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DIETARY, LIFESTYLE, AND GENETIC FACTORS INFLUENCING COLORECTAL CANCER RISK

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ABSTRACT

Cancers of the colon and rectum (colorectum) are complex diseases that form through a combination of both intrinsic (e.g. genetic variation) and extrinsic (e.g. environmental) factors. Currently, colorectal cancer (CRC) is the second leading cancer killer in the United States and the fourth most common form of cancer diagnosed. Many epidemiologic and laboratory studies have found that food and nutrition play an important role in both the incidence and progression of colorectal cancers.

Data for the following studies were collected from adult men and women in central and northeastern Pennsylvania, which has higher CRC incidence and mortality rates than the United States average. Cases were identified from the Pennsylvania cancer registry and population-based controls were identified via random-digit dialing. Dietary information was collected via a modified Diet History Questionnaire (DHQ) developed by Subar and colleagues at the National Cancer Institute (NCI) including supplement use and an additional meat module. Sociodemographic, lifestyle, and buccal cells for genomic DNA analysis were collected during in-person interviews.

The objective of the first study was to examine colorectal cancer risk associated with iron intake (total, dietary, supplemental, and heme). Heme iron content from the diet was calculated using a new heme iron database developed by the NCI using information from a detailed meat module that was added to the food frequency questionnaire. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression. After multivariate adjustment, there were no significant associations between heme iron or total iron intake and CRC incidence. Dietary iron was inversely

associated with colorectal cancer risk in women (OR Q_5 vs. Q_1= 0.45; 95% CI = 0.22-0.92), but not men. Lastly, supplemental iron intake of >18 mg/d vs. none was positively associated with colorectal cancer risk (OR = 2.31; 95% CI = 1.48-3.59; *P*-trend <0.001). In summary, these findings suggest that consumption of >18 mg/d of supplemental iron may increase risk for colorectal cancer.

In the second study, our objective was to examine the associations between calcium and vitamin D intake (total, dietary, and supplemental) and incident colorectal cancer. We found an inverse association between total vitamin D intake and colorectal cancer risk (OR = 0.67; 95% CI = 0.49-0.91; *P*-trend < 0.01). Higher levels of total calcium intake were also inversely associated with colorectal cancer incidence (OR Q5 v. Q1= 0.48; 95% CI = 0.35-0.66; *P*-trend <0.01). In addition, high supplemental intakes of vitamin D (>10 μg/d) and calcium (>500 mg/d) were associated with reduced risk (OR High v. None = 0.68; 95% CI = 0.53-0.89, *P*-trend = 0.05; OR High v. None = 0.61; 95% CI = 0.46-0.81, *P*-trend < 0.01, respectively). Overall, our results indicate reduced risk of colorectal cancer with higher total and supplemental intakes of vitamin D and calcium.

In our final study, we examined the associations between folate intake (total, dietary, and supplemental) and incident colorectal cancer. We also assessed the impact of 35 polymorphisms from three folate-mediated one-carbon metabolism (FOCM)-related genes on colorectal cancer risk. Finally, we examined whether there were any gene-diet interactions between folate intake and FOCM-related polymorphisms that alter colorectal cancer risk within the present multi-site population-based case-control study. After multivariate adjustment, colorectal cancer risk was not significantly associated with dietary or supplemental folate intake overall. Four polymorphisms from three genes had

significant interactions with dietary intake factors associated with colorectal cancer risk. Significant associations were also observed among five polymorphisms related to FOCM. In conclusion, our results suggest that allelic variants in genes involved in FOCM, coupled with dietary intakes modify risk for colorectal cancer.

In conclusion, our research shows several micronutrients may alter colorectal cancer risk. Our research also indicates several polymorphisms that may alter cancer risk and provides a solid foundation for future studies investigating gene-environment interactions.

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Chapter 1

Introduction

Overall Background and Significance

Colorectal cancer (cancer of colon and rectum) ranks fourth in cancer incidence and second in cancer mortality in the United States [1]. Although incidence rates in the United States have been on the decline for the past 25 years, there were still an estimated 143,000 new cases of colorectal cancer in the United States in 2012 with men having slightly higher rates than women [2]. The use of colorectal cancer screening tests can help detect cancer at an earlier stage as well as prevent cancer through the removal of precancerous polyps. Despite advances in screening and treatment, the overall five-year survival rate for colorectal cancer is only 64.3% [3].

The colon extends from the caecum to the rectum where it acts to absorb water, salts, and some nutrients from undigested food and form stool for excretion. Many studies of migrant populations as well as incidence rates from developing countries suggest that diet plays an important role in carcinogenesis [4-7]. Foods that pass through the small intestine into the colon and rectum may contain carcinogens. These carcinogens have the ability to interact with cells lining the lumen of the colon and rectum, resulting in mutations that may lead to cancer.

The study population used in the following studies was comprised of adults of both sexes from central and northeastern Pennsylvania. This study population of Pennsylvanians is important as it is at particularly high risk for colorectal cancer compared to the United States as a whole (50.9 per 100,000 in Pennsylvania vs. 46.3 per 100,000 in the U.S. at the start of this study in 2007) [1]. The reason for the increased

incidence and mortality rates remains unclear, but several hypotheses exploring both intrinsic (e.g. genetic variation) and extrinsic (e.g. environmental) factors have been proposed to explain these high rates. Participants in this study provided dietary information via a modified 137-item food frequency questionnaire. Sociodemographic factors, medical history, alcohol use, lifetime tobacco exposure, physical activity, height, weight, medication use, and other lifestyle-related factorswere collected by trained personnel during in-person interviews. Lastly, oral buccal cell swabs, saliva, and blood samples were collected for genomic DNA isolation. A subset of cases and controls from our study population also provided urine samples. This large multi-site population-based case-control study explored potential environmental and genetic factors among adult men and women from a contiguous 18-county region in Pennsylvania.

Background: Study 1

A recent comprehensive review summarizing the currently available epidemiological studies of red meat intake and colorectal cancer risk reported that there was “convincing” evidence that red meat was a risk factor for colorectal cancer [8]. Red meat has high iron content, especially heme iron. This same report also stated that there was “limited-suggestive” evidence for foods containing iron increasing colorectal cancer risk[8].Animal studies have shown that both heme and nonheme iron are capable of catalyzing free radical formation and DNA damage[9, 10]. Heme iron, in particular, has been associated with increased colonic epithelial proliferation[11] and the promotion of colorectal cancer in rats[12, 13]. Previous population studies have found mixed results for

the association between dietary iron and colorectal cancer risk. Our study population consumes a large amount of red and processed meats and a meat-specific module was added to the food frequency questionnaire to further probe any associations.

Specific Aim and Hypothesis: Study 1

Study 1: Specific Aim

In the present multi-site population-based case-control study in Pennsylvania, we explored the associations between iron intake (total, dietary, supplemental, and heme) and incident colorectal cancer. Heme iron content from the diet was calculated using a new heme iron database developed by the National Cancer Institute (NCI) [14].

Study 1: Hypothesis

Higher intakes of dietary, heme, and total iron intake will be positively associated with an increased risk for colorectal cancer. High levels of supplemental iron intake will also be associated with an increased risk for colorectal cancer.

Specific Aims and Hypothesis: Study 2

Background: Study 2

Several epidemiologic and clinical studies have indicated that diets rich in calcium may have a protective effect against colorectal cancer[15-18]. Calcium is thought to act as a chemopreventative agent by binding secondary bile acids and ionized fatty acids to form insoluble soaps which decrease luminal exposure [19]. Similarly, high intake of vitamin D has also been associated with reduced cancer risk, but the results are less conclusive [20-23]. Vitamin D may protect against carcinogenesis by promoting cell differentiation, apoptosis, cell cycle regulation, and angiogenesis via activation of vitamin D receptors (VDRs) and regulation of calcium metabolism [19, 24]. The majority of studies assessing the association between dietary vitamin D and colorectal cancer have examined vitamin D intake exclusively from food sources, but little research has been done on supplemental vitamin D.

Specific Aim: Study 2

In the present multi-site population-based case-control study in Pennsylvania, we explored the associations between calcium and vitamin Dintake (total, dietary, and supplemental) and incident colorectal cancer.

Hypothesis: Study 2

Higher intakes of total, dietary, and supplemental calcium and vitamin D will be inversely associated with risk for colorectal cancer.

Specific Aims and Hypothesis: Study 3

Background: Study 3

A recent comprehensive review summarizing the currently available epidemiological studies of folate intake and colorectal cancer risk reported that there was "limited, suggestive" evidence that foods containing folate decreased risk for colorectal cancer[25]. Folate is a water-soluble B vitamin that is critical for one-carbon metabolism. Folate-mediated one-carbon metabolism (FOCM) is important for DNA methylation, DNA synthesis and repair, and for producing S-adenosylmethionine (SAM) which is the universal donor of methyl groups [26]. Several studies have shown that insufficient folate intake can lead to DNA damage, via uracil misincorporation or anomalous DNA repair [27]. Low folate intake can also induce epigenetic changes (ie, hypomethylation) that have been associated with colorectal carcinogenesis [27]. Several other dietary factors are also involved in FOCM; micronutrients such as B-vitamins as well as alcohol may alter FOCM and are known cancer risk modifiers.

A number of epidemiologic studies have found conflicting results for folate intake and risk for colorectal cancer[28]. One possible explanation for this heterogeneity in results is polymorphisms located within the FOCM pathway. There is a common disease-

common variant hypothesis whichposits that common diseases such as colorectal cancer may have common mutations between cases and large association studies such as ours provide a powerful strategy for identifying such alleles[29, 30].

Primary Aim: Study 3

The primary aim of study 3 was to examine whether there were any gene-diet interactions between folate intake and FOCM-related polymorphisms that alter colorectal cancer risk within the present multi-site population-based case-control study.

Primary Hypothesis: Study 3

Folate intake levels would interact with FOCM-related genes to significantly alter risk for colorectal cancer.

Secondary Aim: Study 3

In the present multi-site population-based case-control study in Pennsylvania, we explored the associations between folate intake (total, dietary, and supplemental) and incident colorectal cancer. We also assessed the impact of 35 polymorphisms from three FOCM-related genes on colorectal cancer risk.

Secondary Hypothesis: Study 3

Greater intakes of folate would be inversely associated with risk for colorectal cancer. Polymorphisms located within the FOCM pathway would significantly alter colorectal cancer risk.

Summary

The research presented in this dissertation uses data from a large multi-site population-based case-control study in central and northeast Pennsylvania. Three main research questions are addressed: the first in Chapter 3 (Study 1), the second in Chapter 4 (Study 2), and the third in Chapter 5 (Study 3). The first study assesses associations between dietary and supplemental iron intake and colorectal cancer risk (Chapter 3). A manuscript describing this study and our findings was recently accepted for publication (upon revision) [31]. A comprehensive review of the literature on dietary iron intake and colorectal cancer provides context and background for the first study (Chapter 2) and is currently in press [32]. The second study assesses the associations between dietary and supplemental calcium and vitamin D intake and their impact on colorectal cancer risk (Chapter 4). The third study focuses on associations between folate intake and polymorphisms involved in folate metabolism and their impact on colorectal cancer risk (Chapter 5). Chapter 6 summarizes the findings from chapters 3-5, describes the limitations of our research, and provides recommendations for future research.

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Chapter 2

Dietary Iron and Colorectal Cancer Risk: A Review of Human Population Studies[1]

[1] This manuscript was accepted for publication and is in press:
Ashmore JH, Rogers CJ, Kelleher SL, Lesko SM, Hartman TJ. Dietary Iron and Colorectal Cancer: A Review of Human Population Studies. *Critical Reviews in Food Science and Nutrition*.2013 (In Press).

Abstract

Iron is an essential micronutrient that is involved in many redox processes and serves as an integral component in various physiological functions. However, excess iron can cause tissue damage through its pro-oxidative effects, potentiating the development of many diseases such as cancer through the generation of reactive oxidative species. The two major forms of iron in the diet are heme and nonheme iron, both of which are found in several different foods. In addition to natural food sources, intake of nonheme iron may also come from fortified foods or in supplement form. This review summarizes the results of human population studies that have examined the role of dietary iron (heme and nonheme), heme iron alone, and iron from supplements in colorectal carcinogenesis.

Introduction

Iron is an essential mineral in mammals that transports oxygen throughout the body as part of the heme complex, supports growth, plays a key role in the generation of ATP in the electron transport chain, helps maintain immune competence, and is a part of many redox processes [1]. While iron has many beneficial effects, it can also cause tissue damage and disrupt many other vital cell processes and components if excess iron is present.

There are two major types of dietary iron: nonheme iron found in plants, meat, and supplements and heme iron which is only found in meat. Nonheme iron can be divided into two different categories: small iron salts or complexes and the iron mineral ferritin (FTN) [2]. Because of iron's possible toxic effects, iron absorption is tightly regulated. Uptake of nonheme iron in its reduced form occurs via the divalent metal transporter-1(DMT-1) on the apical membrane of enterocytes. Nonheme FTN is absorbed by a receptor-mediated endocytotic process [3]. The exact mechanism of heme iron absorption is less well known, but a heme transporter, heme carrier protein 1 (HCP1), has been characterized in rats [4].

The percentage of iron absorbed from the diet can vary from less than one percent to greater than 50% [5]. The amount of iron stored in the body is the main factor controlling iron absorption. Little nonheme iron is absorbed from the diet; on average, adult men and women absorb 6% and 13% of dietary iron, respectively [5]. The unabsorbed iron travels through the alimentary tract and collects in the colorectum before it is expelled in the feces. Once heme iron is absorbed, the heme protein is catabolized within the enterocyte and released into the bloodstream along with nonheme iron and primarily utilized for erythropoiesis with excessive iron stored in the liver [3, 6].

Body iron stores accumulate with age in all individuals with adequate intake [7, 8], and to a much higher degree in individuals who have hereditary hemochromatosis (HH)[9]. The prevalence rate of HH is approximately one case per 200 persons and often presents with hepatic cirrhosis, hepatocellular carcinoma, and severe fibrosis [10]. The primary cause of HH is a

substitution of a cysteine with a tyrosine amino acid at position 282 in the HFE protein (C282Y) [11]. HFE is an iron regulatory protein that functions to regulate iron absorption. A second mutation in HFE results in a substitution of aspartic acid for histidine at position 63 (H63D) [10]. The H63D mutation is more prevalent than the C282Y mutation in U.S. (13.5% vs. 5.4%, respectively) [12]. Both the C282Y and H63D mutations lead to increased iron absorption, but iron overload is most commonly related to the C282Y mutation. Iron overload is defined as transferrin saturation greater than 50% in premenopausal women, or greater than 55% in postmenopausal women and men, and serum ferritin concentration greater than 200μg/L in women, or greater than 300 μg/L in men [13]. This overload is a problem because there are no direct mechanisms of eliminating excess iron. However, indirect losses occur via sloughing of senescent gastrointestinal cells in feces, menses, parturition following pregnancy, blood loss, and sloughing of skin.

One area that has been widely studied with respect to dietary iron intake is colorectal cancer (cancer of the colon or rectum). Although incidence rates of colorectal cancers have been on the decline for the past 25 years, they still rank third in newly diagnosed cancers and third in cancer deaths [14]. In 2011, there were approximately 140,000 new cases of colorectal cancer in the United States (U.S.) with men having a higher incidence rate than women (57.1 vs. 42.4 per 100,000, respectively) [15]. The mechanisms through which iron may promote colorectal carcinogenesis are varied. Unabsorbed iron from the diet travels through the alimentary tract, eventually ending up in the colon and rectum before it is lost in the feces. Iron in the lumen of the colorectum may interact with other unabsorbed food components, waste, and the cellular membrane of the colon itself. The role of iron in colorectal carcinogenesis has been summarized elsewhere [16, 17]. The pro-oxidative effects of iron can catalyze reactive oxidative species formation that can damage DNA [18]. Several rodent studies have shown that dietary iron increases colonic crypt cell proliferation [19] and enhances colorectal tumor growth [20, 21]. Dietary meat has also been shown to promote colorectal carcinogenesis with an increased effect

directly related to heme iron concentration [22]. Heme iron increases the formation of lipid peroxyl radicals [23] such as malondialdehyde and 4-hydroxynonenal which are potent carcinogens in humans [24, 25].

Epidemiologic studies examining the associations between HFE mutation carrier status and colorectal cancer have found mixed results. A recent international study of over 28,000 individuals found that homozygotes for the C282Y mutation had over twice the risk of colorectal cancer compared to those with no mutation [26]. Individuals with only one mutation (C282Y or H63D) were not at increased risk for colorectal cancer in this study [26]. Two previous studies found positive associations among subjects with any mutation in the HFE gene [27, 28]. Studies only assessing the C282Y mutation found no association with colorectal cancer [29, 30].

Herein, we analyzed the epidemiological studies that have examined the role of dietary iron (heme and nonheme), heme iron alone, and iron from supplements in colorectal carcinogenesis. This review is an analysis of human population-based studies relating dietary iron intake and colorectal cancer risk with a focus on luminal iron exposure. It is meant to be an update to the previous review of case-control studies assessing dietary iron and colorectal cancer [17] as well as a supplement to the recent meta-analysis of cohort studies assessing heme iron and colorectal cancer risk [16].

Methods

Articles were initially located by conducting a literary search on PubMed. Key words in various combinations included the following: “colon, rectum, colorectum, HFE, cancer, adenoma, dietary iron, red meat, and heme.” Reference lists of articles were also scanned for relevant articles. Further criteria limited the search to articles in English from the last 15 years (1996-2012) that measured dietary nonheme or heme iron with respect to adenoma, polyp, or colorectal cancer outcomes. Studies were eliminated from consideration if they did not have dietary intakes

of nonheme iron or heme iron (e.g. included only meat intake or HFE status). Twenty studies were included in this review from Europe [31-36], North America [37-48], Uruguay [49], and Australia [50].

Eighteen of the 20 studies included in this review calculated dietary iron intake which is comprised of both nonheme and heme iron [31-33, 35-43, 45-50]. Nine studies calculated dietary intakes of heme iron [32, 34, 38-42, 44, 48].Two studies analyzed the iron regulatory gene HFEin conjunction with dietary iron intake [38, 45]. The majority of these studies included both men and women. Five studies were female only [34, 38, 41-44] and one study had all male subjects [33]. These studies utilized several experimental designs. Eight of the studies were case-control [31, 35-37, 41, 45, 49, 50], five were nested case-control [33, 38, 40, 43, 46], six were cohort [32, 34, 39, 42, 44, 48], and one was a follow-up of the NHANES I dataset from the National Health Evaluation Follow-up Study (NHEFS) [47].

