Association Between Hemochromatosis (HFE) Gene Mutation Carrier Status and the Risk of Colon Cancer

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Background: **Iron is a pro-oxidant that may promote carcinogenesis. Mutations in the hemochromatosis (HFE) gene are associated with increased total body iron stores in some individuals. We assessed the risk of colon cancer among individuals with and without HFE gene mutations.** ***Methods:*** **We performed a population-based, case–control study in North Carolina. Case patients with colon cancer and control subjects provided information on multiple environmental exposures, including total iron intake and nonsteroidal anti-inflammatory drug (NSAID) use. They also provided a venous blood sample, from which DNA was extracted, amplified, and subjected to diagnostic restriction enzyme mapping to detect two major HFE gene mutations, C282Y and H63D. Data were analyzed with Fisher's exact test and logistic regression. All statistical tests were two-sided.** ***Results:*** **Thirteen hundred and eight subjects participated (475 case patients, 833 control subjects). The allele frequencies of the H63D and C282Y mutations were greater among case patients (0.11 and 0.046, respectively) than among control subjects (0.09 and 0.044, respectively; $P = .14$ and $P = .85$, respectively). When we controlled for age, race, sex, red meat consumption, NSAID use, and total iron intake, subjects with any HFE gene mutation were more likely to have colon cancer than subjects with no HFE gene mutations (adjusted odds ratio [OR] = 1.40, 95% confidence interval [CI] = 1.07 to 1.87). The magnitude of the effect was similar for both the H63D (adjusted OR = 1.44, 95% CI = 1.04 to 1.98) and C282Y mutations (adjusted OR = 1.39, 95% CI = 0.88 to 2.19). The risk of colon cancer associated with an HFE gene mutation was similar for those who did and did not have a family history of colon cancer. Among those with HFE mutations, cancer risk increased with increasing age and total iron intake.** ***Conclusions:*** **HFE gene mutations are associated with an increased risk of colon cancer. Cancer risk is greatest in mutation carriers who are older or consume high quantities of iron. [J Natl Cancer Inst 2003;95:154–9]**

Colon cancer is a prevalent and often fatal disease, with an estimated 107 000 new cases and 48 100 deaths expected in the United States this year *(1)*. The etiology of colon cancer is thought to be multifactorial; both hereditary and environmental factors may contribute to the disease *(2)*. High iron intake is a potential environmental risk factor for colorectal cancer *(3,4)*. Because iron is a pro-oxidant, high iron levels can lead to free radical formation and DNA damage *(5)*. In addition, iron is an element that is essential for the proliferation of neoplastic cells *(6)*.

Hereditary hemochromatosis is an autosomal recessive disease that is characterized by iron overload, which leads to dysfunction of the pancreas, liver, heart, and other organs *(7)*. Mutations in HFE, the gene for hereditary hemochromatosis, which resides on chromosome 6, are strongly associated with the development of hereditary hemochromatosis *(7)*. HFE gene mutations, including the C282Y mutation (in which the cysteine at codon 282 is replaced by a tyrosine) and the H63D mutation (in which the histidine at codon 63 is replaced by an aspartic acid), are found in almost all individuals with phenotypic hemochromatosis *(8)*. Serum ferritin and transferrin saturation, commonly used measures of body iron status, may be elevated in persons who are either heterozygous or homozygous for HFE gene mutations *(9)*, although only homozygotes (C282Y/C282Y) and compound heterozygotes (C282Y/H63D) commonly develop hemochromatosis *(10)*. Both of these HFE gene mutations are extremely common: it has been estimated that approximately 15% of the U.S. population possesses at least one of these mutations *(11)*.

If chronic elevation of total body iron stores is a risk factor for colon cancer, individuals with an HFE gene mutation may be at higher risk for colon cancer than individuals without such mutations. Furthermore, among individuals who have HFE gene mutations, increased levels of iron intake may augment the risk of colon cancer. We tested these hypotheses in a large, population-based case–control study of colon cancer.

