Although cigarette smoking is the major cause of lung cancer, compared with the marginal significantly increased risk of adenocarcinoma polymorphisms, the importance in determining an individual’s susceptibility to lung cancer. To test this hypothesis, we investigated the association of MBD1 –634G>A, –501delT (–501 T/T, T/-, -/-), and Pro401Ala genotypes and their haplotypes with the risk of lung cancer in a Korean population. The MBD1 genotype was determined in 432 lung cancer patients and in 432 healthy control subjects who were frequency matched for age and gender. The –634G genotype was associated with a significantly increased risk of overall lung cancer compared with the –634AA genotype (adjusted odds ratio (OR), 3.10; 95% confidence interval (95% CI), 1.24-7.75; P = 0.016). When analyses were stratified according to the tumor histology, the –634G genotype was associated with a significantly increased risk of adenocarcinoma compared with the –634AA genotype (adjusted OR, 4.72; 95% CI, 1.61-13.82; P = 0.005). For the MBD1 –501delT and Pro401Ala polymorphisms, the –501 T/T genotype was associated with a marginal significantly increased risk of adenocarcinoma compared with the –501delT genotype (adjusted OR, 2.07; 95% CI, 1.02-4.20; P = 0.045), and the Pro/Pro genotype was associated with a significantly increased risk of adenocarcinoma compared with the Ala/Ala genotype (adjusted OR, 3.41; 95% CI, 1.21-9.60; P = 0.02). Consistent with the genotyping analyses, the –634G/–501T/Pro haplotype was associated with a significantly increased risk of overall lung cancer and adenocarcinoma compared with the –634A/–501Ala haplotype (adjusted OR, 1.44; 95% CI, 1.08-1.91; P = 0.012 and P = 0.048; adjusted OR, 1.75; 95% CI, 1.20-2.56; P = 0.004 and P = 0.016, respectively). On a promoter assay, the –634A allele had significantly higher promoter activity compared with the –634G allele in the Chinese hamster ovary cells and A549 cells (P < 0.05 and P < 0.001, respectively), but the –501delT polymorphism did not have an effect on the promoter activity. When comparing the promoter activity of the MBD1 haplotypes, the –634A/–501T haplotype had a significantly higher promoter activity than the –634G/–501T haplotype (P < 0.001). These results suggest that the MBD1 –634G>A, –501delT, and Pro401Ala polymorphisms and their haplotypes contribute to the genetic susceptibility for lung cancer and particularly for adenocarcinoma. (Cancer Epidemiol Biomarkers Prev 2005;14(11):2474–80)

Introduction

Although cigarette smoking is the major cause of lung cancer, only a fraction of smokers develop lung cancer during their lifetime, suggesting that genetic and epigenetic factors are of importance in determining an individual’s susceptibility to lung cancer (1, 2).

DNA cytosine methylation in CpG dinucleotides is a major epigenetic mechanism that regulates chromosomal stability and gene expression (3, 4). Many human cancers, including lung cancer, have both global hypomethylation and regional hypermethylation of CpG islands (5-8). Such aberrant DNA methylation may contribute to carcinogenesis in several ways. Hypomethylation may lead to chromosomal instability, reactivation of transposable elements, and loss of imprinting (6, 9). Methylation of CpG sequences may facilitate C-to-T transition mutations in tumor suppressor genes and/or oncogenes through deamination of 5-methylcytosine to thymine (10). Methylation CpG sequences may also increase susceptibility to attack by some environmental carcinogens (11, 12). Finally, de novo hypermethylation of promoter CpG islands may lead to silencing of tumor suppressor genes and DNA repair genes (4, 6, 9).

Methylated CpG sites are recognized by a family of protein factors containing the methyl-CpG binding domain (MBD); to date, five family members have been identified in mammals: MeCP2, MBD1, MBD2, MBD3, and MBD4 (13-15). Four of these proteins (MeCP2, MBD1, MBD2, and MBD3) play important roles for methylation-mediated transcriptional silencing by recruiting chromatin-modifying factors, such as histone deacetylases, to the methylated promoters (14, 15). In contrast to the other family members, MBD4 protein has a thymine glycosylase activity and binds preferentially to 5mCpG-TpG mismatches, which are the primary products of deamination at methyl-CpG. Therefore, MBD4 protein is thought to function as a DNA repair enzyme to minimize mutation at 5-methylcytosine (16, 17).

