RESEARCH ARTICLE

The DNA Methyltransferase 3B -149 Genetic Polymorphism Modulates Lung Cancer Risk from Smoking

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Abstract

Background: Smoking can cause increase of DNA methylation and hypermethylation of tumor suppressor genes, this possible contributing to subsequent lung cancer development. DNA methyltransferase 3B (DNMT3B) is crucial in regulation of DNA methylation and it has been proposed that green tea might lower cancer risk through inhibiting its activity. Here, we designed a case-control study to investigate whether the DNMT3B -149 genetic polymorphism could modulate lung cancer risk due to smoking. Possible interactions of smoking and green tea consumption with this DNMT3B genetic polymorphism were also assessed. **Materials and Methods:** A total of 190 lung cancer patients and 380 healthy controls were recruited. Questionnaires were administered to obtain data on sociodemographic and lifestyle variables, as well as family history of lung cancer. Genotypes for DNMT3B -149 were identified by polymerase chain reaction. **Results:** Smoking, green tea consumption, exposure to cooking fumes, family history of lung cancer, and the DNMT3B -149 genotype (odds ratio (OR)=2.65; 95% confidence interval (CI) 1.15-6.10) were all significantly associated with the development of lung cancer. Smokers carrying the DNMT3B -149 TT genotype were at elevated risk compared to non-smokers carrying DNMT3B -149 (OR=7.69; 95% CI 2.55-23.14). Interaction of smoking with DNMT3B -149 genotypes was significant regarding lung cancer risk. However, interaction between green tea drinking and DNMT3B -149 genotypes was not. **Conclusions:** The DNMT3B -149 TT genotype might increase the smoking-associated lung cancer risk.

Keywords: Cigarette smoking- lung cancer- DNA methyltransferase 3B- green tea

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Introduction

As is known to all, most cases of lung cancer can be attributed to cigarette smoking (Freedman et al., 2016). Cigarette smoke contains thousands of chemical compounds; in particular, free radicals have been implicated in smoking-related carcinogenesis (Pryor, 1997). In addition, smoking can contribute toward lung cancer development through genetic and epigenetic mechanisms.

In humans, DNA hypermethylation is one of the major epigenetic mechanisms serving various biological functions, such as regulating gene expression (Hamidi et al., 2015). In the processes of DNA methylation, DNA methyltransferase (DNMT) is a key catalyst. At least five DNMT patterns have been identified, including DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L (Hamidi et al., 2015). Importantly, the major cigarette carcinogen, 4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone (NNK), could increase DNMT stability (Lin et al., 2010). The correlation between the promoter hypermethylation of multiple tumor suppressor genes (TSG) and DNMT protein expression in lung cancer tissue has also been demonstrated, especially in smokers with pulmonary squamous carcinoma (Lin et al., 2010). Further, the expressions of DNMT proteins in the lung cancer tissue of smokers are higher, and patients with the hypermethylation of DNMT have a poor prognosis (Lin et al., 2007; Lin et al., 2010). Thus, DNMT might relate to hypermethylation of TSG and subsequent cancer development.

The DNMT3B gene is the locus on chromosome 20q11.2, and has a C to T transition (rs2424913) in the promoter, -149 bp from the transcription start site. This polymorphism can increase the promoter activity by about 30% (Shen et al., 2002). However, it is unclear how this single nucleotide polymorphism affects DNMT3B expression. Even so, previous studies have shown DNMT3B -149 polymorphism was correlated with the occurrence of lung cancer (Shen et al., 2002; Lee et al., 2005). Further, the question of whether there is an

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interaction of smoking and DNMT3B -149 polymorphism on the lung cancer development has not been answered yet.

