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Cancer Genetics and Cytogenetics 185 (2008) 20–27

Cancer Genetics and Cytogenetics

TP53 gene mutations of lung cancer patients in upper northern Thailand and environmental risk factors

Kanokkan Bumroongkit[a], Bruce Rannala[b], Patrinee Traisathit[c], Metawee Srikummool[a], Yannawan Wongchai[a], Daoroong Kangwanpong[a,*]

[a]*Department of Biology, Faculty of Science, Chiang Mai University, 239 Huay Kaew Road, Chiang Mai 50202, Thailand*
[b]*Genome Center and Section of Evolution and Ecology, University of California Davis, Davis, CA 95616*
[c]*Department of Statistics, Faculty of Science, Chiang Mai University, Chiang Mai 50202, Thailand*

Received 24 January 2008; accepted 9 April 2008

Abstract *TP53* mutations are observed in about 40–70% of lung cancer tissues, and the hot spot codon mutations are in exons 5 through 8. Previous studies revealed that the distinct *TP53* mutational pattern between population groups may be due to different racial or exogenous factors. This research aims to identify risk factors that influence *TP53* gene mutation in lung cancer patients residing areas with high lung cancer incidence, in the upper northern part of Thailand. Germline *TP53* mutational analyses were also performed to determine the inherited cancer predisposition. Exons 5–8 of the *TP53* gene were analyzed by sequencing DNA of cancerous tissue and peripheral blood leukocyte samples from 55 non–small lung cell cancer patients. The results showed that the *TP53* germline mutation was not found in all patients, indicating that the *TP53* germline mutations were not exclusively responsible for cancer predisposition in this group of lung cancer patients. A total of 19 somatic mutations were found in 18 patients. Mutations were predominantly found in exons, with only 10.53% observed at the splice sites of intron 7. No characteristic hot spot codons were observed. The data suggest that *TP53* mutations in this study group are induced by exposure to substances other than tobacco smoke. Pesticide exposure or habitation in poorly ventilated houses may instead be related to the tumorigenesis of lung cancer via *TP53* mutations.

1. Introduction

In the upper northern part of Thailand, incidence of lung cancer is much higher than that of other cancers, and it is also significantly higher than in other regions of the country. The dismal outcome of lung cancer has not changed considerably despite substantially therapeutic progression because tumors tend to be detected at a late stage. Better understanding of the molecular mechanisms of lung carcinogenesis could contribute to powerful management of the tumors. The most common histopathologic type of lung cancer in this region is non–small cell lung cancer (NSCLC; 87.6%), whereas small cell lung cancer is found only in 12.4% [1]. Many previous studies have attempted to identify the causes of lung cancer in this region, especially the Chiang Mai province. There is evidence that tobacco smoking, Miang chewing (a typical northern Thai chewing substance made from fermented tea leaves), low fruit and vegetable intake, history of chronic benign respiratory disease, and high levels of particular matter (PM10 and PM2.5), including exposure to indoor radon, were associated with a high incidence of lung cancer in this region [2–7].

Many genetic abnormalities were found in lung cancer cells, including mutations in proto-oncogenes, tumor suppressor genes, and genes regulating apoptosis and DNA repair [8]. One of the most common molecular alterations is a mutation of the *TP53* tumor suppressor gene. *TP53* gene mutations have been detected in preneoplastic lesions of the lung, indicating that they occur at an early stage during the development of cancer [9]. Hence, loss of *TP53* functions is likely to participate in the progression of preneoplastic cells to neoplastic cells. *TP53* mutation occurs in about 40–70% of lung cancers [8]. Most hot spot codon mutations were found within exons 5–8 [10].

There have been several studies suggesting that the first-degree relatives of lung cancer patients have a greater risk

* Corresponding author. Tel.: +66-53-943346, ext. 1305; fax +66-53-892259.
E-mail address: scidkngw@chiangmai.ac.th (D. Kangwanpong).

