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Oxidative Balance and Colon and Rectal Cancer: interaction of lifestyle factors and genes

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Abstract

Pro-oxidant and anti-oxidant genetic and lifestyle factors can contribute to an individual's level of oxidative stress. We hypothesize that diet, lifestyle and genetic factors work together to influence colon and rectal cancer through an oxidative balance mechanism. We evaluated nine markers for eosinophil peroxidase (*EPX*), two for myeloperoxidase (*MPO*), four for hypoxia-inducible factor-1A (*HIF1A*), and 16 for inducible nitric oxide synthase (*NOS2A*) in conjunction with dietary antioxidants, aspirin/NSAID use, and cigarette smoking. We used data from population-based case-control studies (colon cancer n=1555 cases, 1956 controls; rectal cancer n=754 cases, 959 controls). Only *NOS2A* rs2297518 was associated with colon cancer (OR 0.86 95% CI 0.74,0.99) and *EPX*rs2302313 and *MPO*rs2243828 were associated with rectal cancer (OR 0.75 95% CI 0.59,0.96; OR 0.81 95% CI 0.67,0.99 respectively) for main effects. However, after adjustment for multiple comparisons we observed the following significant interactions for colon cancer: *NOS2A* and lutein, *EPX* and aspirin/NSAID use, and *NOS2A* (4 SNPs) and cigarette smoking. For rectal cancer we observed the following interactions after adjustment for multiple comparisons: *HIF1A* and vitamin E, *NOS2A* (3SNPs) with calcium; *MPO* with lutein; *HIF1A* with lycopene; *NOS2A* with selenium; *EPX* and *NOS2A* with aspirin/NSAID use; *HIF1A*, *MPO*, and *NOS2A* (3 SNPs) with cigarette smoking. We observed significant interaction between a composite oxidative balance score and a polygenic model for both colon (p interaction 0.0008) and rectal cancer (p=0.0018). These results suggest the need to comprehensively evaluate interactions to assess the contribution of risk from both environmental and genetic factors.

Keywords

Oxidative balance; colon cancer; rectal cancer; diet; smoking; NSAIDs; *EPX*; *NOS2A*; *MPO*; *HIF1A*

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Introduction

Oxidative stress is caused by an imbalance between pro- and anti-oxidants. Prolonged environmental stress stemming from this imbalance increases and sustains reactive oxygen species (ROS) including free radicals, oxygen ions, and peroxides.¹ High levels of ROS, as well as reactive nitrogen species (RNS) such as nitric oxide (NO), can damage DNA and cell structure and function, resulting in cell proliferation and genomic instability.¹ Several health conditions, including chronic inflammation and cancer initiation and progression, have been linked to oxidative stress. Pro-oxidant and anti-oxidant genetic and lifestyle factors can contribute to an individual's level of oxidative stress. Smoking cigarettes can increase oxidative stress while dietary antioxidants can function to reduce ROS. Vitamins A, C, and E detoxify free radicals directly; vitamin E also plays an important role in cellular defense against lipid peroxidation and degradation of cell membrane by free radicals. Calcium, vitamin D, and folic acid have fairly consistently been associated with colorectal cancer^{2, 3} and have been shown to influence oxidative stress levels.^{4, 5} Genes involved in modulating ROS could directly influence oxidative stress as well as influence disease susceptibility through interactions with pro- and anti-oxidant factors.

Both eosinophil peroxidase (*EPX*) and myeloperoxidase (*MPO*) are located at 17q23. *EPX* encodes a protein that catalyzes the formation of a cytotoxic oxidant that has been implicated in several inflammation-related diseases and cancer.⁶ *MPO* generates reactive oxidant species as part of its function in innate host defense mechanisms that can lead to damage of normal tissue and contribute to inflammatory injury. Polymorphisms in *MPO* have been implicated in lung.⁷ Levels of MPO have been shown to be significantly higher among individuals with ulcerative colitis who develop colorectal cancer versus those without cancer but with ulcerative colitis.⁸ Hypoxia-inducible factor-1A (HIF1A) is one of the transcription factors modulated by ROS⁹ in response to oxidative stress. Inducible nitric oxide synthase (NOS2A) is induced by cytokines and has been reported to play a role in oxidative stress-induced inflammation. NOS2 expression has been associated with *p53* mutations¹⁰; salicylates have been shown to inhibit the expression and function of the enzyme¹¹

In this study we examine the association between genetic variation in the *EPX*, *HIF1A*, *MPO*, and *NOS2A* genes with risk of developing colon and rectal cancer. These genes were assessed as they were originally identified as part of the CHIEF pathway¹² given their involvement in inflammation processes. We evaluate associations in conjunction with diet and lifestyle factors that could alter susceptibility to these genes because of their pro and antioxidant properties. Dietary factors we evaluate are beta carotene, lycopene, lutein, iron, polyunsaturated fat, vitamin D, selenium, tocopherol, vitamin D, calcium, and folic acid. We assess use of aspirin/NSAIDs and cigarette smoking given their association with inflammation and ROS.^{13–15} We create an oxidative balance score (OBS) as described by Goodman and colleagues¹⁶ that summarizes diet and lifestyle oxidative load as a way to consolidate the risk from all diet and lifestyle exposures. We focus not only on the main effects of these genetic factors, but importantly on the interactive effects of these factors with each other and with lifestyle factors. Our underlying hypothesis is genetic and diet and lifestyle factors associated with oxidative balance work together to alter risk of colorectal cancer.

