Point/Counterpoint

Counterpoint: The Myeloperoxidase $^{-463}$G→A Polymorphism Does Not Decrease Lung Cancer Susceptibility in Caucasians[1]

Li-Lian Xu,[2] Geoffrey Liu,[2] David P. Miller, Wei Zhou, Thomas J. Lynch, John C. Wain, Li Su, and David C. Christiani[3]

Occupational Health Program, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts 02115 [L-L. X., G. L., D. P. M., W. Z., L. S., D. C. C.], and Thoracic Surgery Unit, Department of Surgery [J. C. W.], Pulmonary and Critical Unit Medicine [D. C. C.] and Cancer Center, Division of Hematology-Oncology [G. L., T. J. L.], the Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114

Abstract

The myeloperoxidase (*MPO*) *G*-to-*A* substitution polymorphism in the promoter region of the *MPO* gene has been associated with a 40–70% reduction in lung cancer risk in several studies, although a recent nested case-control study disputes these findings. MPO is involved in the activation of a number of procarcinogens, including benzo(*a*)pyrene. The variant *A* allele has been shown to reduce MPO mRNA expression, thus potentially decreasing carcinogen activation. To confirm results from smaller studies, we evaluated this *MPO* polymorphism in 988 incident Caucasian lung cancer cases and 1128 controls. Logistic regression evaluated the association between *MPO* genotype and lung cancer risk, adjusting for age, gender, smoking status, time since quitting smoking, and pack-years of smoking. In the controls, the *A* allele frequency was 21%, and genotype distribution was in the Hardy-Weinberg equilibrium. Compared with the wild-type *G/G* genotype, the adjusted odds ratios for the *A/A* and *A/G* genotypes were 1.15 (95% confidence interval 0.7–1.9, $P > 0.2$) and 1.03 (95% confidence interval 0.8–1.3, $P > 0.20$), respectively. A similar lack of association was seen in analyses stratified by smoking status, median age, a number of smoking variables, disease stage, tumor grade, and histological subtype. These findings are in contrast with earlier studies suggesting a protective effect of carrying the variant *A* allele.

Received 6/22/01; revised 9/12/02; accepted 9/20/02.

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.

[1] Supported by NIH Grants CA74386, ES/CA06409, and ES00002. Dr. Xu was supported by training grant ES T3207069, and Dr. Liu was supported by a Doris Duke Clinical Scientist Development Award and a Charles A. King research fellowship.

[2] L-L. X. and G. L. contributed equally to this work.

[3] To whom requests for reprints should be addressed, at the Occupational Health Program, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115. Phone: (617) 432-3323; Fax: (617) 432-3441; E-mail: dchris@hohp.harvard.edu.

Introduction

The MPO[4] enzyme is a lysosomal hemoprotein found in the primary granules of neutrophils and monocytes/macrophages (1). In the lung, large numbers of neutrophils are recruited after pulmonary injury occurs (2). Such injuries include infection and lung damage caused by exposure to tobacco smoke, asbestos, or ozone (3–7). After neutrophils become activated, MPO is released extracellularly into phagocytic vacuoles (8, 9). MPO catalyzes hypochlorous activity, which acts physiologically as an antimicrobial agent, particularly against bacteria (10). In addition, MPO produces oxygen radicals that can cause DNA damage (11, 12). MPO has Phase I metabolizing activity by converting lipophilic carcinogens into hydrophilic forms (1). During this metabolic biotransformation, MPO converts tobacco smoke precarcinogens, such as benzo(*a*)pyrene, and arylamines are converted into highly carcinogenic intermediates, such as benzo(*a*)pyrene dio-epoxide (13, 14).