Tea is mainly consumed in Asia. Experimental studies have consistently shown tea preparations and tea polyphenols may inhibit the induction of various cancers, including lung cancer (Yang et al., 2013). Our previous study revealed smokers who never drank green tea compared to smokers who drank green tea had a significantly increased lung cancer risk; interaction between green tea consumption and smoking in lung cancer risk was also observed (Lin et al., 2012). Tea polyphenols are strong antioxidants. In particular, DNA methylation in cervical cancer cell lines could be inhibited by epigallocatechin gallate (EGCG), a major component of green tea (Khan et al., 2015). A similar result in prostate cancer cell lines also suggest polyphenols could inhibit DNMT expression (Pandey et al., 2010). Therefore, green tea consumption might alter DNMT activity and reduce cancer development (Henning et al., 2013).

Herein, we designed a case-control study to investigate whether DNMT3B -149 polymorphism could modulate lung cancer risk elicited by smoking, and examine the possible interactions between smoking, green tea drinking with DNMT3B -149 polymorphism in lung cancer occurrence, respectively.

Materials and Methods

Study subjects

The institutional review boards of participating institutions approved the design and final report of this study (Taichung Cheng Ching Hospital: HP140005, Chung Shan Medical University: 1031229, Taichung Tungs' Taichung MetroHarbor Hospital: 104072). Informed consent for participation in this study was obtained from all participants.

A total of 271 lung cancer patients (International Classification of Diseases, 10th revision; ICD cod C33-C34) were recruited from August 2004 to October 2011 from participating institutions in central Taiwan. These hospitals were accessible to patients from all socioeconomic classes. All patients underwent a series of examinations of pathologic stages by board-certified pathologists. Tumor types and stages were determined according to the World Health Organization classification (Anonymous, 1981). Of the patients, 10 patients had severe illness and 37 patients were not the incident cases so they were not interviewed, and 34 patients were excluded due to older age (range = 81-92 years) or incomplete questionnaire data. The excluded cases were comparable with the included cases in regard to demographic characteristics, except age. Among the 190 available patients for matching, their cancer cell types included 108 adenocarcinoma (56.8%), 51 squamous cell carcinoma (26.9%), and 31 others (including small cell carcinoma, neuroendocrine carcinoma, mixed cell carcinoma, and unspecific malignant cell). In this study, two controls were selected for each lung cancer case, matched by gender and age (± 5 years). Therefore, 380 controls were randomly selected from consecutive patients with no history of cancer who were admitted to the same teaching hospitals for physical check-up.

Epidemiological information

Epidemiological information from study subjects was collected by in-person interviews using a standardized questionnaire containing demographic details and lifestyle (Lin et al., 2012). Cumulative smoking dose was calculated by pack-years, defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. In Taiwan, tea is commonly made in a small teapot, and the same tea leaf can be brewed many times. In this study, we defined a standardized cup of tea as 100-120 ml. The period of exposure was assessed when the lung cancer was first diagnosed (for cases) or when the interview was performed (for controls). The frequency of green tea consumption was assessed from five possible answers: every day (more than one cup per day), three to four cups per week, one to two cups per week, one to two cups per month, and seldom. For those consuming tea every day, the number of cups drunk was also further classified: one or two cups per day, three or four cups per day, five to nine cups per day, and 10 or more cups per day. In the present study, the evaluation of green tea consumption was based on a previous study (Tsubono et al., 2001), in which Spearman's correlation between the amount of green tea consumed according to the questionnaire and the amounts consumed according to the three day in one year food records was 0.66, and the correlation between consumption measured by the two questionnaires administered six months apart was also 0.66. In addition, fruits and vegetables intake was measured as the average number of standardized servings per week of fruits and vegetables over the last three years. Past domestic cooking exposure was also evaluated. Subjects were asked about the frequency of using various cooking methods, such as stir-frying. Family history of lung cancer was defined as lung cancer in first-degree relatives of the test subjects.

Genotyping analysis of DNMT3B

Venous blood from all subjects was collected into heparin tubes, prepared into plasma, buffy coat and red blood cells. Genomic DNA was extracted from buffy coat with the Genomic DNA Isolation Kit (Qiagen Inc., Hilden, Germany).