0165-4608/08/$ – see front matter
doi:10.1016/j.cancergencyto.2008.04.009

of developing lung cancer than the general population [11–13]. Others have revealed a distinct *TP53* mutation pattern between population groups [14–16], suggesting the involvement of a potentially distinct mutagenic process in each population. Together, these findings suggest that an inherited genetic factor may be involved in the genesis of lung cancer. Because somatic mutations of the *TP53* gene frequently occur in lung cancer, it is also possible that germline mutation of this gene affects lung cancer susceptibility. Recent reports suggest that carriers of *TP53* gene germline mutations may be at increased risk for carcinoma of the lung [17–19]. The present study aims to identify potential genetic risk factors by determining the *TP53* germline and somatic mutations of lung cancer patients residing in the upper northern part of Thailand. The mutation spectra of *TP53* might provide important clues for cancer risk assessment in molecular epidemiology. Understanding risk factors and genetic predisposition for lung cancer is important to lung cancer prevention.

2. Material and methods

2.1. Patients and samples

The study population comprised of 55 NSCLC patients residing in the upper northern provinces of Thailand (i.e., Chiang Mai, Chiang Rai, Phayao, Lamphun, Lampang, Phrae, and Nan). All patients underwent pulmonary resection with curative intent. There was no chemotherapy or irradiation before surgery. Primary tumor regions were separately excised by experienced pathologists from the Pathology Department, Faculty of Medicine, Chiang Mai University (Chiang Mai, Thailand). Either formalin-fixed paraffin-embedded or fresh-frozen samples from resected tumor were used. Five milliliters of peripheral blood was collected from each patient.

This research project has been approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University. Subjects enrolled in the study were asked to sign informed consent forms before having blood samples taken and being interviewed. A detailed questionnaire was administered by interviewers to all studied participants about personal life, occupation, lifestyle, health and disease, cigarette smoking, alcohol consumption, fermented tea leaves or betel nut chewing, family history of cancer, and environmental exposures.

2.2. DNA preparation, polymerase chain reaction, and sequencing

Genomic DNA from peripheral blood leukocytes and cancerous lung tissues of each patient was extracted by inorganic salting out protocol [20] or phenol-chloroform protocol [21].

DNA sequencing of the *TP53* gene at exons 5–8, including some intronic sequence flanking each exon were amplified by polymerase chain reaction (PCR) according to a standard protocol [22,23] with some modification. Different numbers of intronic primer pairs were used depending upon the quality of DNA, whether they were extracted from formalin-fixed paraffin-embedded tissue, fresh-frozen tissue, or peripheral blood leukocytes (Table 1).

PCR was carried out in 20 μL of reaction mixture that contained 2 μL DNA (50–100 ng), 4 μmol/L of each primer, 1× PCR buffer, 250 μmol/L dNTP, 2.25 mmol/L $MgCl_2$, and 0.8 units AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). The PCR conditions for DNA from blood leukocytes and fresh-frozen tissues consisted of 12 minutes at 95°C for enzyme preincubation and initial denaturation step, followed by 40 cycles of 30 seconds at 94°C, 1 minute at 63°C, 30 seconds frozen at 78°C, and 7 minutes at 72°C for a final extension step.

The PCR reaction for DNA from paraffin-embedded tissues consisted of 12 minutes at 95°C for enzyme preincubation and initial denaturation step, followed by 10 cycles of the TouchDown protocol: 20 seconds at 94°C, 20 seconds at 65°C (decreased by 1°C each cycle), and 20 seconds at 72°C, then 30 cycles of 20 seconds at 94°C, 20 seconds at 55°C, 20 seconds at 72°C, and finally 6 minutes at 72°C for a final extension step.

PCR products were purified by the PCR-M Clean Up System (Viogene, Taipei, Taiwan). The sequencing prereactions were performed using the BigDye Terminator V3.1 Cycle Sequenceing Kit (Applied Biosystems, Foster City, CA) with sequencing primer pairs (Table 1). Prereaction products were sent to Bioservice Unit, BIOTEC (Bangkok) for ABI 3100 automated DNA sequencing or to Macrogen Inc. (Seoul, Korea) for 3730xl DNA sequencing.

DNA sequences from each patient, from both peripheral blood leucocytes and cancerous tissues, were compared with the reference DNA sequence of *TP53* gene from GenBank (accession no. X54156) using the SeqScape program (Applied Biosystems).

