

Effect of SNPs in genes regulating cell cycle control, apoptosis and inflammation on lung cancer susceptibility of northern Thai population

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ABSTRACT: Northern Thai population has experienced a high incidence of lung cancer and greater rates of mortality over the past several decades. In this study, blood samples and personal information were collected from 91 non-small cell lung cancer patients and 84 cancer-free healthy unrelated volunteers living in northern Thailand. Eight functional SNPs, including cell cycle control (*TP53 Pro72Arg*, *MDM2 T309G*, *CCND1 G723A*, *CDKN1A Ser31Arg*), apoptosis (*FASLG C-844T*, *FAS G-1378A*) and inflammation (*TGFBI T-1347C*, *TGFBI Pro10Leu*) were genotyped by multiplex allele-specific polymerase chain reactions. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using logistic regression after making adjustments for age, gender and the smoking status of all participating subjects. No significant association between a single SNP and lung cancer risk was observed. However, a combination of *TGFBI*-1347T and 10Pro allele carriers indicated a significantly increased risk for lung cancer (OR = 2.90, 95% CI = 1.01–8.4, P = 0.049) relative to wild-type genotypes. Among the participants, who had kitchens inside their homes, -1347T allele carriers had significant increase in lung cancer risk (OR = 5.9, 95% CI = 1.32–26.5, P = 0.020) when compared to the *TGFBI*-1347 C/C genotype carriers. In conclusion, we have observed a significant association between *TGFBI*-1347T allele and either *TGFBI* 10Pro allele or having kitchen inside the house on risk elevation of non-small cell lung cancer among the population of northern Thailand.

KEYWORDS: lung cancer, northern Thailand, genetic susceptibility

INTRODUCTION

Lung cancer has been the most common form of diagnosed cancer worldwide and has been associated with the highest rates of global incidence and mortality according to the International Agency for Research on Cancer (IARC) in a 2018 report [1]. In Thailand, lung cancer ranked second in mortality rate among Thai males, preceded by liver cancer in 2015. Lung cancer has high incidence in the north (especially the three provinces: Chiang Mai, Lamphun and Lamphun) while liver cancer is extremely high in the north-east [2, 3]. Based on the most recent volume of the Cancer in Thailand, the annual

age-standardized incidence rates per 100 000 (for the years 2013–2015) of lung cancer for males and females, respectively, were 32.1 and 21.6 in Chiang Mai, 32.9 and 19.5 in Lamphun and 37.9, and 19.8 in Lamphun [3]. Most of these patients were found to have an advanced stage of lung cancer that was either diagnosed as regional or as distant metastases. Non-small cell lung cancer was found to be the most common type of lung cancer with adenocarcinoma representing more than half of the total number of incidences [3]. Notably, more than 80% of the lung cancer patients died within one year after being diagnosed [4]. This evidence clearly suggests that lung cancer is a major health issue for

the population of northern Thailand.

The etiology of lung cancer among the northern Thai population has been associated with exposure to carcinogens stemming from personal habits, occupations and the environment along with certain inherited genetic factors. In accordance with the high prevalence (34.2%) of daily smoking habits among those residing in the northern part of Thailand from 1991 to 2007 [5], tobacco smoking has been identified as the most significant risk factor for lung cancer in this region. Environmental factors, including residential radon [6], outdoor air pollution originating from vegetative burning, seasonal bushfires, engine exhaust emissions [7, 8], and indoor air combustion from the burning of biomass fuels [9], also contributed to the accelerated development of lung cancer among the northern Thai population. An increased risk of *TP53* somatic mutations in lung tissues was found among individuals who used pesticides, had kitchens inside their homes, or lived in concrete homes in comparison with those who were not exposed to any of these risk factors [10]. Recently, a significant association between a high level of indoor radon in the household and a short telomere length has been observed [11]. Interestingly, a combination of genetic polymorphisms within five genes was found to be involved with carcinogen metabolism and DNA repair, and has been significantly associated with an elevated risk of developing lung cancer among northern Thai women [12].

Effects of genetic variations, especially with regard to single nucleotide polymorphisms (SNPs) on the susceptibility to lung cancer, were depended on their location within the genes and the role of proteins encoded from those genes. Previous studies have reported that the SNPs found in genes regulating p53-mediated cell cycle control (i.e. *TP53* Pro72Arg, *MDM2* T309G, *CCND1* G723A, *CDKN1A* Ser31Arg) [13–15], apoptosis (i.e. *FASLG* C-844T, *FAS* G-1378A) [16] and inflammation (i.e. *TGFB1* T-1347C, *TGFB1* Pro10Leu) [17] could affect individual lung cancer susceptibility in Asians. Combinations of these SNPs were also found to be significantly related to an increased risk of developing lung cancer in some studies [15, 16]. In the absence of a smoking habit, several SNPs in these gene groups were significantly associated with an elevated risk of developing lung adenocarcinoma among female non-smokers, who were exposed to certain environmental factors, including cooking oil fumes, fuel smoke and environmental tobacco smoke [18, 19].

This study aimed to evaluate the association between candidate SNPs in genes regulating cell cycle control, apoptosis and inflammation with the risk of developing lung cancer among the population of northern Thailand. Subgroup analyses, involving combinations of two SNPs in the same gene group and individuals exposed to each of the potential environmental risk factors for lung cancer, were also performed.

