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Julia Labadie March 20, 2012

Interaction between oxidative stress-related exposures and genes that encode antioxidant enzymes in a case-control study of colorectal adenoma

By

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Master of Science in Public Health

Epidemiology

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Epidemiology
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Abstract

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CHAPTER I: BACKGROUND

Worldwide, colorectal cancer is the second and third most commonly diagnosed malignancy in women and men, respectively.[1] In the United States alone, an estimated 143,460 new cases will be diagnosed and 51,690 deaths will occur in 2012.[2] Although mortality has decreased, it is primarily due to secondary prevention and early detection. Though progress has been made in the primary prevention of colorectal cancer, further advances are necessary. Mortality and incidence rates for colorectal cancer are higher among individuals 50 years and older, men, and African Americans.[3] Additional non-modifiable risk factors include family history of colorectal cancer, personal history of colorectal polyps, colorectal cancer, or inflammatory bowel disease, and inherited syndromes such as familial adenomatous polyposis and hereditary non-polyposis colon cancer.[3] Several modifiable risk factors have been identified, including smoking, heavy alcohol use, obesity, physical inactivity, and diets high in red or processed meats and low in vegetables, fruits, and whole grains.[3] Convincing evidence indicates that colorectal cancer is a highly preventable disease, with moderate changes in diet and lifestyle estimated to prevent 70% of colorectal cancers.[4] Migration studies have emphasized the importance of environmental influences on colorectal cancer incidence, with immigrant rates approaching the rates of the host country in as little as one generation.[5-7]

The molecular basis of colorectal carcinogenesis is multi-step in nature and requires several genetic modifications before colorectal cancer develops.[8] Among sporadic

colorectal cancer, 85% are estimated to involve, generally as a first step, a mutation in the adenomatous polyposis coli (APC) pathway, which leads to a decrease in cell cycle control. Through this mechanism, cell proliferation increases while apoptosis, cell differentiation, and cell adhesion decrease. The remaining 15% of sporadic colorectal cancers are believed to occur through the mismatch repair (MMR) pathway. Mutations or epigenetic silencing in this pathway decrease cell cycle control through increased cell proliferation and decreased cell differentiation and apoptosis. Early adenomas progress into advanced adenomas through accumulation of mutations, particularly in *K-ras*. Mutations in *p53*, the tumor suppressor gene, promote apoptosis, thereby allowing advanced adenomas to develop into colorectal cancer.[9]

Although the exact etiologic mechanisms for colorectal carcinogenesis are uncertain, oxidative stress is a proposed mechanism that may account for some of the importance of diet and lifestyle. Reactive oxygen species (ROS) have been identified as having a likely role in both the initiation and promotion of cancers of various organs, including cancers of the colon and rectum.[10-17] The role of ROS in carcinogensis is thought to be mediated via three main mechanisms. ROS can directly alter nucleic acids, leading to mutations, exchanges, and aberrations that can lead to cell proliferation, either directly or through compensatory proliferation. ROS can also damage cells by reacting with membrane lipids and denaturing proteins, which can lead to cell death and compensatory hyperproliferation. Last, ROS can modulate gene expression of initiated cells through affecting genes that regulate cell growth and differentiation.

Through neutralization mechanisms, the body can regulate ROS, thereby reducing the extent of damage they can cause.[18] Antioxidant factors, as suggested by their name, work to inhibit or delay the actions of ROS.[19] Antioxidants can be present during all phases of ROS generation, and thus can work by preventing ROS formation or intervening shortly after formation to deactivate or remove ROS. In contrast, pro-oxidants promote ROS production, enabling their action.[19] Oxidative balance, which reflects a favorable ratio of pro-oxidants to antioxidants, thus may play an important role in colorectal carcinogenesis.[20]

Oxidative balance is affected by numerous factors, both endogenous and exogenous. In relation to colorectal cancer, the consequence of oxidative damage may be particularly important. Some studies have estimated substantial quantities of oxygen-derived free radicals in feces, meaning that exposure in the colon is great.[21] Complementary studies have shown that supplemental intake of antioxidants (vitamin E plus ascorbic acid) significantly reduces fecal mutagenicity. This finding is consistent with previously noted inverse associations between colon cancer and high fruit and vegetable intake.[22]

Two pathways may explain the role of oxidative balance in colorectal carcinogenesis.[23] The oxidative stress pathway begins with a defect in the epithelial barrier, resulting in an efflux of potassium and influx of sodium and calcium in epithelial cells. Due to the electrolyte imbalance, ROS are generated as the epithelial crypt cells attempt to restore intracellular electrolytes to their normal levels. The ROS in turn activate cyclooxygenase-2 (COX-2) which forms mitogenic prostaglandins that promote

carcinogenesis. In the inflammatory pathway, a defect in the epithelial barrier causes local irritation, producing an inflammatory response. The inflammation up-regulates COX-2, generating prostaglandins and activating inflammatory cells. Mutagenic and mitogenic ROS are generated from the inflammatory cells, thereby promoting carcinogenesis.[23] Thus, oxidative stress is both the cause and the consequence of inflammation. Either pathway can be blocked by antioxidants, n-3 fatty acids, NSAIDs, or agents that reduce defects in the epithelial barrier.

Consumption of polyunsaturated fatty acids and iron may increase oxidative stress.[24, 25] Iron, which is primarily available from red meat, preferentially catalyzes oxidative reactions through the Haber-Weiss reaction.[26, 27] In the Haber-Weiss reaction, Fe(II) is oxidized to Fe(III) by hydrogen dioxide, producing hydoxyl radicals that are highly reactive and can cause damage to lipids, proteins, DNA, and other nucleic acids.[28] Saturated fatty acids, typically ingested with animal fat, promote tumor growth and inhibit apoptosis.[29] It has been shown that a Western-style diet can produce compensatory hyperproliferation, which may reflect the negative impact of animal fat consumption.[30-32] Basic science evidence supports the role of iron and saturated fatty acids as pro-oxidants, but studies on their association with colorectal cancer have been inconsistent.[33]

Dietary antioxidants such as vitamin C, vitamin E, and various carotenoids have been shown to have chemopreventive properties. For example, in experimental animal studies, they decreased colorectal cell proliferation.[34-37] In addition, rats given a diet low in

fat and protein and high in vitamin E, selenium, vitamin A, and fiber had reduced colorectal epithelial cell proliferation when given chemical carcinogens.[36] In mice, carotene reduced epidermal cell proliferation, skin carcinogenesis, and intestinal carcinogenesis.[37] These animal studies have been supported by small trials in humans. One randomized controlled trial (n=60) gave patients daily supplementation of either vitamin E (160 mg), vitamin C (750 mg), or β-carotene (9 mg) for one month.[38] After comparing colon biopsy specimens from before and after the trial began, it was noted that patients given vitamin C or β-carotene had significantly reduced total proliferation. β-carotene reduced cell proliferation at the base of the crypt only, while vitamin C reduced cell proliferation in all crypt compartments from the apex to the base to values seen in age- and sex-matched controls.[38] Vitamin C, or ascorbic acid, acts as an antioxidant by preventing lipid peroxidation and helping to regenerate α-tocopherol. [39] In a small randomized controlled trial (n=36) in familial polyposis patients, those given 3.0 g ascorbic acid daily experienced a reduction in the labeling index.[40] Vitamin E, a membrane bound antioxidant, also protects against lipid peroxidation.[41]