Subjects and Methods

Subject Selection

We used blood samples that were collected from participants in the North Carolina Colon Cancer Study to compare the prevalence of HFE gene mutations in case patients with colon cancer with that in population-based control subjects. Eligible case patients were between the ages of 40 and 79 years, resided in a 33-county area of North Carolina, and had received a first diagnosis of colon cancer between October 1, 1996, and October 1, 2000. Case patients were identified through the rapid ascertainment system of the North Carolina Cancer Registry. All case patients had histologically proven adenocarcinoma of the colon (International Classification of Diseases, 9th Revision [ICD-9] code 153; http://www.cdc.gov/nchs/icd9.htm). Pathology reports on the colon cancer cases were received from the 35 hospitals in the study region a median of 34 days after the date of diagnosis (interquartile range, 22–63 days). Case patients who had tumors that were surgically localized below the peritoneal

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See "Notes" following "References."

reflection were considered to have rectal cancers and were excluded from our study *(12)*.

We chose our study population to enhance the statistical efficiency of detecting ethnic differences in disease. Therefore, we sampled the case patients to obtain similar numbers of African-American and white participants. Thus, all African-American case patients plus an appropriate random subsample of white case patients were selected and invited to participate. Before contacting a patient with a reported case of colon cancer, we sent a letter and a description of our study to the primary physician requesting permission to invite their patient to participate in our study.

Potential control subjects were sampled from the same geographic area using Medicare rolls (for control subjects aged 65 years or older) and North Carolina Department of Motor Vehicle records of driver's licenses and identification cards (for control subjects younger than 65 years). More than 97% of adults younger than 65 years living in North Carolina have either a driver's license or a state identification card registered through the Department of Motor Vehicles. Potential control subjects with a history of invasive colorectal adenocarcinoma were excluded. We used a two-stage randomized recruitment strategy *(13,14)* for sampling control subjects to control for potential confounding by race, age, and sex, and to facilitate the statistically optimized ethnic ratio. First, we compared the demographic data of the potential control subject with those of patients in North Carolina who had incident colon cancers in the 3 years prior to the study; we used that comparison to calculate a recruitment probability for each subject that represented the likelihood that a subject with the same demographics would be present in the case patient population. Next, a random number between 0 and 1 was generated for the subject and compared with the recruitment probability for the subject's age, race, and sex category to determine whether that potential control subject was recruitable. Although this randomized recruitment strategy is similar to traditional frequency-matched sampling, it has the important advantage that the main effects of matching variables (i.e., race, age, and sex in this study) may be estimated using maximum likelihood estimation in logistic regression models. Appropriate offset terms, which were derived from age-, race-, and sex-specific recruitment probabilities, were added to all logistic models to correct for recruitment probabilities and to allow for the estimation of unbiased odds ratios (ORs) and 95% confidence intervals (CIs) *(14)*. Control subjects were sampled in excess of case patients to improve the statistical power of our study.

Data Collection and Classification

Trained nurse interviewers administered lifestyle and diet questionnaires to all participants in the participants' homes. Lifestyle questionnaires included questions about physical activity, smoking history, occupation, medical history, use of NSAIDs, and family history of cancer. Diet was assessed with the use of a quantitative food frequency questionnaire that was developed at the National Cancer Institute; the questionnaire included questions about the frequency and serving size of more than 100 food items, as well as questions about the use of vitamin and mineral supplements during the previous year (for control subjects) or the year prior to diagnosis (for case patients) *(15)*. With the subject's permission, interviews were recorded on audiotape and later reviewed for quality-control purposes. All data were either double-entered (lifestyle questionnaire data) or scanned (diet questionnaire data) and then checked for consistency. All participants were asked to donate a 40-mL venous blood sample, which was obtained either at the time of interview by the study nurse or at a later date at the participant's physician's office. These samples were centrifuged for serum separation, and buffy coat and serum were frozen separately at –70 °C. This protocol was approved by the University of North Carolina Institutional Review Board and the North Carolina Central Cancer Registry. All participants signed an informed consent to participate.