Methods

Two study populations are included. The first, a population-based case-control study of colon cancer, included cases (n=1,555) and controls (n=1,956) identified between October 1, 1991 and September 30, 1994 living in the Twin Cities Metropolitan Area, Kaiser

Permanente Medical Care Program of Northern California (KPMCP) and a seven-county area of Utah¹⁷. The second study used identical data collection methods as the first study but included population-based cases with cancer of the recto-sigmoid junction or rectum (n=754) and controls (n=959) who were identified between May 1997 and May 2001 in Utah and KPMCP.¹⁸ Eligible cases were between 30 and 79 years old at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of CRC, and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. Controls were matched to cases by sex and by 5-year age groups. At KPMCP, controls were randomly selected from membership lists. In Utah, controls 65 years and older were randomly selected from the Health Care Financing Administration lists and controls younger than 65 years were randomly selected from driver's license lists. In Minnesota, controls were selected from driver's license and state-identification lists. Study details have been reported.^{17, 18}

Interview Data Collection

Data were collected by trained and certified interviewers using laptop computers. All interviews were audio-taped and reviewed for quality control purposes.¹⁹ The referent period for recall of diet was two years prior to diagnosis for cases and prior to selection for controls. Detailed information was collected on diet,²⁰ medical history, cigarette smoking history, regular use of aspirin and non-steroidal anti-inflammatory drugs, and body size. The NCC nutrient database was used to calculate nutrients from reported foods on the extensive diet history questionnaire. The diet history questionnaire obtained detailed information on quantity, frequency, method of preparation, and serving size for each food item eaten.^{20, 21}

TagSNP Selection and Genotyping

TagSNPs were selected using the following parameters: LD blocks were defined using a Caucasian LD map and an $r^2=0.8$; minor allele frequency (MAF) >0.1 ; range= -1500 bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin as described by Carlson²². Additionally, we included SNPs that were putatively functional. This procedure generated nine markers for *EPX*, two for *MPO*, four for *HIF1A*, and 16 for *NOS2A*. All markers were genotyped using a multiplexed bead-array assay format based on GoldenGate chemistry (Illumina, San Diego, California). A genotyping call rate of 99.85% was attained. Blinded internal replicates represented 4.4% of the sample set; the duplicate concordance rate was 100%. Individuals with missing genotype data were not included in the analysis for that specific marker.

Statistical Methods

Statistical analyses were performed for each study independently using SAS® version 9.2 (SAS Institute, Cary, NC) unless otherwise indicated. The linkage disequilibrium (LD) measure, minor allele frequency (MAF) and test for Hardy-Weinberg Equilibrium (HWE) were calculated among white controls using the SAS ALLELE procedure using the International HapMap data. We report odds ratios (ORs) and 95% confidence intervals (CIs) assessed from multiple logistic regression models adjusting for age, center, race/ethnicity, and sex, which were matching variables for the original studies. Analysis for interaction was based on tagSNPs within each gene. All SNPs were evaluated first by comparing the heterozygote and homozygote variant to the homozygote wildtype and subsequently assessing the dominant and recessive models; the best fitting inheritance model is presented in Tables 3 to 5 for those SNPs that were initially significant. SNPs with a minor allele frequency of <0.15 were assigned a dominant model when a recessive model was not indicated for main effect associations. When evaluating interactions a dominant model was used if the minor allele frequency was <0.30 . Diet and lifestyle variables were selected because of their biological plausibility for involvement in this candidate pathway.

Interactions between genes and hypothesized exposures associated with inflammation and oxidative stress included dietary factors with antioxidant properties (including dietary vitamin C, vitamin E, selenium, beta carotene, lycopene, lutein, vitamin D, calcium, and folic acid),²³ and dietary factors with pro-oxidant properties (including dietary iron and polyunsaturated fat). Recent use of aspirin or NSAID was considered an antioxidant given its role as anti-inflammatory drug, and cigarette smoking within two years prior to diagnosis versus not being a recent smoker, was considered a pro-oxidant. Dietary factors were obtained from a detail diet history questionnaire that was linked to the NCC database. Dietary factors were categorized based on sex-specific quartiles among the controls, collapsing the second and third quartiles to form an intermediate group. In addition to the minimal adjustments, logistic regression models involving dietary variables also control for total energy intake. P values for interaction were determined using a 1df likelihood-ratio test comparing a full model that included an interaction term to a reduced model without an interaction term.

To summarize risk associated with both exposures and multiple variants across the pathway, summary scores were developed for an oxidative balance score (OBS) that consisted of diet and lifestyle factors pro- and anti-oxidants as outlined above and a genotype score. In creating the OBS, participants were assigned values of zero for low levels of exposure to anti-oxidants (vitamin C, vitamin E, selenium, beta carotene, lycopene, lutein, vitamin D, calcium, and folic acid and NSAID use) or high exposure to pro-oxidants (high-risk) (dietary iron and polyunsaturated fat and cigarette smoking), one for intermediate levels of exposure, and two for high levels of exposure to anti-oxidants and low exposure to pro-oxidants (low-risk). For dichotomous variables such as NSAID use and smoking, a zero was assigned for those at high-risk (never or non-recent NSAID users; current or recent smokers) and a two for low-risk (current or recent NSAID users; never or non-recent smoker). The summary polygenic score was based on all at-risk genotypes identified as interacting with the OBS was developed similar to those previously reported.²⁴ The high-risk allele and inheritance model for each SNP were based on the main effect risk estimates. For the co-dominant or additive model a score of zero, one, or two was assigned directly correlated to the number of high-risk alleles, while scores of zero or two were assigned for the dominant and recessive models. After assigning a score for each exposure and for each SNP previously identified as being significant, the scores were summed to generate an individual OBS and a polygenic summary score. Individuals missing exposure and/or SNP data were dropped from the analysis. The continuous score variables were then redefined as approximate quartiles.

Adjusted multiple-comparison p values, taking into account all tests run for tagSNPs within the gene, were estimated using the step-down Bonferroni correction (i.e. Holm method²⁵) based on the effective number of independent markers as determined using SNPSpD²⁶ (<http://genepi.qimr.edu.au/general/dateN/SNPSpD>). Wald p values from the main effect models and interaction p values based on likelihood-ratio tests were used for estimates of multiple comparisons. Given our candidate pathway approach, we consider an adjusted p value of 0.10 as potentially important for main effects and 0.15 as potentially meaningful for interactions.