Iron Intake Results

Dietary iron:

The outcomes of the 18 studies that measured dietary iron are mixed (**Tables 1 & 2)**. We included 10 cohort and nested case-control studies in this review (**Table 1**). Of the studies that used cancer incidence as an endpoint, one study [47] found a significant positive association and one study [39] found a significant inverse association between dietary iron and colon cancer. In the follow-up from the NHANES I study [47], men and women with the highest iron intake had an increased risk for colon cancer (RR Q_4 vs. Q_1 = 3.35; 95% CI = 1.74-6.46). Conversely, in the study by Cross et al. [39], both dietary iron and total iron (dietary iron + supplements) had a significant inverse association in each of the 2^{nd}-5^{th} quintiles of intake in the colon, rectum, and colorectum as well as a significant dose-response relationship. In a subsequent sub-analysis of the

NHANES I study [47], men and women with the highest iron intake had an increased risk for proximal colon cancer (RR Q_4 vs. Q_1 = 1.44; 95% CI = 1.23-1.69) , and women alone had an even greater risk (RR Q_4 vs. Q_1 = 1.51; 95% CI = 1.41-1.60). The study by Kato et al. [43] found a positive dose-response in the proximal colon of females (P = 0.04). One study found a significant inverse association between high dietary iron and adenoma recurrence[46]. The majority of the remaining cohort studies found positive, but non-significant associations between dietary iron intake and colorectal cancer or adenoma incidence.

The results of the 8 case-control studies are listed in **Table 2**. Three of the five studies that used cancer incidence as an endpoint found a significant positive association between dietary iron and colorectal cancer [35, 36, 45, 49, 50]. In the Uruguayan study by Deneo-Pellegrini et al. [49] rectal cancer was positively associated with dietary iron in both men and women (OR T_3 vs. T_1 = 3.18; 95% CI = 1.92-5.29), but was particularly evident in men (OR T_3 vs. T_1 = 4.01; 95% CI = 2.09-7.69). In contrast, there were no significant associations between dietary iron and incidence of adenoma. These inconsistent results from both cohort and case-control studies indicate that dietary iron may play a role in modifying colorectal cancer risk.

Supplemental iron:

Of the 20 studies included in this review, only 11 measured supplemental iron intake. The methods used to assess the impact of supplemental iron on colorectal cancer risk varied greatly by study. Many of these studies compared dietary iron and total iron intake (dietary iron + supplements) to evaluate the influence of iron supplements on colorectal risk estimates. Three of the reviewed studies had no data or excluded subjects taking iron supplements because so few reported iron supplement use [33, 38, 40]. Two studies only reported risk associated with total iron intake so no conclusions can be made concerning supplements [43, 45]. The NIH-AARP cohort study, which was also the largest study in this review, reported similar risk estimates for

high dietary and total iron intake and colorectal cancer [39]. Interestingly, a study by Ferrucci et al. [41], showed a potential protective effect of supplemental iron intake on adenoma incidence. In this study, dietary iron was positively associated (OR Q_4 vs. Q_1 = 1.11; 95% CI = 0.57-2.16), total iron intake was inversely associated with adenoma incidence (OR Q_4 vs. Q_1 = 0.91; 95% CI = 0.52-1.59), and supplemental iron had a mild protective effect on adenoma incidence (OR T_3 vs. T_1 = 0.82; 95% CI = 0.54-1.25). While none of these results were significant, overall they suggest a potential benefit on colorectal adenomas from supplemental iron.

In contrast, four studies showed a potential positive association for supplemental iron intake and colorectal cancer or adenoma incidence when comparing dietary iron and total iron intake [31, 37, 46, 48]. In one study conducted by Zhang et al. [48], female subjects consuming high levels of iron from supplements ($\geq$ 25 mg/day) were at an increased risk for rectal cancer compared with women who consumed no supplements (RR = 2.54; 95% CI = 1.43-4.50), but no significant associations were observed among men. The results of these studies assessing supplemental iron intake and colorectal cancer or adenoma are too varied to draw any definitive conclusions; further research needs to be conducted in order to elucidate supplemental iron's impact on colorectal cancer risk.

Heme iron:

The results of the nine studies assessing heme iron intake are presented in **Table 3**. There were significant positive associations with cancer risk in three of the studies with risk estimates ranging from 1.13 to 2.18 [34, 39, 44] for high compared to low heme iron intakes. Five of the remaining six studies reported positive, but non-significant associations between heme iron intake and colorectal cancer [32, 38, 40, 41, 48]. The positive associations observed from the studies assessing heme iron intake strengthen the previous findings that red and processed meats increase colorectal cancer risk. The cohort studies assessing the effect of both dietary iron and heme iron

on colorectal cancer risk suggest that heme iron may play a greater role than iron from non-meat sources on cancer risk.

Mutations in HFE:

Two studies assessed effects of HFE gene status and iron intake on cancer and adenoma incidence. A study conducted by Chan et al. [38]found that women in the highest quartile of dietary iron intake with one or more mutations in the HFE gene (C282Y or H63D) had an OR of 1.14 (95% CI = 0.64-2.03) for adenoma incidence. Similar results were found among women with any HFE mutation with the highest intake of heme iron (OR Q_4 vs. Q_1 = 1.11; 95% CI = 0.64-1.93). The study by Shaheen et al. [45]found that subjects having any mutation in HFE had an OR of 1.40 (95% CI = 1.07-1.87) for colorectal cancer incidence. Among subjects with any mutation in HFE, those with the highest total iron intake had a significant positive association with colorectal cancer (OR Q_4 vs. Q_1 = 1.86; 95% CI = 1.09-3.18). These two studies indicate that HFE mutations have the potential to increase colorectal risk but further studies need to be done to elucidate gene-nutrient interactions, especially large studies that are powered to assess the risk associated with the homozygous C282Y mutation in conjunction with dietary intake.

Discussion

Over the past 15 years a total of 20 human population-based studies have evaluated the associations between dietary iron or heme iron and colorectal cancer or adenoma. Fifteen studies reviewed used cancer as an endpoint while the remaining five used a surrogate for cancer, adenoma or adenoma recurrence, as an endpoint (**Tables 1-3**). Studies that use adenoma or adenoma recurrence as an endpoint are better assessments of early stage colorectal carcinogenesis because adenomas precede cancer, while studies that use cancer as an endpoint typically measure

later stages of carcinogenesis. Previous radiologic evidence has found that 8% of adenomas undergo unrestricted cell division and became carcinomas over 10 years and 24% of adenomas become carcinomas at 20 years [51]. This information may help explain the mixed results between adenoma and cancer studies.

Dietary Iron:

Dietary iron is a measure of total heme and nonheme iron from all food sources. The primary sources of dietary iron are red meat, poultry, beans, leafy vegetables, fruit juice, and fortified breads and cereals. Although these studies are from different populations, many studies found the highest intakes of dietary iron to be 18-20 mg/day with men having slightly higher intakes than women. The results from studies assessing dietary iron intake and colorectal cancer risk were mixed. Two cohort studies that assessed dietary iron intake deserve special mention. In one study of non-institutionalized men and women between 24-74 years of age in the U.S. [47], dietary iron was found to be positively associated with colorectal cancer. This study is particularly important because it is nationally representative and was specifically designed to account for the heterogeneity of the U.S. population and provide a variety of nutritional and health measures. In contrast, a study conducted by Cross et al. [39] found an inverse association between dietary iron and colorectal cancer. This analysis is from the largest study of diet and health ever conducted – the NIH-AARP cohort (also known as the Diet & Health Study) and is comprised men and women age 50-71 years from six states and two major metropolitan areas. The authors of this study point out that many sources of dietary iron are generally healthy (eg. fruit juice, fortified cereals, bread) and highlighted the importance of distinguishing between heme and nonheme iron [39].

These discrepancies between studies are likely due to the complex interaction between foods within the digestive tract as well as the varied sources of iron within the diet. For example,

Tseng et al. [46] found that adenoma recurrence was inversely associated with dietary iron intake. Subjects from this study were part of a clinical trial to assess effects of antioxidant supplementation on adenoma prevention and thus these individuals may have altered their dietary habits during the study. The authors noted that there was very low meat intake in this population and iron intake was highly correlated with dietary fiber (Pearson's $r = 0.70$) which may explain the inverse association [46]. This study is important because it shows that dietary iron may be beneficial if it is derived from fruit and vegetable sources as opposed to meat. Future studies assessing dietary iron need to account for the source of iron to clarify the relation between dietary iron intake and colorectal cancer.

The preponderance of case-control studies found a positive association between dietary iron and colorectal cancer. These associations were strongest in studies that used cancer as an endpoint (**Table 2**). The strongest association between dietary iron and cancer incidence was observed in the rectal cancer study conducted by Deneo-Pellegrini et al. [49]; however, the small sample size and large 95% confidence interval (CI) make it necessary to interpret these findings cautiously. Increased rectal cancer risk was not observed with higher iron consumption in any other case-control study. All but one [50] of these case-control studies asked subjects to provide dietary intake information for the year or two years prior to diagnosis which may have led to recall bias [52]. Also, because colorectal cancer takes many years to develop, it is possible that earlier dietary habits are of greater etiological interest than the reference diet. The study by van Lee et al. [50] asked subjects to provide dietary information for the 10 years prior to filling out the food frequency questionnaire (FFQ) to decrease recall bias and assess a longer exposure period. As a result of the potential recall bias and smaller sample size in many of the case-control studies, more emphasis should be placed on the large cohort studies.

Heme Iron:

A recent consensus report conducted by the World Cancer Research Fund and American Institute for Cancer Research concluded that there was "convincing" evidence that red and processed meat increases the risk for colorectal cancer and individuals should limit their intake of red meat while avoiding processed meat [14]. Red meat has approximately ten-fold the amount of iron as white meat, which may explain the increased risk associated with red meat intake [53]. Heme iron is known to be more bio-available than non-heme iron, but its absorption is still tightly regulated. Unabsorbed heme and nonheme iron from red meat pass through the digestive tract and end up in the lumen of the colon and rectum.

The large cohort study by Cross et al. [39] is especially pertinent because they found a significant inverse association between dietary and total iron intake and colorectal cancer risk but a significant positive association between heme iron intake and colorectal cancer risk. These findings highlight the importance of identifying the source of iron. The number of null associations observed in this review as well as the small risk estimates may be due to the imprecise methods used to assess heme iron content as well as the relatively small range of intakes observed in many studies.

In several studies, the amount of heme iron from meat was estimated using a standard percentage (40%) of total iron from all meat sources [34, 44]. Other studies have applied a percentage according to the animal from which the meat was derived, for example, beef (65%), pork (39%), chicken/fish (26%), or liver (21%) [32, 42]. Studies using this second method have not found an overall association between heme iron intake and colorectal cancer risk. Recently, a heme iron database has been developed to more accurately quantify the amount of heme iron as well as other possible carcinogenic compounds found in unprocessed and processed meats [54]. Three studies [39-41]included in this review used information from the heme database, and two found a significant positive association between heme iron and risk for colorectal cancer [39, 40].

The mechanisms by which heme iron may promote colorectal cancer are poorly understood. Several proposed mechanisms of action are the N-nitrosylation of amines which forms N-nitroso compounds (NOC) and lipid peroxidation [16]. NOCs are known carcinogens which can cause mutations in DNA resulting in cancer if gone unchecked [55, 56]. Heme iron promotes endogenous NOC formation in humans [57]. NOCs can also be introduced exogenously by adding nitrates to meat during processing [39]. In addition, heme iron catalyzes the oxidation of polyunsaturated fats. Free radicals attack membrane lipids producing reactive compounds such as epoxides and aldehydes [16]. One major aldehyde product is malondialdehyde which has been indicated in several human diseases, including colorectal cancer [25, 58, 59]. Cooking of red meat at high temperatures also introduces heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) which are known gastrointestinal carcinogens in animal models [60, 61]. Combined, these data suggest that heme iron may promote carcinogenesis and intake should be limited, especially among individuals who are at increased risk for colorectal cancer.

Supplemental Iron:

Dietary supplement use is increasing in both the U.S. and Europe. Over 40% of U.S. adults used some form of dietary supplement in 1988-1994 and that number grew to over 50% in 2003-2006 [62]. Zhang et al. [48] noted an increase in iron supplement use in both men and women from 1990 to 2004 (men: 14% to 20%; women: 20% to 26%) which is consistent with the overall trend in the U.S. Supplement use in Europe is varied, but many countries document that over 30% of their population takes some form of dietary supplement [63]. Although large numbers of people are taking supplements, little is known about their impact on colorectal cancer risk, especially iron supplements. Four studies in this review noted that a large percentage of their study population used iron supplements [37, 41, 43, 48]. In the study by Kato et al. [43], 69% of the women in the study were taking supplemental iron which amounted to 38% of the total iron

intake. However, many authors of the studies included in this review noted that there were an insufficient number of individuals taking iron supplements to calculate the risk independently associated with iron supplement use. With dietary supplement use on the rise, it is important that future studies measure supplement intake to assess their effectiveness and determine if there are any risks associated with their use.

HFE and Colorectal Cancer:

A long-term determinant of iron status is variation in HFE. The two studies that assessed HFE status in conjunction with dietary iron found conflicting results. These results may be explained by the different outcome measures chosen as well as the limited ability to stratify subjects by HFE status. For example, Chan et al. [38]utilized adenoma incidence as a surrogate for cancer and found no interaction with genotype whereas Shaheen et al. [45] assessed cancer incidence as the endpoint and found a significant effect only among those with any HFE mutation. Both studies lacked the power to stratify subjects as either homozygous or heterozygous for the C282Y HFE mutation which is the primary cause of HH. Instead, they were limited to examining subjects having any or no mutations of the HFE gene (C282Y or H63D). Both mutations cause an increase in iron absorption; however, homozygotes of the recessive C282Y allele accumulate iron to a much greater extent than heterozygotes.

A recent report by Allen and colleagues [10] has shown that 60-80% of individuals homozygous for the C282Y mutation will develop abnormal iron indices, but only 28% of men and 1% of women will develop significant iron overload as a result. The most common explanation for this discrepancy between sexes is the loss of iron through menstruation. Six of the twenty studies in this review were female-only studies [34, 38, 41-44]. Only one of the six studies was comprised solely of post-menopausal women [44] while the rest included both pre- and post-menopausal women. Thus, iron loss from menstruation and pregnancy may help explain the

conflicting results seen in these studies and may also help explain some of the differences in overall incidence rates between men and women. These findings indicate that the risk for colorectal cancer associated with HFE mutation carrier status and iron intake are likely higher for men than women, but individuals with HFE mutations should limit heme iron intake and monitor their iron levels closely.

Strengths and Weaknesses:

Several strengths and weaknesses of the current available studies must be recognized. Self-reported measures of dietary intake may be biased, particularly in case-control studies, which must be taken into consideration when interpreting results. Over- and under-reporting are common problems that occur when participants feel pressured to report consuming more healthy foods (eg. fruits and vegetables) and less unhealthy foods (eg. fried food, foods high in fat, etc.). In addition, due to the limited number of items that may be included in a FFQ, participants have been shown to underestimate actual energy intake [52, 64]. Using this rationale, subjects are likely to under-report meat intake and over-report intake of foods such as salads and fruits which have an inverse association with colorectal cancer risk. To attempt to overcome some of these issues, an additional meat questionnaire was included in three of the studies [39-41]which was aimed at reducing reporting errors and improved calculation of heme iron content of meats using the NCI heme database.

The strengths of this review include the use of both prospective and cross-sectional studies from a variety of different study populations, comparison of different methods for heme iron content across studies, and the use of both cancer and adenoma studies. This is also the first review to date to assess the risk of iron from supplements, but no conclusions could be made due to inconsistent reporting of intake and risk between studies.

Future Directions:

Following our review of the current literature, there are several avenues that require investigation. Further studies need to be conducted to assess the role of iron regulatory genes in conjunction with dietary iron to help elucidate any gene-environmental interactions in iron absorption and cancer formation and progression. A better understanding of the differences between males and females is needed as well as the effect of iron on specific regions of the colon and rectum. Importantly, few studies have investigated the associations between dietary iron intake and colorectal cancer among minorities as well. Studies specifically assessing supplemental iron intake would also add to the current literature where the results are mixed. Finally, a validated heme iron calculation that can be used consistently between studies is also needed to properly ascertain the role of heme iron in colorectal carcinogenesis.

Conclusion

While iron is an essential mineral for proper cellular function, it may be detrimental if consumed in excess. Dietary iron has been widely studied in relation to colorectal cancer with mixed results. Many of the mixed results that have resulted from these studies are a result of diverse study populations or lack of power. Results from some of the larger cohort studies suggest that the primary cause of increased risk is heme iron and red meat intake rather than total dietary iron.

