Published in final edited form as:
Dig Dis Sci. 2008 December ; 53(12): 3095–3102. doi:10.1007/s10620-008-0287-x.

Hemochromatosis Gene Status as a Risk Factor for Barrett's Esophagus

Douglas A. Corley[1,2], **Ai Kubo**[1,3], **T. R. Levin**[1], **Gladys Block**[4], **Laurel Habel**[1], **Gregory J. Rumore**[5], **Charles Quesenberry**[1], and **Patricia Buffler**[4]

1 *Division of Research, Kaiser Permanente, 2000 Broadway, Oakland, CA 94612, USA, e-mail: Douglas.Corley@kp.org*

2 *Department of Medicine and Comprehensive Cancer Center, University of California, San Francisco, CA, USA*

3 *Mailman School of Public Health, Columbia University, New York, NY, USA*

4 *School of Public Health, University of California, Berkeley, CA, USA*

5 *Oakland Medical Center, Kaiser Permanente, Oakland, CA, USA*

Abstract

Conditions causing high iron levels, such as hemochromatosis, are proposed risk factors for esophageal adenocarcinoma. Although this hypothesis is supported by animal models, no human data currently exist. We conducted a case-control study of persons with a new Barrett's esophagus diagnosis (cases), persons with gastroesophageal reflux disease (GERD) (without Barrett's esophagus), and population controls. Subjects completed detailed examinations and assays for hemochromatosis mutations and serum iron stores. We evaluated 317 cases, 306 GERD patients, and 308 population controls. There was no significant association between Barrett's esophagus and any hemochromatosis gene defect (odds ratio [OR] = 1.32, 95% confidence interval [CI]: 0.95–1.84), a moderate or severe mutation (OR = 1.54, 95% CI: 0.94–2.52), or a severe mutation (C282Y homozygote or C282Y/H63D heterozygote; OR = 0.77, 95% CI: 0.24–2.48) compared with the population controls. As expected, gene defects were associated with increased iron stores. We can conclude from our findings that Barrett's esophagus was not associated with hemochromatosis gene defects, although we cannot exclude small effects.

Keywords

Keywords Barrett's esophagus; Esophageal adenocarcinoma; Iron; Hemochromatosis

Background

The incidence of esophageal adenocarcinoma has increased by over 500% within the United States during the last three decades [1]. A proposed modifiable risk factor is a high total body iron concentration, due to diet or from conditions that cause excessive iron absorption, such as mutations in the hemochromatosis gene [2–4]. Defects in this gene are associated with increased total body iron stores, an increase in oxidative stress, and an increased risk of both hepatic and non-hepatic malignancies [5–10]. C282Y homozygotes (i.e., subjects who possess two copies of this mutation) are at the highest risk for iron overload and have an increased

Correspondence to: Douglas A. Corley.

cancer risk. C282Y heterozygotes (who possess a single copy of this abnormal gene) and compound heterozygotes (who possess one copy with a C282Y mutation and one copy with a H63D mutation) may also be at somewhat increased risk [11–15].

Barrett's esophagus, a metaplastic change in the esophageal lining from its usual squamous epithelial lining, may represent an early event in the carcinogenesis of esophageal adenocarcinoma [16]. Persons with Barrett's esophagus have a 30- to 40-fold increased risk of esophageal adenocarcinoma; the evaluation of risk factors for Barrett's esophagus thus permits the investigation of potential early events in the carcinogenic pathway for esophageal adenocarcinoma, and also allows the investigation of risk factors that may be affected by the cancer itself, such as iron stores [16].

The investigation of a potential link between hemochromatosis gene defects, Barrett's esophagus, and esophageal adenocarcinoma is intriguing for several reasons [2]. First, several studies suggest that conditions which increase the total body iron stores may increase the risk of both hepatic and non-hepatic malignancies [5–10]. Second, animal models indicate that iron supplementation before reflux-induced esophageal injury markedly increases the incidence of esophageal columnar metaplasia and esophageal adenocarcinoma [4]. Third, Barrett's esophagus and esophageal adenocarcinoma are much more commonly found among males and among Caucasians [17]. Caucasians are more likely to have hemochromatosis gene defects than African-Americans, and men with hemochromatosis gene defects are more likely to have iron overload than women with similar defects [12–14,18–22]. The potential patterns of hemochromatosis gene defects thus approximate the distributions of Barrett's esophagus and esophageal adenocarcinoma in the population. Fourth, hemochromatosis gene defects are some of the most common genetic disorders in many countries; homozygotes are found in 1–2% of some populations, heterozygotes in 5% of the general population in the United States, and at least one mutation can be found in up to 46% of some populations, although the relevance of some mutations is unclear [11,18,23,24]. Finally, if iron overload from hemochromatosis clearly increased the risk of Barrett's esophagus or esophageal adenocarcinoma, it could be readily identified and treated with iron-reduction strategies.