Additionally, we used a max T permutations procedure²⁷ to further evaluate interactions and correspondingly adjust for multiple comparisons for those variables where confounding was not an issue, including NSAIDs and cigarette smoking. We adapted the logical pattern SNP-SNP interaction framework of Boulesteix et al.²⁸ to further evaluate interactions and correspondingly adjust for multiple comparisons for those variables where confounding was not an issue, including NSAIDs and cigarette smoking. Given the genotypes for a SNP and level of exposure upon a categorical environmental factor, this procedure involved

simultaneously testing the null hypothesis of no association between case-control status and status of a logical pattern involving these explanatory variables, among several logical patterns. For example, consider the logical pattern of individuals who are homozygous for the wild-type allele at a SNP locus and exposed to a binary environmental factor. Rejection of the null hypothesis for this logical pattern would indicate a statistically significant SNP-environment interaction. Adjustment for multiple testing was carried out by using the so-called maxT procedure of Westfall and Young.²⁷ We employed 100,000 maxT permutations upon each SNP-environment pairing. Hypothesis tests involving logical patterns in genotype and level of exposure between an individual SNP and a single environmental factor were mutually adjusted for by comparing each observed test statistic to the permutation null distribution of the maximum test statistic over all tests conducted upon the SNP. This adjustment was then expanded to mutually adjust for all pairings between a single environmental factor and all SNPs within a gene. This method also allowed for partitioning of the data to better identify and categorize the most meaningful units where the interactions occurred. This statistic complements our analyses by more specifically defining the interaction, in addition to using a more robust permutations adjustment for multiple comparisons.

Results

The population is described in Table 1. The majority of participants were non-Hispanic white. The majority of cases were male (56.0% of colon and 59.8% of rectal) and over 60 years of age (73.3% of colon cancer cases and 58.8% of rectal study). A description of candidate genes, *EPX*, *MPO*, *HIF1A*, and *NOS2A* are shown in Table 2. All SNPs were in Hardy Weinberg Equilibrium (HWE). *EPX* rs2302311 was excluded from the analysis as it had a MAF of 0.0055, as was *NOS2A* rs2297516 as it shared an r^2 of 0.84 with rs9906835. A summary of all SNP main effects for colon and rectal cancer can be found in an online supplement.

NOS2A rs2297518 demonstrated a weak association with colon cancer and also interacted with dietary intake of beta carotene and lutein prior to multiple comparison adjustment (Table 3). Prior to adjustment for multiple comparisons, *NOS2A* rs2274894 interacted significantly with vitamin D, rs7406657 with beta carotene, rs4795067 with calcium, rs3794763 and rs944725 with lycopene, and rs2779248 with selenium intake while showing no main effect. *EPX* rs2240815 interacted with vitamin D, folic acid, and selenium, rs12602498 interacted with iron and selenium, rs11739339 with lutein, and rs9892223 with selenium. Two of the four *HIF1A* SNPs, rs11549465 and rs1951795, interacted with polyunsaturated fat. Adjusted p values showed a value of 0.022 for *NOS2A* rs2297518 and lutein and 0.085 for *HIF1A* rs11549465 and rs11549465 and polyunsaturated fat.

We observed few significant main effect associations with rectal cancer, which generally disappeared after adjustment for multiple comparisons (Table 4). *MPO* rs2243828 showed a modest protective effect among those with a variant allele (OR 0.81; 95% CI 0.67,0.99; adjusted p value of 0.072) as did *EPX* rs2302313 (OR 0.75; 95% CI 0.59,0.96; adjusted p value of 0.140). However, we observed numerous interactions, many of which were significant at the 0.15 level or lower after adjustment for multiple testing. One of two SNPs assessed in the *MPO* gene was associated with significant interactions with beta carotene, folic acid, lutein, and selenium. The adjusted p values were 0.096, 0.061, 0.029, and 0.080, respectively. Of the 15 SNPs in *NOS2A* assessed, none were associated with rectal cancer independently, four interacted significantly with vitamin D intake, four with vitamin E intake, five with calcium intake, eight with folic acid intake, one with lutein, four with polyunsaturated fat, and three with selenium intake prior to adjustment for multiple comparisons. All interactions between SNPs in *NOS2A* and calcium had adjusted p values

0.15 (0.016, 0.012, 0.016, 0.056, and 0.056). Although eight SNPs interacted with folic acid (53.3% of SNPs assessed), only one had an adjusted p value <0.15. *HIF1A* interacted significantly with vitamin E and lycopene, also showing no main effect (main effect p value 0.804; interaction p value 0.013 and adjusted p value of 0.026 for vitamin E and interaction p value 0.009 and adjusted p value of 0.018 for lycopene).

EPX interacted with recent aspirin use for rectal cancer (adjusted p value of 0.086) (Table 5). *NOS2A* (4 SNPs for colon and 3 SNPs for rectal) interacted with smoking to alter risk of cancer. However only one of the three SNPs associated with rectal cancer, rs9906835, had an adjusted p value 0.15, adjusted p value 0.043. Both *HIF1A* and *MPO* were associated with significant interactions with cigarette smoking to alter rectal cancer risk (adjusted p value of 0.014 and 0.040 respectively). The maxT statistic was significant for the interaction between *EPX* and aspirin/NSAID where those without recent aspirin/NSAID use and either the wildtype or heterozygote genotypes were at significantly greater risk of colon cancer than the rest of the population. Similar interactions were statistically significant using the maxT statistic for rectal cancer risk, partitioned between those with the heterozygote or homozygote variant and recent aspirin/NSAID use. *NOS2A* also significantly interacted with aspirin/NSAID use using the maxT; those with the wildtype or heterozygote genotype and no aspirin/NSAID use were at the highest risk for rectal cancer. Additionally, smoking interactions with *HIF1A*, *MPO*, and *NOS2A* (rs9906835) were significant with rectal cancer based on the maxT adjustment for multiple comparisons.

Our OBS was associated with reduced risk of both colon and rectal cancer (data not shown in table). For colon cancer the associated ORs and corresponding 95% CI from low quartile (referent) to high OBS were 0.81 (0.67,0.98); 0.69 (0.57,0.85), and 0.52 (95% CI 0.41,0.66). The comparable risk estimates for rectal cancer were 0.64 (0.48,0.84), 0.70 (0.52,0.94), and 0.49 (0.35,0.70). The summary genotype score including *EPX* rs12602498, rs2240815, rs9892223, *NOS2A* rs16949, rs4795067, and rs8072199 was not significantly associated with colon cancer while the score composed of *HIF1A* rs1951795, *MPO* rs2243828, *NOS2A* rs10459953, rs16949, rs2274894, rs2297518, rs2779248, and rs4795067 was modestly associated with rectal cancer risk (linear trend p value of 0.04).. However, the OBS score interacted significantly with the genetic risk score for both colon (p interaction 0.0008) and rectal cancer (p interaction 0.0018) (Figure 1). For both groups those at the highest risk were those with the high risk genotype score and the low OBS. For colon cancer this risk was 2.18 (95% CI 1.36,3.50) while the high-risk genotype score was inversely associated with colon cancer in the presence of a high OBS (OR 0.51, 95% CI 0.30,0.86). Similar trends were observed for rectal cancer with the high-genotype risk having an OR of 1.95 (95% CI 1.02,3.75) in the presence of low OBS and an OR of 0.39 (95% CI 0.19,0.80) when the OBS was high.