MBD1 is known to act as a transcriptional repressor through the cooperation of MBD, cysteine-rich CXJC domains, and a COOH-terminal transcription repression domain (18, 19). Among the MBD family of proteins, MBD1 is characterized by two or three cysteine-rich CXJC domains that were originally found in DNA methyltransferase and human trithorax protein HRX (20). MBD1 has at least five isoforms that are the result of

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Note: S.J. Jang and S.J. Lee contributed equally to this work.

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alternative splicing within the regions of the CXXC domains and the COOH terminus. These MBD1 isoforms preferentially repress transcription from the methylated gene promoters, but the MBD1 isoforms containing three CXXC domains can also repress transcription from the unmethylated promoters, suggesting that MBD1 plays an important role for the establishment and maintenance of local chromatin states to regulate gene activities (18, 21). In addition to transcriptional gene regulation, a recent study suggests that MBD1 is also involved in DNA repair through its interaction with methylpurine DNA glycosylase, which removes the damaged purines produced by methylating or oxidative agents (22).

Single nucleotide polymorphisms are the most common form of human genetic variation and may contribute to the individual susceptibility for lung cancer. We previously showed that some variants in the DNA repair and DNA methyltransferase (DNMT) genes affect either the expression or the activities of enzymes and are therefore associated with the risk of lung cancer (23-27). Several candidate single nucleotide polymorphisms in the MBD1 gene have been recently deposited in the public databases (http://www.ncbi.nlm.nih.gov/SNP). Although the functional effects of these polymorphisms have not been elucidated, we hypothesized that some of these variants, particularly their haplotypes, may influence MBD1 activity on gene regulation and genome stability, thereby modulating the susceptibility to lung cancer. To test this hypothesis, a case-control study was conducted to evaluate the association between MBD1 genotypes/haplotypes and the risk of lung cancer. Among the candidate single nucleotide polymorphisms in the MBD1 gene, we have focused on the amino acid substitution variants (Pro13Leu, 29289281C>G in exon 12; Genbank accession no. NT_010966) because these are the most likely to affect gene functioning.

In the present study, we evaluated the association of −634G>A, −501delT, and 5′-CTTCCAGCAGGTAAGGC-3′ (reverse) and 5′-CTCAGTTAACCTGCGGTGTG-3′ (reverse); and 5′-AAGCAGATTCCGTCTGCGC−3′ (reverse) and 5′-CTTCCAGCAGGTAAGGC-3′ (reverse). The PCR reactions were done in a total volume of 20 µL containing 100 ng genomic DNA, 25 pmol/L of each primer, 0.2 mmol/L deoxynucleoside triphosphates, 75 mmol/L Tris-HCl (pH 9.0), 15 mmol/L ammonium sulfate, 0.1 g/µL bovine serum albumin, 2.5 mmol/L MgCl2, and 1 unit of Taq polymerase (Takara Shuzo Co., Otsu, Shiga, Japan). The PCR cycle conditions consisted of an initial denaturation step at 95°C for 5 minutes followed by 35 cycles of 30 seconds at 94°C; 30 seconds at 56°C for −634G>A, 59°C for −501delT, and 53°C for Pro401Ala; 30 seconds at 72°C; and a final elongation at 72°C for 10 minutes. The PCR products were digested overnight at 37°C with the appropriate restriction enzymes (New England BioLabs, Beverly, MA). The restriction enzymes for the −634G>A, −501delT, and Pro401Ala genotypes were BanII, AvaI, and AvaII, respectively. The digested PCR products were resolved on 6% acrylamide gels and stained with ethidium bromide for visualization under UV light. For quality control, the genotyping analysis was done “blind” with respect to the case/control status. About 10% of the samples were randomly selected to be genotyped again by a different author, and the results were 100% concordant. To confirm the genotyping results, selected PCR-amplified DNA samples (n = 2, respectively, for each genotype) were examined by DNA sequencing, and the results were also 100% concordant.