MATERIALS AND METHODS

Ethics statement

During the course of our research, we protected the rights of the participants along with their identities. We confirmed that all experiments were performed in accordance with the relevant guidelines and regulations established for the experimental protocol on human subjects, as has been approved by the Research Ethics Committee 3, Faculty of Medicine, Chiang Mai University, Thailand (certificate of approval No. 128/2008). Written informed consent was obtained from all participants prior to conducting interviews and collecting blood samples.

Studied population

We performed a case-control study involving a sample population of 91 lung cancer patients and 84 control subjects. All individuals were residents of the northern region of Thailand and were of Thai ethnicity.

Study cases were recruited from patients who had been diagnosed with non-small cell lung cancer, i.e. adenocarcinoma, squamous cell carcinoma, large cell adenocarcinoma, and adenocarcinoma *in situ*, based on pathological and/or cytological reports. Neither chemotherapy nor irradiation had been administered to the participants within six months prior to the date of blood sample collection. The controls were healthy volunteers who had no prior history of any form of cancer. They were recruited as the spouses of lung cancer patients or non-blood-related volunteers who underwent routine annual health check-ups or as those who came along with the cancer patients at the time of their visit to the doctor.

All subjects were interviewed, using questionnaires, to obtain personal information including details of occupation, any history of lung disease, any history of invasive cancers among their first-degree relatives, their personal habits (i.e. tobacco smoking, alcohol consumption, fermented tea leaf or betel nut chewing), and any environmental ex-

posure to known lung carcinogens at their places of residence (i.e. home layout, location of kitchen, burning activities). 'Ever smokers', defined as persons having smoked daily for a year or longer, and 'Never smokers' defined as persons never having smoked regularly for a year or longer.

Peripheral blood samples of each participant were collected by venipuncture at the median cubital vein using a 6 ml vacutainer coated with anticoagulant K2EDTA. All blood samples were processed to obtain cell lysates within 48 h after being collected.

DNA extraction and SNP genotyping

Total genomic DNA from peripheral blood leucocytes was extracted according to the standard inorganic salting out protocol [20]. Genotypes of *TP53* Pro72Arg (rs1042522) and *CDKN1A* Ser31Arg (rs1801270) were investigated by polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP), as has been described by Hishida et al [21]. Genotypes of five SNPs, including *MDM2* T309G (rs2279744), *FAS* G-1378A (rs2234767), *FASLG* C-844T (rs763110), *TGFBI* T-1347C (rs1800469), and *TGFBI* Pro10Leu (rs1800470), were detected by tetra-primer amplification refractory mutation system (T-ARMS-PCR), as has been previously described [15, 22, 23]. Detailed information of the studied SNPs are described in Table 1.

A new set of T-ARMS primers was designed and used for the genotyping of *CCND1* G723A (rs9344) in this study, using the online web service primer design [24]. The T-ARMS primer sequences that were used are listed as follows: forward outer primer, 5' AGT TCA TTT CCA ATC CGC CCT CCATG 3'; reverse inner primer, 5' CTG CCT GGG ACA TCA CCC TCA CTT CCT 3'; forward inner primer, 5' TCC TCT CCA GAG TGA TCA AGT GTG ACACG 3'; reverse outer primer, 5' GTT CTA GGA GCA GTG GAA GAA GCC GGTG 3'. Mismatches between the second bases from 3' terminal of the inner primers and DNA templates were intentionally designed to further enhance specificity. The estimated sizes of three possible amplicons were 214 bp for *CCND1* 723G allele 169 bp for *CCND1* 723A allele and 317 bp for the control products of two outer primers.

The primers and PCR cycling conditions for each SNP genotyping were modified from the relevant manufacturers' protocols according to the specified primers and the size of the amplicons needed to achieve distinguishable allelic-specific products (Table S1). Standard PCR conditions were estab-

lished and initiated with an activation step (95 °C for 12 min), followed by amplification steps (30–40 cycles of 94 °C for 20 s, 60–65 °C for 40 s, 72 °C for 20–60 s), and completed with a final extension step (72 °C for 7 min) (Table S1).

The amplified products were subjected to electrophoresis in agarose gel. The percentage of agarose gel was depended on the size differences among the amplicons (Table S1). After electrophoresis, gels were stained with ethidium bromide and examined under Bio-Rad Gel Doc™ 2000 Gel Documentation Systems using Quality One® program (Hercules, CA, USA). Amplified products were compared with 100 bp DNA GeneRuler (Thermo Scientific, Ontario, Canada), and a genotype of each sample was identified by comparing with three positive controls that had been genotyped previously by direct sequencing.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., NY, USA) following the specific instructions of the statistical methods established for health care research. Continuous variables were transformed into categorical variables using appropriate cut-offs based on the acquired data. For descriptive data of the two subject groups, chi-square tests were used to test for the presence of any statistical differences at a significance level of 0.05 ($\alpha = 0.05$).

Any deviations from the Hardy-Weinberg equilibrium (HWE) of each SNP locus were assessed using the chi-square goodness-of-fit test statistics. Odds ratios (OR) and a 95% confidence interval (CI) were used to evaluate the association between the risk of acquiring lung cancer and the influenced impact of an individual SNP or the combined effects of two SNPs within the same gene group, or the combined effects of the SNPs and any potential environmental risk factor. Stratified analysis for each environmental risk factor was done to determine any lung cancer risk among subjects that were more likely exposed to environmental lung carcinogens (by having kitchens inside their homes, being exposed to burning activities and those living in wooden houses). Using multivariable logistic regression, ORs were adjusted for age, gender and smoking status in the overall analyses of individual SNP genotypes and combinations of two SNPs within the same gene group; while ORs were adjusted for the smoking habits of the participants during the course of the subgroup analyses involving any of the environmental risk factors.

