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Null Results in Brief

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

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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* $C^{262}T$ (rs1001179), *MnSOD* $Val^{9}Ala$ (rs4880), *MPO* $G^{463}A$ (rs2333227), and *eNOS* $Glu^{298}Asp$ (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

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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}
CAT				
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)	
MnSOD				
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)	
MPO				
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)	
eNOS				
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)‡		
CAT								
C/C	180/174	1	139/138	1	49/32	1		
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)	0.41	0.61
MnSOD								
T/T	60/68	1	57/69	1	15/9	1		
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)	0.86	0.61
MPO								
G/G	191/174	1	148/163	1	50/36	1		
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)	0.76	0.52
eNOS								
G/G	141/125	1	102/113	1	40/29	1		
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)	0.96	0.79

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.

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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* $C^{262}T$ or *MnSOD* $Val^{9}Ala$ have been associated with increased ROS levels and, thus, were suggested to increase cancer risk, whereas variant alleles of *MPO* $G^{463}A$ and *eNOS* $Glu^{298}Asp$, 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* $Val^{9}Ala$ and smoking were observed. However, studies done in other cancer sites did not reveal modification of smoking effects by *CAT* $C^{262}T$ (12), *MPO* $G^{463}A$ (13-19), or *eNOS* $Glu^{298}Asp$ (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.

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References

1. Franco R, Schoneveld O, Georgakilas AG, Panayiotidis MI. Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett 2008; 266:6–11.
2. Giovannucci E, Rimm EB, Stampfer MJ, et al. A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. men. J Natl Cancer Inst 1994;86:183–91.
3. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA 2008;300: 2765–78.
4. Verla-Tebit E, Lilla C, Hoffmeister M, Brenner H, Chang-Claude J. Cigarette smoking and colorectal cancer risk in Germany: a population-based case-control study. Int J Cancer 2006;119:630–5.
5. Lilla C, Verla-Tebit E, Risch A, et al. Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. Cancer Epidemiol Biomarkers Prev 2006;15:99–107.
6. Brenner H, Chang-Claude J, Seiler CM, Sturmer T, Hoffmeister M. Does a negative screening colonoscopy ever need to be repeated? Gut 2006;55:1145–50.
7. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 1985;64:111–26.
8. Tamimi RM, Hankinson SE, Spiegelman D, Colditz GA, Hunter DJ. Manganese superoxide dismutase polymorphism, plasma antioxidants, cigarette smoking, and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2004;13:989–96.
9. Slanger TE, Chang-Claude J, Wang-Gohrke S. Manganese superoxide dismutase Ala-9Val polymorphism, environmental modifiers, and risk of breast cancer in a German population. Cancer Causes Control 2006; 17:1025–31.
10. Millikan RC, Player J, de Cotret AR, et al. Manganese superoxide dismutase Ala-9Val polymorphism and risk of breast cancer in a population-based case-control study of African Americans and whites. Breast Cancer Res 2004;6:R264–74.
11. Mitrunen K, Sillanpaa P, Kataja V, et al. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk. Carcinogenesis 2001;22:827–9.
12. Choi JY, Neuhouser ML, Barnett M, et al. Polymorphisms in oxidative stress-related genes are not associated with prostate cancer risk in heavy smokers. Cancer Epidemiol Biomarkers Prev 2007;16: 1115–20.
13. Vineis P, Veglia F, Garte S, et al. Genetic susceptibility according to three metabolic pathways in cancers of the lung and bladder and in myeloid leukemias in nonsmokers. Ann Oncol 2007;18:1230–42.
14. Feyler A, Voho A, Bouchardy C, et al. Point: myeloperoxidase $^{-463}G\rightarrow A$ polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:1550–4.
15. Xu LL, Liu G, Miller DP, et al. Counterpoint: the myeloperoxidase $^{-463}G\rightarrow A$ polymorphism does not decrease lung cancer susceptibility in Caucasians. Cancer Epidemiol Biomarkers Prev 2002;11:1555–9.
16. London SJ, Lehman TA, Taylor JA. Myeloperoxidase genetic polymorphism and lung cancer risk. Cancer Res 1997;57:5001–3.
17. Misra RR, Tangrea JA, Virtamo J, et al. Variation in the promoter region of the myeloperoxidase gene is not directly related to lung cancer risk among male smokers in Finland. Cancer Lett 2001;164:161–7.
18. Cascorbi I, Henning S, Brockmoller J, et al. Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant –463A of the myeloperoxidase gene. Cancer Res 2000;60:644–9.
19. Yang J, Ambrosone CB, Hong CC, et al. Relationships between polymorphisms in NOS3 and MPO genes, cigarette smoking and risk of post-menopausal breast cancer. Carcinogenesis 2007;28:1247–53.

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