We conducted a case-control study of the associations between hemochromatosis gene status, iron stores associated with gene defects, and a new diagnosis of Barrett's esophagus within a non-referral, community-based population.

Design and Methods

Study Population

We conducted a nested case-control study within the Kaiser Permanente, Northern California (KPNC) population, an integrated health services delivery organization. KPNC contains approximately 3.3 million people and its membership demographics closely approximate the underlying census population of Northern California [25]. The eligible subjects were all adult (aged 18–79 years) KPNC members who were continuously enrolled for at least 2 years prior to their index period, met the case or control definitions outlined below, and were able to understand spoken and written English. The study compared cases (subjects with a new diagnosis of Barrett's esophagus) with a population control group and another control group of subjects with a diagnosis of gastroesophageal reflux disease (GERD). The control groups were frequency-matched to the cases by age at the index date, gender, and geographic region (each subject's home facility). The index date for the Barrett's esophagus cases was the date of diagnosis. The index date for the control groups was the midpoint of each 2–3-month selection interval for the cases.

Case Definition

Cases were eligible members who received a new Barrett's esophagus diagnosis between October 2002 and September 2005. Newly diagnosed patients were serially identified during the recruitment period using the International Classification of Disease, 9th revision (ICD-9) code 530.2, which, at KPNC, is uniquely coded on reporting sheets as "Barrett's esophagitis." A single board-certified gastroenterologist (DAC) then reviewed the endoscopy and pathology records. Subjects were included if the endoscopist clearly described a visible length of columnar-type epithelium proximal to the gastroesophageal junction/gastric folds, this area was biopsied, and the biopsies showed specialized intestinal epithelium [16]. Patients were excluded if they had a prior Barrett's esophagus diagnosis, if no pathology evaluations demonstrated intestinal metaplasia, or if, to minimize misclassification, the biopsies were only from an irregular squamocolumnar junction (i.e., an "irregular z-line"). Pathology slides underwent a separate manual review by a gastrointestinal pathologist (GJR).

Population Controls

Population controls were randomly selected from at risk members of the entire Northern California Kaiser membership roster using risk set sampling; this method matches controls who are disease-free (no prior Barrett's esophagus) at the time of the Barrett's esophagus diagnosis in their respective cases [26].

GERD Comparison Group

GERD comparison group members were randomly selected from among persons with the following characteristics prior to the index date: a GERD-related diagnosis code (ICD-9 codes 530.11 [reflux esophagitis] or 530.81 [gastroesophageal reflux]); a prescription for at least 90 days supply of a histamine-2 receptor antagonist or a proton pump inhibitor in the previous year (using electronic pharmacy records); no prior Barrett's esophagus diagnosis; and performance of an esophagogastroduodenoscopy (in proximity to the index date of the case group) that did not demonstrate visible changes of esophageal columnar metaplasia of any type.

Exposure Measurements

All study subjects completed the following: an in-person interview of GERD symptoms, medication use, and medical history; a validated food frequency questionnaire (the Block 1998 full-length, 110 food items) [27–30]; phlebotomy; and anthropometric measurements. Subjects reported symptoms, diet, and exposures in the year prior to their index date.

Hemochromatosis gene status for the C282Y and H63D gene defects were performed at the Kaiser Permanente regional genetics laboratory utilizing commercially available assays.

The serum ferritin and iron saturation levels, two indirect measures of total body iron stores, were measured using standard assays at a regional commercial clinical laboratory [31–33]. Reproducibility over the course of the study was confirmed using serial specimens in a test subject and samples were run in mixed batches of cases and controls.

Confounding and Effect Modification

We evaluated the following as potential confounders of the phenotypic expression (high iron load) of the hemochromatosis phenotype: dietary iron intake, aspirin or nonsteroidal anti-inflammatory drug (NSAID) use, ethnicity (classified as Caucasian vs. non-Caucasian due to the small sample sizes in the ethnic subgroups), smoking status (at least 20 packs of cigarettes over a lifetime vs. never smoked), recent alcohol use, body mass index (BMI), *Helicobacter pylori* antibody status, a comorbidity index (the DxCg score, which creates a predictive

comorbidity score based on demographic data, medical coding, and pharmacy utilization) [34,35], caloric intake, waist circumference, highly sensitive c-reactive protein (a measure of systemic inflammation), and anti-oxidant intake (using an index of beta carotene, vitamin C, vitamin E, beta-carotene, and selenium). We examined the potential impact of non-response bias (differences between participants vs. eligible non-participants) using electronic data (BMI, smoking status, ethnicity, age, gender, DxCg score, and GERD diagnosis) from the databases.