The *MPO* gene contains an untranslated region polymorphism. A single base substitution of *G*-to-*A* at position −463 of the promoter region of the *MPO* gene is associated with a marked decrease in its transcriptional activity, because of the disruption of an SP1-binding site in an *Alu* element (15, 16). This genetically associated lower MPO activity in carriers of the *A* allele may represent a protective host factor. A number of case-control studies has examined the role of this *MPO* polymorphism in lung cancer, with apparent 40–70% reductions in lung cancer risk (17–20). Although Caucasians, African-Americans, Hawaiians, and individuals of Japanese descent were evaluated in these studies, the *MPO A* allele was protective primarily in Caucasians. However, a recent nested case-control study in male smokers from Finland found no protective association (21). The sample sizes of Caucasian cases in all published studies are <400. To confirm the results of these earlier studies showing a protective effect of the variant *A* allele, we conducted a case-control study of this $^{-463}G→A$ *MPO* polymorphism in a study of >900 lung cancer cases and 1100 controls.

Materials and Methods

Details of this population have been published previously (22). In brief, eligible cases from this ongoing molecular epidemiological study consisted of histologically confirmed newly diagnosed lung cancer patients over the age of 18 years, who were treated at Massachusetts General Hospital between January 1993 and 2001. Controls were friends or spouses of patients (with either lung cancer or other cardiothoracic problems), with no matching characteristics. Thus, none of the controls were themselves patients but were recruited from a list of names offered by the case. Where possible, friends were recruited in

[4] The abbreviations used are: MPO, myeloperoxidase; SR-PY, square root pack-years; OR, odds ratio; CI, confidence interval.

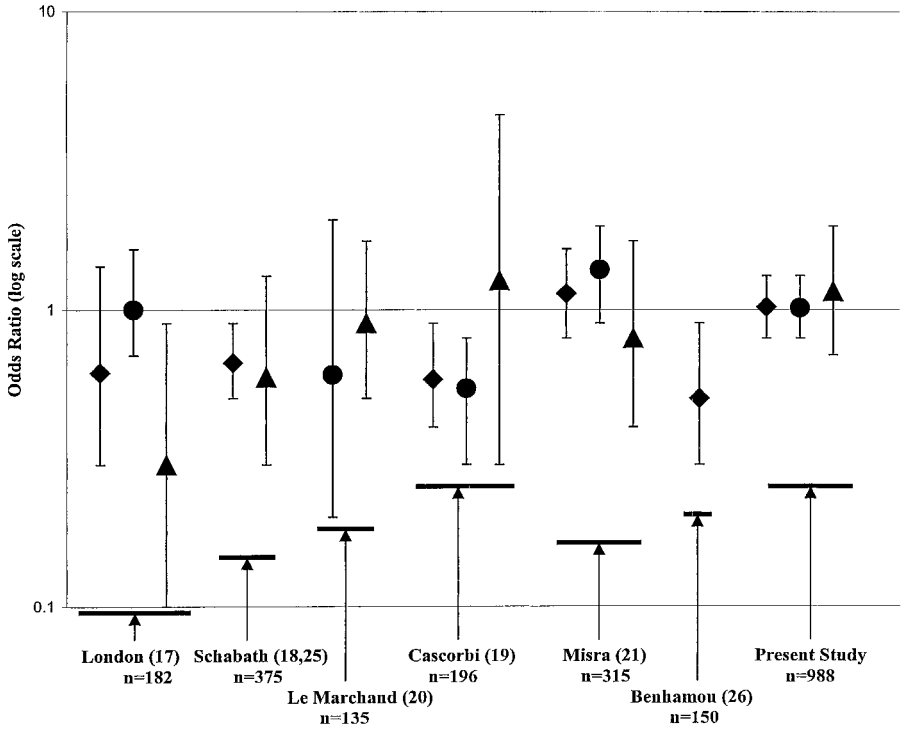

Fig. 1. Comparison of adjusted ORs and 95% CIs from studies of MPO polymorphisms, including only Caucasian populations. ◆, combined A/A + A/G genotypes; ●, A/G genotype alone; ▲, A/A genotype alone. Only first authors are listed for each study (references in parenthesis), and the sample sizes, *n*, represent the number of cases in each study.

favor of spouses. Friends or spouses who carried a previous diagnosis of any cancer (other than nonmelanoma skin cancer) were excluded from participation as a control. Interviewer-administered questionnaires collected information on occupational, dietary, medical, and detailed smoking histories from each subject. Peripheral blood samples were collected from all participants. This study was approved by the Human Subjects Committees of Massachusetts General Hospital and the Harvard School of Public Health (Boston, MA).

