Annals of Epidemiology 23 (2013) 223–226

Contents lists available at SciVerse ScienceDirect

Annals of Epidemiology

journal homepage: www.annalsofepidemiology.org

Brief communication

Associations of oxidative balance-related exposures with incident, sporadic colorectal adenoma according to antioxidant enzyme genotypes

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ARTICLE INFO

Article history:
Received 3 July 2012
Accepted 5 December 2012
Available online 3 January 2013

Keywords:
Colorectal adenoma
Diet
Antioxidant enzymes

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 antioxidant 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: 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 seem to substantially modify associations of environmental exposures related to oxidative balance with risk for sporadic colorectal adenoma.

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 exposures (lifestyle and dietary) 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 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 single nucleotide polymorphisms (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].

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http://dx.doi.org/10.1016/j.annepidem.2012.12.001

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 [12], the Markers of Adenomatous Polyps I study [13], and the Markers of Adenomatous Polyps II study [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 (owing to insufficient sample size and potential genetic differences; 48 cases [5.9%]; 65 controls [6.4%]), those with an implausible reported total energy intake (<600 kcal or >6000 kcal; 11 cases [1.4%]; 28 controls [2.7%]), those on whom we had no genetic data on the SNPs of interest (247 cases [30.6%]; 314 controls [30.8%]), and individuals on whom data were missing on more than 20% of the SNPs of interest (29 [3.6%]; 34 controls [3.3%]). This left a final sample size of 1050 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- and antioxidant 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 scale of 0, 1, or 2 based on study- and gender-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 scale of 2, 1, or 0 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 and aspirin use), regular users were assigned a score of 2 to reflect antioxidant roles, whereas nonusers 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 of 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, and rs8031), 11 for *CAT* (rs1001179, rs11032703, rs11604331, rs12272630, rs16925614, rs499406, rs525938, rs566979, rs7104301, rs7943316, and rs7947841), and 5 for *GSTP1* (rs4147581, rs762803, rs1695, rs749174, and 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% or more 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 and 95% confidence intervals 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 seemed to be unrelated to genetic polymorphisms, beyond what could be expected owing 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 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 *SOD2*, *CAT*, and *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 the OBS 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; therefore, conclusions cannot be drawn about other races. Second, the data used in this study were collected