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Abstract

PURPOSE—Previous research found inverse associations between oxidative balance and risk of colorectal adenoma. However, these measures were limited to extrinsic (dietary and lifestyle) exposures and did not account for intrinsic factors, specifically antioxidant enzymes responsible for cellular defense against oxidative stress. We investigated whether the association between an oxidative balance score (OBS) and colorectal adenoma may vary according to polymorphisms in genes that encode three antioxidant enzymes: manganese superoxide dismutase (*SOD2*), catalase (*CAT*), and glutathione-S-transferase P1 *(GSTP1)*.

METHODS—Using data pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma, we constructed an OBS reflecting pro- and anti-oxidant exposures. We used multivariable logistic regression to assess whether the association between the OBS and colorectal adenoma differed according to polymorphisms in the genes encoding the antioxidant enzymes.

RESULTS—The OBS was inversely associated with colorectal adenoma, adenoma risk was not associated with the genetic polymorphisms, and there was no consistent pattern of effect modification by individual genotypes or combined gene scores.

CONCLUSIONS—Variations in the antioxidant enzyme genes *SOD2, CAT*, and *GSTP1* do not appear to substantially modify associations of environmental exposures related to oxidative balance with risk for sporadic colorectal adenoma.

INTRODUCTION

Oxidative stress, defined as "a disturbance in the pro-oxidant-antioxidant balance in favor of the former," is affected by numerous factors, both endogenous and exogenous[1]. It has

been previously suggested that, because oxidative stress is a multifactorial process affected by multiple exposures and mechanisms, there is need to examine the effects of multiple antioxidant and pro-oxidant agents simultaneously[2–6]. This can be achieved by constructing an oxidative balance score (OBS) that awards points for each high-level antioxidant and low-level pro-oxidant exposure so that higher OBS values are expected to reflect a predominance of antioxidant relative to pro-oxidant exposures.

Previous studies found an inverse association between the OBS and risk of sporadic colorectal adenoma (the precursor to most colorectal cancers)[2, 4]. However, the OBS is limited to extrinsic (lifestyle and dietary) exposures and does not take into consideration intrinsic mechanisms, specifically antioxidant enzymes responsible for cellular defense against oxidative stress.

Antioxidant enzymes such as manganese superoxide dismutase, catalase, and glutathione-S-transferase P1 (encoded by the *SOD2, CAT*, and *GSTP1* genes, respectively) have a role in regulating reactive oxygen species (ROS) levels, and are thus factors in the intrinsic management of oxidative stress. Previous studies have found inverse associations of variants in all three genes with colorectal cancer[7, 8]. Many SNPs encoding these enzymes have been projected to have functional consequences in transcriptional regulation or splicing regulation[9]. Therefore, it is of interest to consider these SNPs in conjunction with the extrinsic factors collectively represented by the OBS.

Herein we report analyses from three pooled colonoscopy-based case-control studies of incident, sporadic colorectal adenoma to investigate whether the association between the OBS and colorectal adenoma may vary according to *SOD2, CAT*, and *GSTP1* genotypes[7, 10–11].

METHODS

Data were pooled from three colonoscopy-based case-control studies of incident, sporadic colorectal adenoma, which have been described in detail elsewhere: the Minnesota Cancer Prevention Research Study (CPRU)[12], the Markers of Adenomatous Polyps I study (MAP I)[13], and the Markers of Adenomatous Polyps II study (MAP II)[14]. All studies were conducted by the same principal investigator using the same or similar protocols that were described in detail previously[12–14]. We excluded participants with hyperplastic polyps only (n=297; 14%), non-white participants (due to insufficient sample size and potential genetic differences; cases n=48, 5.9%; controls n=65, 6.4%), those with an implausible reported total energy intake (<600 kcal or >6,000 kcal) (cases n=11, 1.4%; controls n=28, 2.7%), those on whom we had no genetic data on the single nucleotide polymorphisms (SNPs) of interest (cases n=247, 30.6%; controls n=314, 30.8%), and individuals on whom data were missing on more than 20% of the SNPs of interest (cases n=29, 3.6%, controls n=34, 3.3%). This left a final sample size of 1,050 participants, including 472 cases and 578 controls. Among cases, the median age was 59 years and 61% of subjects were male. Among controls, 38% were male, with a median age of 54 years.

The OBS included 11 pro- or anti-oxidant components selected from the available dietary and lifestyle questionnaire data as described previously[12–14]. Pro-oxidants (tobacco smoking and alcohol, saturated fat, and iron intakes) were categorized on a 0, 1, 2 scale based on study- and sex-specific tertiles among the controls such that high levels of exposure of pro-oxidants received the lowest values. In contrast, antioxidants (total [dietary plus supplemental] 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. Nutrient intakes were energy-adjusted according to the residual regression method[15]. For

dichotomous variables (nonsteroidal anti-inflammatory drug [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 overall OBS was created by summing all 11 components.