Many trials and observational studies have been conducted to examine the relationship between carotenoid intake and colorectal cancer. In a randomized controlled trial (n=864) of β-carotene on colorectal adenoma recurrence, β-carotene supplementation was associated with a significant decrease in risk of recurrence among non-smokers and non-drinkers.[42] However, β-carotene supplementation significantly increased the risk of recurrence among those who smoke and drank. Among postmenopausal women enrolled in the Women's Health Initiative, repeated measures of serum β-carotene were taken and

inverse association between high average measurement and risk of colorectal cancer was observed.[43] This study was based on a combined sample from both the observational study (n=1,062) and the clinical trial (n=4,544). In contrast, another study was conducted using the Women's Health Initiative that only analyzed data from the randomized controlled trial (n=7,627). There, an intention-to-treat analysis showed no evidence of a statistically significant protective effect of β-carotene on risk of colorectal adenoma.[44] Lycopene and lutein, other types of carotenoids, both function as antioxidants.[45] Lycopene is found in tomatoes and tomato products, while lutein is found in dark green vegetables. Studies on the potential cancer preventive effects of lycopene have been conducted in many fields of research, from epidemiology to *in vitro* tissue cultures. Chemoprevention trials on lycopene supplementation have not yet been completed, although some are currently underway.

Non-dietary exogenous factors can also affect oxidative stress balance in the body. Inhaled tobacco smoke contains high concentrations of ROS, making it a powerful pro-oxidant.[46] This effect is exacerbated in the lungs, where inflammatory cells release oxygen radicals.[47] In studies, smoking has been associated with an increase in blood and tissue markers of oxidative stress.[48] Chronic alcohol intake can also result in oxidative stress. Mechanistically, the oxidation of ethanol to acetaldehyde can cause ROS production, nucleic acid oxidation, and decreased activity of antioxidant enzymes.[49, 50]

As discussed previously, inflammation is an important contributor to oxidative stress. When inflammatory cells receive stimuli such as microbial agents, they produce toxic compounds such as nitric oxide, superoxide, hydrogen peroxide, and singlet oxygen. These compounds can react to form peroxynitrite molecules and interact directly with DNA to initiate free radical chain reactions.[51, 52] Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin induce apoptosis of cancer cells, thereby preventing tumor growth.[53] They can also mitigate the effects of the oxidative stress pathway and inflammatory pathway.[23] In a randomized controlled trial (n=1,121), low-dose aspirin had a moderate chemopreventive effect on colorectal adenomas.[54]

In order to prevent damage, the body uses enzymatic cellular defense mechanisms to regulate oxidative stress and ROS levels. Such mechanisms include several antioxidant enzymes, including superodixe dismutases, catalase, and Se-glutathione peroxidase. Superoxide dismutases (SOD) convert superoxide anions ($O_2 \cdot^-$) to hydrogen peroxide, which is then removed by catalase or glutathione peroxidase. When the genes encoding these enzymes are expressed normally, they regulate levels of $O_2 \cdot^-$ in cells and suppress the formation of reactive hydroxyl radicals (OH·).[55] In humans, there are three forms of SOD: cytosolic, mitochondrial, and extracellular.[56] The decreased activity of mitochondrial *MnSOD*, a tumor suppressor gene, has been proposed as a factor in the etiology of colon cancer.[57] A case-control study (n=976) of colorectal adenoma found that a *MnSOD* polymorphism in the mitochondrial targeting sequence was modestly inversely associated with distal colorectal adenomas, but the finding was not statistically significant. The authors speculate that the polymorphism acts by altering the transport of

the enzyme into mitochondria.[58] When at least one variant allele was present, there was a modest inverse association, but there was no evidence for modification by plasma tocopherol.

The role of glutathione-S-transferases (*GST*s) is to catalyze the conjugation of glutathione to various carcinogenic compounds, including ROS.[59] There are four main families of GST enzymes: GST-alpha (*GSTA*), GST-mu (*GSTM*), GST-pi (*GSTP*), and GST-theta (*GSTT*).[60] *GSTP1* has a distinct role in glutathione peroxidase's activity towards lipid peroxides and sensitivity to active oxygen species. A comprehensive meta-analysis on the association between *GSTP1* and colorectal cancer risk yielded no statistically significant findings.[61]

Catalase helps eliminate ROS by breaking down hydrogen peroxide to water.[62] A small case-control study (n=82) on the suitability of catalase as a biomarker of colorectal risk found that catalase activity was significantly lower in colorectal cancer patients compared to controls and participants with colorectal polyps.[63]

Despite strong mechanistic evidence of the importance of pro- and anti-oxidants in colorectal carcinogenesis, observational epidemiological studies of specific determinants of oxidative stress have produced conflicting results.[64-79] This discrepancy is potentially due to the multifactorial nature of oxidative stress. Studies from other areas of chronic disease epidemiology show that a combination of several risk factors may produce an overall large increase in risk even when associations with each individual

factor are weak and inconsistent.[80] Alternative approaches to consider a variety of factors in the same pathway have been applied successfully in nutritional epidemiology.[81-83] Studies of health-related outcomes and Mediterranean diet have evaluated a diet adherence score that mitigates many of the issues encountered when evaluating individual dietary components.[84-88] This assessment has brought new light to the association between dietary patterns and health-related outcomes that may otherwise need much larger sample sizes. Following with this idea, several researchers proposed an oxidative balance score to evaluate the pro-oxidant/antioxidant balance in relation to colorectal adenoma, prostate cancer, and all-cause mortality.[67, 89-92]. However, prior evaluations of this score only considered extrinsic factors such as diet and lifestyle.

It is important to keep in mind that oxidative stress is regulated to a large extent through intrinsic mechanisms, such as the production of the aforementioned antioxidant enzymes. For this reason in the current study we will investigate whether the association between OBS and colorectal adenoma may vary depending on the presence or absence of polymorphisms affecting genes that encode the antioxidant enzymes *MnSOD*, catalase, and *GSTP1*.