Table 2.1: Cohort and nested case-control studies of dietary iron

Year (Author) [ref]	Cases	Controls/ Cohort Size	Result[†]	OR/HR/RR[‡] (*P*-trend)	95% CI	Outcome	Measured Supplements
1996 (Wurzelman)[47]	136	8,876	+	RR=3.35 (*P*<0.001) [Colon]	1.74-6.46	Cancer	N
	65	3,631	+	RR=3.73 (*P*=0.001) [Men]	1.62-8.59		
	72	5,245	+	RR=3.01 (*P*=0.08) [Women]	1.05-8.64		
2010 (Cross)[39]	2,719	300,948	-	HR=0.75 (*P*<0.001)	0.65-0.87	Cancer	Y
2006 (Balder)[32]	869	58,279	NS	RR=1.34 (*P*=0.12) [Men]	0.93-1.93	Cancer	N
	666	62,573	NS	RR=1.08 (*P*=0.90) [Women]	0.72-1.62		
2006 (Cross)[33]	130	260	NS	OR=0.4 (*P*=0.06) [Men]	0.1-1.1	Cancer	Y
2007 (Kabat)[42]	617	49,654	NS	HR=1.07 (*P*=0.94) [Women]	0.75-1.53	Cancer	N
1999 (Kato)[43]	105	523	NS	OR=1.17 (*P*=0.44) [Total Iron; Women]	0.6-2.3	Cancer	Y
2011 (Zhang)[48]	2,114	115,016	NS	RR=1.09 (*P*=0.37)	0.93-1.30	Cancer	Y
	1,035	42,373	NS	RR=1.08 (*P*=0.61) [Men]	0.84-1.38		
	1,079	69,345	NS	RR=1.11 (*P*=0.44) [Women]	0.88-1.41		
1997 (Tseng)[46]	247	419	-	OR=0.37 (*P*=0.005)	0.19-0.73	Adenoma	Y
2005 (Chan)[38]	527	527	NS	RR=1.04 (*P*=0.94) [Women]	0.68-1.57	Adenoma	Y
2011 (Cross)[40]	356	396	NS	OR=0.98 (*P*=0.98)	0.62-1.55	Adenoma	Y

[†] Highest intake quantile vs. lowest; NS = Not significant (95% CI includes 1.0); + means significant positive association; - means significant inverse association

[‡] OR = Odds Ratio; HR = Hazard Ratio; RR = Relative risk

Table 2.2: Case-control dietary iron studies

Year (Author) [ref]	Cases	Controls	Result[†]	Odds Ratios (*P*-trend)	95% CI	Outcome	Measured Supplements
1999 (Deneo-Pellegrini)[49]	216	433	+	OR=3.18 (*P*<0.001) [Rectum]	1.92-5.29	Cancer	N
	141	284	+	OR=4.01 (*P*<0.001) [Men]	2.09-7.69		
	75	149	+	OR=2.61 (*P*=0.02) [Women]	1.12-6.10		
2004 (Levi)[35]	223	491	+	OR=2.43 (*P*<0.05)	1.2-5.1	Cancer	N
2004 (Senesse)[36]	171	309	+	OR=2.3 (*P*=0.02)	1.1-4.7	Cancer	N
2003 (Shaheen)[45]	475	833	NS	OR=1.05 [Total Iron]	0.75-1.49	Cancer	Y
2011 (van Lee)[50]	854	958	NS	OR=0.86 (*P*=0.17)	0.64-1.15	Cancer	N
2001 (Almendingen)[31]	87	35[*]	NS	OR=0.2 (*P*=0.08)	0.1-2.3	Adenoma	Y
1996 (Bird)[37]	467	498	NS	OR=1.4	0.9-2.0	Adenoma	Y
	300	331	NS	OR=1.6 [Men]	0.9-2.8		
	167	167	NS	OR=1.0 [Women]	0.5-2.0		
2008 (Ferrucci)[41]	158	649	NS	OR=1.11 (*P*=0.77) [Women]	0.57-2.16	Adenoma	Y

[†] Highest intake quantile vs. lowest; NS = Not significant (95% CI includes 1.0); + means significant positive association; - means significant inverse association

* ORs for 'Healthy' controls. 'Hospital' controls (n=35): OR=1.3 (0.1-13.8; *P*=0.8)

Table 2.3: Heme iron studies

Year (Author)[ref]	Cases	Controls/ Cohort Size	Result[†]	OR/HR/RR[‡] (*P*-trend)	95% CI	Outcome	Study Design
2010 (Cross)[39]	2,719	300,948	+	HR=1.13 (*P*=0.022)	0.99-1.29	Cancer	Cohort
2005 (Larsson)[34]	547	61,433	+	RR=1.31 (*P*=0.03) [Women]	0.98-1.75	Cancer	Cohort
2004 (Lee)[44]	741	41,836	+	RR=2.18 (*P*=0.01) [Women; Prox. Colon]	1.24-3.86	Cancer	Cohort
2006 (Balder)[32]	869	58,279	NS	RR=1.16 (*P*=0.27) [Men]	0.87-1.55	Cancer	Cohort
	666	62,573	NS	RR=1.22 (*P*=0.22) [Women]	0.89-1.68		
2007 (Kabat)[42]	617	49,654	NS	HR=0.99 (*P*=0.99) [Women]	0.70-1.40	Cancer	Cohort
2011 (Zhang)[48]	2,114	115,016	NS	RR=1.10 (P=0.51)	0.93-1.30	Cancer	Cohort
	1,035	42,373	NS	RR=0.98 (*P*=0.80) [Men]	0.77-1.26		
	1,079	69,345	NS	RR=1.21 (*P*=0.10) [Women]	0.96-1.52		
2005 (Chan)[38]	527	527	NS	RR=1.13 (*P*=0.23)	0.74-1.72	Adenoma	Nested C-C
2011 (Cross)[40]	356	396	NS	OR=1.46 (*P*=0.08)	0.94-2.29	Adenoma	Nested C-C
2008 (Ferrucci)[41]	158	649	NS	OR=1.50 (*P*=0.32) [Women]	0.83-2.73	Adenoma	Case-Control

[†] Highest intake quantile vs. lowest; NS = Not significant (95% CI includes 1.0); + means significant positive association; - means significant inverse association

[‡] OR = Odds Ratio; HR = Hazard Ratio; RR = Relative risk

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Chapter 3

Association of dietary and supplemental iron and colorectal cancer in a population-based study[1]

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Abstract

Objectives: We evaluated the role of dietary iron, heme iron, and supplemental iron on colorectal cancer (CRC) risk in a population-based case-control study in Pennsylvania, including 1005 incident cases and 1062 controls. **Methods**: Diet was assessed through a modified food frequency questionnaire that included supplement use and a meat-specific module. Cases reported intakes for the year before diagnosis while controls reported intakes for the year before interview. Heme iron intake was calculated using a new heme database developed by the US National Cancer Institute (NCI). Odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression. **Results**: After multivariate adjustment, there were no significant associations between heme iron or total iron intake and CRC incidence. Dietary iron intake was inversely associated with CRC among women (OR Q_5 vs. Q_1 = 0.45; 95% CI = 0.22-0.92), but not men. Supplemental iron intake of >18 mg/d vs. none was positively associated with CRC incidence (OR = 2.31; 95% CI = 1.48-3.59; *P*-trend <0.001), an effect that was observed in both men (OR = 2.56; 95% CI = 1.30-5.05) and women (OR = 2.46; 95% CI = 1.34-4.52). **Conclusions**: These findings suggest that consumption of >18 mg/d of supplemental iron may increase risk for CRC.

Introduction

Colorectal cancer (cancer of the colon or rectum; CRC) is the second leading cause of cancer mortality in the United States [1]. Multiple factors contribute to CRC risk including environmental exposures such as excess weight, lack of physical activity, and specific dietary patterns and food and nutrient intake [2]. A recent comprehensive review summarizing the current available epidemiological studies of red meat intake and CRC risk reported that there was "convincing" evidence that red meat was a risk factor for CRC [2]. Red meat has high iron content, especially heme iron. This same report also stated that there was "limited-suggestive" evidence for foods containing iron increasing CRC risk [2].

Iron is an essential mineral that has vital functions throughout the body. It is utilized as a cofactor for a number of enzymes, is involved in oxygen transport, plays a key role in ATP generation, and supports growth [3]. Iron in the diet comes in two different forms, heme and nonheme iron, from multiple sources. Nonheme iron is primarily found in plants such as leafy green vegetables and nuts and is also found in meat. Nonheme iron is the form used for flour enrichment. Heme iron, in the center of a porphyrin ring, is derived from red meat and, to a lesser extent, poultry and fish. Dietary supplements containing iron salts are also major contributors to iron intake among users.

Animal studies have shown that both forms of dietary iron are capable of catalyzing free radical formation and DNA damage [4, 5]. Heme iron, in particular, has been associated with increased colonic epithelial proliferation [6] and the promotion of CRC in rats [7, 8]. Previous population studies have found mixed results for the association between dietary iron and CRC risk. One cohort [9] and several case-control [10-13] studies have found positive associations between dietary iron and CRC while other cohort studies have found inverse [14] or null [15-18]

associations. Heme iron from the diet has traditionally been measured by applying a standard percentage (40% of total iron) to all meats [19, 20] or a percentage based on type of meat [16, 17]. Heme iron can also be converted to nonheme iron depending on the type and duration of cooking method used in preparation [21, 22]. Recently, the US National Cancer Institute (NCI) developed a database that calculates heme iron content based on the type of meat as well as cooking methods used to prepare the meat [23]. This database was used in the present study. Studies using this method have found mixed results for the association between heme iron and colorectal adenoma and cancer incidence [14, 24, 25].

In this study, we investigated the associations between iron (total, dietary, supplemental, and heme) and incident CRC in a population-based case-control study in central and northeast Pennsylvania. This study population is important as it is at particularly high risk for colorectal cancer compared to the U.S. as a whole (54.5 per 100,000 in Pennsylvania vs. 48.8 per 100,000 in the U.S. at the start of this study in 2007).

Subjects and Methods

Sample population

This study included 1196 incident colorectal cancer cases and 1187 healthy controls of both sexes $\geq$18 years of age recruited between June 2007 and May 2011. Cases were identified from the Pennsylvania State Cancer Registry within 15 months of diagnosis. Controls residing in the same region that had no history of CRC were identified by random digit dialing, as described by Waksberg [26]. To be eligible, incident CRC participants had to have a first time, histologically confirmed diagnosis of colon, rectal, or CRC (ICD-10 codes C18.0-18.9; C19.9; C20.9) and be English-speaking. A letter introducing the study was sent to potential participants,

followed by a telephone call from a study coordinator to further explain the study, answer questions, and obtain preliminary informed consent. After oral consent was obtained from a potential case or control, a personal interview was scheduled and a self-administered food frequency questionnaire (FFQ) was mailed with instructions for completion prior to the interview. Written consent and data regarding sociodemographic factors, medical history, alcohol use, lifetime tobacco exposure, physical activity, height, weight, medication use, and other lifestyle-related factors were collected in person by trained interviewers. The returned FFQ was reviewed by study staff for completeness during the interview.

For the present analysis, we excluded participants who were <35 y (n = 14), had missing body mass index (BMI, kg/m^2; n = 29), age (n = 21), energy intake (kcal; n = 218), or tumor site data (n = 6) and those who reported implausible energy intakes [<2093 kJ or >20930 kJ/day; <500 kcal or >5000 kcal/day] (n = 72). Some participants met more than one exclusion criteria (n = 15). After these exclusions, 1005 cases (501 men, 504 women) and 1062 controls (531 men, 531 women) remained for analysis. There were 707 incident colon cancers (cecum through the sigmoid colon) and 298 cancers of the rectum (rectosigmoid junction and rectum). The institutional review boards at the Pennsylvania Department of Health, Northeast Regional Cancer Institute (Scranton, PA), the Penn State College of Medicine (Hershey, PA), and the Lehigh Valley Health Systems (Allentown, PA) reviewed and approved all study procedures.

Assessment of dietary intake and supplement use

Participants completed a modified version of the Diet History Questionnaire (DHQ), a validated FFQ developed by the NCI [27, 28]. The reference period was the past year for controls and the year prior to diagnosis for cases. Modifications to the DHQ were described previously [29]. Briefly, a meat-cooking module was added to capture meat consumption patterns of

Pennsylvania residents as well as a module on supplement use. The meat-cooking module assessed cooking methods and doneness levels which were used to quantify heme iron content of meats using the NCI heme database as described elsewhere [23]. Energy and nutrient intake were calculated with Diet*Calc (version 1.4.3) nutrient analysis software.

Dietary and nutrient intake values were grouped into quintiles based on the distribution among the controls and were entered into models as indicator variables defined by the second through fifth quintiles of intake, with the lowest quintile as the referent group. To conduct a linear trend test across levels of nutrients, variables were created using intake levels based on the median values for each quintile. Dietary levels of nonheme iron and heme iron were adjusted for total energy using the nutrient density method [30]. Total iron intake variables were created by combining residually adjusted dietary intake with supplemental intake.

Supplement variables for iron were characterized in 3 ways for analysis: 1) added as amount of supplement to energy-adjusted dietary data to create total intake variables; 2) used as indicator variables for any vs. no supplement use; and 3) added as four indicator variables of intake (none, below, at, and above the median intake among supplement users), with no use as a referent.

Statistical Analyses

All statistical analyses were performed using SAS statistical software (version 9; SAS Institute, Cary, NC, USA). Demographic characteristics and dietary data from cases and controls were compared using the χ^2-test for categorical variables and Wilcoxon rank sum test for continuous variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed using unconditional logistic regression, with the first quintile as the referent group. Associations stratified by anatomical subsite (colorectum, colon, and rectum) were investigated. Multivariate

models were adjusted for potential confounding variables that were selected *a priori*: age (continuous), sex (male, female), total energy intake (kcal/d, continuous), BMI (kg/m^2, continuous), smoking status (never, current, or former), family history of CRC (yes, no; first degree relative), alcohol (g/d, continuous), physical activity (yes, no; $\geq$ 1 hr/week of vigorous activity), education (no college degree, college degree or above), dietary calcium (mg/1000 kcal, continuous), supplemental calcium (mg/d, continuous), dietary folate (µg/1000 kcal, continuous), supplemental folate (µg/d, continuous), vegetable and fruit intake (svg/1000 kcal, continuous), and regular nonsteroidal anti-inflammatory drug (NSAID) use (yes, no; regular use defined as at least 3 times a week for 1 year prior to diagnosis for cases and 1 year prior to interview for controls). There was no appreciable change (10%) of risk estimates with the addition of vitamin D, vitamin C, or vitamin E from diet and/or supplements to the multivariate model so these variables were not included in the final models. Fiber was highly correlated with dietary iron intake (Spearman's $r = 0.62$) and not included in the models.

Effect modification by age, sex, BMI, alcohol, and smoking was assessed by creating cross-product terms with continuous iron intake variables and including the cross-product term in multivariate models. When significant interactions were found, analyses were repeated by stratifying the effect modifier at the median of controls. All analyses were two-sided and *P*-values were considered significant if <0.05.

Results

Characteristics of cases and controls are presented in Table 1. Controls were more educated, more physically active, and a greater percentage used NSAIDs regularly compared to cases. Cases tended to be older, have a higher BMI, and were more likely to have a first degree relative with CRC. There were no significant differences in sex, race, total energy intake, or

intakes of dietary and total iron between cases and controls in univariate analyses; although the controls were less likely to take iron supplements and were more likely to consume vegetables and alcohol.

There were no significant multivariate-adjusted associations between total iron or heme iron and CRC (Table 2). Dietary iron did not reach statistical significance among all participants (OR Q_5 vs. Q_1= 0.70; 95% CI = 0.44-1.11; *P*-trend = 0.11). When the fully-adjusted model was stratified by sex, there was a significant inverse association between dietary iron and CRC observed among women (OR Q_5 vs. Q_1= 0.45; 95% CI = 0.22-0.92; *P*-trend = 0.02), but not men. There was no evidence of effect modification by BMI, smoking, or alcohol intake for the association between dietary iron, total iron, or heme iron intake and CRC (data not shown). Effect modification by age was observed for the association between dietary iron and CRC ($p = 0.02$); however, after stratifying subjects above and below the control's median (62y), we found no significant associations within strata (>62y OR Q_5 vs. Q_1 = 0.77; 95% CI = 0.43-1.39; $\leq$62y OR Q_5 vs. Q_1 = 0.59; 95% CI = 0.27-1.28).

Because 34% of our study population consumed 18 mg/d of supplemental iron, supplemental iron intake was modeled with four levels: none, <, =, > 18 mg/d. Any supplement use, compared to no use, was not significantly associated with CRC incidence in our study population (OR user vs. non-user = 1.13; 95% CI = 0.86-1.48; data not shown). Supplemental iron intake above the median (18 mg/d) was positively associated with CRC (OR >18mg/d vs. none = 2.31; 95% CI = 1.48-3.59; *P*-trend <0.001) and was significant in both men (OR >18mg/d vs. none = 2.56; 95% CI = 1.30-5.05; *P*-trend = 0.02) and women (OR >18mg/d vs. none = 2.46; 95% CI = 1.34-4.52; *P*-trend = 0.01). There were no significant differences in reported supplement use by sex (p = 0.392; data not shown).

When all colorectal cases were divided into colon and rectum, no significant associations were observed for dietary iron, total iron, or heme iron intakes (Table 3). Supplemental iron

intake above 18 mg/d was positively associated with both colon (OR >18mg/d vs. none = 2.40; 95% CI = 1.48-3.90; *P*-trend <0.01) and rectal (OR >18mg/d vs. none = 2.19; 95% CI = 1.20-4.01; *P*-trend = 0.02) cancers.

Discussion

In a review of 33 case-control studies assessing dietary iron and iron stores, Nelson [31] concluded that high intake of dietary iron was associated with an increased risk for CRC. In our study population, we found a near-significant inverse association for both men and women between dietary iron and CRC incidence (OR Q_5 vs. Q_1 = 0.70; 95% CI = 0.44-1.11; *P*-trend = 0.11) and a significant inverse association between dietary iron and CRC among women. The inverse association observed among women and not men is likely due to the increased iron needs due to iron loss from menstruation. Iron loss would lead to increased iron absorption and consequently less iron in the lumen of the colorectum where it can form free radicals and damage DNA [4, 5].

Our findings for dietary iron are similar to those found in a large prospective cohort study conducted by Cross et al. [14] which found a significant inverse association for dietary iron intake and CRC (HR Q_5 vs. Q_1 = 0.75; 95% CI = 0.66-0.86; *P*-trend <0.001) [14]. It was speculated that this was because nonheme iron was mainly from fortified cereals, fruit juice, and bread which are generally healthy foods that contain other beneficial components such as fiber and antioxidants. Similarly, the biggest contributors to overall dietary iron in our study population were fortified breakfast cereal, meal replacement beverages, oatmeal, and breads and rolls (data not shown), which highlights the importance of identifying the source of iron for epidemiologic studies.

Several studies have assessed heme iron intake in relation to CRC [14, 16-20] and adenoma [24, 25, 32] outcomes. The majority of these studies found a positive association

between high heme iron intake and CRC or adenoma incidence, but only three reached statistical significance [14, 19, 20]. The mixed results observed may be due to the different methods used to calculate heme content from the diet. No significant associations were observed for total iron or heme iron intake in relation to colon or rectum cancer in the present study. The results of our study are similar to those by Ferrucci et al. [25] which calculated heme iron content using both the heme iron database as well as applying the standard 40% of all iron from meat. Our null findings do not support the results of a recent meta-analysis of five prospective cohort studies conducted in the U.S., Sweden, Netherlands, and Canada that included 566,607 individuals with 4,734 cases assessing heme iron and CRC; this meta-analysis found a significant positive association between the highest category of heme iron intake versus the lowest (RR = 1.18; 95% CI = 1.06-1.32) [33].

Most interestingly, a positive association was observed between high levels of supplemental iron intake (>18 mg/d) and risk for CRC in the present study. Results from our study were similar to those from the Nurses' Health Study and Health Professionals Follow-up Study; Zhang et al. [18] found a significant positive association among women taking >25 mg/d of supplemental iron and rectal cancer (RR = 2.54; 95% CI = 1.43-4.50). Increased risk associated with high levels of iron supplement intake as well as high total iron intake may be due to the reduced responsiveness of interferon-gamma (IFN-γ) resulting in decreased immune function and the inability to ward off invading pathogens [34]. However, in the National Institutes of Health (NIH)-AARP Diet and Health Study, a large multi-site prospective cohort of over 500,000 men and women, Cross et al. [14] examined iron intake and colorectal cancer but did not investigate supplemental iron separately from dietary iron. There were no appreciable differences in risk estimates between dietary and total iron intakes, suggesting that supplemental iron had little effect on CRC risk. Iron intake levels from this study as well as those from the nationally representative National Health and Nutrition Examination Survey (NHANES, 2003-2006) [35]

were similar to ours. Further studies need to be done to evaluate the impact of supplemental and total iron intake on CRC risk.