Table 1 General information of the studied SNPs.

Abbrev. ^a	Gene name ^a	dbSNP ID	HGVS nomenclature ^b	Common name (Alternate name)	GMAF ^c
<i>Cell cycle control genes</i>					
<i>TP53</i>	Tumor protein p53	rs1042522	NM_000546.6:c.215C>G (p.Pro72Arg)	Pro72Arg (Arg72Pro)	0.45707 (G)
<i>MDM2</i>	MDM2 proto-oncogene	rs2279744	NM_002392.5:c.14+309T>G	T309G (SNP309)	0.36661 (G)
<i>CDKN1A</i>	Cyclin dependent kinase inhibitor 1A	rs1801270	NM_000389.5:c.93C>A (p.Ser31Arg)	Ser31Arg	0.25619 (A)
<i>CCND1</i>	Cyclin D1	rs9344	NM_053056.2:c.723G>A (p.Pro241=)	G723A (G870A)	0.41354 (A)
<i>Apoptosis genes</i>					
<i>FASLG</i>	Fas ligand	rs763110	NM_000639.2:c.-844C>T	C-844T (T-844C)	0.46965 (C)
<i>FAS</i>	Fas cell surface death receptor	rs2234767	NM_000043.5:c.-1378G>A	G-1378A (G-1377A)	0.18411 (A)
<i>Inflammation cytokine genes</i>					
<i>TGFB1</i>	Transforming growth factor beta 1	rs1800469	NM_000660.6:c.-1347T>C	T-1347C (C-509T)	0.36801 (T)
		rs1800470	NM_000660.7:c.29C>T (p.Pro10Leu)	Pro10Leu (Leu10Pro)	0.45467 (C)

^a According to the HUGO Gene Nomenclature Committee at the European Bioinformatics Institute.

^b According to the recommendations of the Human Genome Variation Society (HGVS).

^c Global minor allele frequency (GMAF) in the ClinVar database.

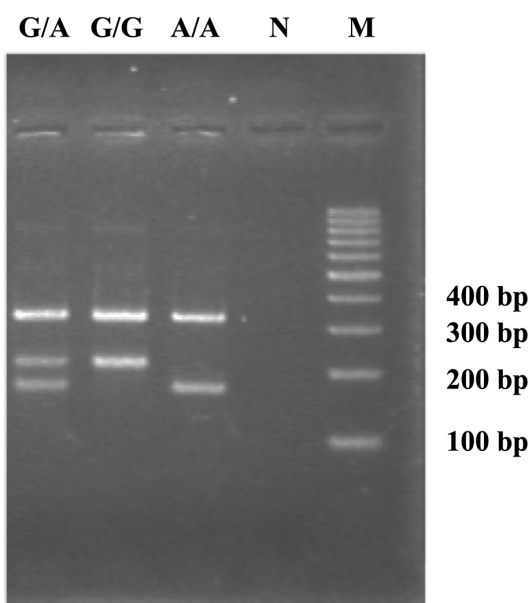


Fig. 1 *CCND1* G723A genotyping by T-ARMS-PCR technique. Three possible genotype products are as follows; G/A (214-bp and 169-bp), G/G (214-bp) and A/A (169-bp). The internal control products are 317-bp amplicons. N: negative control, M: 100-bp DNA ladder.

RESULTS

The demographic characteristics of the patients and the controls, together with their prevalence of exposure to the listed environmental lung cancer risk factors, are shown in Table 2. The mean age of the patients with ever smoking habits were higher than the healthy control group. Among the environmen-

tal factors, significant differences were observed between cancer patients and the control groups only in proportion to their smoking status ($P < 0.01$).

Individual SNP genotyping was successfully done with either PCR-CTPP or T-ARMS-PCR. The establishment of newly designed T-ARMS-PCR primers for the genotyping of *CCND1* G723A is shown in Fig. 1. Using the gel electrophoresis technique, one of three possible genotypes of *CCND1* G723A polymorphism, including G/A (214-bp and 169-bp), G/G (214-bp) and A/A (169-bp), was identified for each sample. SNP genotyping of the randomly selected samples was twice repeated meticulously, and all genotyping results were found to be consistent.

Using the chi-square goodness-of-fit test, we found that the genotype distributions of all 8 selected SNP loci agreed with those of the HWE ($P > 0.05$) in both patients and controls. We then used a dominant comparison model by combining heterozygous and homozygous variants and using the homozygous wild type as a reference group. Multivariate logistic regression analyses (adjusted for age, gender and smoking status) revealed that no significant effects of any variant alleles were observed on the ORs of lung cancer risk at each SNP (Table 3).

We next examined the combined effects of two SNPs within each group of genes regulating the pathway/process on lung cancer risk. According to the ORs and 95% CIs (Table 4), individuals with both -1347T and 10Pro alleles of *TGFB1* had significantly increased their risk of developing lung cancer (OR = 2.90, 95% CI = 1.01–8.4, $P = 0.049$) in comparison with those that did not carry these two alleles. No significant association was found

Table 2 Characteristics of the studied population.