**Promoter-Luciferase Constructs.** To examine the potential effects of the −634G>A and −501delT polymorphisms on the MBD1 transcription activity, the fragments, including the −634G>A (~982 to ~576, transcription start site of exon 1 counted as +1) and the −501delT (~601 to ~290), were synthesized, respectively, by PCR using the genomic DNA from donors that were carrying either the wild-type allele or the polymorphic allele of each polymorphism. The PCR primers used for the −634G>A and the −501delT promoter regions were 5′-GAAGCTTC TCCACATTGCT-3′ (forward) and 5′-CACACCGCAGGTAAACTG-3′ (reverse) and 5′-GGCGTGCCTCGATTACCT-3′ (forward) and 5′-CGGTTTGACCTTACCT-3′ (reverse), respectively. The PCR products were inserted upstream of the SV40 promoter in the pG3-promoter plasmid (Promega, Madison, WI). For comparison of the haplotypes’ promoter activities (haplotype −634G/>501T versus haplotype −634A/>501T), the fragments of the MBD1 promoter region (~994 to +113) were synthesized by PCR using the genomic DNA from donors carrying either the −634G/>501T haplotype or the −634A/>501T haplotype. The PCR primers for the MBD1 promoter were 5′-GCCCATGCGGCGTTATCAG-3′ (forward) and 5′-CAGGCCCAAGGCTGTCTGG-3′ (reverse). The PCR products were inserted upstream of the luciferase gene in the pGL3-basic plasmid (Promega). The correct sequence of all the clones was verified by DNA sequencing.

**Materials and Methods.**

**Study Population.** This case-control study included 432 lung cancer patients and 432 healthy controls. The details of the study population have been described elsewhere (26-29). In brief, the eligible cases included all the patients who were newly diagnosed with primary lung cancer at Kyungpook National University Hospital, Daegu, Korea from January 2001 to February 2002. There were no age, gender, histologic, or stage restrictions, but those patients with a prior history of cancer was defined as one who had stopped smoking at least 1 year before the lung cancer diagnosis (cases) or before the date signed on an informed consent (controls). The cumulative cigarette dose (pack-years) was calculated by using the following formula: pack-years = (packs per day) × (years smoked).

**MBD1 Genotyping.** Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol/chloroform extraction. The MBD1 −634G>A, −501delT, and Pro401Ala genotypes were determined by using a PCR-RFLP assay. The PCR primers were designed based on the Genbank reference sequence (accession no. NT_010966). The PCR primers for the MBD1 −634G>A, −501delT, and Pro401Ala polymorphisms were 5′-CTCTCTGGCGTGGGAGGAGGGT-3′ (forward) and 5′-CCCTGAGGAGGGCGAGGAGT−3′ (reverse); and 5′-TTCGCCAGCCTAACGTAGG-3′ (forward) and 5′-CTCAGTTAACCTGCGGTGTG-3′ (reverse); and 5′-AAGCACAGTTCCGTCTGCGC−3′ (reverse) and 5′-CTTCCAGCAGGTAAGGC-3′ (reverse), respectively. The PCR reactions were done in a total volume of 20 µL containing 100 ng genomic DNA, 25 pmol/L of each primer, 0.2 mmol/L deoxynucleoside triphosphates, 75 mmol/L Tris-HCl (pH 9.0), 15 mmol/L ammonium sulfate, 0.1 g/µL bovine serum albumin, 2.5 mmol/L MgCl2, and 1 unit of Taq polymerase (Takara Shuzo Co., Otsu, Shiga, Japan). The PCR cycle conditions consisted of an initial denaturation step at 95°C for 5 minutes followed by 35 cycles of 30 seconds at 94°C; 30 seconds at 56°C for −634G>A, 59°C for −501delT, and 53°C for Pro401Ala; 30 seconds at 72°C; and a final elongation at 72°C for 10 minutes. The PCR products were digested overnight at 37°C with the appropriate restriction enzymes (New England BioLabs, Beverly, MA). The restriction enzymes for the −634G>A, −501delT, and Pro401Ala genotypes were BanII, AvaI, and AvaII, respectively. The digested PCR products were resolved on 6% acrylamide gels and stained with ethidium bromide for visualization under UV light. For quality control, the genotyping analysis was done “blind” with respect to the case/control status. About 10% of the samples were randomly selected to be genotyped again by a different author, and the results were 100% concordant. To confirm the genotyping results, selected PCR-amplified DNA samples (n = 2, respectively, for each genotype) were examined by DNA sequencing, and the results were also 100% concordant.