The present study was not without limitations. Assessing the diet for the year prior to diagnosis for cases may not be the most relevant exposure period for CRC because of the long development time for CRC. Also, because this was a case-control study, there is a possibility of recall bias. The strengths of the study include our large sample size, the use of population-based controls to better represent our study population, and a detailed meat-specific module that allowed us to utilize the new NCI heme database to more accurately measure exposure. Our study also had a large number of supplement users which allowed for a better assessment of the association between supplemental iron and CRC risk.

Overall, our findings do not support previously observed positive associations between dietary and heme iron and CRC incidence. The inverse association observed for dietary iron in women in our study highlights the importance of identifying the source of iron when examining dietary risk factors for CRC. As high levels of supplemental iron intake were found to be positively associated with CRC, future studies are needed to confirm this finding.

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Table 3.1: Selected characteristics of study participants

Characteristic	Overall (n = 2067)	Case (n = 1005)	Control (n = 1062)	p-value
Age, y	64.2 ± 11.6	66.8 ± 11.8	61.7 ± 10.9	<0.001
BMI, kg/m^2	29.1 ± 6.1	29.7 ± 6.4	28.6 ± 5.8	<0.001
Sex, % female	50.1	50.2	50.0	0.95
Race, % Caucasian	97.3	96.6	97.8	0.10
Education, % college degree or above	29.2	23.9	34.2	<0.001
Family history of CRC, % yes	14.8	17.5	12.2	<0.001
Use NSAIDs, % regular use	51.4	47.5	55.1	<0.001
Physically active, %	32.5	26.9	37.8	<0.001
Smoking status, %				0.04
Never	47	46.8	47.3	
Former	40.7	42.7	38.9	
Current	12.2	10.6	13.8	
Dietary				
Total energy, kcal/d	1822.6 ± 817	1860.4 ± 868	1786.1 ± 762	0.24
Total iron[†], mg/d	23.5 ± 11	23.4 ± 12	23.5 ± 11	0.71
Dietary iron, mg/1000 kcal	8.27 ± 2.5	8.17 ± 2.4	8.36 ± 2.6	0.16
Iron supplements, % any	51.4	49.6	53.1	0.12
Iron supplements[‡], mg/d	17.4 ± 8	18.3 ± 7.9	16.6 ± 7.5	<0.001
Heme iron, µg/1000 kcal	108.3 ± 88	105.1 ± 88	111.4 ± 88	0.05
Dietary calcium, mg/1000 kcal	433.1 ± 150	423.6 ± 142	442.2 ± 157	0.01
Dietary folate equivalents, µg/1000 kcal	263.7 ± 85	261.1 ± 79	266.3 ± 90	0.29
Fruit intake, svg/1000 kcal	1.40 ± 1.1	1.39 ± 1.1	1.41 ± 1.1	0.52
Vegetable intake, svg/1000 kcal	2.03 ± 1.2	1.88 ± 1.1	2.17 ± 1.2	<0.001
Alcohol, g/d	9.51 ± 28	9.10 ± 30	9.90 ± 26	<0.001

[†]Dietary iron residually energy adjusted plus iron from supplements

[‡] Among supplement users

Table 3.2: Age-adjusted and multivariate-adjusted ORs for colorectal cancer risk within quintiles of dietary and supplemental iron intake.

Variable	Q1	Q2	Q3	Q4	Q5	*P*-trend
Total Iron[†] (mg/d)						
Quintile median (range)	11.4 (< 13.0)	14.5 (13.0-16.9)	20.5 (16.9-28.5)	30.9 (28.5-33.1)	37.1 (≥ 33.1)	
Cases/controls	228/212	201/213	176/212	182/213	224/212	
OR[1] (95% CI)	1.0 (ref)	0.80 (0.61-1.06)	0.71 (0.54-0.94)	0.72 (0.55-0.96)	0.85 (0.64-1.11)	0.28
OR[2] (95% CI)	1.0 (ref)	0.93 (0.69-1.26)	0.80 (0.57-1.13)	1.04 (0.70-1.55)	1.22 (0.80-1.85)	0.16
OR[2] (95% CI) - Men (n = 1032)	1.0 (ref)	1.18 (0.77-1.83)	1.00 (0.63-1.59)	1.23 (0.70-2.16)	1.27 (0.70-2.31)	0.45
OR[2] (95% CI) - Women (n = 1035)	1.0 (ref)	0.68 (0.44-1.06)	0.61 (0.36-1.01)	0.87 (0.49-1.55)	1.16 (0.63-2.13)	0.18
Dietary Iron (mg/1000 kcal)						
Quintile median (range)	5.7 (< 6.4)	7.0 (6.4-7.5)	7.9 (7.5-8.5)	9.2 (8.5-10.0)	11.5 (≥ 10.0)	
Cases/controls	202/212	224/213	209/212	197/213	173/212	
OR[1] (95% CI)	1.0 (ref)	1.02 (0.77-1.35)	0.92 (0.70-1.22)	0.83 (0.62-1.10)	0.67 (0.50-0.89)	<0.01
OR[2] (95% CI)	1.0 (ref)	1.02 (0.75-1.38)	1.02 (0.74-1.42)	0.93 (0.65-1.33)	0.70 (0.44-1.11)	0.11
OR[2] (95% CI) - Men (n = 1032)	1.0 (ref)	1.27 (0.84-1.94)	1.30 (0.83-2.03)	1.18 (0.73-1.93)	0.97 (0.53-1.79)	0.62
OR[2] (95% CI) - Women (n = 1035)	1.0 (ref)	0.81 (0.51-1.27)	0.78 (0.48-1.28)	0.68 (0.39-1.18)	0.45 (0.22-0.92)	0.02
Heme Iron[‡] (µg/1000 kcal)						
Quintile median (range)	24.8 (<42.6)	56.3 (42.6-71.9)	88.6 (71.9-109.0)	132.8 (109.0-169.6)	225.3 (≥ 169.6)	
Cases/controls	243/209	188/210	196/210	208/210	170/209	
OR[1] (95% CI)	1.0 (ref)	0.83 (0.63-1.10)	0.95 (0.72-1.26)	1.06 (0.80-1.40)	0.88 (0.66-1.17)	0.78
OR[2] (95% CI)	1.0 (ref)	0.88 (0.65-1.17)	0.96 (0.71-1.29)	1.05 (0.78-1.41)	0.89 (0.65-1.22)	0.76
OR[2] (95% CI) - Men (n = 1019)	1.0 (ref)	0.99 (0.64-1.54)	1.11 (0.72-1.71)	1.07 (0.70-1.64)	0.92 (0.60-1.41)	0.64
OR[2] (95% CI) - Women (n = 1027)	1.0 (ref)	0.80 (0.54-1.20)	0.86 (0.57-1.29)	1.08 (0.71-1.66)	0.88 (0.54-1.44)	0.96
Supplemental Iron[*] (mg/d)						
Median (range)	0 (None)	5.14 (< 18)	18 (18)	34.3 (>18)		
Cases/controls	506/498	86/135	317/385	96/44		
OR[1] (95% CI)	1.0 (ref)	0.75 (0.55-1.02)	0.78 (0.64-0.95)	2.07 (1.40-3.05)		0.30
OR[2] (95% CI)	1.0 (ref)	0.88 (0.63-1.23)	1.04 (0.73-1.48)	2.31 (1.48-3.59)		<0.001
OR[2] (95% CI) - Men (n = 1032)	1.0 (ref)	0.80 (0.49-1.33)	1.00 (0.60-1.68)	2.56 (1.30-5.05)		0.02
OR[2] (95% CI) - Women (n = 1035)	1.0 (ref)	0.94 (0.60-1.49)	1.00 (0.60-1.65)	2.46 (1.34-4.52)		0.01

Table 3.2 legend: [1] OR adjusted for age only. [2] Multivariate adusted OR: model includes age, sex, BMI, total energy intake, family history of CRC, physical activity, education, NSAID use, smoking, alcohol, calcium, folate, vegetable intake, and fruit intake

†Dietary iron residually energy adjusted plus iron from supplements, ‡Heme iron information missing for 7 cases and 14 controls

*Iron from supplements broken into four levels

Table 3.3: Multivariate ORs and 95% CI for colon and rectal cancer risk within quintiles of dietary and supplemental iron intake.

Variable	Q1	Q2	Q3	Q4	Q5	*P*-trend
Total Iron[†] (mg/d)						
Colon cancer, cases/controls	150/212	141/213	125/212	134/213	157/212	
OR[1] (95% CI) for colon cancer	1.0 (ref)	0.91 (0.65-1.28)	0.85 (0.58-1.24)	1.13 (0.72-1.76)	1.29 (0.80-2.07)	0.12
Rectal cancer, cases/controls	76/212	59/213	49/212	48/213	66/212	
OR[1] (95% CI) for rectal cancer	1.0 (ref)	0.92 (0.60-1.42)	0.68 (0.41-1.11)	0.83 (0.46-1.49)	1.05 (0.57-1.95)	0.69
Dietary Iron (mg/1000 kcal)						
Colon cancer, cases/controls	135/212	149/213	149/212	147/213	127/212	
OR[1] (95% CI) for colon cancer	1.0 (ref)	0.95 (0.67-1.34)	1.03 (0.72-1.48)	0.95 (0.64-1.41)	0.70 (0.42-1.17)	0.20
Rectal cancer, cases/controls	67/212	75/213	60/212	50/213	46/212	
OR[1] (95% CI) for rectal cancer	1.0 (ref)	1.09 (0.71-1.66)	0.97 (0.61-1.55)	0.87 (0.52-1.47)	0.68 (0.35-1.33)	0.19
Heme Iron[‡] (µg/1000 kcal)						
Colon cancer, cases/controls	186/209	131/210	125/210	148/210	117/209	
OR[1] (95% CI) for colon cancer	1.0 (ref)	0.80 (0.58-1.10)	0.81 (0.58-1.13)	1.07 (0.77-1.48)	0.85 (0.60-1.20)	0.85
Rectal cancer, cases/controls	57/209	57/210	71/210	60/210	53/209	
OR[1] (95% CI) for rectal cancer	1.0 (ref)	1.11 (0.71-1.73)	1.43 (0.93-2.20)	1.10 90.70-1.73)	1.01 (0.63-1.61)	0.72
Supplemental Iron[*] (mg/d)						
Colon cancer, cases/controls	352/498	61/135	226/385	68/44		
OR[1] (95% CI) for colon cancer	1.0 (ref)	0.91 (0.63-1.32)	1.05 (0.71-1.57)	2.40 (1.48-3.90)		<0.01
Rectal cancer, cases/controls	154/498	25/135	91/385	28/44		
OR[1] (95% CI) for rectal cancer	1.0 (ref)	0.77 (0.46-1.28)	1.01 (0.60-1.69)	2.19 (1.20-4.01)		0.02

[1]Multivariate adusted ORs: model includes age, sex, BMI, total energy intake, family history of CRC, physical activity, education, NSAID use, smoking,alcohol, calcium, folate, vegetable intake, and fruit intake

[†]Dietary iron residually energy adjusted plus iron from supplements, [‡]Heme iron information missing for 4 colon cancer cases and 3 rectal cancer cases

*Iron from supplements broken into four levels

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Chapter 4

Association of Dietary and Supplemental Vitamin D and Calcium with Colorectal Cancer Risk[1]

[1]Ashmore JH, Lesko SM, Miller PE, Muscat JE, Zhu J, Liao J, Harper G, Lazarus P, Hartman TJ.

Abstract

Epidemiological evidence indicates that vitamin D and calcium may decrease the risk of colorectal cancer. In the present study, the associations between intakes of vitamin D and calcium and the risk of incident colorectal cancer was studied in a large case-control study conducted in central and northeastern Pennsylvania including 1011 cases with histologically confirmed colorectal cancer and 1062 population-based controls. Diet was assessed through a modified food frequency questionnaire that included supplement use. Cases reported intakes for the year before diagnosis while controls reported intakes for the year before interview. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression. After adjusting for potential confounding factors including age, sex, total energy intake, body mass index (BMI), smoking, family history of colorectal cancer, alcohol, and non-steroidal anti-inflammatory drug (NSAID) use, the odds ratio (OR) in the highest quintile of total vitamin D intake (Q5) compared to the lowest (Q1) was 0.67 (95% confidence interval [CI] =0.49-0.91; *P*-trend < 0.01). Higher levels of total calcium intake were inversely associated with colorectal cancer incidence (OR Q5 v. Q1= 0.48; 95% CI = 0.35-0.66; *P*-trend <0.01). In addition, high supplemental intakes of vitamin D (>10 μg/d) and calcium (>500 mg/d) were associated with reduced risk (OR High v. None = 0.68; 95% CI = 0.53-0.89, *P*-trend = 0.05; OR High v. None = 0.61; 95% CI = 0.46-0.81, *P*-trend < 0.01, respectively). Overall, our results indicate reduced risk of colorectal cancer with higher total and supplemental intakes of vitamin D and calcium.

Introduction

Several epidemiologic and clinical studies have indicated that diets rich in calcium may have a protective effect against cancers of the colon and rectum (colorectal cancer, CRC)[1-4] which are currently ranked third for both incidence and mortality in the United States[5]. Calcium is thought to act as a chemopreventative agent by binding secondary bile acids and ionized fatty acids to form insoluble soaps which decrease luminal exposure [6]. Similarly, high intake of vitamin D has also been associated with reduced cancer risk, but the results are less conclusive [7-10]. Vitamin D may protect against carcinogenesis by promoting cell differentiation, apoptosis, cell cycle regulation, and angiogenesis via activation of vitamin D receptors (VDRs) and regulation of calcium metabolism [6, 11]. The majority of studies assessing the association between dietary vitamin D and CRC have examined vitamin D intake exclusively from food sources, but little research has been done on supplemental vitamin D.

Our study population is at particularly high risk for CRC. The age-adjusted incidence rate for CRC was 54.5 per 100,000 in Pennsylvania from 2003-2007 (our study began in 2007) which was higher than the US as a whole(48.8 per 100,000) [12]. We investigated the associations between dietary and supplemental vitamin D and calcium, alone and in combination (total intake), with incident colorectal cancer risk in a large population-based case-control study in Pennsylvania.

Subjects and Methods

Sample population

The purpose of this study was to investigate risk factors for colorectal cancer among adults in a contiguous 18-county region in central and northeast Pennsylvania. This study included incident colorectal cancer cases and healthy controls participating in a population-based case-control study. Adult men and women ≥18 years of age were recruited between June 2007 and May 2011. Cases were identified from the Pennsylvania State Cancer Registry within 15 months of diagnosis. Controls residing in the same region that had no history of colorectal cancer were identified by random digit dialing, as described by Waksberg[13]. To be eligible, participants had to have a first time, histologically confirmed diagnosis and be English-speaking. A letter introducing the study was sent to potential participants, followed by a telephone call from a study coordinator to further explain the study, answer questions, and obtain preliminary informed consent. After oral consent was obtained from a potential case or control, a personal interview was scheduled and a self-administered food frequency questionnaire (FFQ) was mailed with instructions for completion prior to the interview. Written consent and data regarding sociodemographic factors, medical history, alcohol use, lifetime tobacco exposure, physical activity behavior, height, weight, medication use, and other lifestyle-related factors were collected by trained interviewers during in-person interviews. The returned FFQ was reviewed by study staff for completeness during the interview.

For the present analysis, we excluded participants who were <35 y (n=13), had missing BMI (kg/m^2) data (n=8), or who reported implausible energy intakes [<2093 kJ or >20930 kJ; <500 kcal or >5000 kcal] (n=73). After these exclusions, we included 1005 cases (501 men, 504 women) and 1062 controls (531 men, 531 women) in this analysis. There were 707 incident colon

cancers, including 440 cancers of the proximal colon (cecum to splenic flexure), 267 cancers of the distal colon (descending to sigmoid colon), and 298 cancers of the rectum (rectosigmoid junction and rectum). The institutional review boards at the Northeast Regional Cancer Institute (Scranton, PA), the Penn State College of Medicine (Hershey, PA), and the Lehigh Valley Health Systems (Allentown, PA) reviewed and approved all study procedures.

Assessment of dietary intake and supplement use

Participants completed a modified version of the Diet History Questionnaire (DHQ), a validated FFQ developed by the National Cancer Institute [14, 15]. The reference period was the past year for controls and the year prior to diagnosis for cases. Modifications to the DHQ were described previously [16]. Briefly, a meat module was added to capture meat consumption patterns of Pennsylvania residents as well as a module on supplement use. Energy and nutrient intake were calculated with Diet*Calc (version 1.4.3) nutrient analysis software.

To develop the categorical variables, nutrient intake values were grouped into quintiles based on the distribution among the controls and were entered into models as indicator variables defined by the second through fifth quintiles of intake, with the lowest quintile as the referent group. To conduct a linear trend test across levels of nutrients, variables were created using intake levels based on the median values for each quintile. Dietary levels of vitamin D and calcium were adjusted for total energy using the residual method [17]. Total vitamin D and calcium intake variables were created by combining adjusted dietary intake with supplemental intake.

Supplement variables for vitamin D and calcium were characterized in 3 ways for analysis: 1) added as amount of supplement to dietary data to create total intake variables; 2) used as indicator variables for any supplement use; and 3) added as 2 indicator variables for the upper

2 tertiles of intake (below and above the median intake among supplement users), with no use as a referent.

Statistics

All statistical analyses were performed using SAS statistical software (version 9.3; SAS Institute, Cary, NC). Demographic characteristics and dietary data from cases and controls were compared using the χ^2-test for categorical variables and Wilcoxon rank sum test for continuous variables. Odds ratios (ORs) and 95% confidence interval s (CIs) were computed using unconditional logistic regression, with the first quintile as the referent group. Associations stratified by anatomical subsite (colorectum, proximal colon, distal colon, and rectum) were investigated. Multivariate models were adjusted for potential confounding variables that were selected *a priori*: age (continuous), sex (male, female), total energy intake (kcal/d, continuous), body mass index (BMI; kg/m^2, continuous), smoking status (never, current, or former), family history of CRC (yes, no; first degree relative), alcohol (g/d, continuous), and regular nonsteroidal anti-inflammatory drug (NSAID) use (yes, no; defined as at least 3 times a week for 1 year prior to diagnosis for cases and 1 year prior to interview for controls). There was no appreciable change (10%) on risk estimates when education was included in the multivariate model so it was not included in the final models.

Effect modification by age, sex, BMI, and smoking was assessed by creating cross-product terms of continuous calcium and vitamin D intake variables and potential modifiers. The cross-product term was then included in multivariate models. When significant interactions were found, analyses were repeated by stratifying the effect modifier at the median. All analyses were two-sided and P-values were considered significant if <0.05.

