Association of polymorphisms in the MTH1 gene with small cell lung carcinoma risk

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Fifty single-nucleotide polymorphisms (SNPs) associated with amino acid changes in 36 genes involved in diverse DNA repair pathways were assessed for associations with risk for small cell lung carcinoma (SCLC) by a case–control study consisting of 211 SCLC cases and 685 controls. An SNP, Val83Met, in the MTH1 (mutT homolog 1) gene encoding a thymidylate synthase that hydrolyzes promutagenic oxidized nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP, showed the strongest and a significant association with SCLC risk (odds ratio (OR) = 1.6, 95% confidence interval (CI): 1.2–2.2, \(P = 0.004\)), while three other SNPs in the TP53, BLM and SNMI genes, respectively, also showed marginal association (0.05 < \(P < 0.1\)). Another SNP, which causes a nucleotide change in the 5'-UTR of MTH1 transcripts leading to alternative translation initiation, was additionally examined and the SNP also showed a significant association (OR = 1.7, 95% CI: 1.2–2.3, \(P = 0.002\)). The two SNPs in the MTH1 gene were in linkage disequilibrium, and the OR for carrying a copy of the haplotype consisting of both the risky SNP alleles was 2.0 (95% CI: 1.2–3.2, \(P = 0.002\)). The present results indicate that inter-individual differences in MTH1 activities due to SNPs are involved in susceptibility to SCLC.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the world, and is comprised of a group of four histologically distinct types: adenocarcinoma (ADC), squamous cell carcinoma (SQC), large cell carcinoma (LCC) and small cell lung carcinoma (SCLC) (1). SCLC accounts for ~20% of all lung cancer cases and has clinical and biological characteristics distinct from non-small cell lung carcinoma (NSCLC). More than 90% of patients at the time of diagnosis are stage III or stage IV owing to its early and wide dissemination. Although, in most cases tumors initially respond to chemotherapy, >95% of patients eventually die from the cancer. Accordingly, the prognosis of patients with SCLC is poor, and 5-year survival of SCLC is <10% (1–3). Thus, SCLC is the most aggressive type of lung cancer. Genes responsible for the susceptibility to SCLC have been searched for to establish novel and efficient ways of preventing the disease. On the basis of the fact that smoking contributes to SCLC development, polymorphisms in metabolic genes encoding enzymes that activate or detoxify carcinogens in tobacco smoke are being studied for association with SCLC risk by case–control studies. Up to the present, a few metabolic genes, such as CYP1A1, CYP2A6 and GSTM1, have been found to be associated with SCLC risk (4–7). Thus, it is possible that polymorphisms in several metabolic genes are involved in SCLC susceptibility.

Polymorphisms in DNA repair genes have been considered to be involved in susceptibility to cancers, since they are thought to cause inter-individual differences in the capacity for preventing mutagenesis (8–12). In fact, single-nucleotide polymorphisms (SNPs) in several DNA repair genes have been shown to be associated with the risk for several types of cancers (12,13). Carcinogens in cigarette smoke are thought to cause a variety of pro-mutagenic DNA adducts, including benzo[α]pyrene-diol-epoxide (BPDE) and 8-hydroxyguanine (8OHG), which are repaired by nucleotide excision repair (NER) and base excision repair (BER) (12). Lung cancer patients were indicated to have lower activities of NER and BER than healthy individuals (9,14). Mice deficient in BER were reported to predispose to lung cancer (15). These results support the fact that the inter-individual variations of DNA repair activity are involved in lung cancer susceptibility. We recently identified 50 non-synonymous (associated with amino acid change) SNPs in 36 DNA repair genes involved in diverse intracellular processes that maintain genome
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Subjects and method