According to a previous study (Shen et al, 2002), DNMT3B -149 genotype was determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism. Primers used for the amplification of DNMT3B gene were 5'-TGC TGT GAC AGG CAG AGC AG-3' and 5'-GGT AGC CGG GAA CTC CAC GG-3'. 0.5 ml of DNA was added to a PCR buffer containing 200 ng of primers, 1.5 mM MgCl2, 0.2 mM dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.1% bovine serum albumin in a final volume of 50 ml. Amplification conditions were the initial denaturation (95ºC for 5 min), followed by 35 cycles of denaturing step (95ºC for 30 s), annealing (63ºC for 90 s), and extension (72ºC for 40 s). The PCR products were digested with BfaI at 16 hours in 37ºC. Homozygous CC individuals had product fragments of 208, 126, and 46 bp, while homozygous TT

individuals had product fragments of 162, 126, and 46 bp, and heterozygous CT individuals had all four fragments.

Rigorous quality control procedures were applied throughout the genotyping processes. After genotyping, 25% of the samples in each genotype group were randomly selected for repeated assays to validate the results.

Statistical analysis

The variables between cases and controls were compared by Student's t test for continuous variables and χ2-test or Fisher's exact test for discrete variables. Further, Goodness-of-fit χ2-test was used to test the genotypes for the Hardy-Weinberg equilibrium. Unconditional and conditional logistic regression models were employed to obtain odds ratio (OR) and 95% confidence interval (95% CI) for each interested variable, respectively. For internal model validation, bootstrap estimates of ORs in conditional logistic regression were conducted. For each bootstrap estimate of OR, 10,000 bootstrap replications were used. Interactions between smoking and DNMT3B -149 genotypes, green tea drinking and DNMT3B -149 genotypes on lung cancer risk were tested using likelihood ratio χ^2-test, respectively. In the test for interaction, the conditional logistic regression model with only the main effect terms was compared to that with both main effect terms and the interaction term. The interaction effect was defined as the difference in the deviance of two models. All tests were two-tailed and a P value less than 0.05 were considered statistically significant. Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

Basic characteristics of the study subjects are shown in Table 1. Compared with healthy controls, more smokers were represented in the lung cancer cases (53.7% vs.

Table 1. Basic Characteristic of Lung Cancer Patents and Healthy Controls

Variables	Cases N = 190	Controls N = 380	Unmatched OR (95% CI)[a]	Matched OR (95% CI)[b]
Gender				
Male	115 (60.5%)	230 (60.5%)	1.00 (0.70-1.43)	1.00 (0.70-1.43)
Female	75 (39.5%)	150 (39.5%)	1	1
Age (years, mean ± SD)	65.5 ± 11.9	64.4 ± 11.8		
Smoking status				
Current and ever smokers	102 (53.7%)	118 (31.1%)	2.57 (1.80-3.68)[c]	4.67 (2.75-7.93)[c]
Never smokers	88 (46.3%)	262 (68.9%)	1	1
Pack-years of smoking				
≥40	65 (34.2%)	60 (15.8%)	3.23 (2.11-4.94)[c]	5.76 (3.22-10.32)[c]
1-39	37 (19.5%)	58 (15.3%)	1.90 (1.18-3.06)[d]	3.41 (1.81-6.41)[c]
0	88 (46.3%)	262 (68.9%)	1	1
Green tea consumption (cups/day)				
0	146 (76.8%)	250 (65.8%)	2.96 (1.64-5.34)[c]	3.02 (1.64-5.55)[c]
< 1	29 (15.3%)	54 (14.2%)	2.72 (1.33-5.56)[d]	2.49 (1.21-5.12)[e]
≥ 1	15 (7.9%)	76 (20.0%)	1	1
Green tea consumption (years)				
0	146 (76.8%)	250 (65.8%)	1.75 (1.03-2.99)[e]	1.92 (1.09-3.36)[e]
≤10	23 (12.1%)	67 (17.6%)	1.03 (0.52-2.04)	1.02 (0.51-2.01)
>10	21 (11.1%)	63 (16.6%)	1	1
Vegetables and fruits intake (servings/week)				
≤14	48 (25.3%)	110 (28.9%)	0.94 (0.62-1.43)	0.89 (0.59-1.37)
15-20	51 (26.8%)	74 (19.5%)	1.48 (0.96, 2.29)	1.41 (0.91-2.18)
≥21	91 (47.9%)	196 (51.6%)	1	1
Exposure to cooking fumes (hours/week)				
≥3	17 (8.9%)	16 (4.2%)	2.41 (1.19-4.89)[e]	2.77 (1.29-5.96)[d]
1-3	19 (10.0%)	15 (4.0%)	2.87 (1.42-5.80)[d]	3.38 (1.57-7.29)[d]
<1	154 (81.1%)	349 (91.8%)	1	1
Family history of lung cancer				
Yes	15 (7.9%)	6 (1.6%)	5.34 (2.04-14.00)[d]	6.80 (2.24-20.65)[d]
No	175 (92.1%)	374 (98.4%)	1	1