Genotyping analyses examined SNPs for the three genes of interest. SNPs were selected based on being common polymorphisms in a pathway and/or having a minor allele frequency greater than 5%, using tagSNPs when available. Using these criteria, 8 SNPs were selected for *SOD2*, 14 for *CAT*, and 6 for *GSTP1*. After excluding polymorphisms that did not pass quality control criteria, the data included 6 SNPs for *SOD2* (rs2842980, rs5746136, rs5746151, rs4880, rs6917589, rs8031), 11 for *CAT* (rs1001179, rs11032703, rs11604331, rs12272630, rs16925614, rs499406, rs525938, rs566979, rs7104301, rs7943316, rs7947841), and 5 for *GSTP1* (rs4147581, rs762803, rs1695, rs749174, rs1138272). Genotyping was conducted using the iPLEX Sequenom genotyping platform at the University of Minnesota's Biomedical Genomics Center. Genotyping of 61 pairs of blinded duplicate samples showed a concordance of 95% for these SNPs. All genotypes were in Hardy-Weinberg equilibrium. After individual assessment of each SNP, we constructed a gene score using two methods. First, we used an *a priori* gene-specific variant allele scoring method in which a value of 0, 1, or 2 was assigned to each SNP based on whether the genotype was homozygous for the common allele, heterozygous, or homozygous for the variant allele, respectively. Second, in an *a posteriori* method, each SNP-specific score was created by assigning each genotype a value of 0, 1, or 2 based on its crude association with colorectal adenoma, using the homozygous common variant as the reference. If the observed association was inverse, the genotype with the strongest inverse association was given a value of 0. However, if the observed association was positive, the genotype with the weakest positive association was given a value of 0. The SNP-specific scores obtained by each method were summed for each gene, and across all three genes.

We used multivariable logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between the OBS and incident, sporadic colorectal adenoma, adjusted for previously established risk factors for colorectal cancer. The OBS was evaluated as a categorical variable, with the lowest OBS category used as the reference group. To assess potential effect modification, the adjusted OBS-adenoma association was then stratified according to the gene scores.

RESULTS

As reported previously[2, 4], there was an inverse association between colorectal adenoma and the OBS. Adenoma risk appeared unrelated to genetic polymorphisms, beyond what could be expected due to multiple comparisons, and examination of interactions between the OBS and individual SNPs revealed no discernible pattern (data not shown, available on request).

As shown in Table 1, there was no consistent pattern of effect modification by the *a priori* variant allele scores for any individual genes. When all three genes were combined in an overall *a priori* variant allele score, there was no pattern consistent with the OBS-adenoma association differing according to the total number of variant alleles. In addition, no OBS-gene-related multiplicative interaction term was statistically significant in the multivariable models. Similar results were noted when using the *a posteriori* overall gene score.

DISCUSSION

The findings from this study do not support the hypothesis that variations in the genes encoding the antioxidant enzymes manganese superoxide dismutase (*SOD2*), catalase

(*CAT*), and glutathione-S-transferase P1 (*GSTP1*) substantially modify associations of environmental exposures related to oxidative balance with risk for sporadic colorectal adenoma.

Based on previous basic science research, it is biologically plausible that antioxidant enzymes may influence the effects of diet and lifestyle on oxidative balance[16–21]. 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[2, 4]. Although there is evidence that most of the SNPs we investigated are predicted (but not proven) 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. Using a source that combines data from multiple sites[9], it was predicted that 6 of the 11 SNPs encoding *CAT* that we investigated (rs1001179, rs12272630, rs499406, rs16925614, rs7104301, and rs566979) may have functional consequences in transcriptional regulation. For *GSTP1*, an estimated three of the five SNPs were predicted to have functional consequences—two involving splicing regulation (rs1695 and rs1138272) and one involving transcriptional regulation (rs749174). Five of the six SNPs encoding *SOD2* were predicted to be functional—four involving transcriptional regulation (rs2842980, rs8031, rs5746151, and rs5746136), and one involving splicing regulation (rs4880). Ideally, the effect (or lack of effect) of each SNP on antioxidant capacity will 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.

This study has several limitations, including those inherent in the case-control study design. First, our analysis was limited to Caucasian participants so conclusions cannot be drawn about other races. Second, 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 adenoma development 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. Third, the factors influencing the development of colorectal adenoma may not be entirely the same as factors influencing the development of colorectal cancer; however, studies conducted on the associations of dietary scores with colorectal cancer found similar associations[22–24]. Fourth, it was not feasible to consider every possible SNP for each of the genes of interest, nor every possible gene of interest. Thus, there may be some influential SNPs that were not evaluated in our analyses. Finally, our sample size, especially considering the multiple comparisons, was relatively small, limiting our power to detect the modest estimated interactions. However, our pooled case-control study reports the first estimates of possible interactions of antioxidant genes with a newly developed oxidative balance score and ranks among the largest reported case-control studies of colorectal adenoma.

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 combined scores. 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 little evidence for substantial effect modification of the OBS-colorectal adenoma association by variation in genes encoding the antioxidant enzymes manganese superoxide dismutase (*SOD2*), catalase (*CAT*), and glutathione-S-transferase P1 (*GSTP1*). However, further, larger, preferably prospective studies may be warranted, especially if and when the functional consequences of polymorphisms in antioxidant enzyme genes are clarified.