Case–control study

All cases and controls were Japanese. The cases consisted of 211 SCLC patients of four hospitals located in the Kanto area of Japan (i.e., Tokyo and surrounding prefectures) from 1999 to 2004. The hospitals were the National Cancer Center Hospital (NCCH) (113 patients), the National Cancer Center Hospital East (NCCHE) (81 patients), the National Nishigunma Hospital (NNGH) (16 patients) and the Gunma Prefecture Cancer Center Hospital (1 patient). All SCLC cases, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any particular exclusion criteria. All the cases were diagnosed by cytological and/or histological examinations according to WHO classification (16). From (1 patient). All SCLC cases, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any particular exclusion criteria. All the cases were diagnosed by cytological and/or histological examinations according to WHO classification (16). From each individual, a 10 or 20 ml whole-blood sample was obtained. Genomic DNAs for all the cases and the controls were isolated from the samples, and 10 ng of genomic DNA was subjected to genotyping by pyrosequencing as described previously (13). Information on the primer sequences and conditions for PCR were described previously (13). Information on the primer sequences and conditions for PCR were described previously (13). Briefly, the controls consisted of patients of two hospitals, NCCH and NNGH, in which SCLC cases were enrolled, and 302 healthy volunteers of Keio University, located in Tokyo. All of the control subjects were selected with a criterion of no history of any cancer.

Smoking history of cases and controls was obtained via interview using a questionnaire. Smoking habit was expressed by pack-years, which was defined as the number of cigarette packs smoked daily multiplied by years of smoking, both in current smokers and former smokers. Smokers were defined as those who had smoked regularly for 12 months or longer at any time in their life, while non-smokers were defined as those who had not. The study was approved by the institutional review boards of the National Cancer Center, the Nishigunma Hospital, the Gunma Prefecture Cancer Center and Keio University.

Results

We conducted a case–control study consisting of 211 SCLC cases and 685 controls (Table I). The SCLC cases consisted of patients enrolled in four hospitals in Tokyo and surrounding prefectures. The 685 controls consisted of patients/outpatients and healthy volunteers without a history of cancer enrolled in two hospitals and a university in the same area. Most of the SCLC cases were males and had a smoking habit, as has been reported (18,19). Therefore, the SCLC cases showed a higher fraction of males and smokers than the controls, and the mean smoking dosage of the SCLC cases was larger than that of the controls.

All the 685 controls were genotyped for the 50 SNPs with a success rate of 99.98% in our previous study (13). The 211 SCLC cases were genotyped for the same 50 SNPs in the present study, and the success rate was 99.94% (Table II). The allele distribution in the SCLC cases was compared with that in the 685 controls. None of the 50 SNPs deviated from HWE in cases and controls ($P > 0.05$). A significant difference in the allele distribution between the controls and cases was observed in one of the 50 SNPs, MTH1-Val83Met (OR for the MTH1-Val83Met allele = 1.6, 95% CI: 1.2–2.2, $P = 0.004$) (Table II). Marginally significant (0.5 \( < P < 0.1 \)) allele differentiations were observed in three SNPs, SNM1- His317Asp, TP53-Arg72Pro and BLM-Thr298Met. Allele distributions for the other 46 SNPs were not significantly different from those of the controls and cases.

