

Null Results in Brief

Effect Modification by Smoking on the Association between Genetic Polymorphisms in Oxidative Stress Genes and Colorectal Cancer Risk

Silvia Funke,¹ Michael Hoffmeister,² Hermann Brenner,² and Jenny Chang-Claude¹

¹Division of Cancer Epidemiology and ²Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany

Introduction

There is growing evidence that oxidative stress induced by reactive oxygen species (ROS) is involved in colorectal carcinogenesis by affecting cellular processes critical in tumor development (1). Crucial genes in the oxidative stress pathway include catalase (*CAT*), manganese superoxide dismutase (*MnSOD*), myeloperoxidase (*MPO*), and endothelial nitric oxide synthase (*eNOS*), coding for enzymes that are related to oxidative stress mechanisms by either neutralizing or generating ROS. Genetic polymorphisms in oxidative stress genes could alter colorectal cancer risk, and this association could be modified by increased ROS exposure following smoking. Tobacco smoke contains highly reactive free radicals and has been associated with colorectal cancer risk in a dose-dependent fashion. In accordance with the biological hypothesis that smoking may act as an initiator of colorectal tumorigenesis and, thus, requires a long induction period (2, 3), we found that long duration of smoking at a high cumulative dose increases colorectal cancer risk (4). In the present study, we assessed potential effect modification by long-term and high-dose smoking on the association between functional genetic polymorphisms in oxidative stress genes [*CAT* C²⁶²T (rs1001179), *MnSOD* Val⁹Ala (rs4880), *MPO* G⁴⁶³A (rs2333227), and *eNOS* Glu²⁹⁸Asp (rs1799983)] and colorectal cancer risk in a German case-control study.

Materials and Methods

Study Population. As described previously, we conducted a population-based case-control study on incident colorectal cancer in the southwest of Germany (4-6). Cases were identified by treating clinicians. Controls were randomly selected from population registers and frequency matched to the cases by sex, 5-y age groups, and county of residence. Eligible participants were of ages ≥ 30 y, resident of the study region, German speaking, and

physically and mentally able to participate in a personal interview. The study was approved by the ethics committee of the University of Heidelberg and the medical boards of Baden-Wuerttemberg and Rhineland-Palatinate. All participants provided written informed consent.

Comprehensive information on colorectal cancer risk factors including lifetime smoking habits was collected. Pathology records and discharge letters were collected for the cases. Between January 2003 and December 2004, 671 cases enrolled, with 643 (96%) providing a biological sample. Of the 1,391 eligible controls, 613 (44.1%) enrolled and provided a biological sample.

Genotyping. According to the manufacturer's instructions, genomic DNA was extracted from whole-blood samples using the FlexiGene DNA Kit (Qiagen) or from mouthwash samples using the QiAmp DNA Mini Kit (Qiagen). DNA isolation was successful for 632 (98.3%) cases and 606 (98.7%) controls. Single-nucleotide polymorphisms in *CAT*, *MnSOD*, *MPO*, and *eNOS* were analyzed using PCR followed by Pyrosequencing technology (Biotage, Uppsala, Sweden). Genotyping was done blinded to case-control status. A 10% random sample was genotyped twice for quality assurance, which yielded 100% concordance.

Statistical Analysis. Conditional logistic regression adjusted for potential confounders was used to assess the association between genetic polymorphisms and colorectal cancer risk and potential effect modification by smoking. Analyses were stratified by sex and 5-y age groups. Test for trend was done by using the Wald statistic.

Statistical interaction was assessed using a multiplicative interaction model. Interaction terms of the dichotomous genotype variable and the dummy-coded ordinal variables for pack-years of smoking (never smoking, 1 to 29 pack-years, ≥ 30 pack-years) were included in the model. Statistical significance of interaction was analyzed by comparing the model with and without the respective interaction terms by means of the likelihood-ratio test with corresponding degrees of freedom. To consider potential effect modification by high cumulative dose solely, we also assessed interaction including only the variable for ≥ 30 pack-years in the model.

