Original Contribution

Interactive Effect of Cigarette Smoking With Human 8-Oxoguanine DNA N-Glycosylase 1 (hOGG1) Polymorphisms on the Risk of Lung Cancer: A Case-Control Study in Taiwan

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Human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) plays an important role in repairing oxidative DNA damage induced by tobacco carcinogens. In this case-control study, the authors examined the interactive effect of hOGG1 gene polymorphisms and cigarette smoking on the risk of lung cancer in Taiwan. A total of 1,096 cases and 1,007 controls were enrolled from 6 medical centers in Taiwan during 2002–2004. hOGG1 Ser326Cys genetic polymorphisms were determined using the MassARRAY system (SEQUENOM, Inc., San Diego, California). Tobacco smoking history was obtained through personal interview according to a structured questionnaire. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95% confidence intervals. The odds of developing lung cancer for persons with the Cys/Cys genotype versus the Ser/Ser genotype were 1.11 (95% confidence interval (CI): 0.74, 1.65) for never smokers, 1.45 (95% CI: 0.74, 2.83) for moderate smokers, and 3.52 (95% CI: 1.54, 8.06) for heavy smokers. The P value for interaction in the logistic model was 0.01. The increased risk associated with the Cys/Cys genotype among heavy smokers remained statistically significant for various histologic types of lung cancer, including adenocarcinoma, squamous cell carcinoma, and small cell carcinoma. The authors conclude that there was a noticeable modifying effect on the association between hOGG1 genotype and lung cancer risk by cigarette smoking status.

case-control studies; DNA damage; DNA repair; genetic predisposition to disease; lung neoplasms; oxoguanine glycosylase 1, human; smoking

Abbreviations: CI, confidence interval; hOGG1, human 8-oxoguanine DNA N-glycosylase 1; OR, odds ratio.

Editor’s note: An invited commentary on this article appears on page 703.

In Taiwan, lung cancer is the leading cause of cancer death among women and the second-leading cause of cancer death among men (1). A number of environmental and genetic risk factors for lung cancer have been identified in Taiwan (2–4). Tobacco smoking is a well-recognized risk factor for lung cancer. Approximately 85% of lung cancers are caused by tobacco smoking (5). Exposure to tobacco smoke may induce oxidative DNA lesions (6, 7), and subsequently an impaired DNA repair mechanism may result in increased risk of smoking-related lung cancer. Therefore, it is critical to include important genetic factors, especially oxidative DNA repair genes, in the investigation of the effects of tobacco smoking on lung cancer.

DNA damage may lead to carcinogenesis through inactivation of tumor suppressor genes or activation of oncogenes (8, 9), and recent studies have focused on the association between genetic polymorphisms in DNA repair genes and risk of lung cancer. The 8-oxoguanine lesion is one of the
major forms of oxidative DNA damage (10, 11), and it can be removed from DNA by human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) (12). This glycosylase has been suggested as a possible suppressor of lung carcinogenesis in OGG1-knockout mice (13), and in a bacterial complementation assay system the hOGG1 Cys326 allele was postulated to reduce the activity of 8-oxoguanine lesion removal (14). In addition, the hOGG1 Ser326Cys genotype has been reported to be associated with the risk of human lung cancer in a number of studies (15–24).

An approximately 2-fold increased risk of lung cancer associated with the Cys/Cys or Ser/Cys genotype of hOGG1 has been observed in many populations, including Chinese, Caucasian, Hawaiian, and Japanese populations (15–17). The Cys/Cys genotype was reported to be associated with an increased risk of lung adenocarcinoma in a recent large-scale study carried out in 6 Eastern European countries (18) and with an increased risk of lung cancer in a meta-analysis (19).

A significant interactive effect of the hOGG1 Ser326Cys polymorphism and cigarette smoking on lung cancer was found in a study of Caucasians (15). However, this finding was not consistent with findings observed in other studies of persons of European descent and Japanese (18, 20, 23, 24). The detection of a possible synergistic effect of cigarette smoking and hOGG1 polymorphisms in these studies might be limited by the small effective sample size in stratification analyses. Only about 200 lung cancer cases and 200 controls were included in 4 previous studies (15, 20, 23, 24). While 2,188 lung cancer cases and 2,198 controls were included in 1 previous study (18), there were only 45 heavy smokers with the Cys/Cys genotype (35 cases and 10 controls) in that study.