Table I. SCLC cases and controls for case–control study

| Subject | Institutiona | No. | Gender (%) | Age (Mean ± SD) | Smoking habit (%) | Pack-years of smokers (Mean ± SD) |
|---------|--------------|-----|------------|----------------|------------------|----------------------------------|
|         |              |     | Male | Female |                 | Non-smoker | Smoker | Unknown |                 | Non-smoker | Smoker | Unknown | Non-smoker | Smoker | Unknown |
| Case    | NCCH         | 113 | 88    | 22     | 25 (22)         | 61 ± 10      | 8 (7)  | 105 (93) | 0 (0)         | 62 ± 31     |
| NNGH    | 81           | 68  | 13    | 16     | 16 (94)         | 68 ± 8      | 0 (0)  | 77 (95)  | 4 (5)         | 57 ± 30     |
|          | 17           | 16  | 94    | 1 (6)  | 16 (94)         | 68 ± 8      | 0 (0)  | 17 (100) | 0 (0)         | 55 ± 25     |
| Total   | 113          | 88  | 22    | 25     | 25 (22)         | 61 ± 10      | 8 (7)  | 105 (93) | 0 (0)         | 62 ± 31     |
| Control | NCCH         | 242 | 129   | 53     | 113 (47)        | 60 ± 16     | 8 (4)  | 199 (94) | 4 (2)         | 59 ± 30     |
| NNGH    | 141          | 100 | 71    | 29     | 41 (29)         | 65 ± 14     | 46 (33) | 91 (65)  | 4 (3)         | 46 ± 35     |
| KEIO    | 302          | 254 | 16    | 48     | 234 (84)        | 48 ± 10     | 202 (67) | 94 (31)  | 6 (2)         | 22 ± 20     |
| Total   | 685          | 483 | 202   | 29     | 202 (29)        | 55 ± 13     | 386 (56) | 287 (42) | 12 (2)        | 35 ± 31     |

aNCCN, National Cancer Center Hospital; NCCH, National Cancer Center Hospital East; NNGH, National Nishigunma Hospital; KEIO, Keio university. 

Statistical analysis

Differences in the allele distributions for the 50 SNPs between the cases and controls were tested by the \( \chi^2 \)-test. Hardy–Weinberg equilibrium (HWE) tests were performed using the TFPGA software (http://bioweb.usu.edu/mmpbio). Calculation of the \( \Delta \) and \( \Delta^2 \) values and haplotype estimation were undertaken using the EM algorithm. The strength of association of MTH1 (mutT homolog 1) genotypes and haplotypes with SCLC risk was measured as crude odds ratios (ORs), and ORs were adjusted for gender, age (49, 50–59, 60–69, 70) and smoking dosage (pack-years: 0, 1–49, 50) with 95% confidence intervals (CIs) by unconditional logistic regression analysis (17). ORs for carrying a copy of a haplotype were also calculated by the bootstrap method with 5000 resampling. All the statistical analyses were performed using the SAS version 9 software (SAS Institute, NC, USA).
ORs, when homozygotes for the 83Val allele were used as a reference, respectively (Table III). On the other hand, ORs of genotypes for the remaining three SNPs, SNM1-His317Asp, TP53-Arg72Pro and BLM-Thr298Met, did not show significant increases or decreases in SCLC cases (data not shown); therefore, these SNPs were not further investigated in the present study.

The *MTH1* gene, whose SNP, Val83Met, showed a significant association as described above, encodes a triphosphatase that hydrolyzes oxidized purine nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP (20). The activity of the MTH1-83Met protein was reported to be more thermolabile than that of the MTH1-83Val protein (20–22). The mitochondrial translocation of the MTH1-83Met protein was reported to be less efficient than that of the MTH1-83Val protein (23). Thus, it was suggested that the MTH1-83Met protein is less active than the MTH1-83Val protein. Previously, another SNP was found in a non-coding exon of *MTH1* (i.e. the T/C SNP in exon 2) 7.0 kb distal to the MTH1-Val83Met SNP, and the C allele in exon 2 leads to the production of an additional translation start site, resulting in the production of a longer MTH polypeptide in addition to commonly produced MTH polypeptides (21).

