Myeloperoxidase Genetic Polymorphism and Lung Cancer Risk

Stephanie J. London, Teresa A. Leeman, and Jack A. Taylor

Abstract

Myeloperoxidase is a lysosomal enzyme found in high concentrations in human lung due to recruitment of neutrophils. Myeloperoxidase activates benzo[a]pyrene as well as aromatic amines in tobacco smoke and generates carcinogen-free radicals. A single base substitution (G to A) in the promoter region of the myeloperoxidase gene has recently been demonstrated to markedly reduce transcription. We developed an RFLP/PCR assay to test the hypothesis that the allele favoring lower transcription (A allele) reduces the risk of lung cancer. Among population controls, 7.8% of 459 Caucasians and 9.4% of 244 African-Americans inherited two copies of the A allele. Caucasians with the A/A genotype were at 70% reduced risk of lung cancer (odds ratio, 0.30; 95% confidence interval, 0.10—0.93; P = 0.04; 182 cases). A lesser reduction in risk was observed for African-Americans with this genotype (odds ratio, 0.61; 95% confidence interval, 0.26—1.41; 157 cases). Individuals who inherited two copies of an allele that reduces transcription of the myeloperoxidase gene may be at decreased risk of lung cancer.

Introduction

MPO is an enzyme found primarily in the lysosomes of neutrophils. Exposure to a variety of pulmonary insults, including cigarette smoke, stimulates recruitment of neutrophils into human lung tissue (1) with local release of MPO (2, 3). MPO activates carcinogens in tobacco smoking including B[a]P (4) and aromatic amines (5) and catalyzes the endogenous formation of carcinogenic free radicals (6). A single base substitution (G to A) in an Alu repeat in the promoter region of the MPO gene, originally reported by Austin et al. (7) as a somatic mutation in acute myelocytic leukemia cells, has recently been shown instead to be an inherited polymorphism with functional significance in vitro (8). The presence of an A rather than a G at this site, 463 bases upstream from the MPO gene, decreases expression, apparently by destroying a binding site for the SP1 transcription factor (8). Given the strong biological rationale for a role of MPO activity in lung cancer etiology, we tested the hypothesis that the presence of an A rather than a G at position —463 in the MPO promoter region decreases the risk of lung cancer. To obviate the need for direct sequencing of DNA used previously (7, 8), we developed a RFLP/PCR method to genotype incident cases of lung cancer and population controls enrolled in a case-control study of African-Americans and Caucasians in Los Angeles County (9).

Materials and Methods

Details of the study population have been published previously (9, 10). In brief, we enrolled incident cases of lung cancer diagnosed at 1 of 35 hospitals in Los Angeles County between September 1, 1990, and January 6, 1994. At enrollment, cases had to be within 7 months of diagnosis to avoid possible bias due to selection of cases with longer survival. Controls under age 65 were sampled from driver’s license lists and those over 65 from lists of Medicare beneficiaries. We frequency matched on age, sex, and ethnicity. Participation was sought by repeated mailings supplemented by phone calls and home visits to nonrespondents. All subjects were residents of Los Angeles County, aged 40—84 years, and able to complete a questionnaire in English. Cases had no prior history of cancer, other than nonmelanoma skin cancer. Subjects completed a questionnaire on risk factors for lung cancer and provided a blood sample. The protocol was reviewed and approved by the Human Subjects Committee at the University of Southern California.

We extracted DNA from peripheral blood lymphocytes as described previously (9). The polymorphic site at position —463 of the MPO gene was amplified using forward primer MPOF (5'-CGG TAT AGG CAC ACA ATG GAC-3') and reverse primer MPOR (5'-GCA ATG GAA CAA GCG ATF-3') by PCR (7). PCR was performed with 200 ng of genomic DNA with 12 pmol of each primer in a 50-μl reaction volume containing 50 mM Tris-HCl (pH 9.0), 20 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, 37.5 μM of each nucleotide, and 2.5 units of Taq polymerase (Life Technologies, Inc., Rockville, MD). The cycling conditions were 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min with a final cycle at 72°C for 7 min. A 5-μl aliquot was digested with 20 units of AciI restriction enzyme (Life Technologies, Inc.) in 2.5 μl of 10× NEB3 buffer [500 mM Tris-HCl, 100 mM MgCl₂, 1 mM NaCl, 10 mM DTT (pH 7.9)] and 15.5 μl water at 37°C for 3 h and separated on a 2.5% agarose gel.

