

*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

A. Joan Levine,<sup>1</sup> Ehab Elkhoully, Anh T. Diep,  
Eric R. Lee, Harold Frankl, and Robert W. Haile<sup>1</sup>

Department of Preventive Medicine, University of Southern California-Keck School of Medicine, Los Angeles, California 90089-9175 [A. J. L., E. E., A. T. D., R. W. H.], and Divisions of Gastroenterology, Kaiser Permanente Medical Centers at Bellflower [E. R. L.], and Sunset [H. F.], Los Angeles, California

### Introduction

MnSOD<sup>2</sup> 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<sub>2</sub>O<sub>2</sub>). 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  $\beta$ -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.  $\alpha$ -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  $\alpha$ -tocopherol level.

### 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  $\alpha$ : $\lambda$  tocopherol ratio as a measure of available  $\alpha$ -tocopherol (10). Alcohol use was dichotomized into  $<20$  and  $\geq 20$  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  $\alpha$ : $\lambda$  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

Received 3/11/02; revised 3/11/02; accepted 6/6/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> To whom requests for reprints should be addressed, at Department of Preventive Medicine, University of Southern California-Keck School of Medicine, Los Angeles, CA 90089-9175.

<sup>2</sup> The abbreviations used are: MnSOD, manganese superoxide dismutase; OR, odds ratio; CI, confidence interval.

Table 1 MnSOD genotype by case status

Genotype	Cases	Controls	OR <sup>a</sup>	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%)	—	

<sup>a</sup> Adjusted for age (continuous), sex, clinic, sigmoidoscopy date, ( $\pm 6$  months), and self-reported racial identification (Hispanic, African American, Asian versus Caucasian).

Table 2 MnSOD genotype by plasma  $\alpha$ -tocopherol availability

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

<sup>a</sup> The plasma  $\alpha$ : $\gamma$  ratio measures an individual's  $\alpha$ -tocopherol availability independently of the type and amount of fat in the body and, thus, is less potentially misclassified than  $\alpha$ -tocopherol. For both measures those with the highest value have the most available  $\alpha$ -tocopherol.

<sup>b</sup> Controlling for age (continuous), sex, race (Hispanic, African American, Asian versus Caucasian), clinic and sigmoidoscopy date ( $\pm 6$  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  $\alpha$ -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.

## References

- Shimoda-Matsubayashi, S., Matsumine, H., Kobayashi, T., Nakagawa-Hattori, Y., Shimizu, Y., and Mizuno, Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. *Biochem. Biophys. Res. Commun.*, 226: 561–565, 1996.
- Rosenblum, J. S., Gilula, N. B., and Lerner, R. A. On signal sequence polymorphisms and diseases of distribution. *Proc. Natl. Acad. Sci. USA*, 93: 4471–4473, 1996.
- Hiroi, S., Harada, H., Nishi, H., Satoh, M., Nagai, R., and Kimura, A. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. *Biochem. Biophys. Res. Commun.*, 261: 332–339, 1999.
- Ambrosone, C. B., Freudenheim, J. L., Thompson, P. A., Bowman, E., Vena, J. E., Marshall, J. R., Graham, S., Laughlin, R., Nemoto, T., and Shields, P. G. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. *Cancer Res.*, 59: 602–660, 1999.
- Mitrunen, K., Sillanpaa, P., Kataja, V., Eskelinen, M., Kosma, V. M., Benhamou, S., Uusitupa, M., and Hirvonen, A. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk. *Carcinogenesis (Lond.)* 22: 827–829, 2001.
- Sachdeva, R. M., Martin, A.-M., Heyworth, M. F., Ziegler-Johnson, C. M., Spangler, E., Walker, A. H., Malkowicz, S. B., and Rebbeck, T. R. American Society for Human Genetics, p. 261. San Diego, CA: ASHG, 2001.
- Wang, L. I., Miller, D. P., Sai, Y., Liu, G., Su, L., Wain, J. C., Lynch, T. J., and Christiani, D. C. Manganese superoxide dismutase alanine-to-valine polymorphism at codon 16 and lung cancer risk. *J. Natl. Cancer Inst.*, 93: 1818–1821, 2001.
- Chow, C. K. Vitamin E regulation of mitochondrial superoxide generation. *Biol. Signals Recept.*, 10: 112–124, 2001.
- Haile, R. W., Witte, J. S., Longnecker, M. P., Probst-Hensch, N., Chen, M. J., Harper, J., Frankl, H. D., and Lee, E. R. A sigmoidoscopy-based case-control study of polyps: macronutrients, fiber and meat consumption. *Int. J. Cancer*, 73: 497–502, 1997.
- Ingles, S. A., Bird, C. L., Shikany, J. M., Frankl, H. D., Lee, E. R., and Haile, R. W. Plasma tocopherol and prevalence of colorectal adenomas in a multiethnic population. *Cancer Res.*, 58: 661–666, 1998.
- Shikany, J. M., Witte, J. S., Henning, S. M., Swendseid, M. E., Bird, C. L., Frankl, H. D., Lee, E. R., and Haile, R. W. Plasma carotenoids and the prevalence of adenomatous polyps of the distal colon and rectum. *Am. J. Epidemiol.*, 145: 552–557, 1997.
- Breslow, N. E., and Day, N. E. *Statistical Methods in Cancer Research. Volume I, the Analysis of Case-Control Studies.* Lyon, France: IARC, 1980.
- Enger, S. M., Longnecker, M. P., Lee, E. R., Frankl, H. D., and Haile, R. W. Recent and past physical activity and prevalence of colorectal adenomas. *Br. J. Cancer*, 75: 740–745, 1997.

## 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

A. Joan Levine, Ehab Elkhoully, Anh T. Diep, et al.

*Cancer Epidemiol Biomarkers Prev* 2002;11:1140-1141.

**Updated version** Access the most recent version of this article at:  
<http://cebp.aacrjournals.org/content/11/10/1140>

**Cited articles** This article cites 11 articles, 3 of which you can access for free at:  
<http://cebp.aacrjournals.org/content/11/10/1140.full#ref-list-1>

**Citing articles** This article has been cited by 3 HighWire-hosted articles. Access the articles at:  
<http://cebp.aacrjournals.org/content/11/10/1140.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://cebp.aacrjournals.org/content/11/10/1140>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.