Discussion

Genetic variation in genes that modulate reactive oxygen species may influence colon and rectal cancer through their interaction with pro-oxidant and anti-oxidant diet and lifestyle factors. Dietary antioxidants including dietary calcium, vitamin D, vitamin E, and folic acid interacted with all genes assessed with more consistent associations for rectal cancer than for colon cancer. The cancer risk associated with cigarette smoking, a pro-oxidant factor, was modified by these genes. Our data suggest that diet, lifestyle, and genetic factors contribute to colon and rectal cancer risk through their influence on oxidative stress. Those individuals that had an inherited genetic profile placing them at higher risk for oxidative stress can reduce their colon and rectal cancer risk by diet and lifestyle factors that offset their inherited susceptibility.

MPO showed both overall and interacting associations with rectal cancer with several diet and lifestyle factors. *MPO* is a marker of activated leucocytes and are related to the production of oxygen free radicals.²⁹ It has been shown that patients with colorectal cancer have higher levels of *MPO*-positive cells than controls.³⁰ Reports of a functional SNP that is in 100% concordance with *MPO* rs2243828, which we analyzed, have shown that variant allele of the SNP result in decreased gene activity and lower myeloperoxidase levels.^{31, 32} Given the harmful effects of myeloperoxidase, one would expect lower risk with the variant allele, such as we observed. Additionally, *MPO* importantly interacted with both pro- and anti-oxidants to alter rectal cancer risk, with the expected pattern of risk. Those with the high-risk genotype (i.e. wildtype) were at reduced risk of rectal cancer in the presence of high anti-oxidants, while in the presence of pro-oxidants experienced increased risk.

Both *HIF-1A* and *NOS2A* have been cited as key molecular players linking inflammation to cancer.¹⁰ *HIF-1A* is one of the transcription factors activated by oxidative stress, which increases expression of inflammatory cytokines, cell-cycle regulatory and anti-inflammatory molecules.⁹ Inflammation is felt to be a key component to colorectal cancer development and progression. Studies have shown increased *HIF-1A* expression in early stages of colorectal cancer.^{33, 34} We observed significant interactions between *HIF1A* and vitamin E and cigarette smoking and rectal cancer and with polyunsaturated fatty acids and colon cancer. Patterns of association were the same as observed for *MPO* with the exposure of high vitamin E intake or smoking cigarettes having the greatest impact on those with the wild-type genotype. Vitamin E has numerous antioxidant properties and unlike other antioxidants is essential for membrane integrity.³⁵ Tocopherols can reduce ROS by reacting with lipid-soluble peroxy radicals to reduce their propagation. Polyunsaturated fatty acids act as pro-oxidants given their role in lipid peroxide formation.³⁶

Inducible nitric oxide synthase (*NOS2*) is induced by inflammatory cytokines and hypoxia and produces large amounts of nitric oxide. Nitric oxide can affect cancer through many ways, it can increase apoptosis and inhibit carcinogenesis or promote carcinogenesis through increasing angiogenesis.³⁷ *NOS2A* and other inflammation-related genes have been associated with micro-RNA 21 expression in colon tumors.³⁸ *NOS2A* codes for inducible nitric oxide 2 and polymorphisms in the gene have been examined in conjunction with colorectal cancer. One study examined the -524T>C polymorphism in *NOS2A* and did not detect an association with colorectal cancer³⁹; others have looked at polymorphisms in the promoter region of *NOS2A* and likewise did not observe significant associations with colorectal cancer.⁴⁰ Failure to account for important effect modifiers such as diet and cigarette smoking could have inhibited detection of an association. This point is further supported by the interaction between dietary antioxidants and *NOS2A* polymorphisms to alter risk of prostate cancer when only marginal associations were observed prior to consideration of this interaction.⁴¹

We and other have shown that associations between dietary calcium, vitamin D, vitamin E, and folic acid are stronger for rectal cancer than for colon cancer.^{2, 3, 42–45} We detected more significant interactions between *NOS2A*, *MPO*, *HIF1A*, and *EPX* and calcium, vitamin D, and folic acid with rectal cancer than colon cancer. We believe that the high proportion of SNPs within these genes showing significant interaction gives support that these observations are more than chance findings. These findings also direct attention to biological mechanism of these dietary factors that have not been fully examined. Calcium signaling is influenced by oxidative stress.⁴⁶ Calcium and vitamin D have been shown to decrease oxidative DNA damage in colorectal mucosa.⁵ Folic acid has been shown to have antioxidative properties.^{4, 47, 48} Folic acid treatment has been shown to inhibit homocysteine-induced oxidative stress and inflammatory responses in macrophages; this could be one of the mechanisms by which folic acid is associated with reduced risk of

colorectal cancer and provide a mechanisms why aspirin and folic acid interact to alter colorectal cancer risk.^{49–52}

The gastrointestinal tract is constantly exposed to ROS from both endogenous and exogenous factors.²⁹ Dietary intake, cigarette smoking, and aspirin/NSAIDs use are involved in the regulation of ROS and RNS and the subsequent inflammation and cellular damage that results from oxidative stress. To capture the effects of these pro- and anti-oxidants, we have created an oxidative balance score, using the methods that have previously been reported by Goodman and colleagues.¹⁶ The OBS was associated with a strong linear trend of protection against both colon and rectal cancer in our study in a similar manner as reported by Goodman and colleagues. However, we also demonstrate that OBS modifies the increased risk associated with genetic factors that increase oxidative stress. This strong modification of risk has clear implications for prevention among those who have inherited genetic susceptibility to increased oxidative stress. These data further support the role of oxidative stress in the development of colon and rectal cancer.