In order to investigate the potential interactive effects of cigarette smoking and hOGG1 genotype on lung cancer, we conducted a large-scale case-control study in Taiwan. In this study, we also examined the association of the hOGG1 Ser326Cys polymorphism with various histologic types of lung cancer, including adenocarcinoma, squamous cell carcinoma, and small cell carcinoma.

**MATERIALS AND METHODS**

**Selection of cases and controls**

The Genetic Epidemiological Study of Lung Adenocarcinoma started in September 2002 and is an ongoing project. The project involves 2 research institutes and 6 medical centers, including the National Health Research Institutes, the Graduate Institute of Epidemiology of National Taiwan University, National Taiwan University Hospital, Taipei Veterans General Hospital, and Chang Gung Memorial Hospital in northern Taiwan; Taichung Veterans General Hospital in central Taiwan; and Kaohsiung Medical University Hospital and National Cheng-Kung University Hospital in southern Taiwan. All lung cancer cases and healthy controls were recruited from the 6 medical centers. We enrolled patients with all histologic types of lung cancer, with the aim of comparing the clinical and etiologic differences in adenocarcinoma and other lung carcinomas.

Cases aged 18 years or older with histologically or cytologically confirmed lung cancer were enrolled in this study. All healthy controls were recruited from persons who were undergoing periodic comprehensive physical examinations at the study medical centers. Controls were selected by frequency matching with cases on ethnicity, sex, education, and 5-year age group. Written informed consent was obtained from each study subject. The Genetic Epidemiological Study of Lung Adenocarcinoma was approved by the institutional review boards of all participating institutions and medical centers.

Both a blood sample (30 mL) and questionnaire information were collected from each participant. The structured questionnaires were administered in face-to-face interviews conducted by well-trained nurses. The information requested in the questionnaire included sociodemographic characteristics, history of exposure to various risk factors for lung cancer, family history of lung cancer (including first-degree, second-degree, and other relatives), and personal history of pulmonary tuberculosis, emphysema, or chronic bronchitis. The database was established at the National Health Research Institutes in Taiwan through a quality control and double-entry process.

The present analysis included participants recruited from September 2002 through March 2004. After exclusion of lung cancer patients who declined to participate (n = 259) or had no histologic/cytologic confirmation (n = 23), a total of 1,096 cases were recruited. Among them, 621 (56.7%) and 475 (43.3%) cases were confirmed by histologic and cytologic examination, respectively. There were 1,007 frequency-matched controls who participated in this study. We were still recruiting cases and controls when the current analyses were conducted. Controls in the present analysis were slightly younger than cases (4.5 years, on average).

**Genotyping assays**

Genomic DNA was extracted from the blood samples of all participants by means of the conventional phenol/chloroform extraction method. For a pilot study, we genotyped the hOGG1 Ser326Cys (rs1052133) polymorphism in 181 cases and 181 controls using a high-throughput genotyping platform, by means of the 5'-nuclease allelic discrimination TaqMan assay in a 96-well format with the ABI Prism 7000HT Sequence Detection System (Applied Biosystems, Foster City, California). The polymerase chain reaction primers (5'-CTCTACAGGTGCTGTTCCAGT-3' and 5'-ACCCTTTCTGCGTTTGTGC-3') and probes (5'-CGCCAATCCCGCAT-3' and 5'-CGCCAATCCCGCAT-3') were designed using the Assays-by-Design Service (Applied Biosystems). We genotyped the hOGG1 Ser326Cys polymorphism in remaining participants using the MassARRAY system (SEQUENOM, Inc., San Diego, California) by means of the National Genotyping Core of the National Research Program for Genomic Medicine in Taiwan.