Results

Characteristics of the 2067 study participants are presented in Table 1. The majority of our study population was Caucasian, 50% were men, and 29% had a college degree or higher. The mean age of participants was 64.2 years and the mean BMI was 29.1 kg/m^2. Over half of the participants reported regular NSAID use. Control subjects were better educated and consumed more alcohol than cases. History of CRC among first degree family members was higher among cases than controls. Energy-adjusted dietary calcium intake was significantly greater among controls than cases. No univariate differences were observed between cases and controls in energy-adjusted dietary vitamin D, but controls had higher total vitamin D intakes compared to cases. Calcium and vitamin D intakes were significantly correlated among all participants (Spearman correlation coefficient: dietary = 0.61; total = 0.62; P <0.001 for both).

Table 2 shows the multivariate ORs for colorectal, colon, and rectal cancer incidence by quintile of vitamin D intake from diet and supplements from logistic regression analyses. The risk estimates (ORs) for the comparison between the highest and lowest quintiles of dietary vitamin D were non-significant. Dietary vitamin D demonstrated a somewhat inconsistent pattern with increasing intakes; trends across increasing quintiles were non-significant for colorectal, colon, and rectal cancers. A high level of vitamin D supplement use (> 10 μg/d) was significantly inversely associated with CRC incidence (OR High v. None = 0.68; 95% CI = 0.53-0.89), and separately for colon cancer incidence (OR High v. None = 0.67; 95% CI = 0.50-0.89) and rectal cancer (OR High v. None = 0.65; 95% CI = 0.45-0.96) with a marginal dose-response relation for each (colorectum P-trend = 0.05, colon P-trend = 0.07, and rectum P-trend = 0.09). There was a consistent trend for reduced risk of CRC (P-trend <0.01), and separately for colon and rectal cancer with increasing levels of total vitamin D (P-trend= 0.02 and P-trend <0.01, respectively).When stratifying the colon into proximal and distal based on tumor location, the

only significant findings that remained were among subjects taking >10 μg/d of supplemental vitamin D (OR High v. None [proximal] = 0.67; 95% CI = 0.47-0.95; OR High v. None [distal] = 0.66; 95% CI = 0.44-0.99, data not shown). There was a marginal inverse dose-response relationship observed in both the proximal and distal colon for total intakes of vitamin D (*P*-trend = 0.07 for both).

A significant interaction was observed between continuous vitamin D supplement intake and age (*P* <0.01; data not shown). Among subjects 62 y and younger (median age of controls), there was a significant inverse association for supplemental vitamin D (OR High v. None = 0.53; 95% CI = 0.35-0.81; *P*-trend <0.01). No meaningful significant associations were found among subjects >62 y (*P*-trend = 0.61). There was also a significant interaction observed between continuous total vitamin D intake and age (*P*<0.01; data not shown). Among subjects ≤62 y, total vitamin D intake was inversely associated with CRC (OR Q5 v. Q1 = 0.51; 95% CI = 0.31-0.85; *P*-trend <0.01). No significant interactions were found among subjects >62 y (*P*-trend = 0.81).

An inverse association was observed between dietary calcium and CRC incidence (Table 3) (OR Q5 v. Q1 = 0.62; 95% CI = 0.46-0.83) and separately for cancer s of the colon and rectum (OR Q5 v. Q1= 0.60; 95% CI = 0.43-0.85; and OR Q5 v. Q1 = 0.64; 95% CI = 0.42-0.97, respectively). An inverse association was also observed with the use of calcium supplements. Compared to no use, use of >500mg/d of supplemental calcium was associated with a lowered risk of CRC (OR High v. None = 0.61; 95% CI = 0.46-0.81; *P*-trend < 0.01) and separately for colon cancer (OR High v. None = 0.63; 95% CI = 0.47-0.86; *P*-trend < 0.01) and rectal cancer (OR High v. None = 0.51; 95% CI = 0.33-0.80; *P*-trend = 0.02). Total calcium intake from diet and supplements was inversely associated with cancer incidence across all sites: CRC (OR Q5 v. Q1= 0.48; 95% CI = 0.35-0.66), colon cancer (OR Q5 v. Q1= 0.53; 95% CI = 0.37-0.76), and rectal cancer (OR Q5 v. Q1 = 0.38; 95% CI = 0.24-0.60). All significant findings for dietary,

supplemental, and total calcium intake remained when stratifying the colon into proximal and distal based on tumor location (data not shown).

A significant interaction effect was observed between continuous supplemental calcium intake and age for CRC (P <0.01; data not shown). Among subjects ≤62 y, there was a significant inverse association for supplemental calcium intake and CRC (OR High v. None = 0.43; 95% CI = 0.26-0.71; P-trend <0.01). There were no significant associations among subjects >62 y, but there was a near significant inverse trend (P-trend = 0.06). There was also a significant interaction between total calcium intake and age (P<0.01; data not shown). Among subjects ≤62 y, total calcium intake was inversely associated with CRC (OR Q5 v. Q1= 0.35; 95% CI = 0.21-0.57; P-trend <0.01). Total calcium was also inversely associated with CRC among subjects >62 y (OR Q_5 v. Q_1 = 0.63; 95% CI = 0.42-0.94; P-trend = 0.02).

There was no significant interaction effect between dietary vitamin D and dietary calcium intake (P = 0.51) on CRC incidence; however, a significant interaction effect was observed between total vitamin D and total calcium intake (P = 0.01). There were no significant findings among subjects with total calcium intakes below the median (824 mg/d, data not shown). A significant dose-response was found among high total calcium consuming subjects (>824 mg/d) with increasing amounts of total vitamin D consumption (P-trend = 0.01). When stratifying subjects as below/above median total vitamin D intake (10.8 μg/d) for total calcium, no significant findings were observed in the low vitamin D group. Among high total vitamin D consumers, high intakes of total calcium showed a significant inverse association with CRC incidence (OR Q5 v. Q1 = 0.39; 95% CI = 0.24-0.64; P-trend < 0.01).

Discussion

In the present analysis, total vitamin D intake was inversely associated with CRC risk. This relationship remained when the colorectum was stratified for both cancers of the colon and rectum. The test for trend was not statistically significant for rectal cancer when stratifying vitamin D by dietary intakes alone. This lack of a significant trend among rectal cancer cases may be due to the smaller numbers and limited power in this sub-analysis. Supplemental vitamin D was inversely associated with CRC, colon cancer, and rectal cancer. Dietary, supplemental, and total calcium were inversely associated with cancer risk in the colorectum, colon, and rectum.

Our results contribute to the growing body of evidence that suggests an inverse association between colorectal cancer risk and vitamin D intake. Previous case-control [7, 18-21] and cohort [22-26]studies have found inverse associations between vitamin D and CRC risk. Other studies have found no associations [4, 8, 27]. Studies that took supplementary vitamin D and fortification into account showed the strongest associations [7, 21, 22, 27]. A 2009 review on global vitamin D status concluded that hypovitaminosis D was widespread and was considered a "major health problem globally" [28]. This report, coupled with the results from our study and other epidemiological studies suggests that increased vitamin D intake from foods and supplements has beneficial effects, including decreased risk of colorectal cancer.

Vitamin D intake was most strongly associated with decreased risk when it was analyzed as total intake rather than dietary or supplemental intake alone. This may be a result of the dietary intakes being too low, even in the highest quintile, to observe a significant association. The Institute of Medicine (IOM) recently updated the dietary references for vitamin D and calcium. The new recommended dietary allowances (RDAs) are 15 μg/d vitamin D and 1,000 mg/d calcium for individuals 19-70 y [29]. The highest quintile of total vitamin D intake in this study (>17.5 μg/d; Table 2) as well as the upper two quintiles of total calcium intake (> 975 mg/d;

Table 3) were significantly inversely associated with CRC which reinforces the IOM recommendations.

Several clinical trials [11, 30-36] as well as some epidemiological studies[2, 10, 22, 37-39], but not all [18, 40, 41] have suggested an inverse association between calcium intake and CRC risk. Overall, many of these studies found a modest protective effect with high intakes of dietary calcium and calcium supplementation. A study conducted by Wu and colleagues [42]on subjects from the Nurses' Health Study and the Health Professionals' Follow-up Study also concluded that diets rich in calcium were protective against distal but not proximal colon cancer (RR >1250 mg/d v. $\leq$ 500 mg/d [distal] = 0.65; 95% CI = 0.43-0.98). The authors of the Polyp Prevention Trial (PPT) concluded that high levels of supplementation (> 1200 mg/d) resulted in a 20% reduction of risk for recurrent colorectal adenomas [31]. However, supplementation at 2000 mg/d in a European trial was modestly, but not significantly associated with a decreased risk in colorectal adenoma recurrence (RR = 0.66; 95% CI = 0.38-1.17) [30]. The authors of the latter European study suggest that poor compliance rate and a smaller than anticipated sample size likely contributed to the modest effect observed. The PPT had over twice as many subjects in each arm as the European study and also had a larger number of more advanced adenomas removed which may increase risk of recurrence [11].

This study had several strengths. One of the primary strengths was the use population-based controls, which allows for better generalization to the overall population. Second, the FFQ used in this study included a detailed supplement questionnaire. Lastly, the large sample size and consistency for dietary, supplemental, and overall intakes of nutrients allows for greater confidence in the results. One limitation of the present study is that estimates of sun exposure, which can also produce vitamin D, were not monitored.In addition, because this is a case-control study, there is the possibility of recall bias. To minimize this, participants were asked to provide dietary and demographic information for the year prior to diagnosis, for cases, or interview, for

controls. This also helped to control for any diet or lifestyle modifications that may have been made as a result of the cancer diagnosis.

In conclusion, the present study adds to the growing body of evidence that high levels of vitamin D and calcium intake are associated with decreased risk of colorectal cancer. Calcium had a more consistent protective effect than vitamin D; however, calcium appears to be more effective in the presence of high vitamin D which supports previous studies [11, 43]. Higher intakes of vitamin D in the presence of high calcium intake were not more protective than vitamin D alone which also supports previous studies [8, 21, 44]. Further research needs to be performed to elucidate the specific mechanisms by which these two micronutrients, alone and in combination, work to decrease colorectal cancer risk.

Table 4.1: Selected characteristics of study participants

Characteristic	Overall (n = 2067)	Controls (n = 1062)	Cases (n = 1005)	p-value
Age, y	64.2 ± 12	61.7 ± 11	66.8 ± 12	<0.001
BMI, kg/m^2	29.1 ± 6	28.6 ± 6	29.7 ± 6	<0.001
Sex, % female	50.1	50.0	50.2	0.946
Race, % Caucasian	97.2	97.8	96.6	0.091
Education, % college degree or above	29.2	34.2	23.8	<0.001
Family history of CRC, % yes	14.8	12.2	17.5	<0.001
Use NSAIDs, % yes	51.4	55.1	47.6	<0.001
Alcohol, g/d	9.54 ± 28	9.90 ± 26	9.15 ± 30	<0.001
Smoking status, %				0.040
Never	47.0	47.3	46.8	
Former	40.7	38.9	42.7	
Current	12.2	13.8	10.6	
Total energy, kcal/d	1822.6 ± 817	1786.1 ± 762	1861.2 ± 869	0.233
Total vitamin D[†], μg/d	11.0 ± 8	11.5 ± 8	10.4 ± 7	0.025
Dietary vitamin D, μg/d	3.94 ± 3	4.02 ± 3	3.85 ± 2	0.554
Vitamin D supplements, % any	66.8	68.1	65.5	0.209
Vitamin D supplements[‡], μg/d	10.5 ± 7	10.9 ± 7	10.1 ± 6	0.033
Total calcium[†], mg/d	958.3 ± 464	999.1 ± 491	915.1 ± 431	<0.001
Dietary calcium, mg/d	776.7 ± 312	796.1 ± 334	756.2 ± 287	0.012
Calcium supplements, % any	36.1	39.3	32.8	0.002
Calcium supplements[‡], mg/d	502.4 ± 352	517.1 ± 367	484.0 ± 331	0.261

[†] Dietary intakes residually adjusted plus supplements

[‡] Among supplement users

Table 4.2: Multivariate-adjusted OR (95% CIs) for colorectal, colon, and rectal cancer incidence with consumption of vitamin D from diet and supplements

Quintile/category	Range	Controls (n = 1062)	Cases (n = 1005)	Colorectum OR[1] (95% CI)	Cases (n = 707)	Colon OR[1] (95% CI)	Cases (n = 298)	Rectum OR[1] (95% CI)
Dietary vitamin D, µg/d								
1	<2.46	212	227	1.0 (ref)	149	1.0 (ref)	78	1.0 (ref)
2	2.46-3.15	213	182	0.81 (0.60-1.10)	126	0.83 (0.59-1.17)	56	0.80 (0.53-1.22)
3	3.15-3.88	212	185	0.78 (0.57-1.05)	134	0.83 (0.59-1.17)	51	0.71 (0.46-1.09)
4	3.88-5.14	213	216	0.85 (0.63-1.15)	157	0.91 (0.65-1.27)	58	0.75 (0.49-1.13)
5	>5.14	212	195	0.80 (0.60-1.07)	141	0.86 (0.62-1.19)	55	0.68 (0.45-1.03)
P-trend				0.259		0.574		0.085
Supplemental vitamin D, µg/d								
None		339	347	1.0 (ref)	235	1.0 (ref)	112	1.0 (ref)
Any		723	658	0.92 (0.76-1.12)	472	0.92 (0.74-1.15)	186	0.86 (0.65-1.14)
None	0	339	347	1.0 (ref)	235	1.0 (ref)	112	1.0 (ref)
Low	≤ 10	441	452	1.05 (0.85-1.30)	320	1.07 (0.85-1.36)	132	0.97 (0.72-1.30)
High	> 10	282	206	0.68 (0.53-0.89)	152	0.67 (0.50-0.89)	54	0.65 (0.45-0.96)
P-trend				0.051		0.066		0.094
Total vitamin D[†], µg/d								
1	<3.58	212	204	1.0 (ref)	130	1.0 (ref)	74	1.0 (ref)
2	3.58-7.76	213	229	1.15 (0.87-1.52)	157	1.23 (0.89-1.70)	72	1.02 (0.69-1.50)
3	7.76-13.14	212	220	1.08 (0.81-1.44)	164	1.23 (0.89-1.69)	56	0.80 (0.53-1.20)
4	13.14-17.53	213	201	0.93 (0.69-1.24)	146	1.00 (0.72-1.39)	55	0.76 (0.51-1.15)
5	>17.53	212	151	0.67 (0.49-0.91)	110	0.70 (0.50-1.00)	41	0.60 (0.39-0.94)
P-trend				0.003		0.019		0.008

[1] Adjusted for age, sex, BMI, total energy intake, NSAID use, family history of CRC, smoking, and alcohol

[†] Dietary vitamin D residually adjusted plus supplements

Table 4.3: Multivariate-adjusted OR (95% CIs) for colorectal, colon, and rectal cancer incidence with consumption of calcium from diet and supplements

Quintile/category	Range	Controls (n = 1062)	Cases (n = 1005)	Colorectum OR[1] (95% CI)	Cases (n = 707)	Colon OR[1] (95% CI)	Cases (n = 298)	Rectum OR[1] (95% CI)
Dietary calcium, mg/d								
1	<600	212	239	1.0 (ref)	159	1.0 (ref)	80	1.0 (ref)
2	600-698	213	208	0.89 (0.67-1.20)	151	0.97 (0.70-1.34)	57	0.82 (0.54-1.24)
3	698-803	212	199	0.81 (0.61-1.10)	146	0.85 (0.61-1.18)	53	0.72 (0.47-1.10)
4	803-964	213	201	0.90 (0.67-1.20)	146	0.98 (0.70-1.36)	55	0.78 (0.51-1.20)
5	>964	212	158	0.62 (0.46-0.83)	105	0.60 (0.43-0.85)	53	0.64 (0.42-0.97)
P-trend				0.003		0.005		0.044
Supplemental calcium, mg/d								
None		645	675	1.0 (ref)	461	1.0 (ref)	214	1.0 (ref)
Any		417	330	0.75 (0.62-0.93)	246	0.77 (0.61-0.97)	84	0.64 (0.47-0.87)
None	0	645	675	1.0 (ref)	461	1.0 (ref)	214	1.0 (ref)
Low	$\leq$ 500	225	195	0.87 (0.68-1.10)	141	0.89 (0.68-1.16)	54	0.73 (0.51-1.04)
High	> 500	192	135	0.61 (0.46-0.81)	105	0.63 (0.47-0.86)	30	0.51 (0.33-0.80)
P-trend				<0.001		0.001		0.022
Total calcium[†], mg/d								
1	<639	212	256	1.0 (ref)	161	1.0 (ref)	92	1.0 (ref)
2	639-761	213	198	0.75 (0.56-1.00)	149	0.84 (0.61-1.16)	53	0.59 (0.39-0.88)
3	761-975	212	221	0.85 (0.64-1.13)	153	0.91 (0.66-1.25)	67	0.73 (0.50-1.08)
4	976-1384	213	188	0.64 (0.48-0.86)	138	0.68 (0.49-0.95)	50	0.51 (0.34-0.78)
5	>1384	212	142	0.48 (0.35-0.66)	106	0.53 (0.37-0.76)	36	0.38 (0.24-0.60)
P-trend				<0.001		<0.001		<0.001

[1] Adjusted for age, NSAID use, education, sex, total energy intake, family history of CRC, BMI, smoking, and alcohol

[†] Dietary calcium residually adjusted plus supplements

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Chapter 5

Association of dietary and supplemental folate intake and polymorphisms of the MTHFR, MTHFD1, and TYMS genes with colorectal cancer in a population-based case-control study[1]

[1]This manuscript was submitted for publication:
Ashmore JH, Lesko SM, Muscat JE, Gallagher CJ, Berg AS, Miller PE, Hartman TJ, Lazarus P. Association of dietary and supplemental folate intake and polymorphisms of the MTHFR, MTHFD1, and TYMS genes with colorectal cancer in a population-based case-control study. *Cancer, Epidemiology, Biomarkers & Prevention*. (March 2013)

Abstract

Several previous studies have found that high levels of dietary folate are associated with reduced risk for colorectal cancer (CRC). In the present study, genetic polymorphisms in genes involved in folate-mediated one-carbon metabolism (FOCM) were examined for their role in altering risk for CRC. The role of dietary and supplemental folate and 35 single nucleotide polymorphisms (SNPs) in three FOCM pathway genes (*MTHFD1*, *MTHFR*, *TYMS*) on CRC risk was performed using a population-based case-control study in Pennsylvania consisting of 686 cases and 740 controls. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression to assess the effect of SNPs as well as folate from the diet and from supplements on CRC risk. Cases and controls reported dietary intakes and supplement use for the year before diagnosis or interview, respectively, using a modified Diet History Questionnaire from the National Cancer Institute. Using a dominant model for the variant allele, several SNPs were significantly associated with CRC including *MTHFD1* rs8003379 (OR=1.65; 95% CI=1.00-2.73) and rs17824591 (OR=1.98; 95% CI=1.14-3.41) and the *TYMS* rs2853533 SNP (OR=1.38; 95% CI=1.05-1.80). Using a non-dominant model, the AA genotype for MTHFR rs1476413 exhibited a marginally significant (OR=1.56; 95% CI=1.00-2.44) association with CRC. Two *TYMS* SNPs (rs16948305 and rs495139) exhibited significant (p=0.024 and p=0.040, respectively) gene-diet interactions with folate intake. One *MTHFD1* and one *MTHFR* SNP exhibited gene-diet interactions with methionine intake. These data suggest that allelic variants in genes involved in FOCM interact with dietary factors including folate and methionine to modify risk for CRC.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer mortality in the United States [1]. Multiple intrinsic (e.g., genetic variation) and extrinsic (e.g., dietary) factors contribute to CRC risk [2]. Folate is a water-soluble B vitamin that is critical for one-carbon metabolism. Folate-mediated one-carbon metabolism (FOCM) is important for DNA methylation, DNA synthesis and repair, and for producing S-adenosylmethionine (SAM) which is the universal donor of methyl groups [3]. Several studies have shown that insufficient folate intake can lead to DNA damage via uracil misincorporation or anomalous DNA repair [4]. Low folate intake can also induce epigenetic changes (i.e., hypomethylation) that have been associated with colorectal carcinogenesis [4].