Participants were asked whether they regularly used NSAIDs (both prescription and over-the-counter forms, including aspirin) at any time during the past 5 years, where "regularly" was defined as 3 or more days per week. On the basis of this response, respondents were categorized as regular users or nonregular users, the latter category including both nonusers and those who used NSAIDS less than 3 days per week. Regular users were further asked to provide details about the frequency (uses per week) and duration of their NSAID use. To characterize the frequency and duration of NSAID use, these responses were converted to a single continuous variable, NSAID-years, which was defined as the average daily NSAID use in number of doses multiplied by the years of NSAID use. Participants were classified according to their use of tobacco as never users, previous users, or current users; cigarette use was further quantified in pack-years. Total iron intake (i.e., dietary intake plus oral vitamin and mineral supplementation) was calculated from responses provided on the diet questionnaire using the Block software program (Dietsys software, version 3.7c; NCI Block Dietary DataSystems, Bethesda, MD) and divided into quartiles. A positive family history was defined as having at least one first-degree relative who was diagnosed with colon cancer at any age. Covariables assessed included sex, age, race, iron intake, red meat consumption, NSAID use, physical activity, family history, fat intake, fiber intake, and smoking.

Allele frequency was calculated according to the following formula *(16)*: Allele frequency $= [a + (2 \times b)]/2 \times c$, where a is the number of subjects heterozygous for the mutation, b is the number of subjects homozygous for the mutation, and c is the total number of subjects.

HFE Genotyping

DNA was extracted from peripheral blood lymphocytes in the buffy coats with the use of a PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). The DNA was resuspended in 750 μL of hydrating buffer (10 m*M* Tris–HCl, 1 m*M* EDTA [pH 7.8]), and aliquots were stored at –70 °C.

To assess HFE genotypes, we used polymerase chain reaction (PCR) to amplify the regions of the HFE gene that contain the C282Y and H63D mutations *(8)*. The resulting DNA fragments were then digested with appropriate restriction endonucleases, and the digestion products were separated by polyacrylamide gel electrophoresis. The C282Y mutation creates a restriction site for the enzyme *Bbr*PI; digestion of the PCR fragment that contains the C282Y mutation results in two fragments of 111 and 29 base pairs (bp). The wild-type allele is not digested by *Bbr*PI at this site; thus, the PCR fragment that contains the wild-type allele remains as a single 140-bp fragment. The H63D mutation destroys a recognition site for restriction enzyme *Dpn*II; diges-

tion of the PCR fragment that contains the H63D mutation results in a single fragment of 172 bp, whereas digestion of the PCR fragment that contains wild-type allele generates two fragments (104 bp and 68 bp) *(8)*. For quality-control purposes, DNA samples from individuals who were known to have the C282Y and H63D mutations by DNA sequence analysis were processed with each batch of samples as positive controls. In addition, 10% of the subjects had their samples re-run to ensure agreement with initial results. The technician who performed the genotyping and the investigator (E. M. Rohlfs) who interpreted the results were blinded to the case or control status of the participant who provided the blood sample.

Statistical Analysis

We investigated the relationship between HFE genotype and risk of colon cancer with the use of SAS statistical software, version 8 (SAS Institute, Inc., Cary, NC). Bivariable associations between primary variables and covariables were assessed with the use of Student's *t* test for continuous variables and Fisher's exact test for categorical variables. Stratified and logistic regression analyses were conducted to assess possible modifiers of the association between genotype and colon cancer risk and to control for potential confounders. The Breslow–Day test for heterogeneity was used to assess potential confounders for nonuniformity of effect before they were inserted into the regression models. All logistic regression models included offset terms to correct for first-stage sampling probabilities. Covariables included sex, age, race, physical activity, smoking, dietary iron intake, red meat consumption, and NSAID use. ORs and 95% CIs were estimated using unconditional logistical regression. Covariables were assessed for interaction by logistic regression with product terms for the variables of interest. The Wald test was used to assess the statistical significance of the cross-product terms. For all associations of genotype with colon cancer risk, those subjects who were homozygous for the wild-type allele served as the comparison group. All tests of statistical significance were two-sided and were considered to be statistically significant at $P<.05$.