Statistical Analysis

The analysis utilized unconditional logistic regression [26,36–38]. A full model was created using possible confounders. Then, each variable was removed singly in a stepwise fashion; those with the weakest statistical association were removed first. After each factor was removed, the model was evaluated for changes in the odds ratio (OR) on the main hemochromatosis–Barrett's esophagus association. Confounding was considered to be present (and the variable retained in the final model) if inclusion altered the OR by >10%. We examined effect modification (e.g., the influence of age or ethnicity on the associations) using cross-product terms in the logistic regression model and generating stratum-specific ORs [38]. Comparisons of proportions used the binomial distribution. The study and analyses were approved by the institutional review board. Analyses used the STATA statistical package (version 8, STATA Corp., College Station, TX).

Results

Study Population

Details of the population have been previously published [39]. We interviewed 953 subjects; whole-blood samples for hemochromatosis gene analysis were completed for 931 cases (98% of the interviewed subjects): 317 cases, 306 GERD patients, and 308 population controls. The general subject characteristics are provided in Table 1. Among the cases, the length of the Barrett's segment was <3 cm in 118 subjects (37%), ≥3 cm in 150 subjects (47%), and the length was not reported in 51 subjects (16%).

Hemochromatosis Gene Status

Hemochromatosis gene mutation status was not consistently associated with the risk of Barrett's esophagus (Tables 2 and 3). Patients with Barrett's esophagus were not statistically significantly more likely to have any gene mutation than the population controls, though Barrett's esophagus cases were somewhat more likely to have any gene mutation than the GERD controls. Moderate and severe mutations, the mutations most often associated with iron overload, were not statistically significantly more common among Barrett's esophagus patients than among the population controls. The main serious mutation associated with iron overload (C282Y homozygote) was found in similar frequencies among cases (two persons, 0.6%), GERD patients (one person, 0.3%), and population controls (two persons, 0.6%).

There was no statistical evidence of differences in the association (effect modification) by race (*P*-value interaction term = 0.47), although whites were more likely to have a moderate or severe gene defect than non-whites (103 [18%] vs. 15 [10%] persons with defects, $P = 0.02$). Moderate or severe mutations were not more common among persons with Barrett's esophagus in either whites (OR = 1.21, 95% CI: 0.72–2.04; cases vs. population controls) or non-whites (OR = 1.70, 95% CI: 0.68–4.22).

There was no difference in the frequency of moderate or severe mutations between men and women (85 [17%] vs. 33 [15%], respectively, $P = 0.42$). There was no statistical evidence of interaction by sex (*P*-value on interaction term for moderate or severe defect = 0.17). However, in analyses stratified by sex, men with Barrett's esophagus were more likely than population

controls to have a moderate or severe mutation (OR = 1.87, 95% CI: 1.03–3.37), whereas women were not (OR = 0.93, 95% CI: 0.37–2.35), although the confidence intervals between the genders overlapped broadly and this association was not present for the most severe mutation most commonly associated with iron overload.

Hemochromatosis Gene Status and Serum Iron Stores

We found the expected increases in iron stores among persons with moderate or severe hemochromatosis gene defects. If hemochromatosis gene defects increase the risk of Barrett's esophagus, the hypothesized mechanism would be through the accumulation of higher iron stores; thus, we would expect that hemochromatosis gene defects would be associated with higher iron stores. We evaluated this hypothesis by assessing whether persons with a hemochromatosis gene mutation were at increased risk of being in the third or fourth quartiles of serum iron stores.

The presence of any mutation, or a moderate or severe mutation, did significantly increase the risk of being in the fourth quartile of transferrin saturation among the population as a whole and among two of the subgroups (the Barrett's esophagus group and the GERD group) (Table 4), though the confidence intervals were broad and overlapping between the groups.

The presence of any hemochromatosis gene defect, or a moderate to severe mutation, was not associated with a significantly increased risk of being in the fourth quartile of serum ferritin concentration in the population as a whole (Table 4), although, when broken down by case and control subgroups, there was an increased risk of being in the fourth quartile among persons with Barrett's esophagus, though the confidence intervals were broad and overlapping between the groups.