CHAPTER II: MANUSCRIPT

Interaction between oxidative stress-related exposures and genes that encode antioxidant enzymes in a case-control study of colorectal adenoma

By Julia Labadie

ABSTRACT

Previous research found inverse associations between oxidative balance and risk of colorectal adenoma. However, all previous measures of oxidative balance were limited to extrinsic (lifestyle and dietary) factors and did not include intrinsic factors, specifically antioxidant enzymes responsible for cellular defense against oxidative stress. Using data pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma (n=1,050) that collected information on participants' medical history, usual dietary intakes, and lifestyle, we considered 11 extrinsic factors with known pro- or anti-oxidant properties. Using these factors, we constructed an oxidative balance score (OBS) in which points were awarded for each pro- and antioxidant exposure so that higher OBS values represented a higher balance of antioxidants to pro-oxidants. We used multivariate logistic regression to assess whether the association between OBS and colorectal adenoma differed according to polymorphisms in genes encoding antioxidant enzymes. For participants in the highest compared to those in the lowest OBS category, the odds ratio (OR) for the OBS-adenoma association was 0.51 (95% confidence interval

[CI] 0.32 - 0.82). For 11 catalase (*CAT*) single nucleotide polymorphisms (SNPs), the OBS-adenoma inverse association was stronger for participants homozygous for the most common allele for two of the SNPs and for those with one or more variant alleles in three, with the strongest being for those homozygous for the rs499406 variant A allele (OR 0.27; CI 0.08 – 0.92). For six manganese superoxide dismutase (*MnSOD*) SNPs, the inverse association was stronger for participants with at least one variant allele in three of the SNPs, with the strongest being for the rs5746151 variant A allele (OR 0.11; CI 0.02 – 0.77). For five gluthathione-S-transferase P1 (*GSTP1*) SNPs, the inverse association was stronger for participants homozygous for the most common allele in one SNP, and for those homozygous for the variant allele in another, with the strongest being for those homozygous for the rs4147581 variant G allele (OR 0.24; CI 0.09 – 0.67). These findings provide limited support for the hypothesis that variation in antioxidant enzyme genes may modify associations of environmental exposures related to oxidative balance and risk for sporadic colorectal adenoma.

INTRODUCTION

Colorectal cancer is the third most commonly diagnosed malignancy in the United States.[2] While many established risk factors are not modifiable (age, race, gender, personal and family history), convincing evidence indicates that colorectal cancer is a highly preventable disease, with moderate changes in diet and lifestyle estimated to prevent 70% of colorectal cancers.[3, 4] It has been suggested that oxidative stress may play a role in the etiology of several chronic diseases, including cancer.[10-17] Oxidative stress, which is defined as "a disturbance in the pro-oxidant-antioxidant balance in favor of the former," is affected by numerous factors, both endogenous and exogenous.[19] Despite convincing biological evidence, epidemiological studies evaluating the associations of various diseases to dietary and lifestyle factors that act as pro- or anti-oxidants have been inconclusive.[64-79]

Studies from other areas of chronic disease epidemiology suggest that a combination of several risk factors may produce an overall large increase in risk even when associations with each individual factor are weak and inconsistent.[80] These observations suggest the potential value of epidemiologic approaches that account for a variety of factors acting along the same etiologic pathway. It has been previously suggested that because oxidative stress is a multifactorial process affected by multiple exposures and mechanisms there is a need to examine the effects of multiple antioxidant as well as pro-oxidant agents simultaneously.[67, 89-92] This can be achieved by constructing an oxidative balance score (OBS), which awards points for each high-level antioxidant and

low-level pro-oxidant exposure so that the higher OBS values are expected to represent a more positive oxidative balance (i.e., higher antioxidant relative to pro-oxidant exposure).

Previous studies found an inverse association between the OBS and risk of sporadic colorectal adenoma (a precursor of cancer).[67, 89] It is important to point out, however, that the OBS is limited to extrinsic (lifestyle and dietary) exposures and does not take into consideration intrinsic mechanisms, specifically antioxidant enzymes, which are responsible for cellular defense against oxidative stress.

Herein we report analyses from three pooled colonoscopy-based case-control studies of incident, sporadic colorectal adenoma on whether the association between OBS and colorectal adenoma may vary according to polymorphisms in genes that encode three antioxidant enzymes which play a role in the destruction of reactive oxygen species (ROS) and their byproducts: manganese superoxide dismutase (*MnSOD*), catalase (*CAT*), and glutathione-S-transferase P1 (*GSTP1*).[55, 59, 62]

METHODS

Study population

Data were pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma. All studies were conducted by the same principal investigator and included patients scheduled for outpatient, elective colonoscopy for screening or clinical indications. Eligible participants were English-speaking, 30-74 years old with no prior history of cancer (except non-melanoma skin cancer), colorectal

adenoma, genetic syndromes associated with colonic neoplasia, or history of inflammatory bowel diseases. The three pooled studies [93], which have been described in detail elsewhere, include the Minnesota Cancer Prevention Research Study (CPRU) [94], the Markers of Adenomatous Polyps I study (MAP I) [95], and the Markers of Adenomatous Polyps II study (MAP II) [96]. The CPRU study was conducted from 1991-1994 in a large, multi-site gastroenterology practice in the Minneapolis, Minnesota metropolitan area. A total of 3,126 patients were identified, of whom 1,500 met final eligibility criteria and consented. MAP I took place from 1994-1997 in Winston-Salem and Charlotte, North Carolina. A total of 420 consenting participants met final eligibility criteria from 2,246 identified. The final study, MAP II, was conducted in 2002 in Columbia, South Carolina. During a five-month period, 251 patients were identified, with 203 eligible participants enrolling. This yielded a starting pooled sample size of 2,123.

Each of the three studies was approved by the Institutional Review Board of the institution where it was conducted. All participants provided informed consent. The present analysis was conducted using de-identified data.

Data collection

Prior to undergoing colonoscopy, all participants completed questionnaires on demographics, medical and family history, body size, lifestyle, hormonal and reproductive history (in women), and diet. Diet was evaluated using modified Willett

food frequency questionnaires (FFQ). In addition, participants had blood drawn for micronutrient analyses and genotyping.

Fasting venous blood samples were drawn into pre-chilled Vacutainer tubes shielded from light. The blood draw and handling protocols were similar for all studies, with the exception of the interval between drawing and processing samples and the use of antioxidant preservatives. For the CPRU study, blood samples were frozen at -70°C within 12 hours of blood draw and no antioxidant preservatives were used. In contrast, for the MAP studies, samples were processed, lipid and aqueous soluble antioxidant preservatives (BHT and salicylic acid, respectively) added to the aliquot vials, and frozen at -70°C immediately after they were drawn.

Single nucleotide polymorphisms (SNPs) for genotyping were selected based on being common polymorphisms in a pathway and/or having a minor allele frequency greater than 5%. When available, tagSNPs were used. Using these criteria, 8 SNPs were selected for *MnSOD*, 14 for *CAT*, and 6 for *GSTP1*. After excluding SNPs that did not pass quality control criteria, genotyping was done on 6, 11, and 5 SNPs, respectively. Genotyping was conducted using the iPLEX Sequenom genotyping platform at the Biomedical Genomics Center, the core genotyping laboratory at the University of Minnesota. Genotyping of 61 pairs of blinded duplicate samples showed a concordance $\geq$ 95% for these SNPs.

Exclusions

After pooling the data from the three studies, some additional exclusions were made. First, all participants who had hyperplastic polyps only were excluded (n=297; 14.0%). Second, due to concerns of genetic differences by race and an insufficient sample size of non-white participants, 113 (5.3%) participants who did not self-identify as white were excluded. Third, participants who reported an implausible total energy intake (<600 kcal or >6,000 kcal) or left ≥ 10% of the food frequency questionnaire blank were excluded (n=39; 1.8%). Fourth, 561 (26.4%) individuals who did not have genetic data on the SNPs of interest were excluded. Last, individuals who were missing data on more than 20% of the SNPs of interest where excluded (n=63; 3.0%). This left a final sample size of 1,050 participants, including 472 cases and 578 controls.