Separate models were generated for the C282Y mutation, for the H63D mutation, and for the presence of either mutation. In addition, our models allowed us to detect a differential effect between the heterozygous and homozygous state of a given mutation. Because the HFE gene product is involved in iron metabolism, and because iron stores may increase with increasing age, we decided *a priori* to examine the possible modifying effects of age and total iron intake on the relationship between HFE gene mutations and colon cancer risk.

Results

In our study, there were 1308 participants (475 case patients and 833 control subjects). Cooperation rates (i.e., the number of individuals who were interviewed divided by the number interviewed plus the number of individuals who refused to be interviewed) were 84% for case patients and 62% for control subjects. Table 1 shows the characteristics of the study group.

Table 2 presents the HFE genotype distributions among case patients and control subjects. The prevalence of any HFE gene mutation was greater among case patients than it was among control subjects (28.8% versus 24.8%; $P = .23$), as was the prevalence of heterozygosity for both the C282Y mutation and the H63D mutation. The allele frequency of the H63D mutation among case patients was greater than that among control subjects (0.11 versus 0.09; $P = .14$). Similarly, the allele frequency of the C282Y mutation among case patients was greater than it was among control subjects (0.046 versus 0.044; $P = .85$). However, neither of these differences was statistically significant.

Table 1. Characteristics of study subjects*

	Case patients (n = 475)	Control subjects (n = 833)
Mean age, y (SD)	63.0 (10.0)	65.2 (9.4)
Female, No. (%)	228 (48)	400 (48)
African-American, No. (%)	204 (43)	317 (38)
Regular NSAID user, No. (%)	178 (37)	383 (46)
Current cigarette smoker, No. (%)	86 (18)	150 (18)
Family history of colon cancer, No. (%)	100 (21)	75 (9)

*SD = standard deviation; NSAID = nonsteroidal anti-inflammatory drug.

Table 2. Distribution of hemochromatosis (HFE) gene mutations among case patients and control subjects

Genotype	Case patients (n = 475)	Control subjects (n = 833)	*P* value*
Wild-type/wild-type, No. (%)	338 (71.1)	626 (75.1)	.12
Any HFE gene mutation, No. (%)	137 (28.8)	207 (24.8)	.12
C282Y/wild-type	39 (8.2)	57 (6.8)	.31
H63D/wild-type	83 (17.5)	124 (14.9)	.18
H63D/H63D	10 (2.1)	12 (1.4)	.37
C282Y/C282Y	0 (0)	3 (0.4)	.56
C282Y/H63D	5 (1.1)	11 (1.3)	.19
Any C282Y mutation, No. (%)	44 (9.3)	71 (8.5)	.68
Any H63D mutation, No. (%)	98 (20.6)	147 (17.6)	.19
Allele frequency, H63D	0.11	0.09	.14
Allele frequency, C282Y	0.046	0.044	.85

*Two-sided Fisher's exact test.

We found large differences in the prevalence of HFE gene mutations between African-American and white study subjects. The prevalence of C282Y mutations and H63D mutations was 13.1% and 25.9%, respectively, among white subjects and 2.1% and 7.7%, respectively, among African-American subjects ($P<.001$ for both). The prevalence of any HFE gene mutation was 37.0% among white subjects and 9.9% among African-American subjects ($P<.001$). Although HFE gene mutations were less common among the African-American subjects than they were among the white subjects, African-Americans who possessed an HFE gene mutation were at greater risk of colon cancer (OR = 2.1, 95% CI = 1.1 to 3.9) than were whites who possessed an HFE gene mutation (OR = 1.2, 95% CI = 0.8 to 1.6).