### Table II. Allele frequencies of 50 SNPs in 36 DNA repair genes in controls and cases

| DNA repair | Gene | SNP | Amino acid change | dbSNP ID | Minor allele frequency<sup>a</sup> |
|------------|------|-----|-------------------|----------|-----------------------------------|
| BER        | PARP/ADPRT | T2444C | Val762Ala | rs1805412 | 0.40 | 0.37 |
|            |       | A2978G | Lys940Arg | rs1136471 | 0.05 | 0.04 |
|            | APEX/APE1 | A395G  | Ile64Val   | rs2307486 | 0.04 | 0.05 |
|            |       | T649G  | Asp148Glu  | rs3136820 | 0.38 | 0.41 |
|            | MBD4  | G1212A | Glu346Lys  | rs140693  | 0.35 | 0.36 |
|            | MTH1/NUDT1 | G273A | Val83Met   | rs4866  | 0.09 | 0.15 (P = 0.004) |
|            | OG1   | C2243G | Ser326Cys  | rs1052133 | 0.48 | 0.46 |
|            | XRCC1  | C685T  | Arg194Tryp | rs1799762 | 0.33 | 0.30 |
|            |       | G944A  | Arg280His  | rs25489 | 0.09 | 0.08 |
| NER        | XPG/ERCC5 | C507G | His1104Asp | rs17655 | 0.42 | 0.46 |
|            | CSB/ERCC6 | G1275A | Gly999Asp | rs2282582 | 0.45 | 0.43 |
|            | XPC   | A2655C | Lys939Gln  | rs228001 | 0.40 | 0.38 |
|            | XPD/ERCC2 | G1615A | Asp312Asn  | rs1799793 | 0.04 | 0.04 |
| Mismatch repair | MLH1 | A676G | Ile219Val  | rs179977 | 0.05 | 0.03 |
|            | MLH3  | C2645T | Pro844Leu  | rs175080 | 0.18 | 0.16 |
|            | MSH2  | C2939T | Thr942Ile  | rs17102999 | 0.05 | 0.06 |
|            | MSH3  | A3122G | Thr1036Ala | rs26279 | 0.24 | 0.27 |
|            | MSH6  | G203A  | Gly39Glu   | rs1042821 | 0.07 | 0.09 |
| DNA double-strand break repair | BRCA2 | A1342C | Asn372His  | rs144848 | 0.22 | 0.21 |
|            | SNM1/KIAA0086 | C1867G | His317Asp | rs375089 | 0.26 | 0.30 (P = 0.08) |
|            | LIG4  | A2245G<sup>c</sup> | Ile658Val | rs2232641 | 0.04 | 0.06 |
|            | NBS1  | C605G  | Gln185Glu  | rs1805794 | 0.50 | 0.46 |
|            | RAD51L3/RAD51D | G501A | Arg165Gln | rs4796033 | 0.04 | 0.03 |
|            | RAD54L | A551G | Lys151Glu  | rs2295466 | 0.02 | 0.01 |
|            | RINT-1 | G33C  | Glu4Gln    | rs818620 | 0.07 | 0.09 |
| DNA damage response | XRCC3 | C1075T | Thr241Met | rs861539 | 0.09 | 0.09 |
| DNA polymerase | TP53 | G466Cc | Arg72Pro   | rs1042522 | 0.33 | 0.38 (P = 0.097) |
| POLD1     | G409A | Arg199His | rs1726801 | 0.20 | 0.22 |
| POLH/XPV/RAD30 | A1840G | Lys535Glu | -        | 0.03 | 0.04 |
| POLI/RAD30B | A2180G<sup>c</sup> | Thr706Ala | rs8305 | 0.25 | 0.24 |
| POL8       | C1683T | Arg438Tryp | rs3730477 | 0.01 | 0.012 |
| REV1       | T982Cc | Phe257Ser | rs3087368 | 0.33 | 0.32 |
| POLZ/REV3  | A1330G | Asn373Ser | rs3087399 | 0.04 | 0.04 |
| Other pathways | BLM | C967T | Thr298Met  | rs2834091 | 0.09 | 0.12 (P = 0.09) |
|            | G4035A | Val1321Ile | rs7162716 | 0.04 | 0.04 |
|            | G827A | Ala266Thr  | rs17232400 | 0.03 | 0.03 |
|            | G1080A | Arg530Gln  | rs17233497 | 0.01 | 0.01 |
|            | A1532G | Ser501Gly  | rs2239359 | 0.17 | 0.16 |
|            | A2457G | Asp809Gly  | rs7195066 | 0.03 | 0.03 |
|            | C3294T | Ser1068Phe | rs7190823 | 0.02 | 0.02 |
|            | G451T  | Arg89Leu   | -        | 0.01 | 0.00 |
|            | G1213A | Arg343Gln  | -        | 0.04 | 0.04 |
|            | A983G  | Lys324Glu  | -        | 0.003 | 0.002 |
|            | C1382T | Thr297Ile  | rs2237857 | 0.12 | 0.13 |
|            | C2573T | Thr781Ile  | rs17847568 | 0.03 | 0.03 |
|            | T4330C | Cys1367Arg | rs1346044 | 0.09 | 0.07 |