2.3. Statistical analysis

Univariate logistic regression analysis was used to examine the association between *TP53* mutations and potential risk factors. Continuous variables were transformed into categorical variables using either cut-off values with limits corresponding to the rounded values of each quartile [24], or most commonly used cut-offs. To control for possible confounding factors, a multivariated logistic regression model was developed using all variables with $P < 0.25$ in the univariated analyses [25]. Odds ratio (OR) and their 95% confidence intervals (95% CI) were estimated. Pairwise interactions between variables independently associated with the outcomes, which had a plausible clinical significance, were evaluated for possible inclusion in the multivariate logistic regression model. Statistical analyses were performed using SPSS statistical software (version 11.5; SPSS Inc., Chicago, IL).

Table 1
PCR and sequencing primers for exons 5–8 of the *TP53* gene

Exons	Product size (bp)	Primers	Primer sequence (5' to 3') [reference no.]
DNA from peripheral blood leucocytes or fresh-frozen tissue			
5–6	546	PCR primers	F: GCC GTG TTC CAG TTG CTT TA [23]
			R: TAA GCA GCA GGA GAA AGC CC [23]
		sequencing primers	F: TGT TCA CTT GTG CCC TGA CT [22]
			R: TTA ACC CCT CCT CCC AGA GA [22]
7–8	701	PCR primers	F: CTT GCC ACA GGT CTC CCC AA [22]
			R: AGG CAT AAC TGC ACC CTT GGT [22]
		sequencing primers	F: AGG CGC ACT GGC CTC ATC TT [22]
			R: TCC ACC GCT TCT TGT CCT GC
DNA from paraffin-embedded tissue			
5	351	PCR primers	F: GCC GTG TTC CAG TTG CTT TA [23]
			R: AGG AGG GGC CAG ACC TAA GA [23]
		Sequencing primers	F: TGT TCA CTT GTG CCC TGA CT [22]
			R: CAG CCC TGT CGT CTC TCC AG [22]
6	311	PCR primers	F: AGC GCT GCT CAG ATA GCG AT [23]
			R: TAA GCA GCA GGA GAA AGC CC [23]
		Sequencing primers	F: GCC TCT GAT TCC TCA CTG AT [22]
			R: TTA ACC CCT CCT CCC AGA GA [22]
7	237	PCR primers	F: CTT GCC ACA GGT CTC CCC AA [22]
			R: AGG GGT CAG CGG CAA GCA GA [22]
		Sequencing primers	F: AGG CGC ACT GGC CTC ATC TT [22]
			R: TGT GCA GGG TGG CAA GTG GC [22]
8	255	PCR primers	F: TAA ATG GGA CAG GTA GGA CC
			R: AGG CAT AAC TGC ACC CTT GGT
		Sequencing primers	F: GAC CTG ATT TCC TTA CTG CCT [23]
			R: TCC ACC GCT TCT TGT CCT GC

Abbreviations: F, forward primer; R, backward primer.

3. Results

3.1. Characteristics of the patients

The characteristics and variable factors of 55 NSCLC patients from the questionnaire are summarized in Table 2. Most patients were male and resided in Chiang Mai province. Among patients who were smokers, roughly half (53.4%) favored Khiyo local hand-rolled cigars, while the rest smoked either commercial cigarettes (23.3%) or a mix of both commercial cigarettes and Khiyo (23.3%). From 31 chewing patients, 93.5% chewed Miang, and only two patients (6.5%) chewed betel nut.

3.2. Molecular analysis

TP53 germline mutations were not found in extracted DNA from peripheral blood leucocytes of any of the 55 patients. A total of 19 somatic mutations were found in 18 lung cancer patients (32.7%), with one patient harboring two mutations (CM106 in Table 3). The most frequent type of mutation was missense (13 cases), followed by nonsense (3 cases), splicing mutation (2 cases), and silent mutation (1 case). The majority of base substitutions found were G→A transitions (5/19; 26.3%). No characteristic hot spot codons were observed, and all the observed mutations were unique and occupied different codons, except at codon 146, where two mutations were observed (Fig. 1). Most of the mutations were found in squamous cell carcinomas (9/18; 42.1%).