Acknowledgments

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Table 1

Association between oxidative balance score (OBS) and incident, sporadic colorectal adenoma, stratified by gene scores

SNP	Model[3]	%	n cases/controls[4]	a priori method[1] OBS Category[5] (vs low) Medium	95% CI	High	95% CI	%	n cases/controls[4]	a posteriori method[2] OBS Category[5] (vs low) Medium	95% CI	High	95% CI
SOD2 score	Overall	100	472/578	0.95	(0.67 – 1.31)	0.64	(0.45 – 0.91)	100	472/578	0.95	(0.69 – 1.30)	0.63	(0.44 – 0.90)
	Low	46.6	228/261	1.01	(0.63 – 1.62)	0.67	(0.40 – 1.14)	41.1	181/250	0.74	(0.45 – 1.22)	0.69	(0.39 – 1.22)
	Medium	34.0	154/203	0.80	(0.46 – 1.39)	0.79	(0.43 – 1.45)	25.6	126/143	1.20	(0.61 – 2.36)	0.56	(0.26 – 1.19)
	High	19.4	90/114	1.36	(0.65 – 2.84)	0.45	(0.18 – 1.12)	33.3	165/185	1.02	(0.59 – 1.78)	0.78	(0.42 – 1.45)
GSTP1 score	Overall	100	472/578	0.96	(0.70 – 1.31)	0.64	(0.45 – 0.91)	100	472/578	0.97	(0.69 – 1.37)	0.64	(0.45 – 0.91)
	Low	42.1	201/241	1.02	(0.62 – 1.67)	0.68	(0.38 – 1.19)	48.6	222/288	0.86	(0.54 – 1.37)	0.60	(0.36 – 1.00)
	Medium	37.2	175/216	0.80	(0.47 – 1.38)	0.77	(0.43 – 1.39)	20.7	95/122	1.28	(0.65 – 2.53)	0.51	(0.22 – 1.17)
	High	20.7	96/121	1.29	(0.64 – 2.61)	0.39	(0.17 – 0.89)	30.8	155/168	0.99	(0.54 – 1.81)	0.77	(0.39 – 1.51)
CAT score	Overall	100	472/578	0.95	(0.67 – 1.31)	0.64	(0.45 – 0.91)	100	472/578	0.96	(0.70 – 1.31)	0.64	(0.45 – 0.91)
	Low	13.1	60/78	0.40	(0.15 – 1.11)	0.67	(0.23 – 1.93)	39.7	177/240	0.68	(0.40 – 1.15)	0.75	(0.42 – 1.33)
	Medium	44.7	207/262	0.95	(0.58 – 1.55)	0.83	(0.47 – 1.45)	37.1	178/212	1.51	(0.90 – 2.56)	0.76	(0.43 – 1.37)
	High	42.2	205/238	1.30	(0.80 – 2.12)	0.55	(0.32 – 0.94)	23.1	117/126	0.92	(0.47 – 1.80)	0.40	(0.18 – 0.89)
Gene score	Overall	100	472/578	0.96	(0.70 – 1.31)	0.64	(0.45 – 0.91)	100	472/578	0.95	(0.69 – 1.30)	0.64	(0.45 – 0.91)
	Low	41.7	201/237	1.02	(0.62 – 1.67)	0.68	(0.38 – 1.19)	49.4	220/299	0.86	(0.54 – 1.37)	0.60	(0.36 – 1.00)
	Medium	29.2	131/176	0.80	(0.47 – 1.38)	0.77	(0.43 – 1.39)	23.5	109/138	1.28	(0.65 – 2.53)	0.51	(0.22 – 1.17)
	High	29.1	140/165	1.29	(0.64 – 2.61)	0.39	(0.17 – 0.89)	27.1	143/141	0.99	(0.54 – 1.81)	0.77	(0.39 – 1.51)

[1] The *a priori* gene-specific variant allele scoring method involved assigning a value of 0, 1, or 2 to each SNP based on whether the genotype was homozygous for the common allele, heterozygous, or homozygous for the variant allele, respectively

[2] The *a posteriori* method involved assigning each genotype a value of 0, 1, or 2 based on its crude association with colorectal adenoma, using the homozygous common variant;

[3] For each gene score, four models were run. The "overall" model included the gene score as a covariate and used all observations, the "low" model included only participants who fell into the low category of the corresponding gene score (e.g., gene score = 0), and the "medium" and the "high" models included participants with gene scores of 1 and

[4] Some study participants did not have data on all SNPs

[5] Adjusted for age, sex, hormone replacement therapy use, family history of colorectal cancer in a first degree relative, body composition (combined BMI and waist-to-hip ratio), total energy intake, physical activity, and total intakes of calcium, dietary fiber, red meat, and total (dietary + supplemental) vitamin D intake. OBS was included in the model as a categorical predictor, with low values expected to reflect higher levels of pro-oxidants relative to anti-oxidants