**Promoter-Luciferase Constructs.** To examine the potential effects of the −634G>A and −501delT polymorphisms on the MBD1 transcription activity, the fragments, including the −634G>A (~872 to ~576, transcription start site of exon 1 counted as +1) and the −501delT (~601 to ~290), were synthesized, respectively, by PCR using the genomic DNA from donors that were carrying either the wild-type allele or the polymorphic allele of each polymorphism. The PCR primers used for the −634G>A and the −501delT promoter regions were 5′-GAAGCTTC TCCACATTGCT-3′ (forward) and 5′-CACACCGCAGGTAAACTG-3′ (reverse) and 5′-GGCGTGCCTCGATTACCT-3′ (forward) and 5′-CGGTTTGACCTTACCT-3′ (reverse), respectively. The PCR products were inserted upstream of the SV40 promoter in the pG3-promoter plasmid (Promega, Madison, WI). For comparison of the haplotypes’ promoter activities (haplotype −634G/>501T versus haplotype −634A/>501T), the fragments of the MBD1 promoter region (~994 to +113) were synthesized by PCR using the genomic DNA from donors carrying either the −634G/>501T haplotype or the −634A/>501T haplotype. The PCR primers for the MBD1 promoter were 5′-GCCCATGCGGCGTTATCAG-3′ (forward) and 5′-CAGGCCCAAGGCTGTCTGG-3′ (reverse). The PCR products were inserted upstream of the luciferase gene in the pGL3-basic plasmid (Promega). The correct sequence of all the clones was verified by DNA sequencing.
**MBD1 Polymorphisms and Haplotypes in Lung Cancer**

**Transient Transfection and Luciferase Assay.** The promoter activity was measured using the Dual-Luciferase Reporter Assay System (Promega) in Chinese hamster ovary cells and A549 lung cancer cells. The Chinese hamster ovary cells were cultured in minimal essential medium with 10% heat-inactivated fetal bovine serum, and the A549 cells were grown in RPMI 1640 that was supplemented with 10% heat-inactivated fetal bovine serum. Cells (1 x 10^7) were plated in a six-well plate the day before transfection so that the cells were ~60% confluent by the next day. The pRL-SV40 plasmid and the pGL3-basic plasmid with the synthesized fragments of the MBD1 promoter region were cotransfected using Lipofectamine reagent (Invitrogen, Carlsbad, CA). The pRL-SV40 vector that provided the constitutive expression of Renilla luciferase was used as an internal control to correct for the differences in transfection and harvesting efficiency. The cells were collected 48 hours after transfection, and the cell lysates were prepared according to Promega’s instruction manual. Luciferase activity was measured using a Lumat LB953 luminometer (EG&G Berthold, Bad Wildbad, Germany), and the results were normalized using the activity of Renilla luciferase. Independent triplicate experiments were done four times, and the results were reported as mean ± SD.

**Statistical Analysis.** The cases and controls were compared using Student’s t test for continuous variables and the \( \chi^2 \) test for categorical variables. Hardy-Weinberg equilibrium was tested for with a goodness-of-fit \( \chi^2 \) test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. We examined the widely used measure of linkage disequilibrium between pairs of biallelic loci, Lewontin’s \( D' \) (1 D'; ref. 30). The haplotypes and their frequencies were estimated based on a Bayesian algorithm using the Phase program (31), which is available at http://www.stat.washington.edu/stephens/phase.html. Unconditional logistic regression analysis was used to calculate the odds ratios (ORs) and 95% confidence intervals (95% CI), with adjustment for possible confounders (gender and family history of lung cancer as a nominal variable and age and pack-years as continuous variables). In addition to the overall association analysis, we did a stratified analysis by age, gender, smoking status, and tumor histology to further explore the association between MBD1 genotypes/haplotypes and the risk of lung cancer in each stratum. To test which one of the three polymorphisms is more likely to be the main cause of the observed association, we compared seven different logistic regression models (each polymorphism alone, any two of the three polymorphisms, and then all the three polymorphisms together). When multiple comparisons are made, the corrected Ps (P*) were also calculated for multiple testing by using Bonferroni’s inequality method. All analyses were done using Statistical Analysis Software for Windows, version 8.12 (SAS Institute, Cary, NC).

**Table 1. Characteristics of the study population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 432)</th>
<th>Controls (n = 432)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61.6 ± 9.0</td>
<td>60.9 ± 9.3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>352 (81.5)*</td>
<td>352 (81.5)</td>
</tr>
<tr>
<td>Female</td>
<td>80 (18.5)</td>
<td>80 (18.5)</td>
</tr>
<tr>
<td>Smoking status †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>317 (73.4)</td>
<td>229 (53.0)</td>
</tr>
<tr>
<td>Former</td>
<td>39 (9.0)</td>
<td>98 (22.7)</td>
</tr>
<tr>
<td>Never</td>
<td>76 (17.6)</td>
<td>105 (24.3)</td>
</tr>
<tr>
<td>Pack-years$</td>
<td></td>
<td>39.9 ± 17.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34.4 ± 17.6</td>
</tr>
</tbody>
</table>

$^*$Column percentage in brackets.

$^p = 0.001$.