Laboratory personnel were blinded to case and control status. DNA was extracted from peripheral blood samples using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). *MPO* polymorphisms were detected using PCR-RFLP methods (17) reported previously, using the forward primer, 5′-CGG TAT AGG CAC ACA ATG GTG AG-3′ and the reverse primer 5′-CAA TGG TTC AAG CGA TTC TTC-3′. A portion (5%) of the samples, randomly chosen, was genotyped a second time for quality control testing.

Over 96% of our cases and controls were non-Hispanic Caucasians by self-report. Although we enrolled individuals of all races, we restricted the primary analysis to Caucasians with complete information on age, gender, smoking status (non, ex-, and current smokers), pack-years of smoking, and years since smoking cessation (for ex-smokers). We limited our primary analysis to Caucasians to diminish confounding attributable to allele frequency variation in different ethnic and racial groups and because previous studies found the strongest associations in Caucasian subgroups. Demographic and genotype data were tabulated for all individuals and separately for cases and controls. Because there were between zero and three controls (median = 1) corresponding to each case (some cases identified more potential controls, although they were not able to identify a single appropriate control subject), we broke the matching process for all analyses. Thus, unconditional logistic regression and generalized additive models (23) were used to examine the relationship between the log odds of lung cancer and each covariate, after adjusting for possible confounding factors, such as age, gender, smoking status, pack-years of smoking, and years since smoking cessation. Years since smoking cessation were defined as zero for both current and nonsmokers. Some variables were transformed to approach normality. Because generalized additive model plots showed a linear association between lung cancer risk and SR-PY, but not for the untransformed variable, we used SR-PY in our logistic regression models. Age, SR-PY, and years since smoking cessation were included as continuous variables. OR and 95% CI were reported, where appropriate. Stratified analyses were possible, given the relatively large sample size of this study; we performed separate analyses by age, gender, and smoking status. We also performed analyses that compared squamous cell carcinoma or adenocarcinoma, separately with all controls; the other histological cell types were considered too rare to be individually analyzed. Where appropriate, we attempted to construct our stratified analyses in a manner that made it directly comparable with analyses from studies published previously.

Results

A group of 2253 samples was successfully genotyped. A group of 2116 (94%) samples was from non-Hispanic Caucasians with complete covariate data. Quality control testing yielded 100% concordance in repeated random samples. Less than 1% disagreement occurred between two reviewers of the agarose gels, primarily about accepting or failing the DNA band quality on a gel. We conducted analyses including and excluding this 1% of samples and found no difference in our conclusions. The genotype distributions of controls (and, incidentally, cases) were in Hardy-Weinberg equilibrium ($P > 0.20$; χ^2 goodness of fit). An *A* allele frequency of 0.21 was found in controls, which was within the range (0.2–0.26) described previously for

Table 1 Demographic information

	Case (988) *n* (%)	Control (1128) *n* (%)
Age		
<50	106 (11)	313 (28)
50–54	88 (9)	128 (11)
55–59	114 (12)	134 (12)
60–64	152 (15)	151 (13)
65–69	184 (19)	191 (17)
≥70	344 (35)	211 (19)
Gender		
Male	538 (54)	525 (47)
Female	450 (46)	603 (53)
Smoking		
Never-smoker	63 (6)	403 (36)
Ex-smoker	519 (53)	506 (45)
Years quit < 15	307 (59)	203 (40)
Years quit ≥ 15	212 (41)	303 (60)
Current smoker	406 (41)	219 (19)
Pack-years < 35	78 (19)	118 (54)
Pack-years 35–64	148 (37)	65 (30)
Pack-years ≥ 60	180 (44)	36 (16)
Type of Control (11 missing)		
Spouse		354 (32)
Friend		763 (68)
Histologic cell type (24 missing)		
Adenocarcinoma	472 (50)	
Squamous cell carcinoma	225 (24)	
Large cell carcinoma	77 (8)	
Small cell carcinoma	88 (9)	
Other/not specified	84 (9)	
Stage (31 missing)		
Early stage (I/II)	529 (56)	
Late stage (III/IV)	410 (44)	

Caucasians (17–21, 25, 26). We evaluated the ethnic subgroups of our Caucasian sample and found that the proportion of each self-reported ethnic subgroup was similar between cases and controls ($P > 0.2$ for each comparison; Ref. 22).