[a], Data were calculated by unconditional logistic regression; [b], Data were matched by age and gender, calculated by conditional logistic regression; [c], P<0.001; [d], 0.001<P<0.01; [e], 0.01<P<0.05.

Table 2. Genotypic Frequency of *DNMT3B* -149 among Lung Cancer Patients and Healthy Controls

Variables	Cases N=190	Controls N=380	Unmatched OR (95% CI)[a]	Matched OR (95% CI)[b]	Adjusted OR (95% CI)[c]
DNMT3B -149 genotypes					
TT	183 (96.3%)	345 (90.8%)	2.65 (1.16-6.09)[d]	2.65 (1.15-6.10)[d]	2.58 (0.98-6.78)[f]
CT	7 (3.7%)	35 (9.2%)	1	1	1
CC	0 (0.0%)	0 (0.0%)			
T allele	373 (98.2%)	725 (95.4%)	2.57 (1.13-5.85)[d]	2.24 (1.03-4.88)[e]	1.9 (0.84-4.28)
C allele	7 (1.8%)	35 (4.6%)	1	1	1

[a], Data were calculated by unconditional logistic regression; [b], Data were matched by age and gender, calculated by conditional logistic regression; [c], Matched data were calculated by conditional logistic regression and adjusted for pack-years of smoking, green tea consumption, exposure to cooking fumes, and family history of lung cancer; [d], P, 0.02; [e], P, 0.04; [f], P, 0.06.

Table 3. The Joint Effects of Cigarette Smoking with DNMT3B -149 Genotypes for Lung Cancer Risk

Variables	Smoking status				Pack-years of smoking					
	Never smokers		Current and ever smokers		0		1-39		≥40	
	CA/CN	OR (95% CI)[a]	CA/CN	OR (95% CI)[a]	CA/CN	OR (95% CI)[a]	CA/CN	OR (95% CI)[a]	CA/CN	OR (95% CI)[a]
DNMT3B -149 genotypes										
TT	83/242	1.12 (0.39-3.22)	100/103	7.69 (2.55-23.14)[b]	83/243	1.12 (0.39-3.21)	37/52	5.62 (1.77-17.89)[b]	63/51	9.57 (3.09-29.66)[b]
CT	5/20	1	2/15	0.89 (0.13-6.16)	5/20	1	0/6	0.41 (0.18-0.93)	2/9	1.43 (0.19-10.80)
TT	83/242	1.09 (0.37-3.21)	100/103	11.03 (1.55-78.50)[b]	83/243	1.09 (0.37-3.21)	37/52	3.38 (0.58-19.75)	63/51	7.93 (0.92-68.43)
CT	5/20	1	2/15	1	5/20	1	0/6	1	2/9	1
Test for interaction	χ^2=4.75 (1 df); P=0.03				χ^2=6.26 (2 df); P=0.04					

[a], Data were matched by age and gender, calculated by (exact) conditional logistic regression and adjusted for green tea consumption, exposure to cooking fumes, and family history of lung cancer; [b], P<0.01.