OBS components

We created an oxidative balance score (OBS) composed of pro- and anti-oxidants using 11 variables determined *a priori* based on expected roles in oxidative processes. Lifestyle variables included alcohol intake (<1 drink/week, 1-6 drinks/week, ≥7 drinks/week), smoking status (never, former, or current), nonsteroidal anti-inflammatory drug (NSAID) use (< or ≥ once/week), and aspirin use (< or ≥ once/week). Dietary intakes were estimated from the FFQ, with the exception of lutein and lycopene for the two MAP studies, for which blood levels were used. Nutrient intakes were energy-adjusted according to the residual regression method and categorized into three groups based on sex- and study-specific tertile cutpoints among the controls. The OBS was constructed based on expected pro- or anti-oxidant roles. Expected pro-oxidants (alcohol

consumption, smoking, saturated fat, iron) were categorized on a 0, 1, 2 scale such that high intakes of pro-oxidants received the lowest values. In contrast, expected antioxidants (vitamin C, vitamin E, lutein/zeaxanthin, lycopene, carotenoids) were categorized on a 2, 1, 0 scale such that high antioxidant intakes received the highest values. For dichotomous variables (NSAID and aspirin use), regular users were assigned a score of 2 to reflect antioxidant roles, while non-users were assigned a score of 0. The OBS was created by summing its 11 components. The score was then categorized into three approximately equal groups.

Gene scores

After individual assessment of each SNP, an *a priori* gene-specific variant allele score was created. In this score, each genotype was assigned a value of 0, 1, or 2 based on whether the genotype was homozygous for the common allele, heterozygous, or homozygous for the variant allele, respectively. The SNPs were then summed to create a continuous score for each gene. The resulting gene-specific scores were then categorized into ordinal variables based on tertile cutpoints. Finally, an overall gene variant allele score was created by summing each categorized gene-specific score, and then the overall gene score itself was categorized into three approximately equal-sized groups.

Colorectal adenoma

Polyps detected and removed during colonoscopy were reviewed by an index study pathologist using diagnostic criteria established by the National Polyp Study [97]. Participants without hyperplastic or adenomatous polyps were identified as controls.

Statistical analysis

All potential covariates were selected *a priori* based on established associations with colorectal adenoma and potential associations with the OBS. Selected characteristics of the cases and controls were compared using pooled two-sample t-tests for continuous variables and chi square tests for categorical variables. Continuous variables were log-transformed to improve normality when necessary. For the CPRU study, lutein and lycopene intakes were available only as servings of lutein- and lycopene-rich fruits and vegetables, whereas in both of the MAP studies, serum lutein and lycopene measurements were available. Consequently, a summary exposure variable was created to reflect study-specific tertiles of lutein and lycopene exposures. All analyses were conducted using SAS version 9.2 or 9.3 (SAS institute, Cary, NC).

Based on the results for the individual potential covariates described above, a multivariate model was built. Multiple established and hypothesized risk factors identified based on previous literature were considered as potential confounding variables. The covariates included in the final model were chosen based on the presence at least one of the following criteria: biological plausibility, whether or not the p-value for the covariate when included in the model was ≤ 0.1, and whether its inclusion in the model affected the odds ratios (OR) for the primary exposure variable by $\geq 10\%$. A correlation analysis was conducted to ensure that highly correlated variables were not included in the model. The covariates selected for the final model included age, sex, hormone replacement therapy use (in women), education, family history of colorectal cancer in a

first degree relative, obesity (BMI <30 kg/m^2 and WHR <1.0 in men or <0.8 in women, either BMI ≥30 kg/m^2 or WHR ≥1.0 in men or ≥0.8 in women, BMI ≥30 kg/m^2 and WHR ≥1.0 in men or ≥0.8 in women), total energy intake, physical activity (moderate plus vigorous in metabolic equivalents), and total intakes of calcium, dietary fiber, red meat, and folate.

Multivariate logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals for the association between the OBS and incident, sporadic colorectal adenoma, adjusted for selected covariates. The OBS was evaluated as a categorical variable, with the lowest OBS category used as the referent group.

Each SNP was assessed on multiple levels. First, preliminary analyses were conducted to evaluate whether individual SNPs were in Hardy-Weinberg equilibrium and whether any linkage disequilibrium was present. Next, crude analyses were conducted to assess whether the genotype for each individual SNP was associated with colorectal adenoma using both chi-square tests and logistic regression. Then, the associations were further examined using multivariate logistic regression, adjusting for all covariates from the gold standard model. Finally, to assess whether the OBS-adenoma association differed according to genotype, the multivariate models were stratified on genotype as well as by including a genotype-OBS interaction term in the model. When too few participants were homozygous for variant allele to ensure a stable model, those with at least one variant allele were combined. Gene-specific scores and the overall gene score were assessed in a similar manner.

RESULTS

Selected characteristics of the cases and controls are summarized in Table 1. Cases were statistically significantly more likely to be male, older, taller, current or former smokers, and have higher waist-hip ratios. They were also more likely to consume more alcohol, report higher energy intakes, and not take NSAIDs regularly. Among women, cases were less likely to report using postmenopausal hormone replacement therapy (HRT).

Crude and adjusted analyses of association of individual SNP genotypes with incident, sporadic colorectal adenoma revealed no substantial or statistically significant associations (data not shown). The adjusted overall OBS-adenoma association indicated that those in the highest tertile relative to those in the lowest tertile of the OBS were at approximately half the risk for incident, sporadic colorectal adenoma (OR = 0.51; 95% CI 0.32 - 0.82). This estimate was essentially unchanged when controlling for individual SNP genotypes.

OBS-adenoma associations according to individual SNP genotypes are shown in Tables 2-4. As noted in Table 2, for 11 SNPs encoding *CAT*, the association was statistically significantly more strongly inverse (about two-thirds lower risk of colorectal adenoma) among individuals who 1) had a rs1001179 variant A allele, 2) had a rs16925614 variant T allele, 3) were homozygous for the rs499406 variant T allele, 4) were homozygous for the rs7943316 common allele, and 5) were homozygous for the rs7947841 common G allele. As shown in Table 3, for the six SNPs encoding *MnSOD*, the risk of colorectal

adenoma was statistically significantly about two-thirds lower for those in the highest category of the OBS among participants 1) with a rs2842980 variant T allele, 2) a rs5746141 variant A allele, and 3) a rs5746136 variant A allele. As shown in Table 4, for the five SNPs encoding *GSTP1* the OBS-colorectal adenoma association was more strongly inverse among participants who were homozygous for 1) the rs4147581 variant G allele and 2) the rs762803 common C allele.

As shown in Table 5, for the three gene variant allele scores, the OBS-adenoma association was more strongly inverse among participants in the highest *GSTP1* variant allele score category (OR = 0.21; 95% CI 0.07 – 0.67), but there was no consistent pattern of effect modification by the variant allele scores for the other genes. When all three genes were considered together in an overall variant allele score, there was no pattern consistent with the OBS-adenoma association differing according to the total number of variant alleles. No OBS-gene-related multiplicative interaction term was statistically significant in the multivariate models.