Table 1 presents the demographic information for cases and controls. Cases were statistically significantly older and more likely to be male, current smokers (as opposed to never-smokers), have a higher number of pack-years, and quit smoking more recently (if an ex-smoker) than controls ($P < 0.05$ for each covariate). The relatively low proportion of small cell carcinoma and advanced stage disease in our sample reflects both the referral nature at our tertiary care hospital and the targeted recruitment of surgically resectable patients between 1993 and 1996.

The *MPO* genotype was not associated with lung cancer risk in the overall sample. The *MPO* genotype distribution did not differ between lung cancer cases and controls (Table 2), regardless of whether covariate adjustments were made for various characteristics (such as smoking status). There were no differences noted regardless of whether we used spouse controls, friend controls, or both. Adjustment by smoking variables did not alter the ORs, and there were no genotype smoking associations seen in either cases or controls. In a secondary analysis, all individuals with complete covariate data, regardless of race, were analyzed, and the ORs were 1.28 (95% CI 0.8–1.9, crude) and 1.17 (95% CI 0.7–1.9, adjusted for age, gender, race, and smoking variables) for *A/A versus G/G*; 0.99 (95% CI 0.8–1.2, crude) and 0.98 (95% CI 0.8–1.2, adjusted) for *A/G versus G/G*; and 1.02 (95% CI 0.9–1.2, crude) and 1 (95% CI 0.8–1.2, adjusted) for *A/A + A/G versus G/G*.

We present the results of selected stratified analyses in Table 3. In total, we performed separate analyses stratified by the median age of controls into age groups (age <50, 50–54, 55–59, 60–64, 65–69, and >70 years) by gender, smoking status, median duration of smoking (for all participants), median number of cigarettes smoked per day (for all participants), and different pack-year categories [either divided by the median pack-year of all participants similar to the studies of London *et al.* (17) and Schabath *et al.* (18) or into the three categories of <30, 30–64, and >64 pack-years similar to Schabath *et al.* (24)]. Neither the crude nor adjusted analyses that evaluated *A/A*, *A/G*, or *A/A + A/G*, each compared with *G/G*, yielded statistically significant results for any subgroup ($P > 0.2$ for all comparisons).

Histologically specific logistic regression analyses were performed. Adjusted ORs of the *A/A*, *A/G*, and combined *A/A + G/G* genotypes, each compared, respectively, with the wild-type *G/G* genotype, were 1.36 (95% CI 0.8–2.5), 1.03 (95% CI 0.8–1.3), and 1.06 (05% CI 0.8–1.4) for adenocarcinomas and 1.82 (95% CI 0.8–4.1), 1.33 (95% CI 0.9–1.9), and 1.38 (95% CI 0.96–2) for squamous cell carcinomas ($P > 0.08$ for each comparison). In each comparison, there were ≥10 individuals in each category being compared; other histological subtypes (such as large cell cancer) did not meet this criterion and were not analyzed separately. Separate analyses for different disease stages (early I/II or late III/IV) and tumor grades (well/moderately or poorly/undifferentiated) were also performed, and no associations were found.

Discussion

Up to the end of 2001, six case-control studies (17–21, 25) have evaluated the relationship between *MPO* genotype and lung cancer risk in Caucasians (Fig. 1). Schabath *et al.* (24) recently expanded their original results to incorporate a larger sample size. Four studies, evaluating a total of 903 lung cancer cases over two continents, found a statistically significant protective effect of either the *A/A* genotype or the combined *A/A + A/G* genotypes (17–19, 24, 25). In addition, Le Marchand *et al.* (Ref. 20; $n = 135$) found a nonsignificant protective trend for the *A* allele, particularly in *A/G* heterozygotes. In contrast, Misra *et al.* (21) reported no association between *MPO* genotype and lung cancer risk in a nested case-control study of 315 cases, all male smokers from Finland who were originally entered into the Alpha-Tocopherol Beta-Carotene Cancer Prevention study. Eligible individuals were all middle-aged Finnish males who lacked significant comorbidities and had been actively smoking at the time of recruitment in the 1980s. Because of these eligibility restrictions, important lung cancer relationships with the *MPO* polymorphism could have been missed if there had been effect modification by gender, specific smoking variables, or smoking-related complications or comorbidities.