31.1%, OR=4.67; 95% CI 2.75-7.93). Further, 34.2% of lung cancer cases had smoked more than 40 pack-years, but only 15.8% of healthy controls had smoked more than 40 pack-years. Lung cancer patients also had a higher proportion of green tea non-drinkers than the controls did (76.8% vs. 65.8%, OR=3.02; 95% CI 1.64-5.55). Besides, compared to healthy controls, lung cancer patients had higher frequencies of exposure to cooking fumes and family history of lung cancer. Unconditional and conditional logistic regression models obtained similar results.

Table 2 shows the genotypic frequency of DNMT3B -149 among lung cancer patients and healthy controls. Compared to healthy controls, cases had a higher prevalence of DNMT3B -149 TT genotype (96.3% vs. 90.8%). After adjusting for the effects of confounding factors, the subjects with DNMT3B -149 TT genotype had a 2.58-fold (95% CI 0.98-6.78, P=0.06) risk of lung cancer compared with those with the CT genotype. The bootstrap estimate of the OR was 2.57 (95% CI 2.52-2.62, P<0.001) in DNMT3B -149 TT, very close to the OR in the above model. Similarly, the bootstrap estimate of the OR in DNMT3B -149 T allele was 1.91 (95% CI 1.88-1.94, P<0.001), close to the OR from the original multivariable model (OR=1.90, 95% CI 0.84-4.28). In our healthy controls, DNMT3B T-149C polymorphism conformed to the Hardy-Weinberg equilibrium (P=0.36).

Subsequently, we evaluated the joint effects of cigarette smoking and DNMT3B -149 genotypes for lung cancer risk (Table 3). Never smokers carrying DNMT3B -149 CT genotype were selected as a reference group. Current and ever smokers carrying DNMT3B -149 TT genotype had an increased lung cancer risk (OR=7.69; 95% CI 2.55-23.14 [bootstrap OR=19.49; 95% CI 19.09-19.89]) compared to the reference group. When current and ever smokers carrying DNMT3B -149 CT genotype were selected as a reference group, current and ever smokers carrying the TT genotype had a more obvious risk of lung cancer (OR=11.03; 95% CI 1.55-78.50 [bootstrap OR=59.96; 95% CI 56.51-63.61]). Further, the interaction of smoking status and DNMT3B -149 genotypes on lung cancer risk was significant (P=0.03). In addition, compared to the reference group, the DNMT3B -149 TT carriers with 1-39 pack-years of smoking had a 5.62-fold (95% CI 1.77-17.89 [bootstrap OR=4.41; 95% CI 4.30-4.52]) risk of lung cancer, and those with ±40

Table 4. The Joint Effects of Green Tea Consumption with DNMT3B -149 Genotypes for Lung Cancer Risk

Variables	Drinking status				Drinking duration in years			
	Drinkers		No drinkers		>10		≤0	
	CA/CN	OR (95% CI)a	CA/CN	OR (95% CI)[a]	CA/CN	OR (95% CI)[a]	CA/CN	OR (95% CI)[a]
DNMT3B -149 genotypes								
TT	42/121	2.33 (0.46-11.80)	141/224	4.20 (0.85-20.87)	19/58	1.06 (0.17-6.50)	23/63	1.37 (0.23-8.32)
CT	2/9	1	May-26	1.41 (0.21-9.37)	2/5	1	0/4	0.52 (0.19-1.46)
TT	42/121	1.91 (0.38-9.51)	141/224	2.84 (0.95-8.54)	19/58	0.82 (0.14-4.78)	23/63	2.48 (0.02-320.47)
CT	2/9	1	5/26	1	2/5	1	0/4	1
Test for interaction	χ^2=0.06 (1 df); P=0.81				χ^2=2.77 (2 df); P=0.25			

Variables	Drinking duration in years	
	0	
	CA/CN	OR (95% CI)[a]
DNMT3B -149 genotypes		
TT	141/224	2.17 (0.38-12.59)
CT	5/26	0.73 (0.10-5.54)
TT	141/224	2.84 (0.95-8.54)
CT	5/26	1
Test for interaction		