The comparison search of *TP53* somatic mutation types in lung cancer reports was undertaken using the most updated International Agency for Research on Cancer (IARC) *TP53* somatic mutation database R12 [26], as well as the UMD p53 database 2007_R1c [27]. Only 3/19 somatic mutations found in this study are novel in lung cancer (i.e., GCC→ACC at codon 138 of exon 5 (position 13091) (GenBank accession no. EF680843); GTG→GTA at codon 217 of exon 6 (position 13411) (GenBank accession no. EF680844); and CTG→CGG at codon 265 of exon 8 (position 14463) (GenBank accession no. EF680845).

3.3. Univariate and multivariate logistic regression

All characteristics of the patients, except provinces (Table 2), were tested for association between potential risk factors and *TP53* mutations in a univariate analysis (Table 4). The potential risk factors associated with *TP53* mutations at the $P < 0.25$ level were pesticide exposure ($P = 0.20$), history of respiratory disease ($P = 0.10$), family history of cancer ($P = 0.23$), house pattern ($P = 0.15$), location of house ($P = 0.24$), and position of kitchen in house ($P = 0.02$).

The risk factors associated with *TP53* mutations after adjustment for confounding variables were presented in Table 5. The factors found to be independently associated with *TP53* mutations were pesticide exposure, cement house, and indoor kitchen. None of the interactions between these variables were significantly associated with *TP53* mutations.

Table 2
Characteristics of lung cancer patients ($N = 55$)

Characteristic	N *(%)*
1. Gender	
Male	37 *(67.3)*
Female	18 *(32.7)*
2. Age (years)	56 *(58.9 ± 10.2)*[a]
3. Histology cell type	
Squamous cell carcinoma	22 *(40.0)*
Adenocarcinoma	28 *(50.9)*
Large cell carcinoma	3 *(5.5)*
Bronchioloalveolar cell	2 *(3.6)*
4. Province	
Chiang Mai	26 *(47.3)*
Chiang Rai	6 *(10.9)*
Lamphun	4 *(7.3)*
Lampang	9 *(16.3)*
Phayao	5 *(9.1)*
Phare	1 *(1.8)*
Nan	4 *(7.3)*
5. Occupation	
Agriculture	34 *(61.8)*
Tread	6 *(19.9)*
Cook	2 *(3.6)*
Teacher	4 *(7.3)*
Traffic police	1 *(1.8)*
Employee	2 *(3.6)*
Officer	4 *(7.3)*
Student	1 *(1.8)*
Masseuse	1 *(1.8)*
6. Pesticide exposure	
No	25 *(45.5)*
Yes	30 *(54.5)*
7. Smoking status	
Never smoking[b]	4 *(7.3)*
Former smoking[c]	21 *(38.2)*
Current smoking[d]	22 *(40.0)*
Passive smoking[e]	8 *(14.5)*
8. Alcohol drinking status	
Never drank[b]	19 *(34.5)*
Previously drank[c]	14 *(25.5)*
Currently drinks[d]	22 *(40.0)*
9. Fermented tea leaves or betel nut chewing status	
Never chewed[b]	24 *(43.6)*
Previously chewed[c]	12 *(21.8)*
Currently chews[d]	19 *(34.5)*
10. History of respiratory diseases	
No	46 *(83.6)*
Yes	9 *(16.4)*
11. History of nonrespiratory disease	
No	27 *(49.1)*
Yes	28 *(50.9)*
12. Family history of cancer [f]	
No cancer	33 *(60.0)*
Lung cancer	9 *(16.4)*
Other cancer	13 *(23.6)*
13. House pattern	
Wood/half cement	23 *(41.8)*
Wood	19 *(34.5)*
Cement	13 *(23.6)*
14. Location of house	
In garden	18 *(32.7)*
Near road	37 *(67.3)*
15. Position of kitchen	
Outside house	24 *(43.6)*
Inside house	31 *(56.4)*
16. Consuming water	
Piped water	27 *(49.1)*
Underground + well	28 *(50.9)*
17. Drinking water	
Piped water	10 *(18.2)*
Underground + well	22 *(40.0)*
bottle	23 *(41.8)*
18. Drinking water preparation	
No processing	15 *(27.3)*
Processing	40 *(72.7)*
19. Burning activity around house	
No	6 *(10.9)*
Yes	49 *(89.1)*
20. Burning activity per month (time)	30 *(8.42 ± 10.3)*[a]

Abbreviation: *N,* number of patients.