Our study, the largest one assessing the $^{-463}G\rightarrow A$ *MPO* genotype and lung cancer, evaluated 988 Caucasian cases and 1128 Caucasian controls. There were roughly equal numbers of males and females in this study, and individuals with a wide range of smoking exposures were included in the analysis. Because this was designed as a confirmatory study, power calculations were performed to ensure that we could make firm conclusions. To our surprise, we found no overall relationship between the *MPO* genotype and lung cancer risk.

A potential limitation in the present study was the lack of matching for age, gender, and smoking characteristics. To address this issue, we relied on covariate adjustments in the final analysis or by analyses stratified by potential confounders. We

Table 2 Genotype lung cancer association in caucasians

	G/G	*A/G*	*A/A*	*A/G + A/A*
n (%)				
Cases	599 (61%)	343 (35%)	47 (5%)	390 (39%)
Controls	697 (62%)	390 (35%)	41 (4%)	431 (38%)
Spouse controls	222 (63%)	119 (33%)	13 (4%)	132 (37%)
Friend controls	467 (61%)	268 (35%)	28 (4%)	296 (39%)
OR (95% CI)				
All controls				
Crude OR	1.0[a]	1.03 (0.9–1.2)	1.34 (0.9–2.1)	1.06 (0.9–1.3)
OR adjusted for age and gender	1.0	1.02 (0.8–1.2)	1.43 (0.9–2.2)	1.06 (0.9–1.3)
OR adjusted for age, gender, smoking variables[b]	1.0	1.01 (0.8–1.3)	1.15 (0.7–1.9)	1.03 (0.8–1.3)
Spouse control				
Crude OR	1.0	1.08 (0.8–1.4)	1.25 (0.7–2.3)	1.09 (0.9–1.4)
OR adjusted for age and gender	1.0	1.06 (0.8–1.4)	1.26 (0.7–2.4)	1.08 (0.8–1.4)
OR adjusted for age, gender, smoking variables	1.0	1.04 (0.8–1.4)	1.08 (0.5–2.1)	1.04 (0.8–1.4)
Friend controls				
Crude OR	1.0	1.00 (0.8–1.2)	1.31 (0.8–2.1)	1.03 (0.8–1.2)
OR adjusted for age and gender	1.0	1.01 (0.8–1.2)	1.46 (0.9–2.4)	1.05 (0.9–1.3)
OR adjusted for age, gender, smoking variables	1.0	0.95 (0.7–1.2)	1.10 (0.6–2.0)	0.97 (0.8–1.2)

[a] Reference group.
[b] Smoking variables include smoking status, pack-years, and years since smoking cessation for ex-smokers.

Table 3 Stratified analyses of *MPO* polymorphism and lung cancer in selected subgroups[a]