Variable	Control group, n(%)	Patient group, n(%)	P value
Sample size	N = 84	N = 91	
Age			
Mean \pm SD	52.5 \pm 9.11	62.2 \pm 9.37	<0.001
(Minimum–Maximum)	26–77	40–84	
Gender			0.22
Female	41 (48.8)	36 (38.7)	
Male	43 (51.2)	55 (61.3)	
<i>Histological type of lung cancer</i>			
Adenocarcinoma (ADC)		58 (63.7)	
Squamous cell carcinoma (SQC)		25 (27.5)	
Large cell carcinoma (LAC)		5 (5.5)	
Adenocarcinoma in situ (AIS)		3 (3.3)	
<i>Smoking status</i>			
Never	49 (58.3)	16 (17.6)	<0.001
Ever	35 (41.7)	75 (82.4)	
<i>Kitchen location</i>			
Outside house	44 (52.4)	51 (56.0)	0.63
Inside house	40 (47.6)	40 (44.0)	
<i>Housing materials</i>			
Concrete house	33 (39.3)	34 (37.4)	0.79
Wooden house	51 (60.7)	57 (62.6)	
<i>Burning activity</i>			
Once a week or none	31 (36.9)	29 (31.9)	0.48
Twice a week or more	53 (63.1)	62 (68.1)	

Table 3 Effects of individual SNPs on the risk of being diagnosed with lung cancer. Odds ratio (OR) and 95% confidence intervals (CI) were estimated using multivariate logistic regression model and were adjusted for age, gender and smoking status.

SNPs	Controls, n(%)	Patients, n(%)	OR	(95% CI)	P value
<i>Cell cycle control genes</i>					
TP53 Pro72Arg	83	91			
Arg/Arg	19 (22.9)	23 (25.3)	1.00	(reference)	
Arg/Pro + Pro/Pro	64 (77.1)	68 (74.7)	0.86	(0.376–1.95)	0.71
MDM2 T309G	83	91			
T/T	16 (19.3)	20 (22.0)	1.00	(reference)	
T/G + G/G	67 (80.7)	71 (78.0)	0.75	(0.307–1.82)	0.52
CDKN1A Ser31Arg	83	91			
Ser/Ser	22 (26.5)	28 (30.8)	1.00	(reference)	
Ser/Arg + Arg/Arg	61 (73.5)	63 (69.2)	1.23	(0.56–2.72)	0.61
CCND1 G723A	80	90			
G/G	7 (8.8)	14 (15.6)	1.00	(reference)	
G/A + A/A	73 (91.2)	76 (84.4)	0.44	(0.138–1.43)	0.17
<i>Apoptosis genes</i>					
FASLG C-844T	81	90			
T/T	11 (13.6)	9 (10.0)	1.00	(reference)	
T/C + C/C	70 (86.4)	81 (90.0)	0.95	(0.320–2.79)	0.92
FAS G-1378A	79	90			
G/G	24 (30.4)	31 (34.4)	1.00	(reference)	
G/A + A/A	55 (69.6)	59 (65.6)	0.93	(0.43–2.04)	0.86
<i>Inflammatory cytokine genes</i>					
TGFB1 T-1347C	73	88			
C/C	18 (24.7)	9 (10.2)	1.00	(reference)	
C/T + T/T	55 (75.3)	79 (89.8)	2.57	(0.90–7.4)	0.079
TGFB1 Pro10Leu	80	90			
Leu/Leu	12 (15.0)	7 (7.8)	1.00	(reference)	
Leu/Pro + Pro/Pro	68 (85.0)	83 (92.2)	2.13	(0.67–6.8)	0.20

Table 4 Combined effects of two SNPs on the risk of being diagnosed with lung cancer. Odds ratio (OR) and 95% confidence intervals (CI) were estimated using multivariate logistic regression model and were adjusted for age, gender and smoking status.

SNPs	Controls, n(%)	Patients, n(%)	OR	(95% CI)	P value
<i>Cell cycle control genes</i>					
<i>TP53</i> Pro72Arg & <i>MDM2</i> T309G	83	91			
The others	32 (38.6)	37 (40.7)	1.00	(reference)	
Pro/- & G/-	51 (61.4)	54 (59.3)	0.83	(0.398–1.71)	0.61
<i>Ser31Arg</i>	83	91			
The others	36 (43.4)	41 (45.1)	1.00	(reference)	
G/- & Arg/-	47 (56.6)	50 (54.9)	1.18	(0.57–2.43)	0.66
<i>TP53</i> Pro72Arg & <i>CCND1</i> G723A	80	90			
The others	20 (25.0)	33 (36.7)	1.00	(reference)	
Pro/- & A/-	60 (75.0)	57 (63.3)	0.51	(0.228–1.13)	0.098
<i>MDM2</i> T309G & <i>CDKN1A</i> <i>Ser31Arg</i>	83	91			
The others	30 (36.1)	42 (46.2)	1.00	(reference)	
G/- & Arg/-	53 (63.9)	49 (53.8)	0.78	(0.375–1.61)	0.49
<i>MDM2</i> T309G & <i>CCND1</i> G723A	80	90			
The others	22 (27.5)	32 (35.6)	1.00	(reference)	
G/- & A/-	58 (72.5)	58 (64.4)	0.51	(0.230–1.14)	0.10
<i>CDKN1A</i> <i>Ser31Arg</i> & <i>CCND1</i> G723A	80	90			
The others	26 (32.5)	39 (43.3)	1.00	(reference)	
Arg/- & A/-	54 (67.5)	51 (56.7)	0.72	(0.341–1.51)	0.38
<i>Apoptosis genes</i>					
<i>FASLG</i> C-844T & <i>FAS</i> G-1378A	79	90			
The others	33 (41.8)	34 (37.8)	1.00	(reference)	
C/- & A/-	46 (58.2)	56 (62.2)	1.10	(0.53–2.32)	0.80
<i>Inflammatory cytokine genes</i>					
<i>TGFB1</i> T-1347C & <i>TGFB1</i> Pro10Leu	72	88			
The others	18 (25.0)	9 (10.2)	1.00	(reference)	
T/- + Pro/-	54 (75.0)	79 (89.8)	2.90	(1.01–8.4)	0.049*

