Null Results in Brief

The MnSOD A16V Mitochondrial Targeting Sequence Polymorphism Is Not Associated with Increased Risk of Distal Colorectal Adenomas: Data from a Sigmoidoscopy-based Case Control Study

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Introduction

MnSOD[2] is one of several intracellular antioxidant enzymes that cooperate with each other and with dietary antioxidants to protect cells from the damage associated with exposure to reactive oxygen species. The MnSOD protein is translated in the cytoplasm and transported to mitochondria where it catalyses the dismutation of superoxide anions to hydrogen peroxide (H_2O_2). Shimoda-Matsubayashi *et al.* (1) described recently a polymorphism in which an alanine is substituted for a valine at codon 16 of the mitochondrial targeting sequence in the human MnSOD gene (*A16V*). The valine at this position was predicted to change the mitochondrial targeting sequence conformation from the preferred amphiphilic helix to a combination of an amphiphilic helix and a β-pleated sheet, possibly interfering with the transport efficiency of the mature MnSOD protein, potentially decreasing its functional effectiveness (2). A decreased transport rate for the valine-containing protein was demonstrated in transient transfection studies, showing that the polymorphism does modify transport efficiency (3). On the other hand, several recent epidemiologic studies suggest that the MnSOD genotype may affect cancer risk but that the high-risk allele may depend on tumor site and be modified by individual pro- and antioxidant status (4–7). In the present study, we assessed the association between the MnSOD *A16V* targeting sequence polymorphism and colorectal adenomas, known precursor lesions for colorectal cancers. α-Tocopherol plays a major role in preventing the propagation of reactive oxygen species-associated damage by lipid peroxidation and is also known to protect against mitochondrial damage associated with excess superoxide anion levels by a variety of mechanisms (8). Therefore, we also assessed potential modification of the MnSOD genotype effect by estimated plasma α-tocopherol level.

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[2] The abbreviations used are: MnSOD, manganese superoxide dismutase; OR, odds ratio; CI, confidence interval.

Materials and Methods

Information on subject eligibility and recruitment is described more completely elsewhere (9). Briefly, subjects were eligible for the study if they underwent screening sigmoidoscopy at either of two Southern California Kaiser Permanente Medical Centers from January 1, 1991, through August 25, 1993, were between the ages of 50–74, had no evidence of prior bowel disease, and had no previous bowel surgery. Cases were subjects diagnosed for the first time with one or more histologically confirmed adenomas. Controls had no polyps of any type at sigmoidoscopy, had no history of polyps, and were individually matched to cases by gender, age (within 5-year category), date of sigmoidoscopy (within 3-month category), and Kaiser Clinic. Data for all of the subjects were anonymized prior to genotyping. Details of plasma nutrient determinations are described more completely elsewhere (10, 11). MnSOD genotype was determined by PCR amplification followed by Alu I digestion using the following primers: forward: 5′-AGC ACC AGC AGG CAG CTG GCT CCA G-3′ and reverse: 5′-CGG TGA CGT TCA GGT TGT TCA CG-3′.

Statistical Power and Analysis. We had 80% power to detect a protective OR of 0.62 or greater or a risk OR of 1.53 or greater at $P < 0.05$. We estimated the main and modified effects of *MnSOD* genotype using unconditional logistic regression controlling for the four matching factors (age, sex, clinic, and sigmoidoscopy date; Ref. 12). Comparison of results from a crude unconditional analysis to those from the matching factor adjusted unconditional analysis yielded similar results. Potential effect modifiers, such as plasma nutrients and exercise, were treated as binomial variables based on their distributions in controls or as specified. We used the α:λ tocopherol ratio as a measure of available α-tocopherol (10). Alcohol use was dichotomized into <20 and ≥ 20 grams/day. For this analysis weekly physical activity was dichotomized as $<$ or $\geq$ to 3 h of sweat-inducing activity per week (13), and subjects were considered regular nonsteroidal anti-inflammatory drug users if they indicated that they had regularly taken aspirin or any other listed products containing either aspirin or other nonsteroidal anti-inflammatory agents during the year before their sigmoidoscopy. We estimated separate ORs at each level of the potential modifier. Statistical interaction terms were used to assess interactions between genotype and potential modifiers.

Results

There was a small, nonsignificant protective effect for the presence of at least one *Ala* allele (Table 1) and no modification of the genotype effect by the α:λ tocopherol ratio (Table 2) or other potential antioxidant exposures including plasma vitamin C, regular nonsteroidal anti-inflammatory drug use, or exercise, or by the potential pro-oxidative exposures smoking as lifetime

Table 1 MnSOD genotype by case status

Genotype	Cases	Controls	OR[a]	95% CI
AA	108 (20.9%)	121 (21.8%)	0.88	0.64–1.20
AV	209 (40.4%)	234 (42.2%)	0.88	0.60–1.27
VV	139 (26.8%)	140 (25.3%)	1.0	Reference
Missing	62 (12%)	59 (10.7%)	—	

[a] Adjusted for age (continuous), sex, clinic, sigmoidoscopy date, (±6 months), and self-reported racial identification (Hispanic, African American, Asian *versus* Caucasian).

Table 2 MnSOD genotype by plasma α-tocopherol availability

Genotype	AA	AV	VV
Plasma α:γ tocopherol ratio			
≤6.05 mg/dl			
Cases/controls	38/37	79/78	51/54
OR[a]	1.08	1.05	1.0
95% CI	0.59–2.00	0.63–1.76	Reference
P_{trend}			0.793
>6.05			
Cases/controls	34/45	74/79	43/45
OR[b]	0.76	0.85	1.0
95% CI	0.39–1.46	0.49–1.47	Reference
P_{trend}			0.407
$P_{interaction}$			0.193

[a] The plasma α:γ ratio measures an individuals α-tocopherol availability independently of the type and amount of fat in the body and, thus, is less potentially misclassified than α-tocopherol. For both measures those with the highest value have the most available α-tocopherol.
[b] Controlling for age (continuous), sex, race (Hispanic, African American, Asian *versus* Caucasian), clinic and sigmoidoscopy date (±6 months).

pack-years or an alcohol intake of >20 grams/day (data not shown).

Discussion

Efficient targeting of the MnSOD protein may be an important part of its functional activity (2), and differential mitochondrial activity for the valine allele has been demonstrated *in vitro* (3). Epidemiologic data suggest that this change may be associated with cancer risk but that the high risk allele may depend on tumor site (4–7). In this study we assessed the association between the MnSOD *A16V* targeting sequence polymorphism and the prevalence of cancer precursor lesions in the distal colon and rectum. The putative high-risk MnSOD codon 16 *Ala* allele was not associated with increased distal adenoma prevalence in this population as a whole or in a subgroup with higher plasma α-tocopherol activity (10). If anything, our data suggested a slightly protective effect for the *Ala* allele and are more consistent with data from a recent lung cancer case control study in which risk was associated with valine homozygosity (7).

Future epidemiologic studies in a variety of tumor sites are needed to clarify whether the MnSOD *A16V* genotype is associated with cancer in a site-specific way and to identify the high-risk allele for various tumor types. One possibility is that the etiologic importance of MnSOD function in neoplasia risk may depend on a differential importance of mitochondrial dysfunction to tumorigenesis in different tissues. For example, increased risks because of a higher postmitotic-cell death rate may not be as readily observed in tissues, such as colorectum, in which postmitotic cell death is already programmed to occur with high frequency. Experimental data regarding how differential MnSOD translocation affects mitochondrial function, nuclear DNA integrity, and cellular survival in various tissues are necessary to clarify hypotheses and to help us interpret the epidemiologic data.

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