DISCUSSION

The findings from this study provide limited support for the hypothesis that variation in antioxidant enzyme genes may modify associations of environmental exposures related to oxidative balance and risk for sporadic colorectal adenoma. Of the 22 SNPs investigated across the three antioxidant enzyme genes, stronger inverse OBS-adenoma associations were found among those with different genotypes for 10 SNPs as well as among those with a higher *GSTP1* variant allele score (although the estimated associations were

statistically significant, the multiplicative interaction terms were not). It is noted that of the 10 SNPs for which differences were found, the inverse OBS-adenoma association was stronger among those homozygous for the common allele for four and among those with one or more copies of the variant allele in six. If these findings represent truth, then combining the SNPs into scores reflecting how the individual SNPs contribute to effect modification (rather than combining them into the *a priori* determined variant allele score we used) would yield much stronger evidence of effect modification from the aggregate contributions of the multiple antioxidant enzyme genotypes. Ideally, the effects (or lack of effects) of each SNP on antioxidant capacity will be ultimately be determined so that the most biologically relevant gene scores can be devised and used to assess gene-environment interactions in larger, prospective studies.

Based on previous basic science research, it is biologically plausible that antioxidant enzymes may influence the effects of diet and lifestyle on oxidative balance. Dietary and lifestyle pro- and anti-oxidants are known to affect the balance of ROS in the body, thereby influencing oxidative balance.[33, 36, 46, 49, 50, 53] This, combined with the role of antioxidant enzymes in regulating oxidative balance and ROS levels, leads to the reasonable hypothesis that antioxidant enzymes can act to modify the impact of dietary and lifestyle pro- and anti-oxidants on oxidative balance and risk for diseases, such as colorectal neoplasms.

Our findings regarding the overall association of the OBS with adenomas are consistent with those reported in other epidemiologic studies, which found inverse associations of

oxidative balance scores with colorectal adenoma, prostate cancer, esophageal cancer, lung cancer, and total cancer mortality.[67, 89, 91, 98, 99] To our knowledge, no other studies to date have considered the combined effects of intrinsic and extrinsic factors as they relate to oxidative balance and cancer risk.[55, 59, 62]

Although there is evidence from bioinformatics programs that most of the SNPs we investigated are predicted to have functional consequences, in our study we found no evidence that any of the individual genotypes alone or in combination were associated with risk for adenoma. Several programs have been developed that use bioinformatics to predict the functional impact of SNPs. Lee and Shatkay designed a program that combines 16 tools and databases to place each SNP into a functional category.[100] This program predicts that six of the 11 SNPs encoding *CAT* (rs1001179, rs12272630, rs499406, rs16925614, rs7104301, and rs566979) that we investigated may have functional consequences in transcriptional regulation. For *GSTP1*, an estimated four of the five SNPs are predicted to have functional consequences, two involving splicing regulation (rs1695 and rs1138272) and two involving transcriptional regulation (rs762803 and rs749174). Five of the six SNPs encoding *MnSOD* were predicted to be functional, four involving transcriptional regulation (rs2842980, rs8031, rs5746151, and rs5746136), and one involving splicing regulation (rs4880).

Although we found some evidence suggesting the possibility that the OBS-adenoma association may differ according to various individual SNP genotypes, we observed less evidence of modification by the gene scores. However, it is unclear whether this was due

to mechanistic or methodological problems that arise when creating a score. Mechanistically, it is possible that functional redundancies in the genome (e.g., related to reducing oxidative stress) could compensate for the effects of variation across multiple relevant SNPs, so that associations may be detected for individual SNPs but not for combinations of them. Methodologically, in the absence of functional studies on the SNPs investigated, *a priori* we calculated our gene scores as variant allele scores with the naive assumption that variant alleles were the risk alleles. Thus, it is possible that this as well as including SNPs that have no effect into the score attenuates any associations involving the gene scores.

Strengths and limitations

This study has several limitations, including ones inherent in the case-control study design. First, there is temporal ambiguity regarding the OBS-colorectal adenoma association. However, in the unlikely event that diet improved due to symptoms of adenoma, this likely would have attenuated our results. Second, limitations of FFQs are known, with issues ranging from seasonal variability in participants' responses to recall bias; however, we used a previously validated FFQ and the questionnaires were completed prior to colonoscopies and diagnoses. Third, our analysis was limited to Caucasian participants so conclusions cannot be drawn about other races. Fourth, the data used in this study were collected before colorectal cancer screening by colonoscopy was common, causing an apparent family history bias such that individuals with a family history of colorectal cancer were more likely to be screened prior to development of adenoma and were thus overrepresented in the control group. However, results stratified

by family history were similar and inclusion of family history in the model had a minimal impact. It is worthwhile to note that this bias would tend to attenuate our results, causing the observed association to be weaker than the true association. Fifth, the factors influencing the development of colorectal adenoma may not entirely be the same as factors influencing the development of colorectal cancer; however, studies conducted on the association of dietary scores with colorectal cancer found similar associations.[101-103] Sixth, our sample size was limited and we made multiple comparisons; however, this study represents the first investigation into possible interactions of antioxidant genes with a newly developed oxidative balance score, providing support for further studies in this area. Finally, it was not feasible to consider every possible SNP for each of the genes of interest. Thus, there may be some influential SNPs that were not evaluated in our analyses.

Strengths of our study include colonoscopy evaluation of both cases and controls and histologically verified adenoma cases, both of which reduce outcome misclassification. In addition, detailed information was collected on covariates, which decreases unmeasured confounding, and questionnaires were administered prior to diagnosis, which reduces recall bias. The in-depth analysis of SNPs encoding antioxidant enzymes included multiple methods of assessing genotypic influence on the association of the OBS with colorectal adenoma, including individual associations, stratified analyses, and stratified scores. This comprehensive approach provided an enhanced ability to investigate the potential influence of genotype. However, our sample size was limited, reducing our ability to detect variations in the association of OBS and colorectal adenoma

according to genotypes. By creating gene scores, we were able to examine the potential overall modifying effect of each antioxidant enzyme.

Our findings, taken in context of the aforementioned limitations and previous literature, provide support for further study of whether antioxidant enzyme genotypes may modify the association of an oxidative balance score (which combines multiple pro- and anti-oxidant environmental exposures) with incident, sporadic colorectal adenoma.