A comprehensive review summarizing the currently available epidemiological studies of folate intake and CRC risk reported that the consumption of foods containing folate decreased risk for CRC [5]. A recent review and meta-analysis of studies assessing folate intake and CRC risk found a 15% decrease in risk among case-control studies and an 8% decrease in risk among cohort studies when comparing high to low folate intakes [6]. Although this systematic review found an overall decrease in risk associated with high folate intake, not all studies found a similar inverse association between folate intake and CRC risk [7-11]. Similarly, a meta-analysis of clinical trials supplementing individuals with folate to prevent colorectal adenomas found no benefit from supplementation [12]. Another important FOCM-related dietary factor is methionine, an essential amino acid, which is utilized in SAM production and has been associated with a decreased risk for CRC [11].

There have been several studies examining genetic variants within the FOCM pathway as possible modifiers of CRC risk. The majority of these studies have focused on a small number of variants from a few genes [13, 14]. More recent studies have investigated associations between

multiple genes within the FOCM pathway and colorectal adenoma or CRC incidence [15, 16]. Studies assessing both genetic variants and dietary intake may reveal gene-diet interactions that can modify cancer risk. Previous studies have investigated gene-diet interactions between polymorphisms in FOCM genes [17-22] and folate intake, but results have been inconsistent. Three enzymes important in the FOCM pathway include *methylenetetrahydrofolate dehydrogenase 1 (MTHFD1), which* encodes a trifunctional enzyme that catalyzes the inter-conversion of one-carbon derivatives of tetrahydrofolate [23], *methylenetetrahydrofolate reductase (MTHFR),*which converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary form of circulating folate and the methyl donor for the conversion of homocysteine to methionine [24], and *thymidylate synthase (TYMS),* which catalyzes the conversion of dUMP to dTMP, which is used in DNA synthesis and repair [25]. In the present study, associations between tagging SNPs in the *MTHFD1, MTHFR* and *TYMS* genes and CRC risk, potential interactions with folate consumption (total, dietary, and supplemental), as well as the consumption of other dietary components (e.g., methionine) in a population-based case-control study in central and northeast Pennsylvania were investigated.

Subjects and Methods

Sample population

This study was performed in subjects drawn from a population of 1196 incident CRC cases and 1187 healthy controls of both sexes ≥18 years of age recruited between June 2007 and May 2011 [26]. Cases were identified from the Pennsylvania State Cancer Registry within 15 months of diagnosis. To be eligible, case participants had to have a first time, histologically confirmed diagnosis of colon, rectal, or CRC (ICD-10 codes C18.0-18.9; C19.9; C20.9) and be

English-speaking. Controls were residents of the same region with no history of CRC and were identified by random digit dialing, as described by Waksberg [27]. A letter introducing the study was sent to potential participants, followed by a telephone call from a study coordinator to further explain the study, answer questions, and obtain preliminary informed consent. After oral consent was obtained from a potential case or control, a personal interview was scheduled and a self-administered food frequency questionnaire (FFQ) was mailed with instructions for completion prior to the interview. Written consent and data regarding sociodemographic factors, medical history, alcohol use, lifetime tobacco exposure, physical activity, height, weight, medication use, and other lifestyle-related factors were collected in person by trained interviewers. The self-administered FFQs were reviewed by study staff for completeness during the interview.

For the present analysis, we excluded participants (n=337) who were <35 y, had missing body mass index (BMI, kg/m^2), age, energy intake (kcal), or tumor site data or those who reported implausible energy intakes [<2093 kJ or >20930 kJ/day; <500 kcal or >5000 kcal/day]. In addition, participants (n=641) were excluded if sufficient genomic DNA was unavailable for genotyping analysis. Some participants met more than one exclusion criteria. After these exclusions, 686 cases and 740 controls remained for analysis. There were 480 incident colon cancers (cecum through the sigmoid colon) and 206 cancers of the rectum (rectosigmoid junction and rectum). The institutional review boards at the participating institutes, the Northeast Regional Cancer Institute (Scranton, PA) and the Penn State College of Medicine (Hershey, PA), reviewed and approved all study procedures.

Assessment of dietary intake and supplement use

Participants completed a modified version of the Diet History Questionnaire (DHQ), a validated FFQ developed by the National Cancer Institute [28, 29]. This DHQ was modified to

best examine the dietary intake of subjects recruited from the population of central Pennsylvania as described previously [30]. Briefly, a meat-cooking module was added to capture meat consumption patterns of Pennsylvania residents as well as a module on supplement use. Energy and nutrient intake were calculated with Diet*Calc (version 1.4.3; Bethesda, MD) nutrient analysis software. The reference period was the year prior to recruitment for controls and the year prior to diagnosis for cases.

All folate values were converted to dietary folate equivalents to account for the different bioavailability between natural folate and synthetic folic acid [31]. Dietary variables were adjusted for total energy using the residual method [32]. Supplement variables for folate were characterized in three ways for analysis: 1) added as amount of supplement to energy-adjusted dietary data to create total intake variables; 2) used as indicator variables for any vs. no supplement use; and 3) added as three indicator variables of intake (none, below, and above the median intake among supplement users), with no use as the referent. Categories of dietary exposures were calculated based on distribution among the control population. Approximate cut points were used to designate categories of dietary intake with the lowest intake level as the referent group. To conduct a linear trend test across levels of nutrients, variables were created using intake levels based on the median values for each category.

Genotyping

Oral buccal cell swabs, saliva, and/or blood samples were collected for genomic DNA isolation. Genomic DNA from oral buccal cell swabs was isolated using standard phenol:chloroform isolation methods. Genomic DNA from saliva was isolated using an Oragene DNA Kit (DNA Genotek Inc, Ontario Canada), while blood genomic DNA was isolated using the QIAamp DNA Blood mini kit (Qiagen Valencia, CA). Picogreen analysis was used to quantify

the amount of double stranded DNA (dsDNA) for each genomic sample (Life Technologies, Grand Island, NY). For a subset of samples that provided a low yield of genomic DNA in the original extraction procedure (185 controls and 99 cases), whole genome amplified (WGA) DNA, prepared by Qiagen's REPLI-g Service according to the manufacturers' protocol (Qiagen Valencia, CA), was used as template DNA for genetic analysis.

A total of 44 tagSNPs were selected for the three genes (*MTHFD1*: 18 SNPs; *MTHFR*: 14 SNPs; *TYMS*: 12 SNPs) by searching Utah residents with Northern and Western European ancestry from the CEPH collection (HapMap.org) using the Tagger program implemented in Haploview software [33] according to the following criteria: (a) SNPs were located in the *MTHFR*, *MTHFD1*, and *TYMS* genes or within their 5-kb 5' or 3' flanking regions, (b) had a minor allele frequency $\geq$ 0.05, and (c) the other unselected SNPs could be captured by one of the tagging SNPs with a linkage disequilibrium (LD) $r^2 \geq 0.80$ (mean r^2: *MTHFD1*=0.99, *MTHFR*=0.96, *TYMS*=0.99). Several SNPs in the flanking region of the *TYMS* gene are located on the *ENOSF1* gene. Genotyping assays for the tagSNPs were designed using the assay design tool available on Illumina's website (www.illumina.com). Goldengate technology (Illumina) was used to genotype the tagSNPs on the CRC cases and controls using 500 ng of DNA on the BeadXpress instrument (Illumina) as per manufacturer's instructions. The output files from the BeadXpress instrument were imported into GenomeStudio software (Illumina) to call genotypes for each SNP. Genotype distributions among controls were analyzed to verify that they fit Hardy-Weinberg Equilibrium (HWE) using a χ^2-test. Failure to obtain unambiguous genotype data resulted in four of the 44 SNPs to be dropped from further analysis (*MTHFD1* rs11158539 and rs2357694; *MTHFR* rs1537514; and *TYMS* rs2260821).

Statistical Analyses

Statistical analyses were performed using SAS statistical software (version 9.3; SAS Institute, Cary, NC). Demographic characteristics and dietary data from cases and controls were compared using the χ^2-test for categorical variables and the Wilcoxon rank sum test for continuous variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were computed using unconditional logistic regression, with the first (lowest) quintile as the referent group. Associations stratified by anatomical sub-site (colorectum, colon, and rectum) were investigated.

For FOCM SNP analysis, heterozygous and homozygous variant genotypes were assessed, (i) separately (i.e. non-dominant model), and (ii) after grouping together (i.e. dominant model) for the analysis of polymorphisms with minor allele frequencies <15%, as compared to the major allele genotype. Interactions between genotypes and diet on CRC risk were evaluated by testing for different slopes associated with nutrient intake across genotype.

In addition to single SNP analysis, we explored haplotype block analyses. Haplotypes were identified using Haploview version 4.2 [33]. Haplotypes in our study population were identified using the EM algorithm [34] in SAS. Only haplotypes with a frequency >0.01 were considered for further analysis. Identified blocks were assessed with odds ratios using the most frequent haplotype as the referent.

Multivariate models were adjusted for potential confounding variables that were selected *a priori*: age (continuous), sex (male, female), total energy intake (kcal/d, continuous), BMI (kg/m^2, continuous), smoking status (never, current, or former), family history of CRC (yes, no; first degree relative), alcohol (g/d, continuous), physical activity (yes, no; $\geq$ 1 hr/week of vigorous activity), education (no college degree, college degree or above), total calcium intake (dietary calcium residually adjusted plus supplemental calcium, mg/d, continuous), dietary fiber (g/d, continuous), dietary methionine (g/d, continuous), and regular non-steroidal anti-

inflammatory drug (NSAID) use (yes, no; regular use defined as at least 3 times a week for 1 year prior to diagnosis for cases and 1 year prior to interview for controls). There was no appreciable change (10%) of risk estimates or improvement in model fit characteristics with the addition of race, vitamin D, vitamin B_6, vitamin B_{12}, or riboflavin from diet and/or supplements to the multivariate model, so these variables were not included in the final models.

Potential effect modification by age, sex, BMI, alcohol, diet, and smoking was assessed by creating cross-product terms with continuous folate intake variables and including the cross-product term in multivariate models. When significant interactions were found, analyses were repeated by stratifying the effect modifier at the median value among controls. All analyses were two-sided and P-values were considered significant if <0.05.

Results

Selected characteristics of the study population are presented in Table 1. Overall, controls were significantly younger, better educated, more physically active, more likely to consume folate supplements, have lower BMI, regularly use NSAIDs, consume more alcohol, have greater methionine intake, and were less likely to have a family history of CRC compared to cases. There was a nearly-significant (p=0.057) higher consumption of total folate among controls vs. cases.

There were no meaningful, significant associations observed between dietary, supplemental, or total folate intake and CRC risk (Table 2). However, when cases were stratified by anatomic sub-site, an inverse association was observed between supplemental folate use and colon cancer (OR any use vs. no use = 0.77; 95% CI = 0.59-1.00); no association was observed with rectal cancer cases.

A significant interaction was observed between total folate intake and age (p = 0.016) for CRC risk. When stratified by the median age of controls (62 y), an inverse association between

total folate and CRC was observed among younger individuals (≤62 y OR Q_5 vs. Q_1 = 0.56; 95% CI = 0.29-1.07; p_{trend} = 0.024) and a positive, but not statistically significant, association was observed among older individuals (OR >62 y Q_5 vs. Q_1 = 1.69; 95% CI = 1.01-2.81; p_{trend} = 0.107). A significant interaction was also observed between supplemental folate intake and age (p = 0.006). Supplemental folate intake >680 µg/day compared to no use was inversely associated with CRC (OR = 0.54; 95% CI = 0.36-0.82; *P*-trend = 0.004) only among younger (≤62 y) individuals.

Of the 40 SNPs examined, all were in HWE among controls except five (*MTHFD1* rs11627525 and rs3783728; *MTHFR* rs2274976 and rs17037390; and *TYMS* rs2612101; Supplemental Table 1), which were not considered for further analysis. Using a dominant model, four of the 35 remaining SNPs were significantly associated with CRC: two in the *MTHFD1* gene (rs8003379: OR = 1.65; 95% CI = 1.00-2.73; and rs17824591: 1.98; 95% CI = 1.14-3.41); two in the *MTHFR* gene (rs1476413: OR = 1.56; 95% CI = 1.00-2.44; and rs1801133: OR = 1.28; 95% CI = 1.01-1.63); and one in the *TYMS* gene (rs2853533: OR = 1.38; 95% CI = 1.05-1.80; Table 3). The variant AA genotype for the rs1476413 SNP within the *MTHFR* gene was also marginally associated with CRC (OR = 1.56; 95% CI = 1.00-2.44) using a non-dominant model. However, for the *MTHFR* rs1801133 SNP, the significant effect observed using a dominant model was driven primarily by the association observed among heterozygotes (no effect was observed in subjects with the homozygous carriers of the variant allele).

Haplotype blocks for each gene were explored to assess their association with CRC. A total of 28 of the 35 SNPs (*MTHFD1:* 10; *MTHFR:* 10; *TYMS:* 8) in HWE combined to form 6 haplotype blocks. No significant associations were observed among any of the three haplotype blocks for the *MTHFD1* gene after multivariate adjustment (Supplemental Table 2). For the *MTHFR* gene, the lowest frequency haplotype was inversely associated with CRC compared to the most frequent haplotype (OR = 0.41; 95% CI = 0.20-0.83; frequency = 0.013). Conversely, a

positive association was observed among the lowest frequency *TYMS* haplotype compared to the most frequent haplotype within the first *TYMS* haplotype block (OR = 3.33; 95% CI = 1.02-10.90; frequency = 0.011).

Two *TYMS* SNPs (rs16948305 and rs495139) exhibited a significant (p=0.024 and 0.040, respectively) interaction with total folate intake (Table 4). After stratifying by levels of total folate intake, the CC genotype for *TYMS* rs16948305 was inversely associated with CRC among individuals with medium (OR = 0.50; 95% CI = 0.50-0.95) or high (OR = 0.71; 95% CI = 0.50-1.00) folate intake.

For the *MTHFD1* rs2295638 SNP, an inverse association with CRC was observed for the wild-type CC genotype in a dominant model for subjects with a medium (OR = 0.43; 95% CI = 0.32-0.58) or high (OR = 0.18; 95% CI = 0.13-0.26) methionine intake (Table 4). While associations with CRC were observed for medium and high methionine intake subjects for all genotypes for the *MTHFR* rs1801133 SNP, this trend was most pronounced for subjects with the CC genotype (OR = 0.42; 95% CI = 0.27-0.67 for medium methionine intake subjects; and OR = 0.16; 95% CI = 0.10-0.28 for high methionine intake subjects).

Discussion

In this population-based study, associations with CRC and polymorphisms in three FOCM genes (*MTHFD1*, *MTHFR*, and *TYMS*) were investigated. A significant association was observed between five FOCM SNPs and CRC. Significant gene-diet interactions were also observed for two *TYMS* SNPs and total folate intake, and one *MTHFD1* SNP and one *MTHFR* SNP and dietary methionine intake. Overall, supplemental folate use was inversely associated with colon, but not rectal, cancer.

Our findings for dietary and total folate intake are contrary to the results of a recent meta-analysis of case-control and cohort studies which suggested that high folate intake decreased risk associated with CRC by 8-15% [6]. In the United States and Canada, the mean folate intake is ~400 µg/d from dietary sources and ~800 µg/d among supplemental folate users [14]. Intakes in the present study population were comparable to these findings, but were higher than most other studies investigating folate and CRC. Data collection for several of the studies included in the meta-analysis was conducted either before or during the mandatory fortification of flour and uncooked cereal-grain products in the United States and Canada which began in 1998 [6]. Over 58% of our study population used folate supplements which also increased intake values to higher levels than most studies.

While significant associations between high dietary and total folate intake and risk for CRC were not observed in the present study, a significant inverse association was observed between colon cancer and supplemental folate use when the colorectum was stratified by sub-site. An Australian study [35] found supplemental folate use reduced risk of left-sided, but not right-sided colon cancers. This is also similar to a Canadian study [36] which found that B-complex supplements reduced colon cancer risk, and findings from the Nurses' Health Study [37] which reported a reduced risk for CRC associated with multivitamin use. It is possible that other nutrients found in these supplements may alter CRC risk.

Significant interactions between total and supplemental folate intake and age were also observed. In both instances, an inverse association was observed among younger individuals and a positive association was observed among older individuals. One possible explanation for these findings is the "dual effect of folate" which posits that folate decreases the risk for cancer in healthy tissues, while it has a promoting effect on the progression of established neoplasms [4, 6, 38, 39]. Within the present study population, high levels of folate intake may be increasing the

risk for CRC for older individuals with undiagnosed neoplasms while protecting younger individuals who are healthy.

Several studies have investigated the interaction between polymorphisms in FOCM genes and dietary factors related to FOCM [15, 20, 40-42]. The present investigation resulted in two significant associations between *TYMS* SNPs and total folate intake. The majority of previous studies assessing *TYMS* in relation to CRC risk have investigated a 6 bp deletion within the 3' untranslated region and a 28 bp repeat in the 5' untranslated region of the gene [25]. These regions were not investigated in the present study. This is the first study demonstrating an interaction between folate intake and *TYMS* rs16848305, suggesting that the dominant allele may decrease risk for CRC, but only among those in the top two-thirds of total folate intake. The interaction between *TYMS* rs495139 and folate intake resulted in no significant findings after stratification by total folate intake; however, individuals homozygous for the variant allele with low folate intakes had a near-significant association with CRC.