		Crude OR			Adjusted OR[b]		
		A/A vs. G/G	*A/G vs. G/G*	*A/A + A/G vs. G/G*	*A/A vs. G/G*	*A/G vs. G/G*	*A/A + A/G vs. G/G*
Age ≤ 59[c]		1.22 (0.6–2.4)	0.92 (0.7–1.3)	0.96 (0.7–1.3)	1.14 (0.5–2.4)	0.85 (0.6–1.2)	0.88 (0.6–1.2)
Cases	*n* = 282	16/171[d]	95/171[e]	111/171[f]			
Controls	*n* = 549	25/327	197/327	222/327			
Age > 59		1.68 (0.9–3.1)	1.11 (0.9–1.4)	1.16 (0.9–1.5)	1.10 (0.5–2.2)	1.10 (0.8–1.5)	1.10 (0.8–1.4)
Cases	*n* = 706	31/427	248/427	279/427			
Controls	*n* = 579	16/370	193/370	209/370			
Females		1.27 (0.7–2.4)	0.97 (0.7–1.3)	0.99 (0.8–1.3)	1.29 (0.6–2.8)	0.99 (0.7–1.4)	1.01 (0.7–1.4)
Cases	*n* = 450	20/273	157/273	177/273			
Controls	*n* = 603	21/365	217/365	238/365			
Males		1.38 (0.8–2.5)	1.10 (0.8–1.4)	1.13 (0.9–1.4)	1.08 (0.5–2.2)	1.05 (0.8–1.4)	1.05 (0.8–1.4)
Cases	*n* = 538	27/325	186/325	213/325			
Controls	*n* = 525	20/332	173/332	193/325			
Current smokers		2.19 (0.9–5.2)	1.16 (0.8–1.7)	1.25 (0.9–1.8)	2.38 (0.9–6.2)	1.25 (0.8–1.9)	1.34 (0.9–2.0)
Cases	*n* = 406	26/234	146/234	172/234			
Controls	*n* = 219	7/138	74/138	81/138			
Former smokers		0.80 (0.4–1.5)	1.01 (0.8–1.3)	0.98 (0.8–1.3)	0.72 (0.4–1.5)	0.95 (0.7–1.3)	0.92 (0.7–1.2)
Cases	*n* = 519	19/322	178/322	197/322			
Controls	*n* = 506	23/312	171/312	194/312			
Nonsmokers		n/a	0.77 (0.4–1.4)	0.79 (0.5–1.4)	n/a	0.79 (0.4–1.4)	0.82 (0.5–1.4)
Cases	*n* = 63	2/42	19/42	21/42			
Controls	*n* = 403	11/247	145/247	156/247			
Adenocarcinomas		1.39 (0.8–2.4)	1.06 (0.8–1.3)	1.09 (0.9–1.4)	1.36 (0.8–2.5)	1.03 (0.8–1.3)	1.06 (0.8–1.4)
Cases	*n* = 473	23/283	167/283	190/283			
Controls	*n* = 1128	41/697	390/697	431/697			
Squamous cell carcinoma		2.02 (1.1–3.8)[d]	1.19 (0.9–1.6)	1.27 (0.95–1.7)	1.82 (0.8–4.1)	1.33 (0.9–1.9)	1.38 (0.96–2.0)
Cases	*n* = 225	15/126	84/126	99/126			
Controls	*n* = 1128	41/697	390/697	431/697			

[a] $P > 0.05$ unless otherwise indicated. n/a, numbers in at least one category or "cells" were too small (<10) for appropriate analysis.
[b] Adjusted analyses include covariates for age, gender, SR-PY, smoking status, and time since smoking cessation.
[c] Median age of controls.
[d] Number of *A/A*/number of *G/G*.
[e] Number of *A/G*/number of *G/G*.
[f] Number of *A/A + A/G*/number of *G/G*.

found no associations between *MPO* genotype and lung cancer risk in different age, gender, and smoking strata and no protective associations regardless of the different combinations of possible covariate adjustments. Even with stratification by combinations of two covariates, no new patterns were observed (stratification by three or more variables was limited by very small numbers). Although we acknowledge the potential for biased point estimates, there was a complete lack of a protective trend for the variant allele in all analyses.

Another potential limitation was the use of controls that

were recruited through the cases, and there is potential for selection bias. However, the controls were evaluated extensively for other polymorphisms, including *GSTM1*, *GSTT1*, *GSTP1*, *NQO1*, *CYP1A1*, *NAT2*, *MnSOD*, and *p53*. For each polymorphism and with the present *MPO* polymorphism, we found that the genotype distributions were similar to other United States-based Caucasian control samples and met Hardy-Weinberg equilibrium conditions when testing was appropriate. Finally, we used a heterogeneous group of Caucasian cases and controls that included those of Anglo-Saxon, French, German, Dutch, Scandinavian, and Southern and Eastern European descent. We found that the proportion of self-reported ethnic subgroups in cases was similar to controls. Furthermore, others have found no significant bias through the use of United States-based controls except in extreme conditions, albeit in controls that were population based (26).