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TABLES

Table 1. Selected characteristics of study population by case-control status

Risk Factors	Controls (n=578)	Cases (n=472)	p-value[6]
Male (%)	38	61	< 0.0001
Education (%)			
Less than high school	7	11	0.06
High School, Vocational School, or Some College	60	57	
College graduate or higher	32	31	
Family history of CRC[1] (%)	29	16	< 0.0001
Age (years)	53.9 (10.4)	57.8 (9.2)	< 0.0001
Height (m)	1.68 (0.10)	1.72 (0.10)	< 0.0001
Body mass index (kg/m^2)	27.4 (5.5)	27.6 (5.4)	0.29
Waist-hip ratio	0.88 (0.11)	0.93 (0.11)	< 0.0001
HRT in women (n=547) (%)	55	45	0.04
Physical activity[2] (METs/wk)	221.9 (203.8)	234.4 (251.0)	0.37
Total[3] **energy intake** (kcal/d)	1,936.6 (694.9)	2,056.9 (776.3)	0.02
Total[3] **calcium intake** (mg/d)	945.6 (498.9)	928.8 (523.2)	0.31
Dietary fiber intake (gm/d)	20.9 (9.6)	21.5 (9.8)	0.40
Red meat intake (servings/wk)	4.6 (3.8)	4.9 (3.6)	0.28
Total[3] **folate intake** (mcg/d)	428.2 (246.9)	422.3 (248.3)	0.70
OBS components			
Smoking status (%)			
Never	49	32	< 0.0001
Former	38	45	
Current	13	24	
Regular[4] **NSAID use** (%)	27	15	< 0.0001
Regular[4] **aspirin use** (%)	34	30	0.27
Alcohol intake (%)			
<1 drink per week	87	79	0.003
1-6 drinks per week	13	21	
7+ drinks per week	0	0	
Lutein exposure[5] (%)			
Low	33	29	0.40
Medium	34	35	
High	32	35	
Lycopene exposure[5] (%)	30	29	0.91
Low	32	32	
Medium	37	39	
High			
Total[3] **vitamin C intake** (mg/d)	275.4 (313.9)	257.9 (312.5)	0.38
Total[3] **vitamin E intake** (mg/d)	70.1 (152.4)	69.1 (157.0)	0.85
Saturated fat intake (gm/d)	22.9 (10.7)	22.8 (12.6)	0.02
Carotenoid intake (IU/d)	9,450.2 (9,377.0)	9,534.2 (9,066.7)	0.53
Total[3] **dietary iron intake** (mg/d)	20.3 (17.8)	19.4 (16.3)	0.66
Oxidative Balance Score (OBS)	11 (3.4)	10 (3.3)	0.04

Mean and standard deviation presented unless otherwise specified

[1]Family history of colorectal cancer in a first degree relative

[2]Moderate and/or vigorous

[3]Total intake = dietary + supplemental

[4]Regular use is defined as > once per week

[5]For CPRU, lutein and lycopene intakes were available as servings of lutein- and lycopene- rich fruits and vegetables; for MAP, lutein and lycopene intakes were available as mg/day

[6]Chi-square p-values given for categorical variables, pooled two-sample t-test p-values for continuous variables

Table 2. Association between OBS and incident, sporadic colorectal adenoma, according to *CAT* genotypes

SNP	Genotype stratified on	n cases/ controls	Overall %	Cases %	Controls %	OBS Category[1] (vs low) Medium	95% CI	High	95% CI
rs1001179	Overall	471/578	99.9	44.9	55.1	0.89	(0.62 - 1.27)	0.51	(0.32 - 0.82)
	GG	274/342	58.7	58.2	59.2	0.93	(0.57 - 1.51)	0.70	(0.37 - 1.32)
	AG	172/209	36.3	36.5	36.2	1.04	(0.57 - 1.91)	0.46	(0.21 - 1.00)
	AA	25/27	5.0	5.3	4.7	-	-	-	-
	AG+AA	197/236	41.3	41.8	40.8	0.83	(0.47 - 1.45)	0.35	(0.17 - 0.74)
rs7943316	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.37 - 0.82)
	TT	215/254	44.7	45.6	43.9	0.79	(0.46 - 1.37)	0.38	(0.19 - 0.77)
	AT	205/256	43.9	43.4	44.3	1.11	(0.62 - 1.97)	0.81	(0.38 - 1.72)
	AA	52/68	11.4	11.0	11.8	1.01	(0.30 - 3.41)	0.56	(0.12 - 2.63)
	AT+AA	257/324	55.3	54.4	56.1	0.98	(0.60 - 1.62)	0.67	(0.35 - 1.29)
rs11604331	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	AA	179/238	39.7	37.9	41.2	0.74	(0.41 - 1.35)	0.68	(0.31 - 1.49)
	AG	227/257	46.1	48.1	44.5	1.10	(0.64 - 1.89)	0.48	(0.24 - 0.96)
	GG	66/83	14.2	14.0	14.4	0.66	(0.25 - 1.72)	0.30	(0.08 - 1.14)
	AG+GG	293/340	60.3	62.1	58.8	1.02	(0.64 - 1.62)	0.45	(0.25 - 0.82)
rs12272630	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	GG	440/543	93.6	93.2	93.9	0.87	(0.60 - 1.27)	0.51	(0.31 - 0.84)
	CG	32/34	6.1	6.8	5.9	3.16	(0.37 - 27.06)	0.39	(0.03 - 5.35)
	CC	0/1	0.1	0.0	0.2	-	-	-	-
	CG+CC	32/35	6.4	6.8	6.1	2.07	(0.28 - 15.52)	0.41	(0.03 - 5.01)
rs525938	Overall	472/577	99.9	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	AA	246/290	51.1	52.1	50.3	0.72	(0.43 - 1.20)	0.38	(0.19 - 0.75)
	AG	185/241	40.6	39.2	41.8	1.35	(0.74 - 2.46)	0.77	(0.35 - 1.68)
	GG	41/46	8.3	8.7	8.0	-	-	-	-
	AG+GG	226/287	48.9	47.9	49.7	1.14	(0.67 - 1.94)	0.70	(0.35 - 1.40)
rs7947841	Overall	472/578	100	45.0	55.0	0.88	(0.61 - 1.26)	0.50	(0.31 - 0.81)
	GG	394/484	83.6	83.5	83.7	0.79	(0.53 - 1.18)	0.37	(0.22 - 0.62)
	AG	71/89	15.2	15.0	15.4	1.70	(0.62 - 4.64)	3.36	(0.80 - 14.14)
	AA	7/5	1.1	1.5	0.9	-	-	-	-
	AG+AA	78/94	16.4	16.5	16.3	1.87	(0.69 - 5.09)	4.57	(1.16 - 18.00)
rs499406	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	GG	158/197	33.8	33.5	34.1	0.75	(0.39 - 1.44)	0.67	(0.28 - 1.58)
	AG	223/275	47.4	47.2	47.6	1.24	(0.73 - 2.12)	0.59	(0.30 - 1.16)
	AA	91/106	18.8	19.3	18.3	0.64	(0.28 - 1.51)	0.27	(0.08 - 0.92)
	AG+AA	314/381	66.2	66.5	65.9	1.01	(0.65 - 1.58)	0.48	(0.27 - 0.85)
rs16925614	Overall	472/577	99.9	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	CC	362/439	76.4	76.7	76.1	0.89	(0.59 - 1.34)	0.55	(0.32 - 0.94)
	CT	101/126	21.6	21.4	21.8	0.89	(0.37 - 2.11)	0.36	(0.12 - 1.13)
	TT	9/12	2.0	1.9	2.1	-	-	-	-
	CT+TT	110/138	23.6	23.3	23.9	0.81	(0.36 - 1.85)	0.34	(0.11 - 0.98)
rs7104301	Overall	569/576	99.5	44.9	55.1	0.91	(0.63 - 1.31)	0.53	(0.33 - 0.85)
	AA	261/317	55.3	55.7	55.0	1.11	(0.68 - 1.82)	0.53	(0.28 - 1.00)
	AG	175/221	37.9	37.3	38.4	0.63	(0.34 - 1.14)	0.49	(0.22 - 1.07)
	GG	33/38	6.8	7.0	6.6	-	-	-	-
	AG+GG	208/259	44.7	44.3	45.0	0.71	(0.41 - 1.24)	0.54	(0.26 - 1.13)
rs566979	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	TT	202/248	42.9	42.8	42.9	0.94	(0.54 - 1.63)	0.52	(0.25 - 1.08)
	GT	206/246	43.0	43.6	42.6	1.09	(0.62 - 1.92)	0.57	(0.27 - 1.21)
	GG	64/84	14.1	13.6	14.5	0.46	(0.16 - 1.33)	0.41	(0.10 - 1.62)
	GT+GG	270/330	57.1	57.2	57.1	0.88	(0.54 - 1.43)	0.51	(0.27 - 0.97)
rs11032703	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	CC	250/440	75.2	74.2	76.1	0.75	(0.49 - 1.15)	0.45	(0.26 - 0.78)
	CT	112/126	22.7	23.7	21.8	1.48	(0.70 - 3.13)	0.62	(0.23 - 1.67)
	TT	10/12	2.1	2.1	2.1	-	-	-	-
	CT+TT	122/138	24.8	25.8	23.9	1.60	(0.79 -3.25)	0.73	(0.28 - 1.90)