Supplemental Analyses

There was no evidence of confounding by caloric intake, BMI, waist circumference, smoking status, alcohol use, aspirin use, nonsteroidal anti-inflammatory agents, *H. pylori* antibody status, socioeconomic status (as measured by either educational level or income), or comorbidity (DxCg score). Serum iron stores may be altered by systemic inflammation [40]; however, adjusting for a sensitive serum marker of inflammation (highly sensitive c-reactive protein) did not alter the observed associations for iron stores.

Discussion

This is the first study to examine the association between hemochromatosis gene defects, their related changes in iron stores, and the risk of Barrett's esophagus. The results indicate that patients with Barrett's esophagus do not have a statistically significantly increased risk of the hemochromatosis gene defects most strongly associated with iron overload. Although some gene defects were somewhat more common among subjects with Barrett's esophagus, this was mainly due to mutations of the H63D gene, which, by itself, is not consistent with iron overload, and these differences were not greater than those attributable to chance alone. As expected, subjects with moderate or severe gene defects were more likely to have increased serum iron transferrin saturations (though not clearly serum iron ferritin concentrations), but since the mutations were not significantly more common among patients with Barrett's esophagus, this did not translate into an average increased risk of iron overload among patients with Barrett's esophagus compared with controls.

There are plausible mechanistic links between iron and esophageal adenocarcinoma. Iron may cause DNA damage (possibly through oxidative stress) [41], enhance cancer cell replication, and down-regulate the immune system surveillance that detects malignant cells [42,43].

Esophageal populations of Barrett's esophagus cells are frequently clonal, thus, iron could theoretically act as both an initiator and promoter of clonal growth in Barrett's esophagus [44].

Human studies, although somewhat conflicting, have generally suggested that persons with hemochromatosis or high iron stores have an increased risk of hepatic and non-hepatic malignancies [5,6,9,10,45–47], and possibly of esophageal carcinoma, although this latter study included only two cancer cases who were not stratified by histology (squamous vs. adenocarcinoma) [9]. It is unclear, however, whether hemochromatosis may increase cancer risk through increased iron loads or whether the association is through other iron-independent interactions with the hemochromatosis gene. Animal studies have indicated that iron supplementation itself markedly increased the risk of esophageal adenocarcinoma [4].

In contrast, a human dietary study suggested that persons with esophageal adenocarcinoma had lower than average dietary iron intakes [48]. A recent study by our group similarly found that persons with Barrett's esophagus had lower dietary iron intakes and lower average iron stores than the population controls. The current study now suggests that, among a large community-based sample, persons with Barrett's esophagus also are not substantially more likely to have certain genetic defects that increase iron stores.

There are plausible reasons for why the current results differ from the animal models of esophageal adenocarcinoma. The animal models used surgery to induce bile reflux and these conditions differ markedly from human reflux physiology. In addition, it is unknown where, if at all, iron may act in the carcinogenic sequence. Iron may increase the risk of neoplastic transformation from Barrett's esophagus to esophageal adenocarcinoma, but may not increase the risk of Barrett's esophagus itself.

There are several strengths of this analysis. First, the subjects came from a population that closely approximates the region's census demographics, thus, the results can likely be generalized to similar large populations. Second, this is the first study to use only patients with a new diagnosis of Barrett's esophagus and the study identified all patients with an incident diagnosis within the population. Prevalent or referral patients may have a different clinical course or may be more compliant with follow-up; prevalent patients may also have initiated changes in supplement use, dietary iron intake, or other behaviors after their Barrett's esophagus diagnosis [49]. The use of incident cases thus minimizes selection bias. Third, the availability of a GERD comparison group provided information on the risk of Barrett's esophagus among patients with GERD. This permitted us to evaluate the question of whether hemochromatosis gene status may help determine why only some persons with GERD develop Barrett's esophagus. Finally, the data were of high quality. The measurements used a systematic protocol, established referral laboratories, and enabled direct review of the endoscopy and pathology results.

There are several potential limitations of this analysis. First, observational studies cannot definitively establish cause and effect [26]. Although the hemochromatosis gene status is fixed for each person, the conditions associated with Barrett's esophagus (i.e., gastroesophageal reflux disease) may cause esophagitis, low levels of blood loss, and lower iron stores that influence the phenotype (i.e., iron overload). However, results from the analyses of iron stores by hemochromatosis gene status that excluded subjects with esophagitis and other disorders at the time of the endoscopy were similar to the results from analyses that included such patients. Second, observational studies are subject to confounding by other factors. Although our analyses provided little evidence of confounding, we cannot exclude incomplete control of confounding from measured or unmeasured factors. Third, the presence of nonresponders may lead to bias; however, the electronic data suggested that nonresponders were, on average,

similar to responders on several demographic variables. The nonresponders were, however, slightly healthier than the responders, with lower comorbidity scores. There are no clear reasons as to why hemochromatosis gene status would lead to a selection bias.