Table 3 shows the results of the logistic regression analysis. We found that after controlling for sex, age, race, iron intake, red meat consumption, and NSAID use, subjects with any HFE gene mutation were more likely to have colon cancer than subjects with no HFE gene mutations (adjusted OR = 1.40, 95% CI = 1.07 to 1.87). When the H63D mutation and the C282Y mutation were considered separately, each was associated with an adjusted OR of colon cancer of similar magnitude (for H63D, adjusted OR = 1.44 and 95% CI = 1.04 to 1.98; for C282Y, adjusted OR = 1.39 and 95% CI = 0.88 to 2.19; data from the full logistic regression models not shown). These estimates did not change statistically significantly when we restricted the analysis to only those persons who were heterozygous for HFE

Table 3. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for logistic regression analysis that assessed the association between colon cancer risk and hemochromatosis (HFE) gene mutations while controlling for sex, age, race, iron intake, red meat consumption, and NSAID use among 475 case patients and 833 control subjects*

Variable	Adjusted OR (95% CI)
No HFE gene mutation	1.00 (referent)
Any HFE gene mutation	1.40 (1.07 to 1.87)
Sex	
Male	1.00 (referent)
Female	0.84 (0.65 to 1.07)
Age, y	
<50	1.00 (referent)
50–69	3.88 (2.44 to 6.16)
⩾70	7.55 (4.67 to 12.21)
Race	
White	1.00 (referent)
African-American	1.35 (1.04 to 1.75)
Quartiles for total iron intake (mg/day)	
1 (0–10.35)	1.00 (referent)
2 (10.36–15.19)	0.94 (0.66 to 1.33)
3 (15.20–26.50)	0.88 (0.61 to 1.26)
4 (>26.50)	1.05 (0.75 to 1.49)
Quartiles for red meat consumption (servings/day)	
1 (0–0.38)	1.00 (referent)
2 (0.39–0.68)	1.15 (0.82 to 1.62)
3 (0.69–1.08)	1.29 (0.91 to 1.82)
4 (>1.08)	1.68 (1.18 to 2.40)
NSAID use	
Nonregular user	1.00 (referent)
Regular user, tertiles (NSAID-years)†	
1 (>0–0.74)	0.54 (0.39 to 0.75)
2 (0.75–4)	0.80 (0.59 to 1.01)
3 (>4)	0.49 (0.34 to 0.71)

*NSAID = nonsteroidal anti-inflammatory drug.

†NSAID-years is defined as the average daily NSAID use in number of doses multiplied by the number of years of NSAID use.

Table 4. Stratified analysis of the effect of total dietary iron intake on colon cancer risk in subjects with a hemochromatosis (HFE) mutation*

Quartile of dietary iron intake (mg/day)	Adjusted OR (95% CI)
1 (0–10.35)	0.97 (0.52 to 1.78)
2 (10.36–15.19)	1.12 (0.65 to 1.92)
3 (15.20–26.50)	1.28 (0.72 to 2.26)
4 (>26.50)	1.86 (1.09 to 3.18)

*All estimates are adjusted for sex, age, race, red meat consumption, and nonsteroidal anti-inflammatory drug use. OR = odds ratio; CI = confidence interval.

Table 5. Stratified analysis of the effect of age on colon cancer risk in subjects with a hemochromatosis (HFE) mutation*

Age, y	Adjusted OR (95% CI)
<50	0.59 (0.22 to 1.56)
50–69	1.29 (0.87 to 1.90)
⩾70	1.90 (1.34 to 2.84)

*All estimates adjusted for sex, race, iron intake, red meat consumption, and nonsteroidal anti-inflammatory drug use. OR = odds ratio; CI = confidence interval.

gene mutations. Physical activity, fat intake, fiber intake, and smoking were included in more comprehensive regression models and did not confound the relationship between HFE gene mutations and colon cancer risk; thus, to improve precision, they were not included in the final models. Possible interaction between HFE mutation status and either total iron intake or age was assessed; neither total iron intake nor age were found to have statistically significant interaction with HFE mutation status ($P = .40$ and .15, respectively).