[1] Adjusted for age, sex, hormone replacement therapy use, education, family history of colorectal cancer in a first degree relative, body composition, total energy intake, physical activity, and total intakes of calcium, dietary fiber, red meat, and folate

Some study participants did not have data for all SNPs

OR estimates were ommitted when sample sizes for homozygous variant genoypes were less than 10%

Table 3. Association between OBS and incident, sporadic colorectal adenoma, stratified on *MnSOD* genotypes

SNP	Genotype stratified on	n cases/ controls	Overall Cases %	Cases %	Controls %	OBS Category[1] (vs low) Medium	95% CI	High	95% CI
rs2842980	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.81)
	AA	299/359	62.7	63.3	62.1	1.19	(0.75 - 1.88)	0.65	(0.36 - 1.18)
	AT	152/198	33.3	32.2	34.3	0.53	(0.28 - 1.00)	0.30	(0.13 - 0.71)
	TT	21/21	4.0	4.4	3.6	-	-	-	-
	AT+TT	173/219	36.4	36.7	37.9	0.54	(0.30 - 0.99)	0.35	(0.16 - 0.78)
rs8031	Overall	470/578	99.8	44.8	55.2	0.90	(0.62 - 1.29)	0.51	(0.32 - 0.83)
	TT	121/151	26.0	25.7	26.1	0.84	(0.39 - 1.78)	0.44	(0.16 - 1.19)
	AT	239/298	51.2	50.9	51.6	0.88	(0.53 - 1.48)	0.39	(0.20 - 0.77)
	AA	110/129	22.8	23.4	22.3	1.07	(0.49 - 2.34)	1.36	(0.50 - 3.72)
	AT+AA	349/427	74.0	74.3	73.9	0.93	(0.61 - 1.43)	0.57	(0.33 - 0.98)
rs5746151	Overall	470/575	99.5	45.0	55.0	0.88	(0.61 - 1.27)	0.50	(0.31 - 0.81)
	GG	425/501	88.6	90.4	87.1	0.85	(0.58 - 1.25)	0.55	(0.34 - 0.91)
	AG	42/72	10.9	8.9	12.5	2.30	(0.62 - 8.45)	0.14	(0.02 - 1.05)
	AA	3/2	0.5	0.6	0.3	-	-	-	-
	AG+AA	45/74	11.4	9.6	12.9	1.80	(0.52 - 6.23)	0.11	(0.02 - 0.77)
rs4880	Overall	468/570	98.9	45.1	54.9	0.93	(0.64 - 1.34)	0.54	(0.33 - 0.86)
	CC	112/134	23.7	23.9	23.5	1.00	(0.46 - 2.18)	1.08	(0.40 - 2.94)
	CT	250/308	53.8	53.4	54.0	0.84	(0.50 - 1.39)	0.42	(0.22 - 0.81)
	TT	106/128	22.5	22.6	22.5	1.33	(0.59 - 3.03)	0.63	(0.21 - 1.84)
	CT+TT	356/436	76.3	76.1	76.5	0.89	(0.59 - 1.36)	0.44	(0.25 - 0.76)
rs5746136	Overall	462/561	97.4	45.2	54.8	0.90	(0.62 - 1.30)	0.52	(0.32 - 0.84)
	GG	242/277	50.7	52.4	49.4	0.87	(0.52 - 1.45)	0.74	(0.38 - 1.44)
	AG	174/236	40.1	37.7	42.1	0.74	(0.40 - 1.36)	0.34	(0.15 - 0.74)
	AA	46/48	9.2	10.0	8.6	-	-	-	-
	AG+AA	220/284	49.3	47.6	50.6	0.96	(0.56 - 1.65)	0.40	(0.19 - 0.81)
rs6917589	Overall	472/576	99.8	45.0	55.0	0.90	(0.63 - 1.29)	0.52	(0.32 - 0.83)
	TT	276/334	58.2	58.5	58.0	0.84	(0.52 - 1.35)	0.60	(0.32 - 1.11)
	CT	164/213	36.0	34.7	37.0	0.76	(0.41 - 1.43)	0.39	(0.17 - 0.87)
	CC	32/29	5.8	6.8	5.0	-	-	-	-
	CT+CC	196/242	41.8	41.5	42.0	0.97	(0.55 - 1.74)	0.46	(0.22 - 0.99)

[1] Adjusted for age, sex, hormone replacement therapy use, education, family history of colorectal cancer in a first degree relative, body composition, total energy intake, physical activity, and total intakes of calcium, dietary fiber, red meat, and folate

Some study participants did not have data for all SNPs

OR estimates were ommitted when sample sizes for homozygous variant genoypes were less than 10%

Table 4. Association between OBS and incident, sporadic colorectal adenoma, stratified on *GSTP1* genotypes