In summary, in a non-referral population, there was no statistically significant association between the presence of hemochromatosis gene defects and the risk of Barrett's esophagus, particularly for the major defects most strongly associated with iron overload. These findings do not provide general support for the hypothesis that a genetic condition associated with increased iron stores acts as a substantial risk factor for Barrett's esophagus, although the infrequency of severe mutations limited the study's power to evaluate for small to moderate effects from severe mutations. Future studies are needed to confirm these results and to evaluate whether iron stores modify the risk of Barrett's esophagus progressing to esophageal adenocarcinoma.

Acknowledgements

United States National Institutes of Health RO1 DK63616 and K08 DK02697.

References

1. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. J Natl Cancer Inst 2005;97:142–146. [PubMed: 15657344]
2. Chen X, Yang CS. Esophageal adenocarcinoma: a review and perspectives on the mechanism of carcinogenesis and chemoprevention. Carcinogenesis 2001;22:1119–1129.10.1093/carcin/22.8.1119 [PubMed: 11470739]
3. Chen X, Ding YW, Yang G, Bondoc F, Lee MJ, Yang CS. Oxidative damage in an esophageal adenocarcinoma model with rats. Carcinogenesis 2000;21:257–263.10.1093/carcin/21.2.257 [PubMed: 10657966]
4. Chen X, Yang G, Ding WY, Bondoc F, Curtis SK, Yang CS. An esophagogastroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. Carcinogenesis 1999;20:1801–1808.10.1093/carcin/20.9.1801 [PubMed: 10469627]
5. Geier D, Hebert B, Potti A. Risk of primary non-hepatocellular malignancies in hereditary hemochromatosis. Anticancer Res 2002;22:3797–3799. [PubMed: 12552996]
6. Elmberg M, Hultcrantz R, Ekbom A, Brandt L, Olsson S, Olsson R, Lindgren S, Lööf L, Stål P, Wallerstedt S, Almer S, Sandberg-Gertzén H, Askling J. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. Gastroenterology 2003;125:1733–1741.10.1053/j.gastro.2003.09.035 [PubMed: 14724826]
7. Petersen DR. Alcohol, iron-associated oxidative stress, and cancer. Alcohol 2005;35:243–249.10.1016/j.alcohol.2005.03.013 [PubMed: 16054986]
8. Stevens RG, Beasley RP, Blumberg BS. Iron-binding proteins and risk of cancer in Taiwan. J Natl Cancer Inst 1986;76:605–610. [PubMed: 3007843]
9. Hsing AW, McLaughlin JK, Olsen JH, Mellemkjar L, Wacholder S, Fraumeni JF Jr. Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. Int J Cancer 1995;60:160–162. [PubMed: 7829208]
10. Fracanzani AL, Conte D, Fraquelli M, Taioli E, Mattioli M, Losco A, Fargion S. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. Hepatology 2001;33:647–651.10.1053/jhep.2001.22506 [PubMed: 11230745]
11. Walsh A, Dixon JL, Ramm GA, Hewett DG, Lincoln DJ, Anderson GJ, Subramaniam VN, Dodemaide J, Cavanaugh JA, Bassett ML, Powell LW. The clinical relevance of compound heterozygosity for the C282Y and H63D substitutions in hemochromatosis. Clin Gastroenterol Hepatol 2006;4:1403–1410.10.1016/j.cgh.2006.07.009 [PubMed: 16979952]
12. McCune CA, Ravine D, Carter K, Jackson HA, Hutton D, Hedderich J, Krawczak M, Worwood M. Iron loading and morbidity among relatives of HFE C282Y homozygotes identified either by

population genetic testing or presenting as patients. Gut 2006;55:554–562.10.1136/gut.2005.070342 [PubMed: 16174659]