[a] Ranges (mean ± standard deviation).

[b] "Never" refers to someone who never used or rarely uses tobacco and/or alcohol, or chews fermented tea leaves or betel nut in his/her lifetime.

[c] "Former" refers to someone who has reported a history of using tobacco, alcohol, or chewing but stopped at least 1 year before being diagnosed with lung cancer.

[d] "Current" refers to someone who is currently using or had stopped less than 1 year prior.

[e] "Passive smoker" refers to a nonsmoker who has smokers in his/her family.

[f] "Family history of cancer" refers to a history of cancer in a first-degree relative or sibling.

4. Discussion

There have been few papers suggesting that the first-degree relatives of lung cancer patients are at greater risk of developing lung cancer than the general population [11–13]. Thus, inherited genetic factors, such as germline mutation, might be involved in the genesis of lung cancer. Tumor suppressor gene mutations can occur both at the somatic and germline levels. Detection of germline mutations in tumor suppressor genes can be useful for identifying an individual with an increased risk for developing cancer. Although the carriers of the *TP53* germline mutations have a high risk of developing the main Li-Fraumeni syndrome (LFS) component cancers [28], several studies have shown that the *TP53* mutation carriers also had increased risk of developing other cancers [17–19]. Nichols et al. [18] revealed that apart from six classic LFS component tumors, carriers of *TP53* mutations appear to be at increased risk for carcinoma of the lung, gastrointestinal tract, female reproductive organs, and lymphoma. Hwang et al. [19] reported that in mutation carriers, lung and colorectal cancers were relatively common and occurred in a higher proportion than did osteosarcoma and brain tumors, which are cancers in the LFS component. In this study, no *TP53* germline mutations were found in extracted DNA from

Table 3
TP53 gene mutations found in 55 lung cancer patients

Patient ID	Exon/intron	Nucleotide mutations	Amino acid mutations	Histology cell type	Predicted mutation
CM001	5	13116G→A	W146X	Adenocarcinoma	Nonsense
CM019	8	14508G→C	R280T	Adenocarcinoma	Missense
CM033	6	13411G→A*	V217V	Adenocarcinoma	Silent
CM054	7	14028A→G	Y234C	Squamous cell carcinoma	Missense
CM106	5	13067C→G	L130V	Bronchioloalveolar cell	Missense
	7	14061G→A	G245D	Carcinoma	Missense
CR008	7	14070G→T	R248L	Squamous cell carcinoma	Missense
CR015	8	14513C→G	R282G	Squamous cell carcinoma	Missense
CR021	5	13197T→C	V173A	Adenocarcinoma	Missense
LPO005	I7-E8	14451G→T	N/A	Squamous cell carcinoma	Splicing
LPA006	8	14550A→G	E294G	Adenocarcinoma	Missense
LPA001	5	13091G→A*	A138T	Squamous cell carcinoma	Missense
LPA013	8	14519A→C	T284P	Squamous cell carcinoma	Missense
LPA035	5	13154G→C	A159P	Adenocarcinoma	Missense
PY001	8	14463T→G*	L265R	Squamous cell carcinoma	Missense
PY008	E7-I7	14410G→T	N/A	Large-cell carcinoma	Splicing
PY009	6	13352G→T	E198X	Squamous cell carcinoma	Nonsense
PY014	8	14517G→C	R283P	Squamous cell carcinoma	Missense
NAN008	5	13116G→A	W146X	Adenocarcinoma	Nonsense

Asterisks denote novel mutations.
Abbreviation: N/A, does not translate to amino acid.

peripheral blood leucocytes of the patients. It can therefore be concluded that the *TP53* germline mutation in exons 5–8 were not responsible for cancer predisposition in this patient group. Only exons 5–8 were studied in this project, however, so germline mutations might be present in other regions of *TP53*. According to the IARC *TP53* germline mutation database R12 [29], the most frequent germline mutation codon is codon 337 of exon 10. Therefore, in the future, we plan to sequence the entire *TP53* gene for germline mutations.