*P*-values by χ²-test against the control population are shown, when they are <0.1.

<sup>a</sup>Determined in our previous study (12).

<sup>b</sup>Significantly associated with SQC and/or ADC risks in our previous study (12).

ORs, when homozygotes for the 83Val allele were used as a reference, respectively (Table III). On the other hand, ORs of genotypes for the remaining three SNPs, SNM1-His317Asp, TP53-Arg72Pro and BLM-Thr298Met, did not show significant increases or decreases in SCLC cases (data not shown); therefore, these SNPs were not further investigated in the present study.

The *MTH1* gene, whose SNP, Val83Met, showed a significant association as described above, encodes a triphosphatase that hydrolyzes oxidized purine nucleoside triphosphates, such as 8-hydroxy-dGTP and 2-hydroxy-dATP (20). The activity of the MTH1-83Met protein was reported to be more thermolabile than that of the MTH1-83Val protein (20–22). The mitochondrial translocation of the MTH1-83Met protein was reported to be less efficient than that of the MTH1-83Val protein (23). Thus, it was suggested that the MTH1-83Met protein is less active than the MTH1-83Val protein. Previously, another SNP was found in a non-coding exon of *MTH1* (i.e. the T/C SNP in exon 2) 7.0 kb distal to the MTH1-Val83Met SNP, and the C allele in exon 2 leads to the production of an additional translation start site, resulting in the production of a longer MTH polypeptide in addition to commonly produced MTH polypeptides (21). This T/C SNP of the *MTH1* gene was
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Table III. MTH1 genotypes and SCLC risk

| SNP        | Genotype          | No. of controls (%) | No. of cases (%) | Crude OR (95% CI, P) | Adjusted OR (95% CI, P) |
|------------|-------------------|---------------------|------------------|----------------------|-------------------------|
| Val83Met   | Val/Val           | 558 (82)            | 154 (73)         | Reference            | Reference                |
|            | Val/Met           | 117 (17)            | 53 (25)          | 1.6 (1.1–2.4, 0.009) | 1.7 (1.1–2.7, 0.03)     |
|            | Met/Met           | 6 (1)               | 4 (2)            | 2.4 (0.7–8.7, 0.2)   | 6.5 (1.3–32.1, 0.02)    |
|            | Val/Met + Met/Met | 123 (18)            | 57 (27)          | 1.7 (1.2–2.4, 0.005) | 1.8 (1.2–2.9, 0.01)     |
| T/C in exon 2 | T/T               | 560 (82)            | 154 (73)         | Reference            | Reference                |
|            | T/C               | 118 (17)            | 53 (25)          | 1.6 (1.1–2.4, 0.009) | 1.7 (1.1–2.7, 0.03)     |
|            | C/C               | 3 (0)               | 4 (2)            | 4.8 (1.1–21.9, 0.04) | 15.7 (2.5–100.6, 0.004) |
|            | T/C + C/C         | 121 (17)            | 57 (27)          | 1.7 (1.2–2.5, 0.004) | 1.9 (1.2–3.0, 0.008)    |

*Adjusted for gender, age and smoking dosage.

not included in our 50 SNP set, because it was located in a non-coding exon. However, the above result prompted us to genotype this SNP in the same SCLC and control subjects. Since genotype data for the MTH1-Val83Met SNP were obtained from 681 of the 685 controls and all 211 cases, the genotypes for the T/C SNP were also determined for the same 681 controls and the 211 SCLC cases. Significant allele differentiations were also observed in the T/C SNP (OR for the C allele = 1.7, 95% CI: 1.2–2.3, P = 0.002). ORs of the heterozygotes, homozygotes for the exon 2-C allele and carriers of the allele were also significantly increased, when homozygotes for the exon 2-T allele were used as a reference, respectively (Table III).