Because any etiologic effect of HFE gene mutations on colon cancer risk may depend on total iron intake, we also performed a stratified analysis to assess the effects of HFE gene mutations within quartiles of total iron intake. Among those subjects who were in the lowest quartile of dietary iron intake, we observed no increased risk of colon cancer associated with having an HFE gene mutation (Table 4). However, the magnitude of the relative risk for colon cancer that was associated with having HFE gene mutations increased with increasing total iron intake.

Because total iron stores may increase with age *(17),* we assessed the effect of HFE gene mutations on colon cancer risk among subjects in three age strata (Table 5). We observed no statistically significant effect of HFE gene mutations among subjects who were younger than 50 years. However, the colon cancer risk that was associated with HFE gene mutations increased successively in the two higher age strata, so that those persons who were older than 70 years and had HFE gene mutations were almost twice as likely to develop colon cancer as were those of the same age without an HFE gene mutation (Table 5).

Because an individual's genetic makeup may be associated with their family history of disease, inclusion of family history of colon cancer as a covariable in logistic regression models that contain the genetic mutation variables might introduce collinearity into the model. Therefore, we performed a separate analysis that stratified individuals on the basis of the presence of a family history of colon cancer. We found, after adjusting for sex, age, race, iron intake, red meat consumption, and NSAID use, that the adjusted OR for colon cancer among those with an HFE gene mutation and a family history of colon cancer (1.58, 95% CI = 0.73 to 3.41) did not differ statistically significantly from the adjusted OR for colon cancer among those with an HFE gene mutation who did not have a family history of colon cancer (1.29, 95% CI = 0.94 to 1.77).

Discussion

In this large, population-based case–control study, we observed that an increased risk of colon cancer was associated with the presence of either of two HFE gene mutations. In addition, the cancer risk that was associated with these HFE gene mutations increased within strata of increasing total iron intake and age. Given that the risk of cancer among those with an HFE mutation was not affected by the subject's family history of colon cancer, other inherited factors appear unlikely to be confounding the observed association between HFE gene mutations and colon cancer risk.

Results of previous studies have suggested that individuals who have higher body stores of iron are at greater risk for colorectal adenomas than those with normal or low body stores *(18,19).* Positive associations between colorectal cancer risk and biochemical markers of increasing body iron stores, such as transferrin saturation and serum iron and ferritin levels, have been found in some studies *(3,4),* but not in others *(20–22).*

Other studies have found that colorectal cancer risk may increase in those who consume high dietary levels of iron *(23),* especially in those who also consume a high-fat diet *(20).*

The mechanism by which elevated iron concentrations might promote carcinogenesis is unclear. Iron is a pro-oxidant that generates free radicals. In animals that are fed an iron-rich diet, levels of natural anti-oxidants in the colon are diminished compared with those in animals receiving lower levels of dietary iron *(24).* Iron also appears to be essential for the proliferation of tumor cells. For example, mice fed an iron-deficient diet display decreased tumor growth after inoculation with colonic adenocarcinoma tumor cells *(25).* Conversely, iron supplementation has been shown to enhance the rate of tumor growth in rodents with chemically induced colonic neoplasia *(26,27).*

The risk of several types of cancer among those with hemochromatosis has been well documented. Subjects with hemochromatosis have an increased risk of malignancies of the liver, which are usually associated with cirrhosis *(28–32).* Some studies have suggested that individuals with hemochromatosis may also have an increased incidence of tumors at other (i.e., extrahepatic) sites *(30,32–34),* but other studies have not found such an association *(35,36).* Because almost all of the subjects in our study who had HFE gene mutations were heterozygous for those mutations, we are unable to reliably estimate the risk of colon cancer among those who are homozygous for HFE gene mutations.