The present study revealed somatic mutations of exons 5–8 of the *TP53* gene in 32.7% of NSCLC cases examined. In NSCLC, the *TP53* mutational frequency is considerably variable among different populations and studies, ranging from 18 to 60% [30]. Among the 19 observed somatic mutations, it is possible that three of them — in exon 5 at

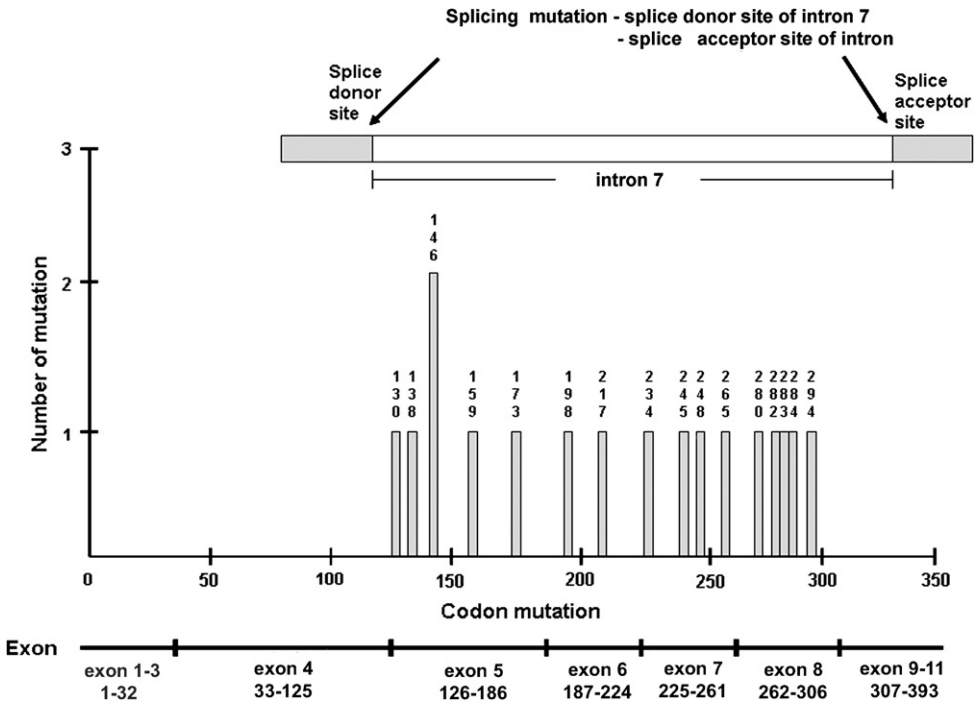

Figure 1. Distribution of mutated codons in exons 5–8 of the *TP53* gene.

Table 4
Univariate analyses for association between confounding factors and mutations of *TP53* gene at exons 5–8 in 55 lung cancer patients