$^\dagger$In current and former smokers (P < 0.001).

**Results**

The demographics of the cases and controls enrolled in this study are shown in Table 1. There were no significant differences between the cases and controls in mean age or gender distribution, suggesting that the matching based on these two variables was adequate. The case group had a higher prevalence of current smokers than the controls (P < 0.001), and the number of pack-years in smokers was significantly higher in the cases than in the controls (17.9 ± 17.9 versus 34.4 ± 17.6 pack-years; P < 0.001). These differences were controlled in the later multivariate analyses.

The genotype and polymorphic allele frequencies of the three MBD1 polymorphisms (–634G>A, −501delT, and Pro401Ala) among the controls and cases are shown in Table 2. The genotype distributions of the three polymorphisms among the controls were in Hardy-Weinberg equilibrium. The distribution of the MBD1 –634G>A genotypes among the cases was significantly different from that among the controls (P = 0.04). When the cases were stratified by histologic type, the distribution of the −634G>A genotypes among the adenocarcinoma cases differed significantly from that among the controls (P = 0.001). The genotype distribution of the −501delT polymorphism among the cases was not significantly different from that among the controls. The genotype distribution among the adenocarcinoma cases differed significantly from that among the controls (P = 0.001).

Table 3 shows the lung cancer risk related to the MBD1 –634G>A, −501delT, and Pro401Ala genotypes, respectively. Adjusted ORs and their 95% CIs were calculated using the more common homozygous variant genotype as the reference group. The −634GG genotype was associated with a significantly increased risk of overall lung cancer compared with the −634AA genotype (adjusted OR, 1.20; 95% CI, 1.14-1.26; P = 0.016). When analyses were stratified according to the tumor histology, the risk associated with the −634G>A genotypes was more pronounced in patients with adenocarcinoma. The −634GG genotype was associated with a significantly increased risk of adenocarcinoma compared with the −634AA genotype (adjusted OR, 1.72; 95% CI, 1.60-1.84; P = 0.005). For the MBD1 −501delT polymorphism, there was no significant association between this polymorphism and the risk of overall lung cancer. When the cases were categorized by tumor histology, however, the −501 T/T genotype was associated with a marginal, significantly increased risk of adenocarcinoma compared with the −501 C/C genotype (adjusted OR, 2.07; 95% CI, 1.02-4.20; P = 0.045). For the Pro401Ala polymorphism, compared with the Ala/Ala genotype, the Pro/Pro genotype was associated with a significantly increased risk of adenocarcinoma (adjusted OR, 3.41; 95% CI, 1.21-9.60; P = 0.02), and the Pro/Ala genotype was associated with a marginal significantly increased risk of adenocarcinoma (adjusted OR, 1.54; 95% CI, 1.00-2.34; P = 0.047).

The −634G>A and −501delT polymorphisms and the −501delT and Pro401Ala polymorphisms were in strong linkage disequilibrium (1 D' = 0.927 and 0.929, respectively) in the study populations. The five common haplotypes accounted for 98.6% of the chromosome of the present study population. Three haplotypes that had a frequency of <1% were excluded [in the controls, 10 (1.2%) and in the cases, 15 (1.7%), respectively] from further analysis to avoid possible errors in the genotyping or the estimation process. Table 4 shows the inferred haplotype distribution for the cases and controls, and the lung cancer risk related to the haplotypes. Consistent with the results of the genotyping analyses, the −634G/A−501T/Pro haplotype was associated with a significantly increased risk of overall lung cancer.
compared with the −634A/−501+/401Ala haplotype (adjusted OR, 1.44; 95% CI, 1.08-1.91; P = 0.012 and \( P_c = 0.048 \)). When analyses were stratified by tumor histology, the −634G/−501T/401Ala haplotype was associated with a significantly increased risk of adenocarcinoma compared with the −634A/−501+/401Ala haplotype (adjusted OR, 1.75; 95% CI, 1.20-2.56; \( P = 0.012 \) and \( P_c = 0.048 \)). When controlling for age, gender, smoking status, pack-years of smoking, and family history of lung cancer.

As a consequence of the strong linkage disequilibrium among the −634G>A, −501delT, and Pro401Ala polymorphisms, it is not easy to determine which polymorphism is more likely to be the cause of the observed association. In an attempt to resolve this, we fitted logistic regression models in which we allowed for the effects of the three polymorphisms, individually and jointly. The models incorporating −501delT, Pro401Ala, or −501delT and Pro401Ala into −634G>A did not fit significantly better than the model with −634G>A alone (all comparisons, \( P > 0.05 \)). However, the model with −501delT alone or Pro401Ala alone fitted less well than any joint models with −634G>A (all comparisons, \( P < 0.05 \)).