To assure data quality, we genotyped duplicate samples (48 cases and 46 controls) with the TaqMan assay, MassARRAY, and autosequence, respectively. The genotype concordance rate between duplicate samples was 100%. Overall, the missing genotyping rate was 0.5% (10/2,013) in our samples.
Statistical analysis

Age, education, and cumulative cigarette smoking were divided into 3 levels. In terms of cumulative cigarette smoking, there were never smokers, smokers who had ever smoked for less than 40 pack-years (moderate smokers), and smokers who had ever smoked for 40 or more pack-years (heavy smokers). The χ2 test was used to test for the statistical significance of different distributions of demographic characteristics and other risk factors between lung cancer cases and healthy controls. The χ2 goodness-of-fit test was used to examine whether the hOGG1 Ser326Cys polymorphism was in Hardy-Weinberg equilibrium in the healthy controls.

For different histologic types of lung cancer, odds ratios and 95% confidence intervals were estimated for each risk factor, using unconditional logistic regression analysis with adjustment for potentially confounding variables, including age, sex, and education. We examined the modifying effect of cigarette smoking (never, moderate, heavy) on the association between lung cancer and hOGG1 polymorphisms through stratification analysis. We evaluated the interactions between hOGG1 polymorphisms and smoking history by including the product term in the unconditional logistic regression models (25). The estimated statistical power for the present analysis was 0.73 (2-sided α = 0.05).

Finally, we assessed the joint effects of hOGG1 Ser326Cys genotype and cigarette smoking history on lung cancer risk by logistic regression analysis after adjusting for age, sex, and education. All data analyses were performed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, North Carolina).

RESULTS

As Table 1 shows, lung cancer cases were significantly younger than controls (a mean age of 63.2 years in cases and 58.7 years in healthy controls) and had lower educational levels than controls. The male:female ratios were similar in cases and controls. For cigarette smoking exposure, cases were more likely to have ever been smokers (52.4%) than were controls (29.9%). In the healthy controls, the prevalence of cigarette smoking was much higher in males (67.1%) than in females (4.8%). The Ser326Cys polymorphism of the hOGG1 gene was in Hardy-Weinberg equilibrium in controls and in the overall sample (P > 0.05). There were 83 cases (9.0%) and 65 controls (7.4%) who had a history of lung cancer among first-degree relatives. There was a higher proportion of cases (8.7%) with a history of pulmonary tuberculosis than of controls (4.2%). The proportion with a history of emphysema or chronic bronchitis was also higher in cases (4.8%) than in controls (3.7%). With regard to histologic type of lung cancer, adenocarcinoma was the most common type (59.8%), followed by squamous cell carcinoma (21.3%) and other cell types (9.3%).

As Table 2 shows, there were significant associations with cigarette smoking for all cell types of lung cancer, in combination or individually. The moderate and heavy smokers had 1.8-fold (95% confidence interval (CI): 1.3, 2.4) and 7.4-fold (95% CI: 5.1, 10.6) higher odds of developing lung cancer than never smokers after adjustment for age, sex, and education. Among various histologic types of lung cancer, cigarette smoking had the strongest effects on small cell carcinoma, followed by squamous cell carcinoma and adenocarcinoma. There were only weak associations with the homozygous variant (Cys/Cys) of hOGG1 Ser326Cys for all cell types of lung cancer combined (odds ratio (OR) = 1.4, 95% CI: 1.0, 1.9) and for adenocarcinoma of the lung (OR = 1.5, 95% CI: 1.0, 2.1), as shown in Table 2.
Table 3 shows findings from stratification analyses of association between hOGG1 Ser326Cys polymorphisms and lung cancer risk, stratified by cumulative cigarette smoking level. For all cell types of lung cancer combined, hOGG1 Ser326Cys genotype was not significantly associated with any cell type of lung cancer among never smokers and moderate smokers. In heavy smokers, however, the Cys/Cys genotype was associated with a significantly increased risk of all lung cancer cell types (OR = 3.52, 95% CI: 1.54, 8.06; P = 0.003), adenocarcinoma (OR = 3.19, 95% CI: 1.20, 8.44; P = 0.019), squamous cell carcinoma (OR = 3.04, 95% CI: 1.15, 8.03; P = 0.025), and small cell carcinoma (OR = 4.53, 95% CI: 1.36, 15.13; P = 0.014). The trend toward an increasing risk of lung cancer, either in combination or individually, with increasing number of Cys alleles (i.e., the highest risk for the Cys/Cys genotype, followed by the Ser/Cys and Ser/Ser genotypes) was also statistically significant (P = 0.002, P = 0.005, P = 0.023, and P = 0.014, respectively). In addition, the P value from testing of the product term in the logistic regression model was 0.01 for all cell types of lung cancer combined. Moreover, the interaction term was statistically significant for small cell carcinoma (P = 0.02) but was not significant for adenocarcinoma (P = 0.21) or squamous cell carcinoma (P = 0.06).