[a], Data were matched by age and gender, calculated by (exact) conditional logistic regression and adjusted for pack-years smoked, exposure to cooking fume, and family history of lung cancer.

pack-years of smoking had a 9.57-fold (95% CI 3.09-29.66 [bootstrap OR=8.16; 95% CI 7.96-8.36]) risk of lung cancer. The interaction of cumulative smoking dose and DNMT3B -149 genotypes on lung cancer risk was also significant (P=0.04). However, the combined effect of green tea consumption and DNMT3B -149 genotypes for lung cancer risk was not significant (Table 4).

Discussion

The present study observed an independent effect of DNMT3B -149 genotypes on the occurrence of lung cancer, and a significant interaction of smoking with DNMT3B -149 genotypes on lung cancer risk. However, the interaction of green tea consumption and DNMT3B -149 genotypes for lung cancer risk was not significant.

The human DNMT3B is required for de novo methylation, and de novo hepermethylation in the promoter CpG islands has been regarded as a key mechanism in silencing TSG expression (Tirado-Magallanes et al., 2017). Importantly, aberrant methylation in the promoter region of the specific TSG is also involved in human pulmonary carcinogenesis, such as p16ink4a (Tam et al., 2013). Taken together, it is biologically reasonable to assert the DNMT3B polymorphism is associated with cancer development by increasing the promoter activity of DNMT3B modulating an aberrant de novo methylation of CpG islands in some TSG (Okano et al., 1999). The effect of DNMT3B -149 C to T on DNMT3B expression is still unclear. A study conducted in the non-Hispanic Caucasian population found DNMT3B -149 T allele was correlated with increased lung cancer risk (Shen et al., 2002). In the present study, a similar result showed individuals with DNMT3B -149 TT genotype compared to those with the CT genotype had a significantly higher lung cancer risk. However, a study conducted in China revealed no association between DNMT3B -149 polymorphism and lung cancer risk; even T allele carriers of DNMT3B -149 polymorphism experienced a reduced risk of stage II lung cancer (Gao et al., 2016). The researchers of the above Chinese study pointed out the inconsistent findings might be due to different ethnic populations, and the gene expressions at distinct tumor stages differed. In this case, a possible selection bias should not be ruled out.

An extended concept is variations in genetic background and/or environmental exposure may result in different results of lung cancer development from distinct ethnicities. Interestingly, the above-mentioned study performed in the non-Hispanic Caucasian population also highlighted the association between the DNMT3B variant and lung cancer risk was more pronounced in younger subjects, suggesting genetic susceptibility (Shen et al., 2002). Further, the present study revealed smokers carrying DNMT3B -149 TT genotype compared to nonsmokers carrying the CT genotype had a higher risk of lung cancer occurrence, and interaction of smoking and DNMT3B -149 genotypes on the lung cancer risk was significant. An important carcinogen from cigarettes named NNK has been suggested to increase DNA methylation and lead to the occurrence of lung cancer (Lin et al., 2010). Reasonable evidence also indicates methylated CpG sequences may increase the susceptibility to attack by some environmental carcinogens like benzo[a] pyrene diol epoxide (Yoon et al., 2001). Regardless, smoking (especially heavy smoking in cumulative dose) elevates lung cancer risk by increasing long-term carcinogen exposure, and simultaneously increased DNA methylation levels provide a further opportunity to induce cancer. The current study presented such epidemiologic evidence for a gene-smoking (DNMT3B and smoking status or pack-years of smoking) interaction, which has not been proven in the past. Simply speaking, smokers with the susceptible DNMT3B -149 genotype are more likely to develop lung cancer.