The association between the MBD1 genotypes/haplotypes and the risk of adenocarcinoma was further examined after stratifying for age, gender, smoking status, and histologic subtypes of adenocarcinoma [adenocarcinoma with/without bronchioloalveolar histology (BAC)]. There was no clear evidence that age, gender, or smoking status modified the effect of the MBD1 genotypes/haplotypes on the risk of adenocarcinoma in the stratified analysis. Moreover, the stratified analysis on the histologic subtypes of adenocarcinoma did not show any difference between adenocarcinomas with and without BAC histology (data not shown).

We investigated the effects of the −634G>A and −501delT polymorphisms on the promoter activity of MBD1 by luciferase assay. The promoter activity of the −634A allele was significantly higher (1.5-fold) compared with the −634G allele in the Chinese hamster ovary cells (\( P < 0.001 \); Fig. 1A), but the −501delT polymorphism did not have an effect on the promoter activity (Fig. 1B). Because the −634G>A and −501delT polymorphisms were in linkage disequilibrium, we also compared the transcription activity of the haplotypes (haplotype −634G/−501T versus haplotype −634A/−501+). In the Chinese hamster ovary cells, the −634A/−501+ haplotype increased transcription activity by 2.1-fold compared with the −634G/−501T haplotype (\( P < 0.001 \)). Similar result was observed in the A549 cells (\( P < 0.01 \); Fig. 1C).

### Table 2. Genotype frequencies of MBD1 polymorphisms in lung cancer cases and controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Variables</th>
<th>Genotype</th>
<th>1/1</th>
<th>1/2</th>
<th>2/2</th>
<th>Polymorphic allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>−634G&gt;A</td>
<td>Controls</td>
<td>(1.6)†</td>
<td>118 (27.3)</td>
<td>307 (71.1)</td>
<td>0.847</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All cases</td>
<td>(3.9)†</td>
<td>134 (31.0)</td>
<td>281 (65.0)†</td>
<td>0.806</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>(2.9)†</td>
<td>62 (29.5)</td>
<td>142 (67.6)†</td>
<td>0.824</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>(6.4)†</td>
<td>46 (32.6)</td>
<td>86 (61.0)†</td>
<td>0.773</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Large cell carcinoma</td>
<td>(0.0)</td>
<td>3 (37.5)</td>
<td>5 (62.5)</td>
<td>0.812</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small cell carcinoma</td>
<td>(2.7)</td>
<td>22 (30.1)</td>
<td>49 (67.1)</td>
<td>0.822</td>
<td></td>
</tr>
<tr>
<td>−501delT</td>
<td>Controls</td>
<td>(6.0)†</td>
<td>171 (39.4)</td>
<td>235 (54.4)</td>
<td>0.742</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All cases</td>
<td>(26.0)†</td>
<td>186 (43.1)</td>
<td>213 (49.2)</td>
<td>0.708</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>(5.2)</td>
<td>95 (45.2)</td>
<td>104 (49.5)</td>
<td>0.721</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>(11.3)</td>
<td>55 (39.0)</td>
<td>70 (49.7)</td>
<td>0.691</td>
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</tr>
<tr>
<td></td>
<td>Large cell carcinoma</td>
<td>(0.0)</td>
<td>4 (50.0)</td>
<td>4 (50.0)</td>
<td>0.750</td>
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<tr>
<td></td>
<td>Small cell carcinoma</td>
<td>(8.2)</td>
<td>32 (43.8)</td>
<td>35 (48.0)</td>
<td>0.699</td>
<td></td>
</tr>
<tr>
<td>Pro401Ala (C→G)</td>
<td>Controls</td>
<td>(9.1)</td>
<td>120 (27.8)</td>
<td>303 (70.1)</td>
<td>0.840</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All cases</td>
<td>(15.3)</td>
<td>138 (31.9)</td>
<td>279 (64.6)</td>
<td>0.806</td>
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<tr>
<td></td>
<td>Squamous cell carcinoma</td>
<td>(2.4)</td>
<td>64 (30.5)</td>
<td>141 (67.1)</td>
<td>0.824</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>(5.7)</td>
<td>49 (34.7)</td>
<td>84 (59.6)</td>
<td>0.770</td>
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<tr>
<td></td>
<td>Large cell carcinoma</td>
<td>(0.0)</td>
<td>3 (37.5)</td>
<td>5 (62.5)</td>
<td>0.812</td>
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<tr>
<td></td>
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<td>49 (67.1)</td>
<td>0.822</td>
<td></td>
</tr>
</tbody>
</table>

*The wild-type allele (−634G, −501T, and 401Pro) is denoted by 1 and the polymorphic allele is denoted by 2.
†Row percentage in brackets.
\( P < 0.05 \).
\( P < 0.01 \).
\( P = 0.001 \).
\( P = 0.0001 \).