The G to A polymorphism at position —463 in the promoter region of the MPO gene destroys (A allele) or creates (G allele) an AciI restriction site within the 350-bp amplification fragment. In addition, there exists within the amplification fragment an invariant AciI restriction site, yielding a 61-bp fragment, which serves as an internal control for digestion. The three possible genotypes are defined by three distinct banding patterns: A/A (289- and 61-bp fragments), A/G (289-, 169-, 120-, and 61-bp fragments), and G/G (169-, 120-, and 61-bp fragments) (Fig. 1).

Results

The mean age was 63.6 years (SD, 9.4) for cases and 62.5 years (SD, 8.4) for controls. The percentage of females was slightly lower...
The 350-bp PCRamplification fragment contains an invariant AcI restriction site yielding A/A, are shown in Table 1. The A allele was relatively common in both ethnic groups. Among Caucasian controls, 39.0% of subjects carried at least one A allele and 9.4% were A/A.

Counts of subjects by the three MPO genotypes, G/G, G/A, and A/A, are shown in Table 1. The A allele was relatively common in both ethnic groups. Among Caucasian controls, 39.0% of subjects carried at least one A allele and 7.8% were homozygotes (genotype A/A). Among African-American controls, 50.4% of subjects carried at least one A allele and 9.4% were A/A.

The association between MPO genotype and lung cancer risk is shown in Table 1. All ORs are calculated relative to subjects with the G/G genotype. Among African-Americans, subjects with the A/A genotype were at decreased risk of lung cancer—70% for smokers and 22.5% current smokers. Current smoking among controls was similar to that observed among persons of comparable age and ethnicity in a contemporary national survey (12).

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The MPO promoter region polymorphism has been implicated in acute myelocytic leukemia in two small series of patients (7, 8). Ours is the first report of this MPO polymorphism in relation to any other disorder. We observed an association between MPO genotype and lung cancer risk in the direction predicted given that the A allele has been shown to reduce transcription of the MPO gene in vitro (8). Subjects homozygous for the A allele, representing about 8—10% of the population, were at decreased risk of lung cancer—70% for Caucasians and 39% for African-Americans.

Although our findings require confirmation in other studies, it is highly biologically plausible that a polymorphism in the MPO promoter region, producing lower MPO activity, would be associated with reduced risk of lung cancer. Most previous studies of genetic variation in metabolism of lung carcinogens have focused on metabolic activation by various cytochrome P-450s, although expression of these enzymes in lung is generally low (13). In contrast, the high levels of MPO activity observed in human bronchi point to the potential importance of this enzyme in pulmonary toxicology (2, 5). MPO activates an intermediate metabolite of B[a]P, a major carcinogen in tobacco smoke, to the ultimate carcinogen, the B[a]P diol-epoxide (4, 14). In addition, MPO enhances binding of B[a]P-7,8 diol to lung DNA in vitro (14) and formation of sister chromatid exchanges (15). MPO also activates aromatic amines contained in tobacco smoke (5, 13). Furthermore, MPO could influence lung cancer risk by an independent mechanism: formation of hypochlorous acid, a potent source of oxidizing free radicals, which can lead to carcinogenesis by directly damaging DNA (16).

It is interesting to note that ozone has been shown to enhance the MPO-dependent activation of B[a]P in rat lung (17). Although this interaction has not been studied in humans, ozone exposure leads to neutrophil accumulation in human lung, and human neutrophils appear to have even higher capacity than those of the rat for B[a]P activation (16). Thus, it is not implausible that ozone might increase MPO-mediated B[a]P activation in humans as well. This speculation could be relevant to our data, which was collected in the Los Angeles metropolitan area and has the highest ozone levels in the United States (18).