* P < 0.05.

Table 5 Effects of individual SNPs on the risk of being diagnosed with lung cancer among individuals having kitchens inside their homes. Odds ratio (OR) and 95% confidence intervals (CI) were estimated using multivariate logistic regression model and were adjusted for age, gender and smoking status.

SNPs	Controls, n(%)	Patients, n(%)	OR	(95% CI)	P value
<i>Cell cycle control genes</i>					
<i>TP53</i> Pro72Arg	40	40			
Arg/Arg	10 (25.0)	12 (30.0)	1.00	(reference)	
Arg/Pro + Pro/Pro	30 (75.0)	28 (70.0)	0.90	(0.285–2.85)	0.86
<i>MDM2</i> T309G	40	40			
T/T	11 (27.5)	9 (22.5)	1.00	(reference)	
T/G + G/G	29 (72.5)	31 (77.5)	1.25	(0.380–4.1)	0.71
<i>CDKN1A</i> <i>Ser31Arg</i>	40	40			
Ser/Ser	9 (22.5)	10 (25.0)	1.00	(reference)	
Ser/Arg + Arg/Arg	31 (77.5)	30 (75.0)	1.03	(0.311–3.40)	0.96
<i>CCND1</i> G723A	39	39			
G/G	2 (5.1)	7 (17.9)	1.00	(reference)	
G/A + A/A	37 (94.9)	32 (82.1)	0.152	(0.023–1.03)	0.053
<i>Apoptosis genes</i>					
<i>FASLG</i> C-844T	38	39			
T/T	7 (18.4)	4 (10.3)	1.00	(reference)	
T/C + C/C	31 (81.6)	35 (89.7)	1.08	(0.250–4.7)	0.91
<i>FAS</i> G-1378A	38	39			
G/G	13 (34.2)	11 (28.2)	1.00	(reference)	
G/A + A/A	25 (65.8)	28 (71.8)	1.52	(0.49–4.7)	0.47
<i>Inflammatory cytokine genes</i>					
<i>TGFB1</i> T-1347C	32	38			
C/C	11 (34.4)	4 (10.5)	1.00	(reference)	
C/T + T/T	21 (65.6)	34 (89.5)	5.9	(1.32–26.5)	0.02*
<i>TGFB1</i> Pro10Leu	37	39			
Leu/Leu	7 (18.9)	4 (10.3)	1.00	(reference)	
Leu/Pro + Pro/Pro	30 (81.1)	35 (89.7)	2.43	(0.54–10.9)	0.25

* P < 0.05.

Table 6 Effects of individual SNPs on the risk of being diagnosed with lung cancer among individuals living in wooden houses. Odds ratio (OR) and 95% confidence intervals (CI) were estimated using multivariate logistic regression model and were adjusted for age, gender and smoking status.

SNPs	Controls, n(%)	Patients, n(%)	OR	(95% CI)	P value
<i>Cell cycle control genes</i>					
<i>TP53</i> Pro72Arg	50	57			
Arg/Arg	9 (18.0)	12 (21.1)	1.00	(reference)	
Arg/Pro + Pro/Pro	41 (82.0)	45 (78.9)	1.18	(0.338–4.1)	0.80
<i>MDM2</i> T309G	50	57			
T/T	9 (18.0)	13 (22.8)	1.00	(reference)	
T/G + G/G	41 (82.0)	44 (77.2)	0.64	(0.189–2.15)	0.47
<i>CDKN1A</i> Ser31Arg	50	57			
Ser/Ser	10 (20.0)	21 (36.8)	1.00	(reference)	
Ser/Arg + Arg/Arg	40 (80.0)	36 (63.2)	0.75	(0.247–2.26)	0.61
<i>CCND1</i> G723A	48	57			
G/G	5 (10.4)	3 (5.3)	1.00	(reference)	
G/A + A/A	43 (89.6)	54 (94.7)	3.13	(0.41–23.7)	0.27
<i>Apoptosis genes</i>					
<i>FASLG</i> C-844T	49	47			
T/T	10 (20.4)	7 (12.3)	1.00	(reference)	
T/C + C/C	39 (79.6)	40 (87.7)	1.37	(0.353–5.3)	0.65
<i>FAS</i> G-1378A	48	57			
G/G	15 (31.2)	20 (35.1)	1.00	(reference)	
G/A + A/A	33 (68.8)	37 (64.9)	1.24	(0.43–3.58)	0.69
<i>Inflammatory cytokine genes</i>					
<i>TGFB1</i> T-1347C	43	56			
C/C	12 (27.9)	5 (8.9)	1.00	(reference)	
C/T + T/T	31 (72.1)	51 (91.1)	4.2	(0.95–18.5)	0.058
<i>TGFB1</i> Pro10Leu	49	57			
Leu/Leu	7 (14.3)	4 (7.0)	1.00	(reference)	
Leu/Pro + Pro/Pro	42 (85.7)	53 (93.0)	4.1	(0.77–21.6)	0.099