Results

All single-nucleotide polymorphisms were in Hardy-Weinberg equilibrium and the observed allele frequencies

Cancer Epidemiol Biomarkers Prev 2009;18(8):1-3

Received 5/27/09; accepted 6/1/09; published OnlineFirst 7/21/09.

Grant support: German Research Foundation (grants BR 1704/6-1, BR 1704/6-3, and CH 117/1-1). S. Funke has a scholarship from the Deutsche Forschungsgemeinschaft, Graduiertenkolleg 793.

Requests for reprints: Jenny Chang-Claude, Division of Cancer Epidemiology, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Phone: 49-6221-422373; Fax: 49-6221-422203. E-mail: j.chang-claude@dkfz.de
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doi:10.1158/1055-9965.EPI-09-0507

Table 1. ORs for colorectal cancer according to polymorphisms in *CAT*, *MnSOD*, *MPO*, and *eNOS*

Genetic polymorphism	n (cases/controls)	Crude model* [OR (95% CI)]	Adjusted model† [OR (95% CI)]	P _{trend}
<i>CAT</i>				
C/C	374/348	1	1	0.64
C/T	235/231	0.95 (0.75–1.20)	0.95 (0.73–1.22)	
T/T	23/26	0.82 (0.46–1.47)	0.92 (0.49–1.73)	
T carriers		0.94 (0.75–1.18)	0.94 (0.74–1.21)	
<i>MnSOD</i>				
T/T	136/146	1	1	0.4
C/T	321/294	1.18 (0.89–1.57)	1.18 (0.86–1.60)	
C/C	166/163	1.1 (0.80–1.52)	1.17 (0.83–1.65)	
C carriers		1.15 (0.88–1.51)	1.17 (0.88–1.57)	
<i>MPO</i>				
G/G	396/373	1	1	0.92
G/A	202/206	0.92 (0.72–1.17)	0.94 (0.73–1.22)	
A/A	29/24	1.25 (0.71–2.20)	1.24 (0.68–2.27)	
A carriers		0.95 (0.76–1.20)	0.97 (0.76–1.25)	
<i>eNOS</i>				
G/G	289/271	1	1	0.98
G/T	285/272	0.99 (0.78–1.25)	1.02 (0.79–1.31)	
T/T	58/61	0.90 (0.60–1.33)	0.99 (0.64–1.52)	
T carriers		0.97 (0.78–1.22)	1.01 (0.79–1.29)	

NOTE: *MnSOD*, T = Val, C = Ala; *eNOS*, G = Glu, T = Asp.

*Conditional logistic regression stratified by sex and age in 5-y age groups.

†Conditional logistic regression stratified by sex and age in 5-y age groups adjusted for education level, endoscopic screening, family history of colorectal cancer, body mass index, regular use of nonsteroidal anti-inflammatory drugs, frequency of red meat consumption, pack-years of smoking, and average lifetime daily alcohol consumption.

were comparable to those reported for Caucasians in the dbSNP database. Consistent with previous reports, differences between cases and controls were observed with respect to body mass index, endoscopic screening examinations, regular use of nonsteroidal anti-inflammatory drugs, and education level. Smoking status was not associated with colorectal cancer risk in multivariate analyses. However, an increased risk of colorectal cancer was observed in smokers who had smoked for ≥ 30 pack-years [odds ratio (OR), 1.33; 95% confidence interval (95% CI), 0.87–2.04]. None of the genetic polymorphisms were associated with colorectal cancer risk (Table 1). No significant interaction was observed between pack-years of smoking and genetic polymorphisms for the two models that included two levels of cumulative dose (1–29 pack-

years, ≥ 30 pack-years) or smoking ≥ 30 pack-years only (Table 2).