Since both the case and control subjects in the present case–control study were enrolled in several institutions, it was possible that differences in the institutions lead to the observed allele differentiations due to population stratification. Therefore, we compared allele frequencies for the MTH1-Val83Met and exon 2-T/C SNPs among SCLC cases and controls of each institution (Figure 1). Allele frequencies for the MTH1-Val83Met SNP had been also reported in two other populations consisting of healthy Japanese volunteers (21,24); therefore, the frequencies in those studies were also compared. Frequencies of the 83Met and exon 2-C alleles in any SCLC populations were higher than those in any of the control populations. Allele frequencies for these SNPs were not significantly different among control populations and among case populations (P > 0.05 by χ²-test). We also compared the frequencies of genotypes for the two SNPs, and they were not significantly different among control populations and among case populations, either (P > 0.05 by χ²-test). Thus, it was indicated that the 83Met and exon 2-C alleles were associated with the SCLC risk beyond institutional differences.

Both the SNPs of MTH1 were found to be in linkage disequilibrium with each other (D’ = 0.97, r² = 0.91). Thus, we further evaluated the haplotype differentiation between the SCLC cases and the controls (Table IV). The haplotype consisting of the two risky alleles (i.e., haplotype #2 consisting of the 83Met and C alleles in Table IV) was significantly over-represented in the SCLC population (OR = 1.7, 95% CI: 1.2–2.4, P = 0.001), and the OR for haplotype #2 was similar to those for individual 83Met and C allele, respectively. In addition, by taking into account the estimation error of haplotype frequency, crude and adjusted ORs for carrying one copy of haplotype #2 were calculated on the basis of the estimated number of haplotypes for each subject by the bootstrap method, and they were 1.8 (95% CI: 1.2–2.5, P = 0.0004) and 2.0 (95% CI: 1.2–3.2, P = 0.002), respectively.

Fig. 1. Frequencies of the MTH-83Met and exon 2-C alleles in cases and controls. Allele frequency is shown with its sampling variations estimated by 95% CI. Frequencies of the MTH-83Met allele in two control populations reported by Yoshimura et al. (24) and Oda et al. (21) are also shown.

We next assessed the effect of smoking on the contribution of the MTH1-Val83Met and exon 2-T/C SNPs to the SCLC risk. ORs in light (PY < 50) smokers and heavy (PY ≥ 50) smokers were compared (Table V). The number of non-smokers in the case subjects was small (i.e. N < 10); therefore, they were excluded from the analysis. Increases of ORs for the 83Met and exon 2-C alleles were more evident in light smokers than in heavy smokers, and the ORs were statistically significant in light smokers but not in heavy smokers. P-values for interaction of the Val83Met and exon 2-T/C genotypes on the SCLC risk with smoking were 0.15 and 0.11, respectively. P-value for interaction of haplotype #2 on the SCLC risk by smoking was calculated as being 0.095.