Table 4 presents the joint effects of the functional Ser326Cys polymorphism of hOGG1 and cigarette smoking history on the risk of lung cancer. We found that heavy smokers carrying the Cys/Cys genotype (as compared with never smokers carrying the Ser/Ser genotype) had significantly increased odds of developing any histologic type of lung cancer, including adenocarcinoma (OR = 6.20, 95% CI: 2.94, 13.10), squamous cell carcinoma (OR = 31.01, 95% CI: 10.73, 89.62), and small cell carcinoma (OR = 191.79, 95% CI: 20.67, 1999). The wide 95% confidence interval for small cell carcinoma was due to its small sample size (n = 99).

The effects of cigarette smoking level on the association between hOGG1 genotype and lung cancer risk are also shown in Table 4. Increasing odds of all histologic types of lung cancer (all types combined, adenocarcinoma, squamous cell carcinoma, and small cell carcinoma) with increasing number of Cys alleles were observed only in heavy smokers, not in moderate or never smokers. These findings provided empirical evidence for synergistic effects of hOGG1 genotype and cigarette smoking on lung cancer risk, and the interactions were observed for all histologic types of lung cancer.

**DISCUSSION**

To the best of our knowledge, this study was one of the largest-scale case-control studies to date to investigate the synergistic effects of hOGG1 polymorphisms and cigarette smoking on lung cancer risk. The present study had more statistical power to detect this effect modification because of the high frequency of the Cys allele (60.9%) in the Taiwan population as compared with other populations. Our results indicate that heavy smoking is a modifier of the effect of hOGG1 polymorphisms on the development of lung cancer.
Table 3. Odds Ratios for Major Subtypes of Lung Cancer According to Cigarette Smoking History and hOGG1 Ser326Cys Single Nucleotide Polymorphism (Obtained by Logistic Regression With Adjustment for Age, Sex, and Education), Taiwan, Republic of China, 2002–2004

<table>
<thead>
<tr>
<th>Smoking History (Pack-Years) and Genotype</th>
<th>All Cell Types (n = 1,096)</th>
<th>Adenocarcinoma (n = 655)</th>
<th>Squamous Cell Carcinoma (n = 233)</th>
<th>Small Cell Carcinoma (n = 99)</th>
<th>Controls (n = 1,007)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>OR</td>
<td>95% CI</td>
<td>No.</td>
</tr>
<tr>
<td>Never smoker (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>60</td>
<td>12.0</td>
<td>1.00</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>207</td>
<td>41.5</td>
<td>1.11</td>
<td>0.74, 1.65</td>
<td>172</td>
</tr>
<tr>
<td>Moderate smoker (0–&lt;40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>29</td>
<td>13.6</td>
<td>1.00</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>109</td>
<td>51.2</td>
<td>1.05</td>
<td>0.56, 1.96</td>
<td>66</td>
</tr>
<tr>
<td>Heavy smoker (≥40)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>46</td>
<td>13.7</td>
<td>1.00</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>155</td>
<td>46.1</td>
<td>1.78</td>
<td>0.86, 3.67</td>
<td>55</td>
</tr>
</tbody>
</table>

* P < 0.05; **P < 0.01.