We believe the study is informative for others interested in assessment of candidate pathways and disease. An analytic issue is the possibility of chance findings and therefore an enforcement of a penalty for making multiple comparisons is made. To minimize comparisons, identification of an optimal cut-point for main effect p values that can guide further interaction analysis is desirable. Some programs have used a p value of 0.15 as a standard to take SNPs into interaction assessment, feeling that at that level of main effect, important interaction associations will not be missed.⁵³ Our data suggest that the main effect association has limited correlation with interaction effects. This is reasonable if one considers that to detect statistically significant interactions the associations are different across categories of exposure. In most instances, our data suggest that associations are biased to null when interaction is not considered. Our data support the need to evaluate interactions with all SNPs to detect important interactions. For instance, roughly 1/3 to 1/2 of *NOS2A* SNPs showed interaction with dietary variables such as folate and calcium for rectal cancer, while none were associated independently with rectal cancer. *MPO* was the only gene that showed both an independent association with rectal cancer and interacted with multiple pro- and anti-oxidants. Thus, to determine the impact of a gene on disease risk, it is essential to consider both the independent effects as well as the interactive effects with other genes and lifestyle and environmental factors that share the biological pathway of interest.

We used two methods to adjust for multiple comparisons, the Bonferroni correction which takes into account the correlated nature of the SNP data and the maxT which relies on permutation methods to adjust for multiple comparisons. Several interactions were significant after adjusting for multiple comparisons by both methods. However the maxT method appears to be more robust for many interactions. This could stem from the partitioning of data into categories that best describe the interaction. The traditional Bonferroni statistic is based on our results from logistic regression models that rely on a common referent point and difference in effects across cells of environmental and genetic exposures. We believe that these two methods are complementary, reinforcing the associations that are significant after multiple testing adjustment and helping to define the elements of the data that are interacting. While the maxT provided an additional robust assessment of important interactions for those variables where confounding was not an issue, we were unable to utilize this method for our dietary variables, where considerable confounding from calories and other variables existed.

Major strengths of our study were the hypothesis-driven approach, the large and extensive data set that includes information on genetic, diet, and lifestyle data, and our ability to

examine colon and rectal cancer separately. While we believe that the data we present is both thorough and informative, we acknowledge that limitations exist. Dietary data are based on recall and since SNPs assessed were tagSNPs to assess variation across the gene could lead to missing important associations. Likewise, the majority of SNPs assessed had a MAF of >0.10 so that we would have sufficient power to look at interactions, which could lead to our missing associations with rarer genotypes. For instance, while we have detected associations we have minimal information on the functionality of the SNPs evaluated. Additional lab-based experiments are needed to determine functionality. Through our analysis we have made many comparisons. Although we have provided adjusted p values to take into account multiple comparisons, chance findings may exist and therefore replication of these findings is critical. A hazard of multiple testing adjustments is the increased likelihood of rejecting a finding that is true. Thus, we believe that adjusted p values greater than 0.05 are important especially for interactions and merit replication in other large sample sets to validate these findings. We also believe that the number of SNPs associated within a gene adds further to the likelihood that the gene is associated with colorectal cancer.

In summary, this study supports the hypothesis that oxidative stress increases risk of colon and rectal cancer. Genetic and diet and lifestyle factors that contribute to oxidative balance influence risk. This risk associated with genetic factors is greatest among those with low levels of factors contributing to oxidative balance. Our findings of strong interactions with dietary calcium and folic acid with genetic factors related to oxidative stress suggest that the biological mechanisms through which these dietary factors influence colon and rectal cancer relate to those involving oxidative stress. These findings need replication by other large studies of colon and rectal cancer given the potential of modifying risk from genetic factors by diet and lifestyle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Colon Cancer

Rectal Cancer

Figure 1.

A. Combined effect of polygenic score and oxidative balance score (OBS) and risk of colon cancer.

B. Combined effect of polygenic score and oxidative balance score (OBS) and risk of rectal cancer.

Table. 1

Description of Study population

	Colon		Rectal	
	Controls n (%)	Cases n (%)	Controls n (%)	Cases n (%)
Age	30–39	40 (2.0)	23 (1.5)	19 (2.5)
	40–49	128 (6.5)	102 (6.6)	96 (12.7)
	50–59	326 (16.7)	290 (18.7)	196 (26.0)
	60–69	673 (34.4)	538 (34.6)	250 (33.2)
	70–79	789 (40.3)	602 (38.7)	193 (25.6)
Center	Utah	378 (19.3)	249 (16.0)	365 (38.1)
	KPMCP	787 (40.2)	744 (47.9)	274 (36.3)
	Minnesota	791 (40.4)	562 (36.1)	480 (63.7)
	NHW	1828 (93.5)	1428 (91.8)	625 (82.9)
Race /Ethnicity	Hispanic	75 (3.8)	59 (3.8)	61 (8.1)
	Black	53 (2.7)	68 (4.4)	29 (3.9)
	Asian			29 (3.0)
Sex	Male	1047 (53.5)	870 (56.0)	451 (59.8)
	Female	909 (46.5)	685 (44.1)	303 (40.2)