It was important to perform separate analyses by disease stage at diagnosis, tumor grade, and histological cell type to account for potential selection bias in the present study because cases in this present study were not entirely consecutive, although annual participation rates were excellent (85–92%). Furthermore, cases were recruited in a tertiary care center and probably reflected skewed referral patterns. Stratified analyses yielded no significant differences (either statistically or in the magnitude of association) between different stages or grades, although we found a nonsignificant trend for an increased risk for both adenocarcinoma and squamous cell carcinoma in the presence of an *A* allele, suggesting either a weak effect or that the effect may be present in a subset of patients.

In conclusion, we were not able to confirm an association between lung cancer risk and the *G*-to-*A* substitution polymorphism in the promoter region of the *MPO* gene in a large sample. Our results are consistent with the lack of association reported by Misra *et al.* (21), in contrast to at least five other studies finding a protective effect (statistically significant or by trend) conferred by the variant allele (17–20, 25).

Acknowledgments

We thank the following staff members of the Lung Cancer Susceptibility Group: Barbara Bean, Andrea Solomon, Jessica Shin, Linda Lineback, Salvatore Mucci, Richard Rivera-Massa, Lucy Ann Principe, Lisa I. Wang, Yang Sai, Stephanie Shih, and Maria Fragoso.

References

1. Klebanoff, S. J. Myeloperoxidase: occurrence and biological function. *In:* J. Everse, K. E. Everse, and M. B. Grisham (eds.), Peroxidases in Chemistry and Biology, Vol. 1, pp. 1–35. Boca Raton, FL: CRC Press, Inc., 1991.

2. Hunninghake, G. W., and Crystal, R. G. Cigarette smoking and lung destruction. Accumulation of meutrophils in lungs of cigarette smokers. Am. Rev. Respir. Dis., *128:* 833–838, 1983.

3. Schmekel, B., Karlsson, S. E., Linden, M., Sundstrom, C., Tegner, H., and Venge, P. Myeloperoxidase in human lung lavage. A marker of local neutrophil activity. Inflammation, *14:* 447–454, 1990.

4. Schmekel, B., Hornblad, Y., Linden, M., Sunstrom, C., and Venge, P. Myeloperoxidase in human lung lavage. Internalization of myeloperoxidase by alveolar macrophages. Inflammation, *14:* 455–461, 1990.

5. Hunninghake, G. G., Gadek, J. E., Kawanami, O., Ferrans, V. J., and Crystal, R. G. Inflammatory and immune process in the human lung in health and disease: evaluation by bronchoalveolar lavage. Am. J. Pathol., *97:* 149–206, 1979.

6. Petrushka, J. M., Leslie, K. O., and Mossman, B. T. Enhanced lipid peroxidation in lung lavage of rats after inhalation of asbestos. Free Radical. Biol. Med., *11:* 425–432, 1991.

7. Mustafa, M. G. Biochemical basis of ozone toxicity. Free Radic. Biol. Med., *9:* 245–265, 1990.

8. Oldeberg, H., Olofsson, T., and Olsson, I. Myeloperoxidase-mediated extracellular iodination during phagocytosis in granulocytes. Scand. J. Hematol., *12:* 155–160, 1974.

9. Bradley, P. P., Christensen, R. D., and Rothstein, G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood, *60:* 618–622, 1982.

10. Nichols, B. A., Bainton, D. F., and Farquar, M. G. Differentiation of monocytes: origin, nature, and fate of their azurophil granules. J. Cell Biol., *50:* 498–515, 1971.

11. Reynolds, W. F., Chang, E., Douer, D., Ball, E. D., and Kanda, V. An allelic association implicates myeloperoxidase in the etiology of acute promyelocytic leukemia. Blood, *90:* 2730–2737, 1997.