13. Lazarescu A, Snively BM, Adams PC. Phenotype variation in C282Y homozygotes for the hemochromatosis gene. Clin Gastroenterol Hepatol 2005;3:1043–1046.10.1016/S1542-3565(05)00413-1 [PubMed: 16234052]
14. Barton JC, Acton RT, Dawkins FW, Adams PC, Lovato L, Leiendecker-Foster C, McLaren CE, Reboussin DM, Speechley MR, Gordeuk VR, McLaren GD, Sholinsky P, Harris EL. Initial screening transferrin saturation values, serum ferritin concentrations, and HFE genotypes in whites and blacks in the Hemochromatosis and Iron Overload Screening Study. Genet Test 2005;9:231–241.10.1089/gte.2005.9.231 [PubMed: 16225403]
15. Beutler E, Felitti V, Gelbart T, Ho N. The effect of HFE genotypes on measurements of iron overload in patients attending a health appraisal clinic. Ann Intern Med 2000;133:329–337. [PubMed: 10979877]
16. Sharma P, McQuaid K, Dent J, Fennerty MB, Sampliner R, Spechler S, Cameron A, Corley D, Falk G, Goldblum J, Hunter J, Jankowski J, Lundell L, Reid B, Shaheen NJ, Sonnenberg A, Wang K, Weinstein W. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. Gastroenterology 2004;127:310–330.10.1053/j.gastro.2004.04.010 [PubMed: 15236196]
17. Kubo A, Corley DA. Marked multi-ethnic variation of esophageal and gastric cardia carcinomas within the United States. Am J Gastroenterol 2004;99:582–588.10.1111/j.1572-0241.2004.04131.x [PubMed: 15089886]
18. Hoppe C, Watson RM, Long CM, Lorey F, Robles L, Klitz W, Styles L, Vichinsky E. Prevalence of HFE mutations in California newborns. Pediatr Hematol Oncol 2006;23:507–516.10.1080/08880010600751918 [PubMed: 16849282]
19. McClung JP, Marchitelli LJ, Friedl KE, Young AJ. Prevalence of iron deficiency and iron deficiency anemia among three populations of female military personnel in the US Army. J Am Coll Nutr 2006;25:64–69. [PubMed: 16522934]
20. Beutler E, West C. Hematologic differences between African-Americans and whites: the roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume. Blood 2005;106:740–745.10.1182/blood-2005-02-0713 [PubMed: 15790781]
21. Ganji V, Hampl JS, Betts NM. Race-, gender- and age-specific differences in dietary micronutrient intakes of US children. Int J Food Sci Nutr 2003;54:485–490.10.1080/09637480310001622341 [PubMed: 14522694]
22. Fleming DJ, Jacques PF, Tucker KL, Massaro JM, D'Agostino RB Sr, Wilson PW, Wood RJ. Iron status of the free-living, elderly Framingham Heart Study cohort: an iron-replete population with a high prevalence of elevated iron stores. Am J Clin Nutr 2001;73:638–646. [PubMed: 11237943]
23. Adams PC, Kertesz AE, McLaren CE, Barr R, Bamford A, Chakrabarti S. Population screening for hemochromatosis: a comparison of unbound iron-binding capacity, transferrin saturation, and C282Y genotyping in 5,211 voluntary blood donors. Hepatology 2000;31:1160–1164.10.1053/he.2000.6984 [PubMed: 10796893]
24. Aranda N, Viteri FE, Fernández-Ballart J, Murphy M, Arija V. Frequency of the hemochromatosis gene (HFE) 282C→Y, 63H→D, and 65S→C mutations in a general Mediterranean population from Tarragona, Spain. Ann Hematol 2007;86:17–21.10.1007/s00277-006-0179-0 [PubMed: 17013646]
25. Krieger N. Overcoming the absence of socioeconomic data in medical records: validation and application of a census-based methodology. Am J Public Health 1992;82:703–710. [PubMed: 1566949]
26. Rothman, KJ.; Greenland, S. Modern epidemiology. Vol. 2. Lippincott-Raven; Philadelphia: 1998.
27. Block G, Hartman AM. Issues in reproducibility and validity of dietary studies. Am J Clin Nutr 1989;50:1133–1138. [PubMed: 2683721]discussion 1231–1235
28. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol 1986;124:453–469. [PubMed: 3740045]
29. Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc 1992;92:686–693. [PubMed: 1607564]

30. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol 1990;43:1327–1335.10.1016/0895-4356(90)90099-B [PubMed: 2254769]

31. Massey AC. Microcytic anemia. Differential diagnosis and management of iron deficiency anemia. Med Clin North Am 1992;76:549–566. [PubMed: 1578956]

32. Hillman, RS. Iron deficiency and other hypoproliferative anemias. In: Fauci, AS.; Braunwald, E.; Isselbacher, KJ.; Wilson, JD.; Martin, JB.; Kasper, DL.; Hauser, SL.; Longo, DL., editors. Harrison's principles of internal medicine. McGraw-Hill; New York: 1998. p. 638-643.