Discussion

The MTH1 gene was cloned as a human homolog for the Escherichia coli mutT gene, encoding an enzyme hydrolyzing 8-hydroxy-dGTP, an oxidized dNTP causing A:T to C:G transversion (20). It has been shown that MTH1 protein hydrolyzes not only 8-hydroxy-dGTP but also several other oxidatively damaged dNTPs, such as 2-hydroxy-dATP, thereby preventing multiple mutations including A:T to C:G, G:C to T:A and G:C to A:T mutations (20). Mth1 nullizygous mice are susceptible to tumor development in lung and other tissues (25). Thus, it has been assumed that inter-individual differences in MTH1 activity are associated with risks for cancers by causing inter-individual differences.
in the capacity to prevent mutations of the cancer-related genes caused by incorporation of oxidatively damaged dNTPs during DNA replication (20). In the present study, SNPs in the MTH1 gene were found to be associated with SCLC risk. To the best of our knowledge, SNPs in the MTH1 gene were found for the first time as being associated with risks for human cancers by a case–control study. However, the possibility of false positives (type I statistical errors) must be considered. We performed 50 separate tests of significance in the analysis. A consecutive Bonferroni adjustment to yield an experiment-wide type I error rate of 0.05 would demand a test-wise \( P \)-value of 0.001. Therefore, the association of the MTH1-Val83Met SNP would not be considered significant on an experiment-wide level after Bonferroni adjustment. Thus, the association requires confirmation in other population samples, although the present study proposed MTH1 as a candidate gene responsible for SCLC susceptibility.

The two MTH1 SNPs, Val83Met and exon 2 T/C, examined in the present study were suggested to cause functional differences, although their effects on mutation suppression efficiency against oxidative DNA damages are unknown (20–22). These two SNPs were in linkage disequilibrium, and the risky allele of each SNP (i.e. the 83Met and exon 2 C alleles) was on the same haplotype (haplotype #2) in most of the Japanese population. Thus, at present, it is unclear whether both or one of the two SNPs are responsible for the SCLC susceptibility. It is also possible that other SNPs consisting of the haplotype are responsible. Further biological and genetic analyses of the MTH1 SNPs will elucidate the issue.

Interestingly, ORs for carriers of the 83Met and C alleles were more evidently increased in light smokers than in heavy smokers. Tobacco smoke is known to cause oxidative damages on genomic DNA and nucleoside triphosphates (26). Therefore, individuals carrying the 83Met and C alleles might be more prone to acquiring gene mutations even by a low-dose exposure of carcinogens, and therefore, the effects of MTH SNPs might have more prominently appeared under the condition of a low-dose exposure of tobacco smoke. On the other hand, the effects of the SNPs might be masked under the condition of a high-dose exposure of tobacco smoke, since, under such a condition, environmental factors (i.e. carcinogens in tobacco smoke) rather than genetic factors predominantly determine the risk for SCLC. However, the interaction of MTH1 SNPs with smoking on SCLC risk in the present study was not statistically significant; therefore, further case–control studies are necessary to elucidate how MTH1 SNPs contribute to SCLC risk of smokers.

We previously examined the same 50 SNP set for associations with lung SQC and ADC risk using the same controls (13). In the study, frequencies of the MTH1-83Met allele in SQC and ADC cases, respectively, were slightly higher than that in controls. However, ORs of the carriers of the allele was not significantly increased (Figure 2). Thus, the MTH1-Val83Met SNP was thought to be associated with SCLC risk but not with NSCLC risk. In the previous study, an SNP, TP53-Arg72Pro, in the p53 gene was associated with SQC risk, and the association remained significant after Bonferroni adjustment. Association of the SNP with NSCLC and overall lung cancer risks have been observed in several other case–control studies (28–31). The association was also supported by a report that TP53-Arg72Pro protein has a weaker activity than TP53-Arg72Arg protein in inducing apoptosis of human cells suffering from DNA damages (32). Interestingly, the TP53-72Pro allele was marginally significantly over-represented in SCLC cases in the present study. ORs of the homozygotes for the carriers of the TP53-72Pro allele were increased in SCLC cases, although the increase was not statistically significant (Figure 2). Thus, it is possible that the TP53-72Pro allele confers increased susceptibility both to SCLC and NSCLC. In the present study, marginally significant associations with SCLC risk were observed for two other SNPs, BLM-Thr298Met and SMN1-His317Asp. However, such associations were not detected in ADC and