### Table 3. Adjusted ORs (95% CIs) for lung cancer associated MBD1 genotypes

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>All cases</th>
<th>Squamous cell carcinoma</th>
<th>Adenocarcinoma</th>
<th>Small cell carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>−634G&gt;A</td>
<td>G/G</td>
<td>3.10 (1.24-7.75)†</td>
<td>2.13 (0.68-6.60)</td>
<td>4.72 (1.61-13.82)†</td>
<td>2.26 (0.44-11.55)</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>1.26 (0.93-1.71)¢</td>
<td>1.13 (0.77-1.65)</td>
<td>1.46 (0.95-2.24)</td>
<td>1.28 (0.74-2.21)</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>−501delT</td>
<td>T/T</td>
<td>1.47 (0.84-2.57) ¢</td>
<td>0.93 (0.43-1.98)</td>
<td>2.07 (1.02-4.20) ¢</td>
<td>1.59 (0.60-4.21)</td>
</tr>
<tr>
<td></td>
<td>T/–</td>
<td>1.17 (0.86-1.56)</td>
<td>1.25 (0.88-1.78)</td>
<td>1.08 (0.71-1.63)</td>
<td>1.24 (0.73-2.11)</td>
</tr>
<tr>
<td></td>
<td>–/–</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Pro401Ala (C→G)</td>
<td>Pro/Pro</td>
<td>2.12 (0.89-5.05)</td>
<td>1.49 (0.47-4.71)</td>
<td>3.41 (1.21-9.60)</td>
<td>1.89 (0.38-9.39)</td>
</tr>
<tr>
<td></td>
<td>Pro/Ala</td>
<td>1.24 (0.92-1.68)</td>
<td>1.11 (0.76-1.62)</td>
<td>1.54 (1.00-2.34)</td>
<td>1.14 (0.65-1.99)</td>
</tr>
<tr>
<td></td>
<td>Ala/Ala</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age, sex, smoking status, pack-years of smoking, and family history of lung cancer.

\( P = 0.016 \).
\( P = 0.005 \).
\( P = 0.045 \).
\( P = 0.002 \).
\( P = 0.047 \).
MBD1 Polymorphisms and Haplotypes in Lung Cancer

Discussion

We investigated the potential association of three MBD1 polymorphisms (−634G>A, −501delT, and Pro401Ala) with the risk of lung cancer. In addition, we estimated the MBD1 haplotypes of the three polymorphisms and compared their frequency distributions in the lung cancer cases and controls. Compared with the −634A/−501T/Pro haplotype, the −634G/−501T/Pro haplotype was associated with an increased risk of lung cancer and especially adenocarcinoma. This finding suggests that the MBD1 −634G>A, −501delT, and Pro401Ala polymorphisms and their haplotypes could be used as a marker for genetic susceptibility to adenocarcinoma. Of three major histologic types of lung cancer, the proportion of adenocarcinoma is increasing worldwide. Thus, identification of genetic factors that are responsible for the susceptibility to adenocarcinoma is indispensable for establishing novel and efficient ways of preventing the disease. This is the first case-control study of MBD1 polymorphisms and haplotypes to examine their relation to lung cancer.

In the present study, carriers with the −634G/−501T/Pro haplotype were at increased risk of lung cancer compared with individuals having the −634A/−501T/Pro haplotype. To determine whether the association between the MBD1 polymorphisms and the risk of lung cancer is due to differences in the transcriptional activity of MBD1, we compared the promoter activity of the wild-type allele or the polymorphic allele of these two polymorphisms by luciferase assay. In vitro promoter assay revealed that the −634A allele had a significantly higher transcriptional activity than the −634G allele, and the −501delT polymorphism did not have an effect on the transcriptional activity of the MBD1 promoter. When we compared the promoter activity of the MBD1 haplotypes, the −634A/−501T haplotype had significantly higher transcriptional activity than the −634G/−501T haplotype. These results suggest that the genetic effects of MBD1 polymorphisms on the risk of lung cancer can be mainly attributed to the −634G>A polymorphism, and these findings also suggest that the −634G>A change influences MBD1 expression, thus contributing to the genetic susceptibility to lung cancer. The mechanism by which the MBD1 −634A/−501T haplotype leads to higher promoter activity is currently unknown.