33. Beutler E, Hoffbrand AV, Cook JD. Iron deficiency and overload. Hematology (Am Soc Hematol Educ Program) 2003;2003:40–61.10.1182/asheducation-2003.1.40 [PubMed: 14633776]

34. Zhao Y, Ash AS, Ellis RP, Ayanian JZ, Pope GC, Bowen B, Weyuker L. Predicting pharmacy costs and other medical costs using diagnoses and drug claims. Med Care 2005;43:34–43. [PubMed: 15626932]

35. Zhao Y, Ellis RP, Ash AS, Calabrese D, Ayanian JZ, Slaughter JP, Weyuker L, Bowen B. Measuring population health risks using inpatient diagnoses and outpatient pharmacy data. Health Serv Res 2001;36:180–193. [PubMed: 16148968]

36. Breslow, NE.; Day, NE. Statistical methods in cancer research. Volume 1, the analysis of case–control studies; International Agency for Research on Cancer; Lyon, France. 1980.

37. Kleinbaum, DG.; Kupper, LL.; Morgenstern, H. Principles and quantitative methods. Van Nostrand Reinhold; New York: 1982. Epidemiologic research.

38. Hosmer, DW.; Lemeshow, S. Applied logistic regression. Vol. 2. Wiley; New York: 2000.

39. Corley DA, Kubo A, Levin TR, Block G, Habel L, Zhao W, Leighton P, Quesenberry C, Rumore GJ, Buffler PA. Abdominal obesity and body mass index as risk factors for Barrett's esophagus. Gastroenterology 2007;133:34–41.10.1053/j.gastro.2007.04.046 [PubMed: 17631128]

40. Preziosi P, Hercberg S, Galan P, Devanlay M, Cherouvrier F, Dupin H. Iron status of a healthy french population: factors determining biochemical markers. Ann Nutr Metab 1994;38:192–202. [PubMed: 7832579]

41. Walker EM Jr, Walker SM. Effects of iron overload on the immune system. Ann Clin Lab Sci 2000;30:354–365. [PubMed: 11045759]

42. Luo Y, Henle ES, Linn S. Oxidative damage to DNA constituents by iron-mediated fenton reactions. The deoxycytidine family. J Biol Chem 1996;271:21167–21176.10.1074/jbc.271. 35.21167 [PubMed: 8702887]

43. Hann HW, Stahlhut MW, Hann CL. Effect of iron and desferoxamine on cell growth and in vitro ferritin synthesis in human hepatoma cell lines. Hepatology 1990;11:566–569.10.1002/hep. 1840110407 [PubMed: 2158479]

44. Barrett MT, Sanchez CA, Prevo LJ, Wong DJ, Galipeau PC, Paulson TG, Rabinovitch PS, Reid BJ. Evolution of neoplastic cell lineages in Barrett oesophagus. Nat Genet 1999;22:106–109.10.1038/8816 [PubMed: 10319873]

45. Nelson RL. Iron and colorectal cancer risk: human studies. Nutr Rev 2001;59:140–148. [PubMed: 11396694]

46. Macdonald GA, Tarish J, Whitehall VJ, McCann SJ, Mellick GD, Buttenshaw RL, Johnson AG, Young J, Leggett BA. No evidence of increased risk of colorectal cancer in individuals heterozygous for the Cys282Tyr haemochromatosis mutation. J Gastroenterol Hepatol 1999;14:1188–1191.10.1046/j.1440-1746. 1999.02027.x [PubMed: 10634155]

47. Nelson RL, Davis FG, Persky V, Becker E. Risk of neoplastic and other diseases among people with heterozygosity for hereditary hemochromatosis. Cancer 1995;76:875–879.10.1002/1097-0142 (19950901)76:5<875::AID-CNCR2820760523>3.0.CO;2-Q [PubMed: 8625192]

48. Zhang ZF, Kurtz RC, Yu GP, Sun M, Gargon N, Karpeh M Jr, Fein JS, Harlap S. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. Nutr Cancer 1997;27:298–309. [PubMed: 9101561]