Table 7 Effects of individual SNPs on the risk of being diagnosed with lung cancer among individuals exposed to burning activities. Odds ratio (OR) and 95% confidence intervals (CI) were estimated using multivariate logistic regression model and were adjusted for age, gender and smoking status.

SNPs	Controls, n(%)	Patients, n(%)	OR	(95% CI)	P value
<i>Cell cycle control genes</i>					
<i>TP53</i> Pro72Arg	53	62			
Arg/Arg	9 (17.0)	12 (19.4)	1.00	(reference)	
Arg/Pro + Pro/Pro	44 (83.0)	50 (80.6)	0.58	(0.180–1.87)	0.36
<i>MDM2</i> T309G	53	62			
T/T	11 (20.8)	16 (25.8)	1.00	(reference)	
T/G + G/G	42 (79.2)	46 (74.2)	0.58	(0.195–1.71)	0.32
<i>CDKN1A</i> Ser31Arg	53	62			
Ser/Ser	16 (30.2)	16 (25.8)	1.00	(reference)	
Ser/Arg + Arg/Arg	37 (69.8)	46 (74.2)	1.94	(0.70–5.4)	0.21
<i>CCND1</i> G723A	51	61			
G/G	5 (9.8)	9 (14.8)	1.00	(reference)	
G/A + A/A	46 (90.2)	52 (85.2)	0.44	(0.100–1.96)	0.28
<i>Apoptosis genes</i>					
<i>FASLG</i> C-844T	51	61			
T/T	7 (13.7)	5 (8.2)	1.00	(reference)	
T/C + C/C	44 (86.3)	56 (91.8)	1.11	(0.266–4.6)	0.89
<i>FAS</i> G-1378A	51	61			
G/G	16 (31.4)	20 (32.8)	1.00	(reference)	
G/A + A/A	35 (68.6)	41 (67.2)	1.07	(0.390–2.95)	0.89
<i>Inflammatory cytokine genes</i>					
<i>TGFB1</i> T-1347C	46	59			
C/C	11 (23.9)	9 (15.3)	1.00	(reference)	
C/T + T/T	35 (76.1)	50 (84.7)	1.35	(0.397–4.6)	0.63
<i>TGFB1</i> Pro10Leu	51	61			
Leu/Leu	8 (15.7)	7 (11.5)	1.00	(reference)	
Leu/Pro + Pro/Pro	43 (84.3)	54 (88.5)	1.44	(0.384–5.4)	0.59

between any other combination of two SNPs within the same gene groups and the risk of acquiring lung cancer.

Lung cancer susceptibility risks were evaluated among individuals who were exposed to each of the three environmental risk factors, including those having kitchens inside their houses (Table 5), those living in wooden houses (Table 6), and those exposed to burning activities in and around their homes (Table 7). Interestingly, a significant association was observed between a combined effect of SNPs and the location of the kitchen for lung cancer risk. Among participants who had kitchens inside their houses, *TGFB1*-1347T allele carriers were associated with a significant increase risk of lung cancer (OR = 5.9, 95% CI = 1.32–26.5, $P = 0.020$, adjusted for age, gender and smoking status) in comparison with the *TGFB1*-1347 C/C genotype carriers. Albeit somewhat non-significant, two SNPs in the *TGFB1* gene revealed potential associations with an elevated risk for developing lung cancer among those participants residing in wooden houses ($P < 0.10$) (Table 6). However, there was no significant association observed between SNP and lung cancer risk among those exposed to burning activities around their homes (Table 7).

DISCUSSION

In previous studies, the variant alleles of all eight studied SNPs in this study had been reported to affect changes in either protein functions or gene expression and were found to be significantly associated with lung cancer risk. However, effects of these SNP variations and the potential environmental risk factors for susceptibility to lung cancer among the people of northern Thailand has not yet been evaluated. Although, there were no significant associations between each of the studied SNPs and a risk for lung cancer in the multivariate analyses, a potential association between the *TGFB1* T-1347C polymorphism and a risk of acquiring lung cancer was observed. It was likely that individuals carrying -1347T alleles would have a 2-fold increased risk of developing lung cancer (Table 3). In a stratified analysis of smoking history, Park et al [16] found that having at least one -1347T allele was associated with a 3.8-fold increased risk of acquiring lung cancer among heavy smokers (≥ 30 pack-years). Moreover, a 1.2-fold increased risk of acquiring lung cancer was found among Asian individuals who carried at least one *TGFB1*-1347T allele in the meta-analysis [25]. However, a significant inverse association was observed between the *TGFB1* T-1347C

polymorphism and a lung cancer risk in several previous studies conducted among Asian populations. Kang et al [25] found that the *TGFB1* -1347T allele carriers displayed 27% and 37% reduced risks of acquiring lung cancer and lung adenocarcinoma, respectively. These results were obtained from multivariate analyses after being adjusted for any potential confounding factors, including age, gender, smoking status, and, especially, pack-years of smoking [26].