The risk of extrahepatic cancer among those heterozygous for HFE gene mutations is not well-described but, given the ubiquity of heterozygosity for these mutations, may be of great importance. Heterozygosity for either the C282Y mutation or the H63D mutation is common; at least 20% of most white populations possess one or more HFE gene mutations *(9,11).* Therefore, even relatively small increases in the risk of colon cancer that might arise from the presence of a single HFE gene mutation could potentially account for a large number of cases of colon cancer each year. In a large case–control study that examined cancer risk among parents of individuals with hemochromatosis, the parents (who were presumed to be heterozygous for HFE gene mutations) were found to display increased risks for colorectal neoplasia, hematologic malignancies, and gastric cancer compared with population norms *(37).* However, two smaller studies that examined the association between HFE gene mutations and colorectal cancer failed to demonstrate a difference in the prevalence of HFE gene mutations between case patients and control subjects *(38,39).*

The mechanism by which heterozygosity for an HFE gene mutation might predispose individuals to colon cancer is not known. One possible explanation is that chronic subclinical increases in total body iron stores may promote increased oxidative stress and induce DNA damage. However, other mechanisms may also contribute to the association between HFE gene mutations and colon cancer risk observed in our study population. The HFE gene encodes a transmembrane protein that has homology to the β-2 microglobulin receptor *(8).* This protein is ubiquitously expressed in the body and may have functional roles in addition to iron homeostasis. We found that the association between colon cancer risk and HFE gene mutations strengthened as both dietary iron intake and age increased. Because individuals in Western societies may accumulate increased total body iron stores as they age *(22,40),* our findings suggest that the association between HFE gene mutations and colon cancer risk may reflect the accumulation of total body stores of iron among older individuals. Although heterozygosity for the C282Y mutation has been associated with more severe abnormalities in serum studies of iron status than has heterozygosity for the H63D mutation *(9),* we found that heterozygosity for either mutation conferred similar increases in colon cancer risk. The reasons for this finding are unclear, as are the reasons for our finding that HFE gene mutations are associated with a higher risk of colon cancer among African-Americans than among whites.

Our study has several strengths. First, our study was population-based, and both the case patients and the control subjects were drawn from a 33-county area of North Carolina. As such, our study does not suffer from the potential referral and selection biases that occur in hospital- or clinic-based studies. Second, participation rates among the case patients and the control subjects were good. Third, case definition was rigorous, and exposure information was obtained by trained nurses who interviewed participants in their homes. Finally, DNA analyses were performed in a technically proficient laboratory by investigators who were blinded to the case or control status of the participants.

There are several factors that limit the interpretation of our data. Although our study was large, with more than 1300 participants, the number of participants in some HFE genotype subgroups (especially those who were homozygous for the HFE gene mutations) was limited, making it difficult to draw unambiguous conclusions about those subgroups. In addition, the retrospective design of our study precluded use of traditional measurements of iron, such as serum iron, ferritin, and transferrin saturation, because such measurements in case patients might have been confounded by anemia associated with their cancers. Although blood losses associated with menses may explain the attenuated effect of HFE gene mutations in younger individuals, data regarding menses were not available in our study. These measures may be useful in prospective settings to more fully explain the mechanisms that contribute to the effects we observed.

Our findings have several implications. If subsequent studies confirm that mutations in the HFE gene are a risk factor for colon cancer, testing for such mutations may allow the identification of a subgroup of individuals that might benefit from intensified colorectal cancer screening. In addition, if high iron intake among those with HFE gene mutations predisposes such individuals to the development of colon cancer, dietary modifications may be considered as an appropriate intervention in an attempt to lower this risk. Clearly, however, these possibilities are highly speculative, given the preliminary nature of the present findings.

In conclusion, after controlling for multiple confounders, we found that subjects who had HFE gene mutations had an increased risk of colon cancer. This effect was most pronounced among subjects with a high iron intake, as well as among subjects older than 70 years. The HFE gene may be one that contributes to the heritability of colon cancer.

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NOTES

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