SNP	Genotype stratified on	n cases/ controls	Overall %	Cases %	Controls %	OBS Category[1] (vs low) Medium	95% CI	High	95% CI
rs1695	Overall	468/569	98.8	45.1	54.9	0.89	(0.62 - 1.28)	0.50	(0.31 - 0.81)
	AA	210/250	44.4	44.9	43.9	0.98	(0.55 - 1.74)	0.60	(0.28 - 1.27)
	AG	209/260	45.2	44.7	45.7	0.76	(0.44 - 1.33)	0.54	(0.27 - 1.10)
	GG	49/59	10.4	10.5	10.4	0.93	(0.28 - 3.07)	0.23	(0.04 - 1.26)
	AG+GG	258/319	55.6	55.1	56.1	0.80	(0.49 - 1.29)	0.45	(0.24 - 0.84)
rs1138272	Overall	468/563	98.2	45.4	54.6	0.88	(0.61 - 1.27)	0.49	(0.31 - 0.80)
	CC	385/470	83.8	82.3	85.1	1.02	(0.68 - 1.52)	0.57	(0.34 - 0.96)
	CT	78/76	14.9	16.7	13.5	0.57	(0.19 - 1.66)	0.54	(0.14 - 2.18)
	TT	5/8	1.3	1.1	1.4	-	-	-	-
	CT+TT	83/84	16.2	17.7	14.9	0.51	(0.18 - 1.42)	0.43	(0.11 - 1.68)
rs4147581	Overall	468/575	99.3	44.9	55.1	0.93	(0.64 - 1.34)	0.53	(0.33 - 0.85)
	CC	112/154	25.5	23.9	26.8	1.04	(0.48 - 2.22)	0.70	(0.25 - 1.95)
	CG	239/293	51.0	51.1	51.0	1.13	(0.67 - 1.90)	0.64	(0.33 - 1.25)
	GG	117/128	23.5	25.0	22.3	0.50	(0.22 -1.14)	0.24	(0.09 - 0.67)
	CG+GG	356/421	74.5	76.1	73.2	0.91	(0.59 - 1.39)	0.48	(0.28 - 0.83)
rs762803	Overall	471/577	99.8	44.9	55.1	0.90	(0.62 - 1.29)	0.52	(0.32 - 0.83)
	CC	156/186	32.6	33.1	32.2	0.72	(0.37 - 1.40)	0.34	(0.15 - 0.79)
	AC	241/284	50.2	51.2	49.2	1.17	(0.70 - 1.95)	0.79	(0.40 - 1.54)
	AA	74/106	17.2	15.7	18.4	0.69	(0.26 - 1.84)	0.28	(0.07 - 1.05)
	AC+AA	315/390	67.4	66.9	67.6	0.99	(0.64 - 1.55)	0.64	(0.36 -1.15)
rs749174	Overall	466/569	98.6	45.0	55.0	0.90	(0.62 - 1.30)	0.51	(0.31 - 0.82)
	CC	207/248	44.0	44.4	43.6	0.73	(0.41 - 1.32)	0.42	(0.20 - 0.89)
	CT	212/260	45.6	45.5	45.7	0.94	(0.55 - 1.61)	0.66	(0.33 - 1.35)
	TT	47/61	10.4	10.1	10.7	1.19	(0.35 - 4.08)	0.35	(0.06 - 1.96)
	CT+TT	259/321	56.0	55.6	56.4	1.00	(0.62 - 1.62)	0.57	(0.30 - 1.09)

[1] Adjusted for age, sex, hormone replacement therapy use, education, family history of colorectal cancer in a first degree relative, body composition, total energy intake, physical activity, and total intakes of calcium, dietary fiber, red meat, and folate

Some study participants did not have data for all SNPs

OR estimates were ommitted when sample sizes for homozygous variant genoypes were less than 10%

Table 5. Association between OBS and incident, sporadic colorectal adenoma, stratified on gene scores

SNP	Model[2]	n cases/ controls	Overall %	Cases %	Controls %	OBS Category[1] (vs low) Medium	95% CI	High	95% CI
MnSOD score	Overall	472/578	100	45.0	55.0	0.90	(0.62 - 1.29)	0.51	(0.32 - 0.83)
	Low	238/278	49.1	50.4	48.1	0.81	(0.49 - 1.36)	0.70	(0.36 - 1.39)
	Medium	150/191	32.5	31.8	33.0	1.03	(0.53 - 2.01)	0.45	(0.20 - 1.05)
	High	84/109	18.4	17.8	18.9	1.32	(0.51 - 3.40)	0.49	(0.14 - 1.71)
GSTP1 score	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	Low	205/252	43.5	43.4	43.6	0.93	(0.52 - 1.66)	0.57	(0.27 - 1.21)
	Medium	175/210	36.7	37.1	36.3	1.01	(0.55 - 1.87)	0.90	(0.41 - 1.97)
	High	92/116	19.8	19.5	20.1	0.80	(0.35 - 1.83)	0.21	(0.07 - 0.67)
CAT score	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	Low	62/81	13.6	13.1	14.0	0.78	(0.23 - 2.59)	0.91	(0.20 - 4.22)
	Medium	206/260	44.4	43.6	45.0	0.83	(0.48 - 1.45)	0.45	(0.22 - 0.92)
	High	204/237	42.0	43.2	41.0	1.01	(0.58 - 1.76)	0.51	(0.37 - 1.07)
Gene Score	Overall	472/578	100	45.0	55.0	0.89	(0.62 - 1.28)	0.51	(0.32 - 0.82)
	Low	212/252	44.2	44.9	43.6	0.65	(0.36 - 1.16)	0.53	(0.25 - 1.12)
	Medium	129/170	28.5	27.3	29.4	0.86	(0.43 - 1.71)	0.38	(0.15 - 0.92)
	High	131/156	27.3	27.8	27.0	1.21	(0.61 - 2.42)	0.55	(0.21 - 1.40)

[1] Adjusted for age, sex, hormone replacement therapy use, education, family history of colorectal cancer in a first degree relative, body composition, total energy intake, physical activity, and total intakes of calcium, dietary fiber, red meat, and folate

[2]For each gene score, 4 models were run. The first included all obsrvations, the next three included only participants who fell into the low, medium, or high category for the corresponding score, respectively

Some study participants did not have data for all SNPs

CHAPTER III: SUMMARY

Public health implications

With the completion of the human genome project, there has been a push to develop applications of these rich data to clinical medicine. Extensive research has been conducted to evaluate associations between genes and chronic diseases, with varying success. With this research has come a drive toward what has been coined "personalized medicine," a model that proposes using genetic data to tailor interventions and medical care at the individual level. This study can be seen as a preliminary analysis that may help lead to providing personalized medicine one day for preventing colorectal adenoma and cancer. By understanding how genotype may influence the effects of dietary and lifestyle exposures, we may be able to evaluate individual risk for colorectal adenoma and thus tailor medical care and advice accordingly.

Possible future directions

Ideally, this study would be expanded upon by conducting analyses in a larger study population. This would allow us to gain a greater understanding of how the homozygous variant genotypes may modify the OBS-colorectal adenoma association. In addition, it would be of interest to conduct a similar analysis using a biomarker-driven OBS to determine if the associations are altered. It is possible that biomarkers of oxidative stress and inflammation could provide a better understanding of how antioxidants can modify the influence of dietary and lifestyle pro- and anti-oxidants. Last, it would be valuable to conduct analyses in a more ethnically diverse study population. This would increase

external validity, and would allow the assessment of potential evolutionary differences in expression of antioxidant enzymes.

By exploring this research question using various study designs, valuable information can be gained. A prospective study where dietary and lifestyle data are collected at multiple points throughout the lifetime would allow us to investigate whether there is a critical point in someone's life where diet and lifestyle have a stronger influence on risk of colorectal cancer. In addition, it would allow comparison of dietary patterns throughout the lifetime. A clinical trial using a dietary intervention that emphasizes a more positive oxidative balance in the diet could be conducted to determine whether results differ according to genotype. Finally, biological studies should be conducted to investigate whether there are functional consequences to any of the SNPs. Then, repeat analyses could be conducted using a hypothesis-driven gene score that includes only those SNPs with a functional consequence.