Table 2

SNPs analyzed in *EPX*, *HIF1A*, *MPO*, and *NOS2A*

Symbol	Alias	Chromosome Location	SNP	Coordinate	Major/Minor Allele	MAF ¹	FDR HWE
<i>EPX</i>	<i>EPO</i>	17q23.1	rs12602891	53625120	T /C	0.45	0.95
	<i>EPP</i>		rs11079339	53625441	A /G	0.15	1.00
			rs10853004	53626496	A /G	0.32	1.00
	<i>EPX-PEN</i>		rs2240815	53627389	A /G	0.46	1.00
<i>HIF1A</i>	<i>HIF-1alpha</i>	14q21-q24	rs12602498	53628207	A /G	0.33	0.97
			rs9892223	53633265	A /G	0.45	1.00
			rs8077426	53635956	G /A	0.22	1.00
			rs2302313	53638025	G /A	0.11	1.00
			rs1951795	61241179	C /A	0.19	0.86
			rs2301113	61276301	A /C	0.23	0.95
<i>MPO</i>	<i>PASD8</i>	17q23.1	rs11549465	61277310	C /T	0.10	1.00
	<i>MOPI</i>		rs6573399	61285478	G /T	0.14	1.00
			rs2759	53703105	A /G	0.03	1.00
			rs2243828	53713883	A /G	0.20	0.96
<i>NOS2A</i>	<i>HEP-NOS</i>	17q11.2-q12	rs7406657	23107817	G /C	0.24	1.00
<i>INOS</i>	rs9906835		23113501	A /G	0.39	0.91	
	rs2297516		23119857	A /C	0.39	0.85	
<i>NOS2</i>			rs2297518	23120724	G /A	0.20	1.00
<i>NOS2B</i>	<i>NOS2B</i>	17q23.1	rs2274894	23123298	G /T	0.40	0.82
			rs2314810	23128237	G /C	0.05	1.00
			rs4795067	23130802	A /G	0.35	0.95
			rs3729508	23133157	G /A	0.41	1.00
			rs3730017	23133229	C /T	0.02	1.00
			rs944725	23133698	C /T	0.39	1.00
<i>NOS3</i>	<i>NOS3</i>	17q23.1	rs3794763	23135353	G /A	0.21	0.90
			rs8072199	23140975	C /T	0.46	0.96
			rs16949	23148826	T /C	0.24	1.00
			rs3730013	23150045	C /T	0.32	1.00

Symbol	Alias	Chromosome Location	SNP	Coordinate	Major/Minor Allele	MAF _I	FDR HWE
			rs10459953	23151645	G / C	0.37	1.00
			rs2779248	23151959	T / C	0.38	0.89

I Minor Allele Frequency (MAF) and FDR-adjusted Hardy-Weinberg Equilibrium (FDR HWE) based on white control population.

Table 3

Main and interactive effects of *EPX*, *HIF1A*, and *NOS2A* and diet and risk of colon cancer

Gene SNP (Model)	Main Effect ¹			Interaction Effect ²						Adjusted P Value	
	Heterozygote ³ OR (95% CI)	Variant (95% CI)	Wald P Value	Wildtype ⁴		Variant ³		Interaction P Value			
				OR	(95% CI)	OR	(95% CI)				
									High		Low
<i>EPX</i>											
rs2240815 (D)	0.88 (0.76, 1.02)		0.102	0.58 (0.40, 0.83)	0.68 (0.51, 0.91)	0.61 (0.45, 0.82)	0.043	0.260			
<i>NOS2A</i>											
rs2274894 (A)	1.02 (0.88, 1.18)	1.16 (0.95, 1.42)	0.330	1.01 (0.72, 1.41)	1.61 (1.08, 2.40)	0.98 (0.63, 1.50)	0.043	0.442			
rs2297518 (D) ⁵	0.86 (0.74, 0.99)		0.033	0.74 (0.58, 0.95)	0.66 (0.49, 0.88)	0.76 (0.57, 1.02)	0.029	0.284			
rs7406657 (D)	1.01 (0.88, 1.16)		0.876	0.71 (0.54, 0.93)	0.82 (0.62, 1.07)	0.89 (0.67, 1.18)	0.028	0.284			
rs4795067 (A)	0.98 (0.85, 1.13)	0.98 (0.78, 1.22)	0.950	0.52 (0.38, 0.72)	0.64 (0.41, 0.99)	0.61 (0.37, 0.99)	0.019	0.190			
<i>EPX</i>											
rs2240815 (D)	0.88 (0.76, 1.02)		0.102	0.51 (0.35, 0.73)	0.66 (0.49, 0.89)	0.54 (0.39, 0.75)	0.022	0.130			
rs9892223 (D)	0.96 (0.83, 1.12)		0.624	0.52 (0.36, 0.75)	0.72 (0.54, 0.97)	0.59 (0.43, 0.81)	0.032	0.160			
rs12602498 (D)	0.94 (0.82, 1.08)		0.379	0.69 (0.50, 0.95)	0.76 (0.58, 1.00)	0.78 (0.57, 1.08)	0.036	0.216			
rs11079339 (D)	0.98 (0.84, 1.14)		0.756	0.64 (0.51, 0.82)	0.78 (0.58, 1.05)	0.78 (0.57, 1.06)	0.041	0.247			
<i>NOS2A</i>											
rs2297518 (D)	0.86 (0.74, 0.99)		0.033	0.59 (0.46, 0.76)	0.63 (0.48, 0.83)	0.69 (0.51, 0.92)	0.002	0.022			
rs3794763 (D)	1.05 (0.92, 1.21)		0.445	1.08 (0.83, 1.41)	1.64 (1.23, 2.17)	1.14 (0.84, 1.53)	0.031	0.283			
rs944725 (D)	1.04 (0.90, 1.20)		0.581	1.23 (0.88, 1.72)	1.59 (1.19, 2.11)	1.2 (0.89, 1.63)	0.020	0.204			
<i>HIF1A</i>											
rs11549465 (D)	0.88 (0.74, 1.04)		0.139	1.36 (1.03, 1.79)	1.09 (0.77, 1.54)	0.9 (0.61, 1.32)	0.047	0.085			
rs1951795 (D)	0.96 (0.84, 1.11)		0.603	1.45 (1.08, 1.95)	1.32 (0.99, 1.76)	1.25 (0.90, 1.74)	0.042	0.085			

Gene SNP (Model)	Main Effect ¹				Interaction Effect ²								Adjusted P Value
	Heterozygote ³		Variant		Wald P Value	Wildtype ⁴		Variant ³		Interaction P Value			
	OR	(95% CI)	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)				
EPX													
rs12602498 (D)	0.94	(0.82, 1.08)			0.379	0.76	(0.54, 1.07)	0.74	(0.56, 0.98)	0.87	(0.62, 1.21)	0.028	0.169
rs2240815 (D)	0.88	(0.76, 1.02)			0.102	0.69	(0.46, 1.03)	0.63	(0.47, 0.86)	0.71	(0.50, 1.01)	0.029	0.169
NOS2A													
rs2779248 (R)			0.88	(0.72, 1.07)	0.212	0.89	(0.67, 1.17)	0.63	(0.41, 0.97)	1.03	(0.66, 1.59)	0.037	0.380

¹ Main effect ORs are relative to wildtype/heterozygote genotype if recessive (R) or wildtype genotype if additive (A) or dominant (D) models