12. Whiteman, M., Spencer, J. P., Jenner, A., and Halliwell, B. Hypochlorous acid-induced DNA base modification: potentiation by nitrite: biomarkers of DNA damage by reactive oxygen species. Biochem. Biophys. Res. Commun., *257:* 572–576, 1999.

13. Mallet, W. G., Mosebrook, D. R., and Trush, M. A. Activation of (±) *trans*-7, 8-dihydroxy-7, 8-dihydrobenzo-[a]-pyrene to diolepoxides by human polymorphonuclear leucocytes or myeloperoxidase. Carcinogenesis (Lond.), *12:* 521–524, 1991.

14. Kadubar, F. F., Butler, M. A., Kaderlik, K. R., Chou, H. C., and Lang, N. P. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. Environ. Health Perspect., *98:* 69–74, 1992.

15. Austin, G. E., Lam, L., Zaki, S. R., Chan, W. C., Hodge, T., Hou, J., Swan, D., Zhang, W., Racine, M., Whitsett, C., and Brown, B. Sequence comparisons of putative regulatory DNA of the 5′ flanking region of the myeloperoxidase gene in normal and leukemic bone marrow cells. Leukemia (Baltimore), *7:* 1445–1450, 1993.

16. Piedrafita, F. J., Molander, R. B., Vansant, G., Orlova, E. A., Pfahl, M., and Reynolds, W. F. An Alu element in the myeloperoxidase promoter contains a composite SPI-thyroid hormone-retinoic acid response element. J. Biol. Chem., *271:* 14412–14420, 1996.

17. London, S. J., Lehman, T. A., and Taylor, J. A. Myeloperoxidase genetic polymorphism and lung cancer risk. Cancer Res., *57:* 5001–5003, 1997.

18. Schabath, M. B., Spitz, M. R., Zhang, X., Delcros, G. L., and Wu, X. Genetic variants of myeloperoxidase and lung cancer risk. Carcinogenesis (Lond.), *21:* 1163–1166, 2000.

19. Cascorbi, I., Henning, S., Brockmuller, J., Gephart, J., Meisel, C., Muller, J. M., Loddenkemper, R., and Roots, I. Substantially reduced risk of cancer of the aerodigestive tract in subjects with variant −G463A of the myeloperoxidase gene. Cancer Res., *60:* 644–649, 2000.

20. Le Marchand, L., Seifried, A., Lunn, A., and Wilkens, L. R. Association of the myeloperoxidase −463G→A polymorphism with lung cancer risk. Cancer Epidemiol. Biomark. Prev., *9:* 181–184, 2000.

21. Misra, R. R., Tangrea, J. A., Virtamo, J., Ratnasinghe, D., Andersen, M. A., Barrett, M., Taylor, P. R., and Demetrius, A. Variation in the promoter region of the myeloperoxidase gene is not directly related to lung cancer risk among male smokers in Finland. Cancer Lett., *164:* 161–167, 2001.

22. Zhou, W., Thurston, S. W., Liu, G., Xu, L. L., Miller, D. P., Wain, J. C., Lynch, T. J., Su, L., and Christiani, D. C. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. Cancer Epidemiol. Biomark. Prev., *10:* 461–466, 2001.

23. Hastie, T. J., and Tibshirani, R. J. (eds.). Generalized Additive Models. New York: Chapman and Hall, Inc., 1990.

24. Schabath, M. B., Spitz, M. R., Delclos, G. L., Gunn, G. B., Whitehead, L. W., Wu, W. X. Association between asbestos exposure, cigarette smoking, myeloperoxidase (MPO) genotypes, and lung cancer risk. Am. J. Ind. Med. *42:* 29–37, 2002.

25. Feyler, A., Voho, A., Bouchardy, C., Koukkanen, K., Dayer, P., Hirvonen, A., and Benhamou, S. Myeloperoxidase −463 G→A polymorphism and lung cancer risk. Cancer Epidemiol. Biomark. Prev., *11:* , 2002.

26. Wacholder, S., Rothman, N., and Caporaso, N. Population stratification in epidemiologic studies of common genetic variants and cancer: quantification of bias. J. Natl. Cancer Inst. (Bethesda), *92:* 1151–1158, 2000.