Our previous study showed smoking induced pulmonary carcinogenesis could be modulated by green tea consumption (Lin et al., 2012). Previous studies also suggested the EGCG of tea polyphenols had an inhibited DNMT ability to reduce tumor development in different tissue or cancer cells (Pandey et al., 2010; Khan et al., 2015). Polyphenol inhibition of DNA methylation might occur through directly inhibiting DNMT activity or indirectly decreasing the universal methyl donor (S-adenosyl-L-methionine) to lower DNMT activity mediated by catechol-O-methyltransferase (COMT). Unfortunately, the current study could not detect a significant interaction of green tea consumption and DNMT3B -149 genotypes on lung cancer risk. A possible explanation is the small sample size lowers the statistical power, especially in the analysis of subgroups. Besides, the bioavailability of polyphenols from green tea consumption might be a differential in the subjects carrying different COMT genotypes. Further studies are needed to clarify the role of COMT gene for green tea inhibiting DNA methylation and decreasing lung cancer risk.

The present study revealed individuals with longer time spent stir-frying experienced an elevated lung cancer risk. Among those complicated ingredients in cooking oil fumes, aromatic heterocyclic amines were the major carcinogens related to lung cancer (Seow et al., 2000). Our lung cancer cases were also more likely than controls to report having a family history of lung cancer, indicating family risk of lung cancer could be due to genetic or shared environmental factors. Many studies suggest consumption of fruits and vegetables is beneficial in preventing lung cancer (Vieira et al., 2016). However, the current study showed no association with fruits and vegetables intake and lung cancer risk. Estimation of fruits and vegetables consumption derived from a questionnaire is usually biased. The relationship between fruits and vegetables consumption and lung cancer risk still needs to be clarified.

Among our healthy controls, the -149 T allele frequency for DNMT3B (95.4%) was similar to that in the Taiwanese population (97.8%) (Chen et al., 2008). Our genotype distribution was also in the Hardy-Weinberg equilibrium. However, selection bias might exist in the current study. Our control subjects were healthy persons and might have healthier behavior. Even so, among the 190 patients available for analysis, the frequency of major cell types (adenocarcinoma, 56.8%; squamous cell carcinoma, 26.9%) was similar to that reported in a previous Taiwanese study (Chen et al., 2005). The male-to-female ratio of lung cancer in Taiwanese is approximately 2:1 (Chen et al., 2005). In the present study, the male proportion of lung cancer was 60.5%. The mean age of our lung cancer patients at recruitment was comparable to that found in a previous study of Taiwanese (65.5 vs. 63.4 years) (Chen et al., 2005). Smoking prevalence in our subjects (cases, 53.7%; controls, 31.1%) was also consistent with the findings of a previous study in Taiwanese (cases, 52.4%; controls, 29.9%) (Chang et al., 2009). Therefore, our participants could be regarded as representative of the general population of Taiwan. In the current study, recall bias may be another potential problem. Thus, misclassification of exposure may influence the effect of cigarette smoking and consumption of green tea on lung cancer risk. However, such misclassification should be non-differential. Besides, we could not be measured in vivo content of green tea, because it has short half-life. Again, it should be mentioned the small numbers of subjects might generate a false positive result, especially in the analysis of each subgroup. Even though the bootstrap analysis, which is used with increasing popularity in models, was used for internal model validation in the present study. However, once the original sample size is small, the resampling analysis is possible not to be done well because the set of bootstrap samples may not be rich enough. For example, when current and ever smokers carrying the DNMT3B -149 CT genotype were selected as a reference group in Table 3, the OR of lung cancer was 11.03 (95% CI 1.55-78.50) in current and ever smokers carrying DNMT3B -149 TT genotype, but bootstrap OR was 59.96 (95% CI 56.51-63.61). Such result might be due to the small sample size of the reference group. More studies on larger populations and of more varying ethnicities should be conducted to further examine our results. Our current observation still needs to be confirmed in future functional studies.

In conclusion, DNMT3B -149 TT genotype which had higher promoter activity might increase the lung cancer risk caused by smoking.

Authors' Contributions

CYL, CCH, and RHW designed the study. CYL, CCH, CHT, JYW, CLK, YYC, YWC, and RHW acquired data; CYL, CCH, and RHW analyzed and interpreted data.

Statement competing of Interests

None.

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