Few studies have assessed polymorphisms in *MTHFD1* in relation to risk for CRC [15, 20, 43, 44]. A significant interaction was observed between *MTHFD1* rs2295638 and methionine intake in the present study. High levels of dietary methionine intake were inversely associated with CRC in a previous study [45]. Liu et al [20] observed a gene-diet interaction between *MTHFD1* rs2236225 and methionine in their study, but found no significant associations after stratification. Both the rs2295638 and rs2236225 SNPs are located within the same haplotype block, but no significant associations were observed for *MTHFD1* rs2236225 alone, or for any *MTHFD1* haplotypes, in the present study.

MTHFR is the most widely-studied FOCM gene. The majority of studies assessing *MTHFR* and CRC risk have focused on the rs1801133 SNP. The variant allele in this SNP causes an increase in thermolability of the MTHFR enzyme [46] which is associated with decreased plasma folate and increased plasma homocysteine [47, 48]. The *MTHFR* rs1801133 SNP was

associated with decreased risk for CRC among individuals with high methionine intake. After stratification by genotype, individuals with the variant allele had a higher risk within each strata compared to the dominant allele, except for the TT genotype in the low methionine group. These findings mirror the results for the individual SNP analysis which found the variant allele to be associated with an increased risk for CRC in the present study. The present findings for the *MTHFR* rs1801133 SNP are in contrast to those from a recent meta-analysis of 25 independent populations which concluded that the TT genotype was inversely associated with CRC compared to the CC genotype (OR = 0.83; 95% CI = 0.75-0.93) [49]. The inconsistent findings for *MTHFR* rs1801133, coupled with the interaction with methionine, suggest the possibility of incompletely controlled confounding and warrant further investigation.

Strengths of the present case-control study include the relatively large sample size in conjunction with both dietary and genetic information which made the assessment of gene-environment interactions possible. This study also had a large number of dietary supplement users, making it possible to investigate associations between dietary, supplemental, and total folate intake. One drawback of the present study was the small number of individuals within some strata, resulting in imprecise estimates. For the gene-diet analysis, no adjustments for multiple comparisons were made, which may have resulted in spurious findings. Colonoscopic confirmation that controls harbored no occult cancers would have strengthened this study, but this is not practical in a population-based study.

In summary, the results from the present case-control study indicate that dietary intakes and three genes involved in FOCM may interact to modify CRC risk. Variants in two TYMS SNPs interacted significantly with folate intake to alter risk for CRC. Furthermore, one MTHFD1 and one MTHFR SNP modified susceptibility to CRC based on methionine intake. Folate intake alone was not associated with CRC, but folate supplement use did decrease risk for CRC. Further

research is needed to test the present results and determine what the optimal level of folate intake is to minimize risk for CRC.

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Table 5.1: Selected characteristics of study participants

Characteristic	Overall (n = 1426)	Case (n = 686)	Control (n = 740)	p-value
Age, y	64.3 ± 11	66.6 ± 12	62.1 ± 11	<0.001
BMI, kg/m^2	29.1 ± 6	29.7 ± 6	28.6 ± 6	<0.001
Sex, % female	48.3	48.4	48.1	0.913
Education, % college degree or above	29.9	25.1	34.4	<0.001
Family history of CRC, % yes	15.6	19.4	12.0	<0.001
Physical activity, % ≥1hr/wk	32.8	27.3	38.0	<0.001
Use NSAIDs, % yes	51.3	47.4	54.9	0.005
Alcohol, g/d	9.90 ± 29	9.28 ± 29	10.48 ± 29	<0.001
Smoking status, %				0.017
Never	47.8	46.2	49.2	
Former	41.0	44.3	37.8	
Current	11.3	9.5	13.0	
Total energy, kcal/d	1843.7 ± 821	1888.2 ± 868	1802.4 ± 773	0.166
Total Folate†, mg/d	812.2 ± 374	791.9 ±376	830.9 ± 373	0.057
Dietary Folate†, mg/d	468.4 ± 145	464.9 ± 144	471.6 ± 146	0.551
Folate supplements, % any	58.4	54.7	61.8	0.007
Folate supplements‡, mg/d	589.2 ± 210	598.2 ± 207	581.8 ± 213	0.257
Methionine†, mg/d	1.55 ± 0.45	1.51 ± 0.5	1.58 ± 0.4	<0.001
Fiber, g/d	17.1 ± 9	17.0 ± 9	17.2 ± 9	0.615

† Dietary intakes were adjusted for total energy intake by the residual method

‡ Among supplement users

Table 5.2: Age-adjusted and multivariate-adjusted ORs1 for CRC risk within quintiles of dietary and supplemental folate intake.

Variable	Q1	Q2	Q3	Q4	Q5	*P*-trend
Total Folate[†] (µg/d)						
Range (quintile cut-points)	<440	440-621	621-1050	1050-1172	>1172	
Cases/controls	167/148	140/148	126/148	119/148	134/148	
OR (95% CI) - CRC	1.0 (ref)	1.01 (0.71-1.43)	0.91 (0.64-1.30)	0.86 (0.560-1.24)	1.15 (0.78-1.70)	0.993
OR (95% CI) - Colon	1.0 (ref)	1.00 (0.68-1.49)	0.88 (0.59-1.31)	0.84 (0.56-1.27)	1.10 (0.72-1.70)	0.836
OR (95% CI) - Rectum	1.0 (ref)	0.97 (0.58-1.62)	0.93 (0.56-1.54)	0.89 (0.51-1.54)	1.25 (0.70-2.23)	0.749
OR[a] (95% CI) - Young (≤62 y)	1.0 (ref)	0.81 (0.47-1.38)	0.78 (0.47-1.31)	0.49 (0.26-0.90)	0.56 (0.29-1.07)	0.024
OR[a] (95% CI) - Old (>62 y)	1.0 (ref)	1.09 (0.68-1.76)	0.93 (0.56-1.54)	1.13 (0.70-1.82)	1.69 (1.01-2.81)	0.107
Dietary Folate (µg/d)						
Range (quintile cut-points)	<364	364-425	425-480	480-575	>575	
Cases/controls	135/148	150/148	132/148	154/148	115/148	
OR (95% CI) - CRC	1.0 (ref)	1.29 (0.89-1.86)	1.33 (0.90-1.95)	1.54 (1.04-2.27)	1.37 (0.88-2.13)	0.154
OR (95% CI) - Colon	1.0 (ref)	1.21 (0.80-1.83)	1.31 (0.85-2.02)	1.56 (1.01-2.42)	1.41 (0.87-2.30)	0.122
OR (95% CI) - Rectum	1.0 (ref)	1.47 (0.88-2.47)	1.28 (0.72-2.25)	1.56 (0.89-2.76)	1.37 (0.71-2.64)	0.383
Supplemental Folate (µg/d)						
Range	0 (None)	Use	0 (None)	≤ 680	> 680	
Cases/controls	283/311	375/457	283/311	85/120	290/337	
OR (95% CI) - CRC	1.0 (ref)	0.83 (0.66-1.05)	1.0 (ref)	0.73 (0.52-1.03)	0.87 (0.68-1.12)	0.316
OR (95% CI) - Colon	1.0 (ref)	0.77 (0.59-1.00)	1.0 (ref)	0.66 (0.45-0.98)	0.81 (0.61-1.08)	0.170
OR (95% CI) - Rectum	1.0 (ref)	0.96 (0.68-1.35)	1.0 (ref)	0.83 (0.50-1.38)	1.01 (0.70-1.47)	0.937
OR[b] (95% CI) - Young (≤62 y)	1.0 (ref)	0.65 (0.45-0.93)	1.0 (ref)	0.87 (0.54-1.40)	0.54 (0.36-0.82)	0.004
OR[b] (95% CI) - Old (>62 y)	1.0 (ref)	1.01 (0.74-1.38)	1.0 (ref)	0.61 (0.37-1.01)	1.17 (0.84-1.63)	0.295

[1] Multivariate-adjusted OR: model includes age, sex, total energy intake, BMI, physical activity, NSAID use, family history of CRC, education, calcium, fiber, methionine, smoking, and alcohol.

[†]Dietary folate residually energy adjusted plus folate from supplements

a) Age x total folate: *P*-interaction = 0.016; b) Age x supplemental folate: *P*-interaction = 0.006

Table 5.3: Summary of significant ORs for single SNP analysis

		Case/Control	OR[a] (95% CI)	OR[b] (95% CI)	OR[b,c] (95% CI)
MTHFD1					
rs8003379	AA	354/405	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AC	246/236	1.21 (0.96-1.53)	1.24 (0.97-1.58)	1.29 (1.02-1.63)
	CC	44/36	1.47 (0.92-2.35)	1.65 (1.00-2.73)	
rs17824591	GG	398/449	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	233/227	1.19 (0.94-1.50)	1.26 (0.99-1.61)	1.33 (1.05-1.68)
	AA	39/27	1.73 (1.03-2.91)	1.98 (1.14-3.41)	
MTHFR					
rs1476413	GG	361/404	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	241/253	1.07 (0.85-1.34)	1.03 (0.81-1.31)	1.10 (0.88-1.39)
	AA	57/43	1.49 (0.97-2.28)	1.56 (1.00-2.44)	
rs1801133	CC	241/263	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	309/259	1.34 (1.05-1.71)	1.35 (1.05-1.74)	1.28 (1.01-1.63)
	TT	75/81	1.04 (0.72-1.49)	1.06 (0.72-1.55)	
TYMS					
rs2853533	GG	474/513	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GC	165/150	1.26 (0.98-1.64)	1.34 (1.02-1.76)	1.38 (1.05-1.80)
	CC	15/9	1.91 (0.81-4.50)	2.03 (0.84-4.94)	

a) Age-adjusted only; b) Multivariate ajusted for: age, sex, kcal, bmi, NSAID use, physical activity, family history of CRC, smoking, education, alcohol, methionine, fiber, calcium, and folate; c) Dominant model - homozygous and heterozous individuals for variant allele combined

Table 5.4: Significant associations between polymorphism genotypes and colorectal cancer, stratified by dietary factors

	Tertile 1		Tertile 2		Tertile 3	
	case/cont	OR (95% CI)	case/cont	OR (95% CI)	case/cont	OR (95% CI)
Total folate intake[a,b,c]						
TYMS rs16948305						
CC	218/179	1.0 (ref)	144/180	0.69 (0.50-0.95)	136/183	0.71 (0.50-1.00)
CT+TT	55/57	0.65 (0.42-1.02)	55/58	0.83 (0.53-1.30)	63/54	1.05 (0.67-1.66)
P Interaction = 0.024						
TYMS rs495139						
CC	84/82	1.0 (ref)	77/77	1.22 (0.76-1.97)	72/76	1.20 (0.74-1.95)
CG	128/121	1.13 (0.75-1.72)	90/121	0.80 (0.52-1.25)	102/125	0.99 (0.64-1.55)
GG	58/35	1.69 (0.98-2.94)	36/47	0.83 (0.47-1.46)	25/38	0.79 (0.42-1.49)
P Interaction = 0.040						
Dietary methionine intake[d,e]						
MTHFD1 rs2295638						
CC	343/183	1.0 (ref)	165/193	0.43 (0.32-0.58)	80/189	0.18 (0.13-0.26)
CT+TT	18/16	0.77 (0.37-1.62)	11/9	0.69 (0.27-1.79)	10/12	0.44 (0.18-1.07)
P Interaction = 0.019						
MTHFR rs1801133						
CC	146/92	1.0 (REF)	64/83	0.42 (0.27-0.67)	31/88	0.16 (0.10-0.28)
CT	175/76	1.45 (0.98-2.16)	88/93	0.55 (0.36-0.84)	46/90	0.26 (0.16-0.43)
TT	37/31	0.63 (0.35-1.13)	25/28	0.59 (0.31-1.12)	13/22	0.32 (0.14-0.71)
P Interaction = 0.042						

a) Adjusted for age, sex, BMI, physical activity, total energy intake, family history of CRC, education, dietary calcium, dietary fiber, dietary methionine, smoking status, and alcohol

b) Dietary folate intake residually adjusted plus supplemental folate

c) Tertile cutpoints: 555/1095 µg/d

d) Adjusted for age, sex, BMI, physical activity, total energy intake, family history of CRC, education, dietary calcium, dietary fiber, dietary folate, smoking status, and alcohol

e) Tertile cutpoints: 1.55/1.91 g/d

Chapter 6

Conclusions

Summary of research findings and implications

The Pennsylvania colorectal cancer study is a large multi-site population-based case-control study of >2,400 adult men and women living in central and northeast Pennsylvania. This study was designed to assess environmental and genetic factors that may explain the increased incidence and mortality for colorectal cancer within Pennsylvania.

Research presented in this dissertation expands on the findings of Miller et al. [1] which found that individuals with diets with higher meat-related mutagen exposure were at increased risk for colorectal cancer compared to those with low exposures. In our related study (Study 1), looking at dietary iron and colorectal cancer, we found that high levels of heme iron, which is specific to meat, was not associated with increased risk for colorectal cancer. Dietary iron was inversely associated with colorectal cancer risk in women, but not men. Total iron intake was positively associated with colorectal cancer, but none of the results reached statistical significance. Lastly, high levels of supplemental iron intake were found to be positively associated with colorectal cancer risk within our study population.

In our second study, we assessed dietary and supplemental calcium and vitamin D intake and colorectal cancer risk (Chapter 5). We found significant inverse associations between dietary, supplemental, and total calcium intakes and colorectal cancer risk. Results were also similar between the colon and rectum. For the vitamin D analysis, only high levels of total vitamin D and supplemental vitamin D were associated with reduced colorectal cancer risk.

Chapter 6 explored the relationship between folate intake and folate-related genes and how they interact to alter colorectal cancer risk. In this study, high levels of total folate intake were positively associated with colorectal cancer among older individuals. A near-significant inverse association was observed among younger individuals with high levels of total folate

intake. Similarly, supplemental folate was inversely associated with colorectal cancer among younger, but not older,individuals. These two findings suggest that folate may act differently on younger and older individuals. Sources of dietary folate were similar between both young and older individuals within our study population, as well. One possible explanation for these findings is the "dual effect of folate" which posits that folate decreases the risk for cancer in healthy tissues, while it has a promoting effect on the progression of established neoplasms [2-5]. Among our study population, high levels of folate intake may be increasing the risk for colorectal cancer for older individuals with undiagnosed neoplasms while protecting younger individuals who are healthy. Four polymorphisms from three genes had significant gene-diet interactions within our study population. There were also five polymorphisms from the same three genes that significantly altered colorectal cancer risk. Many of these associations between polymorphisms and colorectal cancer are the first reported and should be validated in future human population studies.

Limitations

The studies presented in this dissertation were not without limitations. The case-control study design utilized by all three studies may lead to recall bias; cases may alter their reporting or intake of foods that are perceived to be risk factors for cancer, such as red meat or foods high in fat, which may lead to spurious findings. Another drawback of this study was the lack of histological confirmation of healthy tissues for controls. Controls in our population may have had precancerous polyps or asymptomatic colorectal cancer, but the large sample size helps to reduce this possible confounder. The study population utilized in these studies was also predominately white and from rural areas in Pennsylvania which makes our findings difficult to generalize to a wider population. Lastly, we attempted to control for potential confounders through multivariate

models, but residual or unknown confounders still remain possible and must be taken into consideration when interpreting results.

Individuals who survive cancer are likely to become more health conscious as a result. Many people believe supplements to be 'healthy' and start to take them as a result of cancer diagnosis. Cases from our study could have potentially reported supplement use they began after diagnosis, not prior to, which would have altered our findings. Previous studies have shown that cancer survivors will also begin taking new supplements after diagnosis, and many consume more than one supplement. With the increase in fortified foods, supplement use may cause users to have nutrient intakes above the tolerable upper-intake values established by the Institute of Medicine (IOM). Supplement users also tend to engage in other 'healthy' behaviors such as exercise and tend to have lower obesity rates. These issues must also be taken into consideration when assessing the impact of supplement use on CRC risk.

Future Directions

We explored the relationship between various micronutrient intakes as well as the interaction between nutrient intakes and genetics and colorectal cancer risk. Continued research involving sequenced genes and micronutrients will strengthen our findings and help to elucidate the complex interplay between environmental exposures and genetics. Future studies that analyze the urine collected from a large subset of study participants will also help strengthen findings and clarify the different metabolic profiles and risk factors of cases and controls. With the decreasing cost of sequencing, future studies may also be able to sequence the entire genome, allowing for genome-wide association studies which will greatly increase the ability to detect potential risk-modifying polymorphisms.

