Association of the Myeloperoxidase $\text{G}^{-463}\text{A}$ Polymorphism with Lung Cancer Risk

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Abstract

Myeloperoxidase, which is released from neutrophils in response to various pulmonary insults including tobacco smoke, is suspected to play a carcinogenic role in the lung. A G-to-A substitution polymorphism in the promoter region of the MPO gene has been suggested in in vitro studies to decrease gene transcription. We tested the association of this polymorphism with lung cancer in a population-based case-control study of 323 cases and 437 controls of Caucasian, Japanese, or Native Hawaiian ancestry in Hawaii. We found a marked difference in the frequency of the variant A allele among Caucasians (26%), Japanese (17%), and Hawaiians (13%). Overall, the variant allele was somewhat less frequent in cases than controls ($P = 0.13$). Individuals with the A/A genotype were found to be at a 50% decreased risk compared to those with two G alleles (95% confidence interval, 0.2–1.3). Although not statistically significant, this inverse association was suggested in both sexes and two of the three ethnic groups studied. Heterozygotes were at no decreased risk. Further work needs to clarify the functional relevance of the A allele in vivo and to confirm the inverse association of the A/A genotype with lung cancer in large epidemiological studies.

Introduction

The MPO³ enzyme functions as an antimicrobial agent in neutrophils by catalyzing the production of genotoxic hypochlorous acid and other reactive oxygen species (1). Upon activation of the neutrophils, MPO is released into phagocytic vacuoles and in the extracellular milieu (2, 3). MPO may also play a role in the bioactivation of tobacco smoke carcinogens in the lung, such as benzo[a]pyrene and arylamines (4, 5). Because neutrophils are recruited in large numbers to the lung in response to various pulmonary insults, including infection, tobacco smoke particulates, asbestos, and ozone (6–10), which are all risk factors for lung cancer, MPO is suspected to play a role in lung carcinogenesis (4, 11).

A G→A substitution at position −463 in the promoter region of the MPO gene has been associated in cellular transfection assays with a decreased transcriptional activity due to the disruption of an SP1 binding site (12). The high activity allele was originally described as being associated with acute myeloid leukemia (13). Individuals who inherit two copies of the low activity allele have subsequently been found to be at a decreased risk of lung cancer in a case-control study of Caucasians and African Americans in Los Angeles (14). We sought to replicate this finding in a population-based case-control study of lung cancer conducted among Caucasians, Japanese, and Native Hawaiians in Hawaii.

Materials and Methods

Details of the study population have been published previously (15). Lung cancer patients were identified by the rapid-reporting system of the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. Eligible cases were all patients with histologically confirmed primary lung cancer who were newly diagnosed between January 1, 1992, and March 31, 1997 in all main medical centers on the island of Oahu, Hawaii. Other eligibility criteria included age between 18 and 79 years, Oahu residency, no previous history of lung cancer, and ethnicity (≥75% Japanese, ≥75% Caucasian, any Hawaiian/part-Hawaiian heritage). An interview was completed for 64% of the eligible cases. The main reasons for nonparticipation were patient refusal (17%), physician refusal (2%), and death with absence of a suitable surrogate for interview (17%).

Controls were randomly selected from a list of Oahu residents interviewed by the State of Hawaii Department of Health as part of a health survey of a 2% random sample of households in the state. This source was supplemented with controls over age 65 from the Health Care Financing Administration participants on Oahu. One control was matched to each case on sex, ethnicity, and age (±2 years). The overall participation rate for the controls was 62% percent. Reasons for nonparticipation included refusal (25%), inability to locate (10%), serious illness (1%), and death (2%). A total of 341 cases (76% of interviewed cases) and 456 population controls (80% of interviewed controls) donated a blood specimen for the study. Eighteen cases and 19 controls were excluded from the present analysis because the DNA extracted from their blood sample had been depleted.

In-person interviews were conducted at the subjects’ homes by trained interviewers. On average, cases were interviewed within 4 months of diagnosis. The questionnaire included detailed demographic information, including ethnic origin of each grandparent, a lifetime history of tobacco and alcohol use, a quantitative food frequency questionnaire, vari-
Gotes have all four bands (the Lane 4), whereas those homozygous for the A allele show three bands, at 169, 120, and 61 bp (Lanes 1 and 2). Heterozygotes have all four bands (Lanes 3 and 5–8). Lane M, Hinfl-digested ϕ-X174 DNA molecular weight marker.

Fig. 1. Gel showing the three genotypes for the polymorphism at position 463 of the MPO gene. An invariant AciI restriction site in the 350-bp amplification fragment yields a 61-bp fragment in all samples. The G-to-A substitution leads to a loss of an additional AciI restriction site. Individuals homozygous for the A allele have two bands, at 289 and 61 bp (Lane 4), whereas those homozygous for the G allele show three bands, at 169, 120, and 61 bp (Lanes 1 and 2). Heterozygotes have all four bands (Lanes 3 and 5–8). Lane M, Hinfl-digested ϕ-X174 DNA molecular weight marker.