Although we observed a significant association between a combination of the two SNPs in the *TGFB1* gene, namely -1347T and 10Pro alleles, these two SNPs were found to be in strong linkage disequilibrium [17, 18]. Both *TGFB1* T-1347C and Pro10Leu are known to be functional polymorphisms that affect the levels of *TGFB1* transcription and TGF- β 1 secretion [27]. However, there has been no experimental evidence showing the effect of combining *TGFB1* T-1347C and Pro10Leu polymorphisms on the levels of TGF- β 1 [28]. Based on the functional studies of each SNP, it has been hypothesized that synergy between *TGFB1* upregulation (caused by -1347T allele) together with high levels of TGF- β 1 secretions (caused by 10Pro allele) might lead to a substantial increase in the plasma levels of TGF- β 1. Together with an inactivation of key mediators in TGF- β 1 signaling (e.g. SMAD4), the role of TGF- β 1 was switched from acting as a tumor-suppressive, by inducing cell cycle arrest and apoptosis, to serving as a tumor-promoting factor via immunosuppression by highly secreted TGF- β 1 proteins in the tumor microenvironment [29, 30]. However, our observations of significant increases in the risk of developing lung cancer among individuals who carry both -1347T and 10Pro alleles of *TGFB1* were inconsistent with those of a previous study conducted upon the Korean population [26]. Kang et al [26] found that the *TGFB1* haplotype, with -1347T and 10Pro, was significantly associated with a 25% reduction in the risk of being diagnosed with lung adenocarcinoma. However, neither overall lung cancer nor squamous cell carcinoma subtypes were related to the *TGFB1* haplotype with -1347C and 10Leu after adjustments were made for age, gender, smoking status, and pack-years of smoking.

We have observed a significant association between the *TGFB1*-1347T allele and a risk of developing lung cancer among individuals who had kitchens inside their houses. *TGFB1*-1347T allele carriers were associated with about a 5-fold increased risk of being diagnosed with lung cancer in comparison

with the *TGFB1*-1347C allele carriers. Having a kitchen inside the house was found to have significantly contributed to a high prevalence (45.6%) of *TP53* somatic mutations that were observed in lung cancer patients in comparison with lung cancer patients who had kitchens outside their houses (16.7%) [10]. The severity of this factor with regard to an increased risk of lung cancer may depend upon certain characteristics or features of the subject's home, such as the number of windows, the presence of a chimney, proper air ventilation, and any cooking activities that occur inside the kitchen that involve fuel used for cooking [31]. Moreover, the use of biomass fuels for cooking was recently found to be associated with long-term exposure to cancer-causing substances, including polycyclic aromatic hydrocarbons, such as benz[a]anthracene, benzo[k]fluoranthrene, benzo[a]pyrene, and their nitro derivatives, that were found to have accumulated in the homes of lung cancer patients residing in Chiang Mai Province [9]. Undeniably, inhalation of these substances contributes to the development of lung cancer. On the other hand, the longer one spends inside their home, the greater the risk they appear to have of developing lung cancer. *Trans, trans*-2,4-decadienal (*tt*-DDE or 2,4-De), a specific type of dialdehyde in cooking oil fumes was found to be associated with increased cell proliferation and enhanced production of tumour necrosis factor- α (TNF- α) in human bronchial epithelial cells [32]. This TNF- α in cooperation with TGF- β 1, which is up-regulated by the -1347T allele, could promote cancer progression through epithelial-mesenchymal transition (EMT) [29, 33]. Thus, individuals who had kitchens inside their houses while carrying *TGFB1*-1347T alleles could have an increased risk of developing lung cancer.

At first, we expected that the participants who were exposed to burning activities around their homes would have a greater likelihood of long-term exposure to airborne particulate matters that would subsequently lead to lung cancer. Vegetative burning was the main source of particulate matters, with an aerodynamic diameter of smaller than 10 μm (46–82% of the total PM_{10} concentration), during the period of June 2005 to June 2006 in Chiang Mai and Lamphun Provinces [8]. Burning in open fields is a convenient way to get rid of agricultural residues after harvesting crops. Smoke from bush fires in this region and from surrounding countries during the dry season are brought here by southwesterly winds that also contribute to the accumulation of particulate matters in the air of Chiang Rai Province,

the northern most province of Thailand [34, 35]. For every 10- $\mu\text{g}/\text{m}^3$ change in the exposure of PM_{10} , the risk of developing lung cancer and lung adenocarcinoma would increase by 9% and 29%, respectively [36]. However, we did not observe any significant association between each of the studied SNPs and the risk of being diagnosed with lung cancer among participants who were exposed to burning activities around their homes. However, having or not having exposure to burning activities around the home may depend upon a number of other factors, such as the location of the house (urban or rural area), the weather, the crop seasons, and the amount of agricultural waste or household waste that is being produced by members of that household. More detailed information about the exposure to burning activities may be useful in defining this parameter and in the application of the stratified analyses by a subgroup of the burning activities.