² Interaction ORs are relative to non-smoker/low dietary intake and wildtype/heterozygote genotype if recessive -R- or non-smoker/low dietary intake and wildtype genotype if additive (A) or dominant (D) models

³ Risk estimates are for heterozygote/variant genotypes if dominant model

⁴ Risk estimates are for wildtype/heterozygote genotype if recessive- R- model

⁵ Main effect adjusted *p* value for multiple comparisons of 0.33

Table 4
Main and interactive effects of *EPX*, *HIF1A*, *MPO*, and *NOS2A* and diet and risk of rectal cancer

Gene SNP (Model)	Main Effect ¹				Interactive Effect ²								Adjusted P Value	
	Heterozygote ³		Variant (95% CI)	Wald P Value	Wildtype ⁴				Variant ³					Interaction P Value
	OR	(95% CI)			OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)		
<div>HighLowHigh</div>														
Vitamin D														
<i>EPX</i>														
rs10853004 (A) ⁵	1.02	(0.83, 1.26)	1.41	(1.02, 1.95)	0.109	0.61	(0.39, 0.94)	1.22	(0.57, 2.63)	1.79	(0.92, 3.48)	0.047	0.280	
rs12602891 (A)	0.99	(0.80, 1.23)	1.21	(0.92, 1.58)	0.289	0.59	(0.36, 0.97)	0.94	(0.54, 1.63)	1.27	(0.71, 2.26)	0.049	0.280	
rs2302313 (D) ⁶	0.75	(0.59, 0.96)			0.023	0.79	(0.55, 1.12)	0.83	(0.51, 1.36)	0.54	(0.30, 0.96)	0.600	1.000	
<i>NOS2A</i>														
rs10459953 (A)	0.90	(0.73, 1.11)	0.99	(0.74, 1.33)	0.589	0.96	(0.61, 1.52)	1.34	(0.75, 2.41)	0.41	(0.21, 0.80)	0.025	0.206	
rs16949 (D)	1.03	(0.85, 1.25)			0.789	0.6	(0.40, 0.91)	0.83	(0.56, 1.21)	0.92	(0.59, 1.44)	0.031	0.211	
rs2779248 (A)	0.99	(0.81, 1.22)	1.05	(0.78, 1.41)	0.932	0.57	(0.35, 0.93)	0.7	(0.39, 1.26)	1.03	(0.54, 1.95)	0.029	0.211	
rs3729508 (R)			1.12	(0.87, 1.45)	0.388	0.89	(0.63, 1.27)	1.58	(0.93, 2.70)	0.57	(0.33, 1.01)	0.016	0.164	
rs944725 (R)			1.06	(0.82, 1.38)	0.637	0.67	(0.47, 0.96)	0.58	(0.34, 0.98)	0.95	(0.54, 1.66)	0.017	0.164	
<i>HIF1A</i>														
rs1951795 (D)	0.97	(0.80, 1.19)			0.804	0.52	(0.33, 0.82)	0.76	(0.51, 1.12)	0.8	(0.49, 1.30)	0.013	0.026	
<i>NOS2A</i>														
rs10459953 (A)	0.90	(0.73, 1.11)	0.99	(0.74, 1.33)	0.589	0.91	(0.55, 1.51)	2.14	(1.15, 4.00)	0.49	(0.26, 0.94)	0.005	0.055	
rs2779248 (A)	0.99	(0.81, 1.22)	1.05	(0.78, 1.41)	0.932	0.54	(0.32, 0.92)	0.67	(0.37, 1.21)	1.05	(0.50, 2.21)	0.044	0.357	
rs3729508 (A)	1.03	(0.84, 1.28)	1.14	(0.86, 1.51)	0.661	0.86	(0.50, 1.47)	2.1	(1.13, 3.90)	0.69	(0.38, 1.25)	0.045	0.357	
rs4795067 (A)	0.95	(0.78, 1.17)	0.87	(0.64, 1.19)	0.674	0.5	(0.31, 0.83)	0.66	(0.37, 1.21)	0.78	(0.35, 1.78)	0.048	0.357	
<i>MPO</i>														
rs2243828 (D) ⁷	0.81	(0.67, 0.99)			0.038	0.54	(0.37, 0.78)	0.62	(0.42, 0.92)	0.59	(0.39, 0.89)	0.051	0.096	
<i>NOS2A</i>														
rs16949 (D)	1.03	(0.85, 1.25)			0.789	0.43	(0.28, 0.66)	0.66	(0.45, 0.96)	0.68	(0.43, 1.09)	0.002	0.016	
rs2274894 (R)			1.17	(0.90, 1.53)	0.247	0.74	(0.51, 1.08)	2.31	(1.31, 4.07)	0.47	(0.26, 0.86)	0.001	0.012	
rs2297518 (D)	1.06	(0.87, 1.30)			0.572	0.45	(0.30, 0.68)	0.64	(0.43, 0.94)	0.71	(0.44, 1.17)	0.002	0.016	

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Gene SNP (Model)	Main Effect ¹				Interactive Effect ²								Adjusted P Value
	Heterozygote ³		Variant		Wald P Value	Wildtype ⁴		Variant ³		Interaction P Value			
	OR	(95% CI)	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)				
<i>NOS2A</i>													
rs2243828 (D)	0.81	(0.67, 0.99)			0.038	0.76	(0.50, 1.16)	0.66	(0.44, 0.98)	0.88	(0.55, 1.41)	0.042	0.080
rs3730013 (D)	0.99	(0.82, 1.20)			0.936	0.62	(0.39, 0.99)	0.59	(0.40, 0.87)	0.83	(0.53, 1.30)	0.003	0.030
rs944725 (A)	0.92	(0.75, 1.14)	1.02	(0.76, 1.35)	0.668	0.67	(0.40, 1.11)	0.55	(0.31, 0.98)	0.86	(0.45, 1.64)	0.029	0.267
rs9906835 (D) ⁸	0.83	(0.68, 1.01)			0.068	0.64	(0.39, 1.07)	0.61	(0.41, 0.90)	0.71	(0.45, 1.14)	0.033	0.267

¹Main effect ORs are relative to wildtype/heterozygote genotype if recessive (R) or wildtype genotype if additive (A) or dominant (D) models