Discussion

We observed no effect modification either by low- or high-level smoking on the association between genetic polymorphisms in *CAT*, *MnSOD*, *MPO*, or *eNOS* and colorectal cancer risk. Focusing particularly on smoking for a long duration and at high intensity, which has been shown to contribute to colorectal carcinogenesis, we observed also no significant effect modification. We had more than 80% power to detect an interaction OR of 3.0 between ≥ 30 pack-years of smoking and *CAT*, *MnSOD*,

Table 2. ORs for colorectal cancer according to polymorphisms in *CAT*, *MnSOD*, *MPO*, and *eNOS* by pack-years of smoking

Genetic polymorphism	Never smokers		1–29 pack-years		≥ 30 pack-years		P*	P†
	Ca/Co	OR (95% CI)‡	Ca/Co	OR (95% CI)‡	Ca/Co	OR (95% CI)‡		
<i>CAT</i>								
C/C	180/174	1	139/138	1	49/32	1	0.41	0.61
T carriers	120/112	0.98 (0.69–1.41)	103/121	0.76 (0.51–1.12)	32/24	1.53 (0.52–4.57)		
<i>MnSOD</i>								
T/T	60/68	1	57/69	1	15/9	1	0.86	0.61
C carriers	237/216	1.18 (0.77–1.81)	180/190	1.28 (0.81–2.02)	65/47	0.82 (0.23–2.90)		
<i>MPO</i>								
G/G	191/174	1	148/163	1	50/36	1	0.76	0.52
A carriers	108/110	0.86 (0.60–1.23)	90/96	0.95 (0.63–1.42)	31/20	1.19 (0.43–3.24)		
<i>eNOS</i>								
G/G	141/125	1	102/113	1	40/29	1	0.96	0.79
T carriers	159/160	1.01 (0.71–1.44)	140/146	1.08 (0.73–1.61)	41/27	1.35 (0.53–3.43)		

NOTE: *MnSOD*, T = Val, C = Ala; *eNOS*, G = Glu, T = Asp.

Abbreviations: Ca, cases; Co, controls.

*P value for interaction of conditional logistic regression model between genotype and two categories of smoking (2 degrees of freedom).

†P value for interaction of conditional logistic regression model between genotype and ≥ 30 pack-years of smoking (1 degree of freedom).

‡Conditional logistic regression stratified by sex and age in 5-y age groups adjusted for education level, endoscopic screening, family history of colorectal cancer, body mass index, regular use of nonsteroidal anti-inflammatory drugs, frequency of red meat consumption, and average lifetime daily alcohol consumption.

MPO, or *eNOS* genotype and nearly 70% to detect an interaction OR of 2.5. Because the effect of smoking on colorectal cancer risk was quite modest, only strong interaction with oxidative stress gene polymorphisms would have been detectable in this study.

As tobacco smoke has the potential to generate oxidative stress (7), smoking could modify the association between polymorphisms in oxidative stress genes. Variant alleles of *CAT* C²⁶²T or *MnSOD* Val⁹Ala have been associated with increased ROS levels and, thus, were suggested to increase cancer risk, whereas variant alleles of *MPO* G⁴⁶³A and *eNOS* Glu²⁹⁸Asp, both related to low activity of the corresponding enzymes, have been proposed to reduce cancer risk. Epidemiologic studies considering effect modification by smoking on the association between genetic polymorphisms in oxidative stress genes and cancer risk are scarce. Despite a biological plausible hypothesis, this has thus far not been assessed for colorectal cancer. With regard to breast cancer risk, both significant (8) and nonsignificant (9-11) interactions between *MnSOD* Val⁹Ala and smoking were observed. However, studies done in other cancer sites did not reveal modification of smoking effects by *CAT* C²⁶²T (12), *MPO* G⁴⁶³A (13-19), or *eNOS* Glu²⁹⁸Asp (19). Large studies will be required to investigate potential interaction between smoking and polymorphisms in oxidative stress genes with respect to colorectal cancer, which is likely to be modest.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Cancer Epidemiol Biomarkers Prev Published OnlineFirst July 21, 2009.

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doi:[10.1158/1055-9965.EPI-09-0507](https://doi.org/10.1158/1055-9965.EPI-09-0507)

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