49. Morrison, AS. Screening in chronic disease. Vol. 2. Oxford University Press; New York: 1992.

Table 1

Characteristics of the study groups

	Cases Number or mean (% or standard deviation)	GERD controls Number or mean (% or standard deviation)	Population controls Number or mean (% or standard deviation)
Number of subjects	317	306	308
Age (years)			
20–39	9 (3)	12 (4)	9 (3)
40–59	119 (38)	110 (36)	104 (34)
60–79	189 (60)	184 (60)	195 (64)
Race			
White	275 (87)	244 (80)	260 (84)
Hispanic	25 (8)	20 (7)	13 (4)
Black	4 (1)	20 (7)	16 (5)
Asian/Pacific islander	4 (1)	11 (4)	11 (4)
Others	7 (2)	8 (3)	6 (2)
Unknown	2 (1)	2 (1)	1 (0)
Sex			
Male	232 (73)	211 (69)	211 (69)
Smoking status (ever smoked)	209 (66)	181 (59)	171 (56)
Mean serum ferritin	116 (±132)	130 (±135)	157 (±138)
Mean transferrin saturation	21% (±10)	22% (±10)	22% (±8)
Mean BMI (kg/m^2)	30 (±6)	29 (±5)	29 (±6)

Table 2

Distribution of hemochromatosis gene mutations between Barrett's esophagus cases and controls

Hemochromatosis gene patterns	Cases Number (%)	GERD controls Number (%)	Population controls Number (%)
Wild type/wild type	187 (59.0)	207 (67.7)	202 (65.6)
Wild type/H63D	81 (25.6)	63 (20.6)	73 (23.7)
Wild type/C282Y	33 (10.4)	27 (8.8)	22 (7.1)
H63D/H63D	11 (3.5)	6 (2.0)	4 (1.3)
C282Y/H63D	3 (1.0)	2 (0.7)	5 (1.6)
C282Y/C282Y	2 (0.6)	1 (0.3)	2 (0.7)
Total	317	306	308

Table 3

Association between hemochromatosis gene status and Barrett's esophagus

Mutation status	Number of Barrett's esophagus/GERD/ population	Barrett's esophagus vs. population controls OR (95% CI)[a]	Barrett's esophagus vs. GERD patients OR (95% CI) [a]
No mutation	187/207/202	1.0	1.0
Mild mutation[b]	81/63/73	1.21 (0.83–1.77)	1.39 (0.94–2.05)
Moderate mutation[c]	44/33/26	1.75 (1.03–2.97)	1.40 (0.85–2.31)
Severe mutation[d]	5/3/7	0.77 (0.24–2.48)	1.90 (0.45–8.12)
Any mutation[e]	130/99/106	1.32 (0.95–1.84)	1.41 (1.01–1.97)
Moderate or severe mutation	49/36/33	1.54 (0.94–2.52)	1.45 (0.90–2.33)

[a] Odds ratios are adjusted for gender, age, ethnicity, and smoking status

[b] Wild type/H63D

[c] C282Y/wild type, H63D/H63D

[d] C282Y/H63D, C282Y/C282Y

[e] Any subject without wild type/wild type

Table 4

Evaluation of whether subjects with hemochromatosis gene defects are at increased risk of higher iron stores: hemochromatosis gene status, serum iron saturation, and serum ferritin concentration

Mutation status	Barrett's esophagus/GERD/population	Among all subjects	Among Barrett's esophagus	Among GERD group	Among controls
Odds of being in fourth quartile of iron saturation (95% confidence interval)[a,b]					
No mutation	187/207/202	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Any mutation	130/99/106	1.99 (1.36–2.91)	1.61 (0.86–3.02)	3.03 (1.54–5.99)	1.74 (0.85–3.55)
Moderate or severe mutation[c]	49/36/33	2.88 (1.63–5.06)	2.40 (1.00–5.78)	5.45 (1.84–16.18)	1.99 (0.68–5.81)
Odds of being in fourth quartile of ferritin (95% confidence interval)[a,d]					
No mutation	187/207/202	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Any mutation	130/99/106	1.25 (0.84–1.87)	1.88 (0.91–3.87)	0.79 (0.37–1.68)	1.74 (0.80–3.77)
Moderate or severe mutation[c]	49/36/33	1.39 (0.78–2.48)	2.62 (1.04–6.56)	1.22 (0.42–3.51)	1.34 (0.38–4.76)

[a] Odds ratios are adjusted for gender, age, ethnicity, and smoking status

[b] Odds of being in the fourth quartile (median 31%) vs. first quartile (median 12%) of percent transferrin saturation; quartiles are based on the control population distributions

[c] C282Y/wild type, H63D/H63D, C282Y/H63D, C282Y/C282Y

[d] Odds of being in the fourth quartile (median 291 ng/ml) vs. first quartile (median 38 ng/ml) of ferritin concentration; quartiles are based on the control population distributions