²Interaction ORs are relative to non-user/non-smoker/low dietary intake and wildtype/heterozygote genotype if recessive (R) or non-user/non-smoker/low dietary intake and wildtype genotype if additive (A) or dominant (D) models

³Risk estimates are for heterozygote/variant genotypes if dominant (D) model

⁴Risk estimates are for wildtype/heterozygote genotype if recessive (R) model

⁵Similar associations for rs11652709 (p: 0.039)

⁶Main effect adjusted p value of 0.140

⁷Main effect adjusted p value of 0.072

⁸Similar associations for rs2297516 (p: 0.042)

Table 5

Main and interactive associations between NSAID use and cigarette smoking and *EPX*, *MPO*, *HIF1A*, and *NOS2A* and risk of colorectal cancer

Gene SNP (Model)	Main Effect ¹			Interaction Effect ²						Interaction P Value	Adjusted P Value	maxT P Value ⁵	Interaction Level (L) ⁶	maxT P Value ⁷		
	Heterozygote ³		Wald P Value	Wildtype ⁴		Variant ³										
	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)	OR	(95% CI)							
Colon Cancer																
EPX				Recent Aspirin/NSAID		No Recent Aspirin/NSAID		Recent Aspirin/NSAID								
rs10853004 (A) ⁸	0.97	(0.84, 1.12)	0.87	(0.69, 1.09)	0.478	0.56	(0.46, 0.69)	0.73	(0.55, 0.98)	0.66	(0.45, 0.95)	0.034	0.205	<0.00001	G={0,1} and E = ₌₀	<0.00001
NOS2A				Recent Smoker		Non-Smoker / Non-Recent		Recent Smoker								
rs2274894 (A)	1.02	(0.88, 1.18)	1.16	(0.95, 1.42)	0.330	0.88	(0.66, 1.18)	1.04	(0.83, 1.31)	1.63	(1.08, 2.45)	0.017	0.156	0.024	G={1,2[mls1]} and E = 1	0.185
rs3729508 (R)			1.03	(0.86, 1.23)	0.751	1.07	(0.88, 1.29)	0.93	(0.76, 1.14)	1.57	(1.09, 2.25)	0.044	0.312	0.056	G = 2 and E = ₌₁	0.360
rs7406657 (D)	1.01	(0.88, 1.16)			0.876	1.36	(1.09, 1.70)	1.08	(0.93, 1.26)	1.02	(0.78, 1.32)	0.035	0.290	0.041	G = 0 and E = ₌₁	0.293
rs944725 (A)	1.05	(0.91, 1.22)	1.00	(0.81, 1.22)	0.740	1.44	(1.09, 1.89)	1.14	(0.91, 1.43)	0.84	(0.56, 1.27)	0.013	0.127	0.042	G={0,1} and E = ₌₁	0.288
Rectal Cancer																
EPX				Recent Aspirin/NSAID		No Recent Aspirin/NSAID		Recent Aspirin/NSAID								
rs11079339 (D)	0.95	(0.76, 1.19)			0.644	0.8	(0.64, 1.01)	1.21	(0.90, 1.61)	0.54	(0.38, 0.77)	0.014	0.086	0.006	G={1,2} and E = ₌₁	0.032
NOS2A				Recent Smoker		Non-Smoker / Non-Recent		Recent Smoker								
rs4795067 (D)	0.93	(0.77, 1.13)			0.483	0.55	(0.41, 0.74)	0.78	(0.61, 1.00)	0.67	(0.51, 0.87)	0.028	0.285	0.001	G={0,1} and E = ₌₀	0.012
HIF1A																
HIF1A				Recent Smoker		Non-Smoker / Non-Recent		Recent Smoker								

Gene SNP (Model)	Main Effect ¹				Interaction Effect ²								maxT P Value ⁵	Interaction Level (L) ⁶	maxT P Value ⁷	
	Heterozygote ³		Wald P Value	Wildtype ⁴		Variant ³		Interaction P Value	Adjusted P Value							
	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)									
rs1951795 (D)	0.97	(0.80, 1.19)			0.804	1.71	(1.23, 2.36)	1.11	(0.88, 1.38)	0.93	(0.63, 1.37)	0.007	0.014	0.009	G = 0 and E = 1	0.023
<i>MPO</i>																
rs2243828 (D)	0.81	(0.67, 0.99)			0.038	1.65	(1.19, 2.28)	0.91	(0.73, 1.13)	0.81	(0.55, 1.21)	0.021	0.040	0.003	G = 0 and E = 1	0.004
<i>NOS2A</i>																
rs2779248 (A)	0.99	(0.81, 1.22)	1.05	(0.78, 1.41)	0.932	1.86	(1.22, 2.82)	1.19	(0.86, 1.66)	1.06	(0.54, 2.08)	0.032	0.265	0.037	G = 0 and E = 1	0.262
rs3730013 (A)	1.00	(0.82, 1.23)	0.96	(0.70, 1.32)	0.966	1.00	(0.69, 1.46)	0.84	(0.59, 1.18)	2.22	(1.01, 4.89)	0.029	0.265	0.057	G={1,2} and E =1	0.380
rs9906835 (D) ⁹	0.83	(0.68, 1.01)			0.068	0.81	(0.54, 1.22)	0.73	(0.59, 0.91)	1.26	(0.90, 1.78)	0.004	0.043	0.002	G={1,2} and E =0	0.021

¹ Main effect ORs are relative to wildtype/heterozygote genotype if recessive (R) or wildtype genotype if additive (A) or dominant (D) models

² Interaction ORs are relative to non-smoker/low dietary intake and wildtype/heterozygote genotype if recessive (R) or non-smoker/low dietary intake and wildtype genotype if additive (A) or dominant (D) models

³ Risk estimates are for Heterozygote/variant genotype if dominant (D) model

⁴ Risk estimates are for Wildtype/heterozygote genotype if recessive (R) model

⁵ Adjusted p-value within a single SNP-environment pairing (100K permutations)

⁶ G = numerical coding (i.e., 0, 1, 2) for the SNP; E = numerical coding (i.e., 0, 1) for the environmental factor.

⁷ Adjusted p-value for multiple SNPs (within the same gene) each paired with a single environment factor (100K permutations)

⁸ Similar associations for rs11652709 (p: 0.022)

⁹ Similar associations for rs2297516 (p: 0.011)