### Table IV. Association of MTH1 haplotypes and SCLC risk

| Haplotype SNP | Haplotype frequency | OR (95% CI) | P |
|---------------|---------------------|-------------|---|
| Val83Met T/C in exon 2 | Control (95% CI) | Case (95% CI) | |
| 1 | Val | T | 0.90 (0.89–0.92) | 0.85 (0.82–0.89) | Reference |
| 2 | Met | C | 0.089 (0.073–0.10) | 0.14 (0.11–0.18) | 1.7 (1.2–2.4) | 0.001 |
| 3 | Met | T | 0.0067 (0.0023–0.011) | 0.0024 (0–0.0070) | 0.4 (0.05–3.0) | 0.3 |
| 4 | Val | C | 0.0030 (0–0.0059) | 0.0024 (0–0.0070) | 0.9 (0.1–7.7) | 0.9 |

### Table V. OR for MTH1 genotypes by smoking dosage and age

| SNP | Stratification | No of controls (%) | No of cases (%) | Crude OR | Adjusted OR* | P for interaction* |
|-----|---------------|-------------------|-----------------|----------|--------------|------------------|
|     | Major homozygote | Minor allele carrier | Major homozygote | Minor allele carrier |          | (95% CI, 𝑃) | (95% CI, 𝑃) |
| Val83Met T/C in exon 2 | py = 0 | 319 (83) | 67 (17) | 5 (63) | 3 (38) | 2.8 (0.7–12.2, 0.16) | 2.9 (0.7–12.7, 0.16) |
|     | 0 < py < 50 | 178 (82) | 38 (18) | 59 (69) | 26 (31) | 2.1 (1.2–3.7, 0.014) | 2.3 (1.2–4.4, 0.011) |
|     | py ≥ 50 | 54 (78) | 15 (22) | 88 (77) | 26 (23) | 1.1 (0.5–2.2, 0.87) | 1.1 (0.5–2.3, 0.85) |
|     | py = 0 | 316 (82) | 68 (18) | 5 (63) | 3 (38) | 2.8 (0.7–12.0, 0.16) | 2.8 (0.6–12.3, 0.17) |
|     | 0 < py < 50 | 181 (84) | 35 (16) | 59 (69) | 26 (31) | 2.3 (1.3–4.1, 0.006) | 2.6 (1.3–4.9, 0.005) |
|     | py ≥ 50 | 54 (78) | 15 (22) | 88 (77) | 26 (23) | 1.1 (0.5–2.2, 0.87) | 1.1 (0.5–2.3, 0.85) |

*Adjusted for gender and age.
SQC (Table II). SNPs that showed association with SQC or ADC risk, such as LIG4-Ile658Val, POLI-Thr706Ala and REV1-Phe257Ser, were not associated with SCLC risk in this study. Thus, genes involved in the susceptibility might be overlapped but different between SCLC and NSCLC.

In the present and previous studies (13), we examined the associations of 50 SNPs in 36 DNA repair genes with SCLC and NSCLC risks. The studies led us to identify several DNA repair genes commonly or specifically involved in the susceptibility to SCLC and NSCLC. The results supported the idea that inherited variations in DNA repair genes are involved in susceptibility to lung cancer of each individual. Further examination of SNPs in DNA repair genes in the present and also in other sets of subjects will help us understand genetic factors responsible for the susceptibility to lung cancer. In addition, studies up to the present suggested that polymorphisms of genes involved in metabolism of carcinogens in cigarette smoke, such as CYP1A1, CYP2A6 and GSTM1, are also responsible for the susceptibility to lung cancer (4–7). It is possible that such polymorphisms modify the effect of SNPs in DNA repair genes on risk for lung cancer. Therefore, combined effects of polymorphisms in DNA repair genes and metabolic genes on risks for SCLC and NSCLC should be also further investigated.

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