Results

Forty % of subjects were Caucasian, 36% were Japanese, and 24% were Hawaiian. No significant differences were noted in the sex and ethnic distributions of the cases and controls contributing a blood sample. The distribution of subjects by case-control status, ethnicity, and MPO genotype (G/G, G/A, A/A) is presented in Table 1. Based on the controls, the frequency of the A allele was estimated to be 26% in Caucasians, 17% in Japanese, and 13% in Hawaiians in our population. Overall (P for χ2 test for association = 0.13) and in Caucasians (P = 0.10) and Japanese (P = 0.19), but not in Hawaiians (P = 0.56), the A allele was more common in controls than in cases. The unadjusted lung cancer OR for the G/A and A/A genotypes, compared to the G/G genotype, was 0.8 (95% CI, 0.6–1.2) and 0.6 (95% CI, 0.3–1.1), respectively, with a trend of decreasing risk with the number of A alleles that was close to statistical significance (P = 0.07; Table 1). This trend did not persist after adjustment for other lung cancer risk factors in this study, although the point estimate of the OR for the A/A genotype did not materially change (0.5). The 95% CI for this OR was 0.2–1.3. Very similar risk estimates were found for males [1.0, 1.1 (0.6–1.8), 0.6 (0.2–2.1)] and females [1.0, 1.1 (0.6–2.3), 0.6 (0.2–2.0)]. The ethnic-specific analysis showed a similar, nonsignificant decrease in risk for the A/A genotype in Caucasians and Hawaiians, whereas no clear association was suggested in Hawaiians (Table 1). No cell type specificity was suggested for the association because similar ORs were obtained for squamous cell carcinomas and adenocarcinomas. The respective adjusted ORs for the G/A and A/A genotypes were 1.4 (0.7–2.9) and 0.6 (0.1–3.6) for squamous cell carcinoma, and 0.9 (0.6–1.6) and 0.5 (0.2–1.5) for adenocarcinoma. Finally, the interactions of the MPO genotype with pack-years and total vegetable intake (≤median versus >median), as well as with GSTM1 genotype (15), were investigated. No suggestion of interaction was detected, although, due to the relatively low frequency of the A allele, the power was limited.

Discussion

In this population-based case-control study of lung cancer, we found weak evidence that a polymorphism suggested to reduce transcription of the MPO gene confers a reduced risk of lung cancer. Although the risk of individuals with the A/A genotype was decreased by a sizable 50% compared to those who inherited two G alleles, this risk estimate was not statistically significantly different from 1.0. Overall, we interpret our data as being supportive of an association because they are internally consistent (the inverse association was suggested in both sexes and in two of the three ethnic groups studied) and because they are also consistent with the only previous study of this association.

London et al. (14) reported the results of a population-
based case-control study of MPO and lung cancer conducted among 182 Caucasian and 157 African American cases and 459 Caucasian and 244 African American controls in Los Angeles. They found that Caucasians with the A/A genotype were at a 70% decreased risk of lung cancer (OR = 0.3; 95% CI, 0.1–0.9) compared to those with G/G. The decreased risk with this genotype in African Americans was present but of a smaller magnitude (OR = 0.6; 95% CI, 0.3–1.4).

Possible reasons for the somewhat weaker ORs found in the present study, compared to the findings by London et al. (14), include the lower frequency of the variant allele in two of the ethnic groups studied (17% for Japanese and 13% for Hawaiians in Hawaii compared to 23% for Caucasians and 30% for African Americans in Los Angeles). However, in Caucasians, despite a very similar allele frequency in both populations (26% versus 23%), the risk estimate for the A/A genotype was closer to one in our study, implicating other factors. One such factor may be the greater cigarette smoking exposure in the Los Angeles study, where the median for pack-years was 35 compared to 28 in the present study. However, no evidence of a stronger association with lung cancer at higher levels of cigarette smoking was found for the A/A genotype in either study.

Thus, the somewhat stronger association between MPO and lung cancer in Los Angeles may result from unmeasured contributing exposures. One such exposure may be high ozone levels leading to the recruitment of neutrophils in the airways and the release of MPO (10). As pointed out by London et al. (14), Los Angeles has the highest ozone levels in the United States. In contrast, air quality is excellent on Oahu.

An association of MPO with lung cancer is biologically plausible. MPO is released from neutrophils in the lung as part of the inflammatory response to a variety of local exposures, such as infectious agents, tobacco smoke particulates, ozone, and asbestos. MPO and its reactive by-products have been linked to oxidative stress (17), DNA-strand breakage (18), bioactivation of carcinogens (4, 5), and inhibition of DNA repair (19). The G-to-A substitution in the promoter region of the MPO gene is located in a cluster of nuclear receptor sites in an Alu element. The presence of an A instead of a G at position −463 disrupts the core binding site for a SP1 transcription factor, as well as a retinoic acid response element. The A allele has been associated with a severalfold less transcriptional activity than the G allele in cellular transfection assays (12). Finally, the G/G genotype was found to be over represented in acute promyelocytic leukemia, a subtype of acute myelocytic leukemia in which MOP is highly expressed (13). Given our results and those of London et al. (14), the significant concentration and possible carcinogenic role of MPO in the lung, and the in vitro data suggesting a lower gene transcription with the A allele, the possibility of an association between the A allele and lung cancer is a strong possibility. Further work needs to clarify the functional relevance of the A allele in vivo and to confirm the inverse association between the A/A genotype and lung cancer in large epidemiological studies.

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References

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<sup>a</sup> P for the genotype variable assigned the values 1, 2, or 3 according to the subject’s number of A alleles (0, 1, and 2, respectively).
<sup>b</sup> Unadjusted OR and exact 95% CI.
<sup>c</sup> OR and 95% CI adjusted for age, sex, ethnicity (when appropriate), smoking status, years of smoking, (years of smoking)<sup>2</sup>, number of cigarettes/day, and saturated fat and vegetable intakes.


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