in the earlier PLCO study, the current investigation examined additional SNPs from the same eight genes as well SNPs from additional genes. Moreover, the earlier PLCO investigation examined the association of gene variants and serum indices of iron on a smaller subset of 356 incident adenoma cases and 396 controls, and genotyping in the earlier PLCO study was performed on a subset of 770 adenoma cases and 777 controls. Diet–gene interactions were not investigated in the earlier study. A total of 664 cases and 704 controls from the previous PLCO genotyping study are included in the present investigation. Lastly, the case definition in the earlier PLCO study included individuals with any colorectal adenoma rather than only those with advanced adenoma, which are clinically more relevant, as was the case for the dietary analyses in the current study.

Prior to the earlier PLCO investigation, genetic studies of iron and colorectal neoplasia in humans had focused on polymorphisms in hemochromatosis (*HFE*) and transferrin receptor-1 (*TFR1*) with mixed results (25–30) but did not consider dietary intake. The present study is the first to address the interaction between dietary factors and a comprehensive set of polymorphisms relating to iron homeostasis. We found some effect modification for the association between dietary iron and colorectal adenoma by *HEPHL1* (rs7946162 and rs2460063), and heme iron and colorectal adenoma by *ACO1* (rs1041320), as well as between heme iron and colorectal cancer by *ACO1* rs10970985 and *HMOX1* rs737777. *HEPHL1*, which is thought to function as a ferroxidase and facilitate the transfer of iron to transferrin and *HMOX1* (encodes for the enzyme heme oxygenase 1, an essential enzyme in heme catabolism). Both of these genes could potentially modify iron exposure from dietary sources (50). *ACO1* codes for iron regulatory element binding protein 1 (50); however, there were only small number of individuals (n = 7) in the highest quartile of intake carrying the C allele for rs1041320, so this result should be interpreted with caution. Although the associations were attenuated with correction for multiple comparisons, our findings for effect modification with SNPs in these genes are intriguing and merit further investigation.

The role of *HEPHL1*, *ACO1* and *HMOX1* in the development of colorectal cancer is not well documented and the expected direction of the SNPs' effect modification is not known. A recent study indicated overexpression of *ACO1* in human rectal carcinoma cells relative to normal rectal cells (51). In addition, the earlier PLCO study reported variation in serum ferritin by variation in *ACO1* genotype (18), although the earlier study did not investigate the *ACO1* SNPs that were suggestive of modifying the effect of iron intake in the present study, and none of the *ACO1* SNPs associated with serum ferritin in the earlier study were correlated ($r^2 < 0.80$) with the *ACO1* SNPs (rs1041320 and rs10970985) suggested to modify the effect of iron intake on neoplasia risk in the current study.

Strengths of the present study include the large sample size with a comprehensive number of candidate genes related to iron homeostasis analyzed, as well as prospectively collected detailed dietary data. Unlike most studies of heme iron, which have employed standard factors to calculate heme content from meat, we used values from a quantitative iron database that takes into account the type of meat, cooking method and doneness level, which affect the conversion of heme to non-heme iron (24). Although the study of advanced colorectal adenoma investigated prevalent cases at baseline, the FFQ was completed prior to screening for over 80% of the PLCO cohort, and since participants with adenoma are usually asymptomatic, participants were unlikely to have made dietary changes related to their undetected adenoma. An additional strength includes the use of screening with sigmoidoscopy, which enabled us to accurately define case and control status, although it does not exclude the possibility that neoplasia was present in the proximal regions of the colon. Furthermore, we were able to use advanced adenomas as the outcome measure, which is potentially more relevant to the development of colorectal cancer than non-advanced lesions as advanced adenomas are more likely to progress to colorectal cancer within a person's lifetime. Finally, we used a two-step approach to identify the SNPs involved in G–E interactions, which seeks to reduce the number of false positives (39), and tag SNPs were selected to comprehensively evaluate each gene. Despite our relatively large sample size for advanced adenoma, we

were still somewhat underpowered to detect modest effects of single gene variants as well as G–E interactions. In addition, the number of colorectal cancer cases in our study limited our statistical power for this outcome. A total of 664 adenoma cases and 704 adenoma controls were included in a previously published PLCO investigation (18); however, the previous study did not examine the interaction of total, dietary or heme iron with a genetic variation in 21 genes involved in iron homeostasis. Differences in our results between advanced adenoma and colorectal cancer are surprising, given the role of advanced adenoma as a precursor lesion for colorectal cancer. These differences could also be attributed to issues of sample size and chance or it may be possible that the cancers in this study are atypical since they were diagnosed in a heavily screened population.

In summary, these results suggest that genetic factors may be important in understanding the role of iron in colorectal neoplasia and could help to explain the mixed findings reported in other observational studies. However, our results did not withstand adjustment for multiple comparisons testing, and thus these signals should be interpreted with caution. Future studies should target the specific genes and SNPs for which the association was significant prior to multiple comparison corrections.

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