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Appendix A

Additional Tables

Additional Table 1:40 SNPs from three FOCM-related genes

Cases						Controls					
	Genotypes (%)			MAF	P-HWE		Genotypes			MAF	P-HWE
MTHFD1											
rs7151163	GG (20.6)	GT (46.2)	TT (33.3)	G = 0.437	0.131	rs7151163	GG (20.5)	GT (48.0)	TT (31.5)	G = 0.445	0.483
rs8003379	AA (55.0)	AC (38.2)	CC (6.8)	C = 0.259	0.887	rs8003379	AA (60.0)	AC (34.9)	CC (5.3)	C = 0.228	0.832
rs11627525	CC (83.6)	CT (15.4)	TT (1.0)	T = 0.087	0.372	rs11627525	CC (84.8)	CT (13.8)	TT (1.4)	T = 0.083	**0.013**
rs2236225	CC (31.5)	CT (51.4)	TT (17.3)	T = 0.428	0.208	rs2236225	CC (32.8)	CT (46.3)	TT (20.9)	T = 0.441	0.106
rs2295638	CC (93.8)	CT (6.1)	TT (0.2)	T = 0.032	0.640	rs2295638	CC (93.9)	CT (6.0)	TT (0.2)	T = 0.032	0.594
rs11629135	GG (84.2)	AG (14.9)	AA (0.9)	A = 0.084	0.491	rs11629135	GG (85.3)	AG (13.8)	AA (1.0)	A = 0.079	0.172
rs3783728	TT (84.0)	CT (15.3)	CC (0.7)	C = 0.084	0.895	rs3783728	TT (84.2)	CT (14.3)	CC (1.5)	C = 0.087	**0.008**
rs3818239	AA (78.3)	AG (20.5)	GG (1.2)	G = 0.115	0.754	rs3818239	AA (80.0)	AG (18.3)	GG (1.7)	G = 0.109	0.151
rs2281603	AA (58.4)	AG (34.9)	GG (6.8)	G = 0.242	0.203	rs2281603	AA (56.7)	AG (38.3)	GG (5.0)	G = 0.242	0.236
rs11158542	TT (47.0)	CT (43.3)	CC (9.8)	C = 0.314	0.917	rs11158542	TT (48.5)	CT (42.8)	CC (8.7)	C = 0.301	0.656
rs745686	AA (42.8)	AG (45.9)	GG (11.3)	G = 0.343	0.612	rs745686	AA (46.1)	AG (42.0)	GG (12.0)	G = 0.329	0.185
rs1256146	GG (67.2)	AG (29.9)	AA (2.9)	A = 0.179	0.598	rs1256146	GG (66.7)	AG (28.8)	AA (4.5)	A = 0.189	0.109
rs1956545	AA (86.6)	AG (12.5)	GG (0.9)	G = 0.072	0.143	rs1956545	AA (85.8)	AG (13.7)	GG (0.6)	G = 0.074	0.982
rs17751556	TT (88.3)	CT (11.3)	CC (0.5)	C = 0.061	0.702	rs17751556	TT (87.3)	CT (12.2)	CC (0.6)	C = 0.067	0.536
rs17824591	GG (59.4)	AG (34.8)	AA (5.8)	A = 0.232	0.528	rs17824591	GG (63.9)	AG (32.3)	AA (3.8)	A = 0.200	0.799
rs2295640	CC (80.5)	CG (18.9)	GG (0.6)	G = 0.101	0.293	rs2295640	CC (80.5)	CG (18.8)	GG (0.7)	G = 0.101	0.335
MTHFR											
rs4846047	GG (50.0)	CG (41.1)	CC (9.4)	C = 0.299	0.590	rs4846047	GG (51.0)	CG (39.6)	C (9.4)	C = 0.292	0.267
rs4846049	GG (46.0)	GT (42.7)	TT (11.3)	T = 0.327	0.447	rs4846049	GG (48.5)	GT (40.2)	TT (11.4)	T = 0.314	0.074
rs2274976	GG (90.9)	AG (9.0)	AA (0.2)	A = 0.046	0.715	rs2274976	GG (91.6)	AG (7.7)	AA (3.4)	A = 0.046	**0.002**
rs1476413	GG (54.8)	AG (36.6)	AA (8.7)	A = 0.269	0.069	rs1476413	GG (57.7)	AG (36.1)	AA (6.1)	A = 0.242	0.687
rs17375901	CC (90.5)	CT (9.1)	TT (0.5)	T = 0.050	0.279	rs17375901	CC (91.3)	CT (8.5)	TT (0.3)	T = 0.045	0.642
rs12121543	CC (57.8)	AC (35.0)	AA (7.2)	A = 0.247	0.131	rs12121543	CC (59.8)	AC (34.3)	AA (5.9)	A = 0.230	0.396
rs6541003	AA (36.3)	AG (46.4)	GG (17.4)	G = 0.406	0.326	rs6541003	AA (37.1)	AG (45.4)	GG (17.5)	G = 0.402	0.137
rs1801133	CC (38.6)	CT (49.4)	TT (12.0)	T = 0.367	0.110	rs1801133	CC (43.6)	CT (43.0)	TT (13.4)	T = 0.349	0.178
rs17421462	GG (85.8)	AG (13.0)	AA (1.2)	A = 0.077	**0.024**	rs17421462	GG (84.9)	AG (14.4)	AA (0.7)	A = 0.079	0.689
rs2066471	GG (67.1)	AG (30.1)	AA (2.8)	A = 0.179	0.523	rs2066471	GG (69.0)	AG (27.6)	AA (3.4)	A = 0.172	0.428
rs17037390	GG (73.5)	AG (24.6)	AA (2.0)	A = 0.142	0.886	rs17037390	GG (74.5)	AG (22.0)	AA (3.4)	A = 0.145	**0.004**
rs9651118	TT (64.1)	CT (32.5)	CC (3.5)	C = 0.197	0.503	rs9651118	TT (64.8)	CT (31.7)	CC (3.5)	C = 0.193	0.648
rs17037425	GG (75.5)	AG (23.2)	AA (1.3)	A = 0.129	0.445	rs17037425	GG (77.4)	AG (20.4)	AA (2.2)	A = 0.124	0.097
TYMS											
rs2853532	CC (46.3)	CT (43.9)	TT (9.9)	T = 0.318	0.760	rs2853532	CC (44.5)	CT (46.1)	TT (9.5)	T = 0.325	0.189
rs1004474	AA (30.7)	AG (49.3)	GG (20.0)	G = 0.446	0.960	rs1004474	AA (30.8)	AG (50.3)	GG (18.9)	G = 0.441	0.595
rs2847153	GG (62.2)	AG (34.2)	AA (3.6)	A = 0.207	0.279	rs2847153	GG (62.9)	AG (33.7)	AA (3.4)	A = 0.203	0.247
rs9948583	CC (46.2)	CT (44.4)	TT (9.4)	T = 0.316	0.474	rs9948583	CC (47.8)	CT (42.9)	TT (9.4)	T = 0.308	0.890
rs3744962	TT (80.8)	CT (18.4)	CC (0.8)	C = 0.100	0.494	rs3744962	TT (82.5)	CT (16.4)	CC (1.2)	C = 0.094	0.353
rs16948305	CC (74.2)	CT (24.4)	TT (1.3)	T = 0.136	0.271	rs16948305	CC (76.2)	CT (21.2)	TT (2.5)	T = 0.132	0.061
rs2298582	AA (80.1)	AC (18.4)	CC (1.5)	C = 0.107	0.342	rs2298582	AA (77.0)	AC (21.6)	CC (1.4)	C = 0.122	0.834
rs495139	CC (34.7)	CG (47.6)	GG (17.7)	G = 0.415	0.615	rs495139	CC (32.6)	CG (50.8)	GG (16.6)	G = 0.420	0.247
rs2612101	CC (65.9)	CT (28.6)	TT (5.6)	T = 0.198	**0.011**	rs2612101	CC (69.9)	CT (24.2)	TT (6.0)	T = 0.181	**<.0001**
rs2853533	GG (72.5)	CG (25.2)	CC (2.3)	C = 0.149	0.886	rs2853533	GG (76.3)	CG (22.3)	CC (1.3)	C = 0.125	0.597
rs2853741	CC (49.7)	CT (41.1)	TT (9.2)	T = 0.298	0.687	rs2853741	CC (51.9)	CT (41.4)	TT (6.8)	T = 0.275	0.342

Additional Table 2: Associations for SNPs from three FOCM-related genes

SNP		OR[a] (95% CI)	OR[b] (95% CI)	OR[b,c] (95% CI)
MTHFD1				
rs7151163	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GT	0.96 (0.71-1.30)	0.94 (0.68-1.29)	0.96 (0.71-1.30)
	TT	1.02 (0.74-1.42)	1.00 (0.71-1.41)	
rs8003379	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AC	1.21 (0.96-1.53)	1.24 (0.97-1.58)	1.29 (1.02-1.63)
	CC	1.47 (0.92-2.35)	1.65 (1.00-2.73)	
rs2236225	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.16 (0.90-1.48)	1.13 (0.87-1.47)	1.04 (0.82-1.33)
	TT	0.87 (0.63-1.19)	0.84 (0.60-1.17)	
rs2295638	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.09 (0.67-1.76)	1.26 (0.77-2.07)	1.26 (0.77-2.06)
	TT	0.69 (0.04-11.57)	1.18 (0.06-23.88)	
rs11629135	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	1.08 (0.79-1.47)	1.03 (0.75-1.42)	1.03 (0.75-1.40)
	AA	0.80 (0.26-2.42)	0.98 (0.31-3.09)	
rs3818239	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	1.17 (0.89-1.54)	1.19 (0.90-1.59)	1.16 (0.88-1.53)
	GG	0.63 (0.25-1.57)	0.77 (0.30-2.00)	
rs2281603	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	0.90 (0.72-1.13)	0.92 (0.72-1.17)	0.97 (0.77-1.22)
	GG	1.41 (0.88-2.26)	1.40 (0.85-2.29)	
rs11158542	TT	1.0 (ref)	1.0 (ref)	1.0 (ref)
	TC	1.04 (0.83-1.30)	1.04 (0.82-1.31)	1.05 (0.84-1.32)
	CC	1.14 (0.78-1.67)	1.14 (0.76-1.70)	
rs745686	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	1.19 (0.94-1.50)	1.19 (0.94-1.51)	1.17 (0.93-1.47)
	GG	1.03 (0.72-1.47)	1.10 (0.75-1.60)	
rs1256146	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	0.98 (0.77-1.25)	0.98 (0.76-1.26)	0.93 (0.73-1.19)
	AA	0.65 (0.36-1.18)	0.62 (0.33-1.16)	
rs1956545	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	0.93 (0.68-1.283)	0.95 (0.68-1.32)	0.96 (0.69-1.32)
	GG	1.49 (0.40-5.47)	1.15 (0.31-4.31)	
rs17751556	TT	1.0 (ref)	1.0 (ref)	1.0 (ref)
	TC	0.89 (0.63-1.25)	0.85 (0.60-1.22)	0.85 (0.60-1.21)
	CC	0.74 (0.16-3.47)	0.81 (0.17-4.02)	

rs17824591	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	1.19 (0.94-1.50)	1.26 (0.99-1.61)	1.33 (1.05-1.68)
	AA	1.73 (1.03-2.91)	1.98 (1.14-3.41)	
rs2295640	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CG	0.99 (0.74-1.331)	0.98 (0.72-1.32)	0.97 (0.72-1.31)
	GG	0.94 (0.23-3.82)	0.84 (0.20-3.53)	
MTHFR				
rs4846047	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GC	1.06 (0.85-1.34)	1.09 (0.86-1.39)	1.08 (0.86-1.35)
	CC	1.05 (0.71-1.54)	1.02 (0.68-1.53)	
rs4846049	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GT	1.13 (0.89-1.42)	1.14 (0.90-1.46)	1.12 (0.89-1.41)
	TT	1.05 (0.73-1.50)	1.06 (0.73-1.54)	
rs1476413	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	1.07 (0.85-1.34)	1.03 (0.81-1.31)	1.10 (0.88-1.39)
	AA	1.49 (0.97-2.28)	1.56 (1.00-2.44)	
rs17375901	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.05 (0.72-1.54)	1.01 (0.68-1.50)	1.05 (0.71-1.55)
	TT	1.82 (0.30-11.06)	2.37 (0.37-15.34)	
rs12121543	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CA	1.08 (0.85-1.36)	1.05 (0.82-1.34)	1.09 (0.86-1.38)
	AA	1.30 (0.82-2.05)	1.34 (0.84-2.15)	
rs6541003	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	1.06 (0.83-1.35)	1.08 (0.84-1.39)	1.07 (0.85-1.35)
	GG	1.02 (0.74-1.40)	1.05 (0.76-1.46)	
rs1801133	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.34 (1.05-1.71)	1.35 (1.05-1.74)	1.28 (1.01-1.63)
	TT	1.04 (0.72-1.49)	1.06 (0.72-1.55)	
rs17421462	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	0.89 (0.65-1.23)	0.88 (0.63-1.22)	0.93 (0.67-1.28)
	AA	1.61 (0.52-5.06)	2.23 (0.66-7.53)	
rs2066471	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	1.13 (0.89-1.44)	1.14 (0.89-1.46)	1.12 (0.88-1.42)
	AA	0.94 (0.50-1.76)	0.93 (0.48-1.80)	
rs9651118	TT	1.0 (ref)	1.0 (ref)	1.0 (ref)
	TC	1.03 (0.81-1.30)	0.98 (0.77-1.26)	0.97 (0.77-1.23)
	CC	0.94 (0.52-1.71)	0.86 (0.46-1.61)	
rs17037425	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)

	AG	1.15 (0.89-1.49)	1.16 (0.88-1.52)	1.11 (0.85-1.44)
	AA	0.68 (0.29-1.56)	0.62 (0.26-1.49)	
TYMS				
rs2853532	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	0.93 (0.74-1.17)	0.94 (0.74-1.19)	0.97 (0.77-1.22)
	TT	1.05 (0.72-1.54)	1.11 (0.74-1.67)	
rs1004474	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	0.96 (0.75-1.23)	0.98 (0.76-1.27)	1.01 (0.79-1.29)
	GG	1.05 (0.77-1.43)	1.10 (0.79-1.53)	
rs2847153	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	1.07 (0.85-1.35)	1.10 (0.87-1.41)	1.11 (0.88-1.41)
	AA	1.16 (0.64-2.10)	1.22 (0.66-2.27)	
rs9948583	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.07 (0.85-1.35)	1.10 (0.87-1.40)	1.11 (0.88-1.39)
	TT	1.09 (0.74-1.61)	1.14 (0.76-1.72)	
rs3744962	TT	1.0 (ref)	1.0 (ref)	1.0 (ref)
	TC	1.17 (0.88-1.56)	1.15 (0.85-1.56)	1.12 (0.83-1.51)
	CC	0.71 (0.23-2.20)	0.68 (0.21-2.21)	
rs16948305	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.13 (0.87-1.46)	1.08 (0.83-1.42)	1.04 (0.80-1.35)
	TT	0.54 (0.24-1.23)	0.60 (0.25-1.44)	
rs2298582	AA	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AC	0.79 (0.60-1.03)	0.79 (0.60-1.06)	0.83 (0.63-1.10)
	CC	1.09 (0.44-2.68)	1.55 (0.59-4.04)	
rs495139	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CG	0.87 (0.69-1.11)	0.87 (0.67-1.11)	0.89 (0.70-1.13)
	GG	0.99 (0.72-1.37)	0.95 (0.68-1.33)	
rs2853533	GG	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GC	1.26 (0.98-1.64)	1.34 (1.02-1.76)	1.38 (1.05-1.80)
	CC	1.91 (0.81-4.50)	2.03 (0.84-4.94)	
rs2853741	CC	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	1.03 (0.81-1.31)	1.03 (0.80-1.33)	1.08 (0.85-1.38)
	TT	1.42 (0.91-2.20)	1.41 (0.89-2.23)	

a) Age-adjusted only; b) Multivariate ajusted for: age, sex, kcal, bmi, NSAID use, physical activity, family history of CRC, smoking, education, alcohol, methionine, fiber, calcium, and folate; c) Dominant model - homozygous and heterozous individuals for variant allele combined

Additional Table 3: Haplotype associations with CRC risk

	Haplotype Block	Freqency	OR (95% CI)*
		MTHFD1	
Block 1[a]	T-A	0.559	1.0 (ref)
	G-C	0.238	1.16 (0.83-1.63)
	G-A	0.203	0.78 (0.43-1.43)
Block 2[b]	C-T-C-A	0.444	1.0 (ref)
	C-C-C-A	0.430	1.12 (0.81-1.54)
	T-C-C-G	0.084	1.11 (0.79-1.55)
	C-C-T-G	0.025	0.97 (0.43-2.16)
Block 3[c]	A-T-A-G	0.467	1.0 (ref)
	G-C-G-G	0.223	1.01 (0.78-1.31)
	A-T-A-A	0.185	0.67 (0.41-1.10)
	A-C-G-G	0.083	0.90 (0.28-2.83)
	A-T-G-G	0.029	1.78 (0.96-3.29)
	G-T-A-G	0.011	1.23 (0.54-2.81)
		MTHFR	
Block 1[d]	G-G-G-C-C-A-T-G-G-T	0.357	1.0 (ref)
	G-G-G-C-C-A-C-G-G-C	0.192	0.79 (0.56-1.12)
	C-T-A-C-A-G-C-G-A-T	0.144	0.99 (0.55-1.81)
	C-G-G-C-C-G-C-A-G-T	0.079	1.63 (0.46-5.75)
	G-T-A-T-A-G-C-G-G-T	0.050	0.91 (0.60-1.39)
	G-T-A-C-A-G-C-G-G-T	0.043	1.37 (0.86-2.16)
	C-T-G-C-C-G-C-G-G-T	0.037	0.65 (0.30-1.42)
	G-G-G-C-C-A-C-G-G-T	0.036	0.54 (0.27-1.10)
	C-T-A-C-C-G-C-G-A-T	0.026	0.89 (0.31-2.59)
	G-T-G-C-C-G-C-G-G-T	0.013	0.42 (0.21-0.85)
		TYMS	
Block 1[e]	C-A-G-C-T-C	0.449	1.0 (ref)
	T-G-A-T-T-C	0.205	1.02 (0.75-1.38)
	C-G-G-C-T-T	0.123	0.87 (0.61-1.26)
	T-G-G-T-T-C	0.097	0.94 (0.66-1.35)
	C-A-G-C-C-C	0.092	1.24 (0.31-4.96)
	T-G-G-C-T-C	0.013	0.72 (0.40-1.31)

	C-G-G-T-T-T	0.011	3.33 (1.02-10.90)
Block 2[f]	C-A	0.466	1.0 (ref)
	G-A	0.419	0.88 (0.66-1.16)
	C-C	0.113	0.97 (0.64-1.48)

*Multivariate ajusted for: age, sex, kcal, bmi, NSAID use, physical activity, family history, smoking, education, alcohol, methionine, fiber, calcium, and folate

a) rs7151163 rs8003379

b) rs11627525 rs2236225 rs2295638 rs3818239

c) rs2281603 rs11158542 rs745686 rs1256146

d) rs4846047 rs4846049 rs1476413 rs17375901 rs12121543 rs6541003 rs1801133 rs17421462 rs2066471 rs9651118

e) rs2853532 rs1004474 rs2847153 rs9948583 rs3744962 rs16948305

f) rs495139 rs2298582

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SELECTED PUBLICATIONS

1. **Ashmore JH**, Lesko SM, Miller PE, Cross AJ, Muscat JE, Zhu J, Liao J, Harper G, Lazarus P, Hartman TJ (2012). Association of dietary and supplemental iron and colorectal cancer in a population-based study. *European Journal of Cancer Prevention.* (In Press).
2. **Ashmore JH**, Rogers CJ, Kelleher SL, Lesko SM, Hartman TJ (2012). Dietary iron and colorectal cancer: a review of human population studies. *Critical Reviews in Food Science and Nutrition*. (In Press).
3. Larson MK, Shearer GC, **Ashmore JH**, Anderson-Daniels JM, Graslie EL, Tholen JT, Vogelaar JL, Korth AJ, Nareddy V, Sprehe M, Harris WS (2011). Omega-3 acids modulate collagen signaling in human platelets. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 84(3-4), 93-98. PMID: 21177087.
4. Larson MK, **Ashmore JH**, Harris KA, Vogelaar JL, Pottala JV, Sprehe M, Harris WS (2008).Effects of omega-3 acid ethyl esters and aspirin, alone and in combination, on platelet function in healthy subjects. *Thrombosis and Haemostasis, 100*(4), 634–641. PMID: 18841286

HONORS AND AWARDS

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