Analysis of the potential transcription factor binding sites by the Alibaba2 program (32) showed that the −634G>A change leads to the creation of a cytoplasmic polyadenylation element binding site and eliminates an activator protein 2 α binding site and thyroid hormone receptor-α binding site and eliminates an activator protein 2 leads to the creation of a cytoplasmic polyadenylation element.

Table 4: Distribution of MBD1 haplotypes, predicted by Bayesian algorithm, in controls and cases

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls (n = 854)</th>
<th>All cases (n = 849)</th>
<th>Histologic type of lung cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td>222</td>
<td>631 (73.9)</td>
<td>597 (70.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>111</td>
<td>104 (12.2)</td>
<td>137 (16.1)</td>
<td>1.44 (1.08-1.91)</td>
</tr>
<tr>
<td>212</td>
<td>70 (8.2)</td>
<td>68 (8.0)</td>
<td>1.00 (0.70-1.43)</td>
</tr>
<tr>
<td>211</td>
<td>25 (2.9)</td>
<td>26 (3.1)</td>
<td>1.03 (0.58-1.83)</td>
</tr>
<tr>
<td>112</td>
<td>24 (2.8)</td>
<td>21 (2.5)</td>
<td>0.98 (0.54-1.81)</td>
</tr>
</tbody>
</table>

*The order of polymorphisms for the haplotypes is as follows: −634G>A, −501delT, and Pro401Ala. The wild-type allele (−634G, −501T, and 401Ala) is denoted by 1 and the polymorphic allele is denoted by 2.

Three haplotypes that had a frequency of <1% were excluded from analysis: controls 10 and cases 15 (squamous cell carcinoma, n = 6; adenocarcinoma, n = 7; large cell carcinoma, n = 1; and small cell carcinoma, n = 1), respectively.

Eight large cell carcinoma cases were excluded from analysis.

*Adjusted for age, sex, pack-years of smoking, and family history of lung cancer.

1P = 0.012 and P= 0.048 (Bonferroni corrected P).

1P = 0.004 and P = 0.016.
with nonmucinous BAC histology, and the East Asian populations, whereas the K-ras mutations are more frequent in smokers, males, adenocarcinomas with mucinous BAC histology, and the Western populations (40, 41). These observations suggest that adenocarcinomas arising in the never smokers and smokers may be caused by different etiologies, not only in relation to environmental risk factors but also in relation to genetic susceptibility factors (40-42). Therefore, we did a stratification analysis to examine if the MBD1 genotypes/haplotypes may have differential effects on the risk of adenocarcinoma according to age, gender, and smoking status and the histologic subtypes of adenocarcinoma (adenocarcinoma with/without BAC histology). In the current study, no risk modification was found with regard to age, gender, and smoking status. Moreover, the stratified analysis on the histologic subtypes of adenocarcinoma did not show any difference between adenocarcinomas with and without BAC histology (data not shown). However, because the number of subjects in the subgroups was small, our findings from the stratified analyses should be interpreted with caution before being confirmed in larger studies.

In the current study, the MBD1 Pro<sup>13</sup>Leu, −870G>A, and −750C>A polymorphisms were not detected in the preliminary study that included 27 healthy controls. These samples included 54 chromosomes, which provides at least a 95% confident level to detect alleles with frequencies >5%. Thus, it is very likely that if these polymorphisms exist, they may not play a major role in the genetic susceptibility to lung cancer in the Korean population (43, 44).

In conclusion, we found that the MBD1 −634G>A, −501delT, and Pro<sup>40</sup>Ala polymorphisms and their haplotypes were significantly associated with the risk of lung cancer and particularly adenocarcinoma. These results suggest that the MBD1 gene may be involved in the development of lung cancer, although additional studies having larger sample sizes are required to confirm our findings. Future studies on the other MBD1 sequence variants and their biological function are also needed to understand the role of the MBD1 polymorphisms in determining the risk of lung cancer. Moreover, because genetic polymorphisms often vary between different ethnic groups, further studies are needed to clarify the association of the MBD1 polymorphism with lung cancer in diverse ethnic populations.

References


Methyl-CpG Binding Domain 1 Gene Polymorphisms and Risk of Primary Lung Cancer

Jin-Sung Jang, Su Jeong Lee, Jin Eun Choi, et al.


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