Although we have observed some association between the candidate SNPs in genes regulating pathways/processes and a risk of developing lung cancer in the population of northern Thailand, we were faced with some limitations during the course of conducting this research. The limitations were solely due to the small sample size. The limited number of samples in both patient and control groups affected the statistical power in the analyses of multivariate logistic regression, especially with regard to the stratified subject groups who were exposed to the designated environmental risk factors. Although the sample size of this study is acceptable for multivariate logistic regression analyses (at least ten samples per one independent variable) [37], it was still less than the suggested level (at least two controls per one case) [38], resulting in low statistical power. The expansion of the population size of the study by recruiting more lung cancer patients and even more unrelated control subjects from the same district may be beneficial for an incoming case-control study. With an adequate sample size, consideration of other risk factors for lung cancer, such as education level, occupation, exposure to second-hand smoke, previous experience with lung diseases, the use of household solid fuels, and a family history of lung cancer, as well as menstrual and reproductive factors in women, can be evaluated for any association with lung cancer development in further studies.

CONCLUSION

In conclusion, we have observed significant associations between *TGFB1*-1347T allele and either *TGFB1* 10Pro allele or having kitchen inside the house with an elevated risk of non-small cell lung cancer among the population of northern Thailand. Our results suggest that there are mutual contributions of individual genetic susceptibility and exposure to environmental carcinogens for the development of lung cancer in this region.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at <http://dx.doi.org/10.2306/scienceasia1513-1874.2020.079>.

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Appendix A. Supplementary data

Table S1 Primer sequences and PCR conditions for SNP genotyping using PCR-CTPP or T-ARMS-PCR technique.

SNP	Primer sequence (from 5' to 3')	Final conc. (µM)	Allele-specific amplicon size (bp)	Common amplicon size (bp)	No. of cycles	Annealing temp. (°C)	Extension time (s)	Agarose (%)	Ref.
TP53 Pro72Arg (rs1042522)	FO: CCCCTTGGCGTCCCAAGC	0.4	135	278	30	62	60	3	[21]
	RI: CTGTGGAGGGGCCACGG	0.4	(Pro allele)						
	FI: GCCAGAGGTGCTCCCG	0.4	178						
	RO: CGTGCAGAGTCACAGACTTGGGCTG	0.4	(Arg allele)						
MDM2 T309G (rs2279744)	FO: GGCAGTCGCCCGCAGGGAGGGGG	0.4	158	224	30	64	60	3	[15]
	RI: ACCTGGATCATCGGACCTCCGGGCTGC	0.4	(G allele)						
	FI: GGGGGCGGGGCTGGGGCGGTTT	0.4	122						
	RO: TGCCCACTGAACGGGGCCCAATCCCGCCAG	0.4	(T allele)						
CDKN1A Ser31Arg (rs1801270)	FO: TCACCTGAGGTGACACAGCAAAGCC	0.4	334	498	30	62	60	2	[21]
	RI: CATTAGCGATCACAGTCGGG	0.4	(Ser allele)						
	FI: CAGTGGACAGCGAGCACTGAGA	0.4	211						
	RO: CCAGTCCACCTGGGGACCC	0.4	(Arg allele)						
CCND1 G723A (rs9344)	FO: AGTTCATTTCCAAATCCGCCCTCCATG	0.2	169	327	30	62	60	2	This study
	RI: CTGCTGGGACATCACCTCAGTTCCT	0.2	(A allele)						
	FI: TCCTCCAGATGATCAAGTGTGACACG	0.2	214						
	RO: GTTCTAGGAGCAGTGGAAAGAACCGGTG	0.2	(G allele)						
FAS G-1378A (rs2234767)	FO: CCTTCCCTCACAGCCCTTTTCCTTCC	0.2	216	507	30	64	60	2	[22]
	RI: TTAGTGCCATGAGGAAGACCCTGTGC	0.2	(G allele)						
	FI: AGTGTGTGCACAGGCTGGCCCA	0.2	340						
	RO: CTTTGGCATCTCCACCAAGCTCTG	0.2	(A allele)						
FASLG C-844T (rs763110)	FO: TGCAGTTAAGTACGATAGCACCACTGCA	0.1	145	284	35	63	20	3	[22]
	RI: AACCCACAGAGTGCCTTTGTAATGCA	0.4	(T allele)						
	FI: GGCAACAATGAAATGAAACATGGC	0.4	192						
	RO: AGGAGGAAAACATCTGTTGCCATCATCT	0.1	(C allele)						
TGFB1 T-1347C (rs1800469)	FO: AGTAAATGATGGGTGGCAGGGTGTG	0.1	141	295	30	60	60	2	[23]
	RI: GAGGAGGGGCAACAGGACACCTTAG	0.2	(C allele)						
	FI: GGTGTGCTGCTGAGCCCTTCCATACT	0.2	207						
	RO: AAAGAGGACCGGGGAGAGGGCTTAAT	0.1	(T allele)						
TGFB1 Pro10Leu (rs1800470)	FO: CAGCTTCCCTCGAGGCCCTCCTACCTT	0.2	180	255	30	65	40	2	[23]
	RI: CTCGGGCTGGGCTGCTTCT	0.2	(Pro allele)						
	FI: AGTAGCCACAGCGGGTAGCAGCATCG	0.2	123						
	RO: TTCGGCTTACAGGTCATGTCGATAG	0.2	(Leu allele)						

FO = forward outer primer; RI = reverse inner primer; FI = forward inner primer; RO = reverse outer primer.