Abbreviations: CI, confidence interval; hOGG1, human 8-oxoguanine DNA N-glycosylase 1; OR, odds ratio.
A weak association between hOGG1 polymorphisms and lung cancer was observed in the present study. The finding was similar to findings reported in some previous studies (15–19) but inconsistent with those reported in other studies (20–24). No association between the homozygous Cys/Cys genotype and lung cancer was reported in a Japanese study (20), a German study (21), or a meta-analysis (22). In another 2 Japanese studies (23, 24), a decreased lung cancer risk associated with the Cys/Cys genotype and an increased lung cancer risk associated with the Ser/Cys genotype were observed. These inconsistent findings might be due to insufficient statistical power in some studies. Other reasons for the inconsistency might include genetic heterogeneity across study populations or a failure to study effect modifiers such as cigarette smoking (19). The prevalences of cigarette smoking could be very different across populations. If tobacco smoking does interact with the hOGG1 gene to influence risk of lung cancer, the failure to include cigarette smoking in exploring the main effect of the hOGG1 gene on lung cancer may result in bias. In a population with a high prevalence of cigarette smoking, the hOGG1 gene might have a much greater impact on lung cancer than in populations with a low smoking prevalence. To better characterize the effect of the DNA repair gene on lung cancer risk, it is necessary to incorporate influential environmental factors like cigarette smoking into the study.

The present study had several limitations. Firstly, our controls were younger and had more education than cases, which may have resulted in biased odds ratio estimates. However, such a bias would have occurred only if younger controls with more education were more likely to have a particular genotype, which is an unlikely scenario. Furthermore, we tried to control the confounding effects of age and education by including them in the logistic regression models in this study.

Since the genotype was considered unrelated to the participation of cases and controls, the effect of gene-environment interaction should not have been influenced (34).

Secondly, the prevalence of cigarette smoking in female controls was consistent with that (4.9%) in a national survey of female adults (http://olap.bhp.doh.gov.tw/) carried out by the Bureau of Health Promotion of the Taiwan Department of Health, but the prevalence of cigarette smoking in male controls was slightly higher than that (45.5%) of male adults in Taiwan. The effect of cigarette smoking on lung cancer risk in males may have been slightly underestimated in this study. However, cigarette smoking was significantly associated with all histologic types of lung cancer, with stronger associations with squamous cell carcinoma and small cell carcinoma than with adenocarcinoma, in this study. These findings were consistent with those observed in previous studies (35). In addition, our findings on the gene-smoking interaction were not affected by the slightly higher prevalence of cigarette smoking in controls, because genotype was considered unrelated to the participation of study subjects (34).

Thirdly, the odds ratios for small cell carcinoma had wide confidence intervals because of the small number of subjects in that group. Future studies with a large sample of small cell carcinoma patients are needed to clarify the interactions between hOGG1 genotype and cigarette smoking in small cell carcinoma.

Squamous and small cell carcinomas of the lung, which are related to smoking, may be regarded as diseases that are distinct from lung adenocarcinoma, which is less related to smoking. They have strikingly different molecular profiles, as reported in a previous study (36). However, the interactions of hOGG1 genotype with heavy smoking were observed in both smoking-related and less-smoking-related.
lun cancers in this study. Although the biologic mechanism is not clear, it is possible that the hOGG1-involved base excision repair pathway may be one of the common pathogenic mechanisms for major types of lung cancer.

In summary, we found no strong main effects of hOGG1 polymorphisms on the development of lung cancer, but there were significant interactive effects of the hOGG1 Ser326Cys genotype with cigarette smoking on various histologic types of lung cancer. Although cigarette smoking is the major cause of lung cancer, only a small fraction of smokers (10%) ever develop lung cancer. This may be due to the variable quantity of cumulative smoke exposure among ever smokers. It may also be attributable to some genetic factor(s) which may reduce the effect of cigarette smoking on the risk of lung cancer (37). The interactive effects of other DNA repair genes and cigarette smoking history on lung cancer risk are worth additional investigation in future studies.

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Conflict of interest: none declared.

REFERENCES