Interpretation of data on the potential role of this MPO gene polymorphism in lung cancer etiology is limited by the lack of published information on the functional significance of the polymorphism on MPO-mediated reactions relevant to carcinogenesis. However, the in vitro data supporting the functional relevance of this polymorphism are strong (8). The G to A base difference is located in an Alu sequence, which encodes a hormone response element, upstream from the human MPO gene. There is substantial evidence that such Alu-hormone response elements influence the expression of nearby genes, and for this reason, the G to A substitution was chosen for detailed transcription studies by Piedrafita et al. (8). Several pieces of evidence suggest that this polymorphism is important for MPO gene expression (8). The Alu sequence containing the polymorphism is a functional retinoic acid receptor binding site, and the presence of an A rather than a G reduces transcription in a CV-1 cell chloramphenicol acetyltransferase reporter gene assay severalfold. Furthermore, the presence of an A instead of a G eliminates the 10-bp core

Discussion

The MPO promoter region polymorphism has been implicated in acute myelocytic leukemia in two small series of patients (7, 8). Ours is the first report of this MPO polymorphism in relation to any other disorder. We observed an association between MPO genotype and lung cancer risk in the direction predicted given that the A allele has been shown to reduce transcription of the MPO gene in vitro (8). Subjects homozygous for the A allele, representing about 8—10% of the population, were at decreased risk of lung cancer—70% for Caucasians and 39% for African-Americans.

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![Fig. 1. Ethidium bromide gel showing three possible genotypes from a single nucleotide polymorphism (G or A) in the promoter region 463 bases upstream of the MPO gene. The 350-bp PCR amplification fragment contains an invariant AcI restriction site yielding A/A, are shown in Table 1. The A allele was relatively common in both lanes. Individuals homozygous for the A allele have no additional AcI restriction sites and consequently show only two bands, at 289 bp and the invariant band at 61 bp. The G allele creates an additional AcI restriction site such that homozygous G individuals have three bands at 169, 120, and 61 bp. Heterozygous individuals show all four bands.](image)

Table 1 MPO genetic polymorphism in relation to risk of lung cancer among African-Americans and Caucasians in Los Angeles County

<table>
<thead>
<tr>
<th>MPO genotype</th>
<th>African-Americans</th>
<th>Caucasians</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
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</tr>
<tr>
<td>G/A</td>
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<td></td>
</tr>
<tr>
<td>A/A</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*OR and 95% CI adjusted for frequency matching factors, age, and sex. Also adjusted for smoking modeled as terms for the natural logarithm of pack-years and the product of the natural logarithms of pack-years and years since quit smoking. G/G genotype served as reference category.*
binding site for the general transcription factor SP1, decreasing SP1 binding severalfold. Additionally, abolishing this SP1 site negates the increased transcription seen in the chloramphenicol acetyltransferase reporter gene assay.

We did not observe an altered risk of lung cancer for G/A heterozygotes. There are no data to allow prediction of MPO activity in heterozygotes. However, one can speculate that the G to A polymorphism for MPO might be analogous to polymorphisms described for heterozygotes. However, one can speculate that the two copies of an allele containing an inactivating mutation (19). The concentration of B(α)P and other tobacco-related carcinogens might be low relative to doses administered in drug metabolism studies; thus, it would not be surprising that a single functional copy of the gene may suffice. Alternatively, because other enzymes are capable of metabolism of MPO, other differences in activity of one enzyme that occur in G/A heterozygotes might not be sufficient to influence disease risk.

To analyze our samples, we developed an PCR/RFLP based assay using the restriction enzyme AcI to avoid the need to directly sequence all 1042 subjects. The assay allows relatively rapid genotyping of large numbers of subjects and will facilitate further studies of this MPO gene polymorphism in relation to lung cancer risk as well as other disorders for which a role for MPO in pathogenesis is biologically plausible.

Acknowledgments

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References

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