Variable	*n/N (%)*	*P*	Odds ratio estimation (95% CI)
1. Gender			
Male	12/37 *(32.4)*	0.95	1.0
Female	6/18 *(33.3)*		1.0 (0.3–3.5)
2. Age (years)			
⩽ 50	4/13 *(30.8)*	0.46	1.0
51–60	7/17 *(41.2)*		1.6 (0.3–7.2)
61–70	4/19 *(21.1)*		0.6 (0.1–3.0)
> 70	3/6 *(50.0)*		2.3 (0.3–16.4)
3. Histopathology cell types			
Adenocarcinoma	7/28 *(25.0)*	0.5	1.0
Squamous cell carcinoma	9/22 *(40.9)*		2.1 (0.6–6.9)
Other types	2/5 *(40.0)*		2.0 (0.3–14.5)
4. Occupation[a]			
Employee/tread/officer/ student/reception/masseuse	4/14 *(28.6)*	0.81	1.0
Agriculture	11/34 *(32.4)*		1.2 (0.3–4.7)
Cook/ teacher/ traffic police	3/7 *(42.9)*		1.9 (0.3–12.5)
5. Pesticide exposure			
No	6/25 *(24.0)*	0.20	1.0
Yes	12/30 *(40.0)*		2.1 (0.7–6.8)
6. Smoking status[b]			
Former smoker	7/21 *(33.3)*	0.65	1.0
Current smoker	7/22 *(31.8)*		0.9 (0.3–3.3)
Passive smoker	4/8 *(50.0)*		2.0 (0.4–10.5)
7. Alcohol drinking status			
Nondrinker	6/19 *(31.6)*	0.60	1.0
Former drinker	6/14 *(42.9)*		1.6 (0.4–6.8)
Current drinker	6/22 *(27.3)*		0.8 (0.2–3.1)
8. Fermented tree leaves or betel nut chewing status			
Nonchewer	6/24 *(25.0)*	0.25	1.0
Former chewer	3/12 *(25.0)*		1.0 (0.2–4.9)
Current chewer	9/19 *(47.4)*		2.7 (0.7–9.8)
9. History of nonrespiratory disease			
Yes	8/28 *(28.6)*	0.50	1.0
No	10/27 *(37.0)*		1.5 (0.5–4.6)
10. History of respiratory diseases			
Yes	1/9 *(11.1)*	0.10	1.0
No	17/46 *(37.0)*		4.7 (0.5–40.8)
11. Family history of cancer			
Lung cancer	1/9 *(11.1)*	0.23	1.0
Other cancer	4/13 *(30.8)*		3.5 (0.3–38.8)
No cancer	13/33 *(39.4)*		5.2 (0.5–46.6)
12. House pattern			
Wood/half cement	5/23 *(21.7)*	0.15	1.0
Wood	6/19 *(31.6)*		1.7 (0.4–6.6)
Cement	7/13 *(53.8)*		4.2 (0.9–18.3)
13. Location of house			
In garden	4/18 *(22.2)*	0.24	1.0
Near road	14/37 *(37.8)*		2.1 (0.6–7.8)
14. Position of kitchen			
Outside house	4/24 *(16.7)*	0.02	1.0
Inside house	14/31 *(45.2)*		4.1 (1.1–14.9)
15. Consuming water			
Piped water	9/27 *(33.3)*	0.93	1.0
Underground/ well	9/28 *(32.1)*		0.9 (0.3–2.9)
16. Drinking water			
Piped water	4/10 *(40.0)*	0.33	1.0
Underground/ well/ rain	9/22 *(40.9)*		1.0 (0.2–4.8)
Bottle	5/23 *(21.7)*		0.4 (0.1–2.1)
17. Drinking water preparation			
Processing	12/40 *(30.0)*	0.49	1.0
No processing	6/15 *(40.0)*		1.6 (0.5–5.3)
18. Burning activity around house			
Yes	15/49 *(30.6)*	0.35	1.0
No	3/6 *(50.0)*		2.3 (0.4–12.6)
19. Burning activity per month (times)			
0	2/6 *(33.3)*	0.9	1.0
1–5	10/27 *(37.0)*		1.2 (0.2–7.6)
6–15	3/12 *(25.0)*		0.7 (0.1–5.7)
>15	3/10 *(30.0)*		0.9 (0.1–7.5)

Abbreviation: *n/N*, number of mutations/number of patients.

[a] In the occupation categories, the number of patients in each occupation was small, so teacher, cook, and traffic police were combined in the same category because these workers usually inhaled either cooking fumes, dust, or vehicle emission. The rest of the occupations, except for agriculture, were also combined into one category.

[b] Since there is no mutation in the nonsmoking group, only three categories (former smoker, current smoker, and passive smoking) were used for calculating former the association between smoking status and mutations.

position 13091, in exon 6 at position 13411, and in exon 8 at position 14463 —are novel in lung cancer [26,27].

Although *TP53* point mutations were scattered along the coding sequence, they tended to cluster at certain locations in exons 5–8 as hot spot codons. The hot spot codons in NSCLC are codons 157, 158, 175, 245, 248, 249, and 273 [27], which are associated with smoking [10]. In this research project, mutations at these hot spot codons were found in only two patients. One is a smoker and the other is a nonsmoker, but her career as a cook exposed her to cooking fumes. No characteristic hot spot codon was observed in this study. Most of the mutations occurred only once and at different codons, except for codon 146, where two mutations occurred (2/19; 10.5%) (Fig. 1). Mutations at codon 146 in the IARC *TP53* somatic mutation database R12 [26] is found in only 9/2,709 lung cancer studies. Factors of ethnicity and specific environmental carcinogens might contribute to pattern differences.

Table 5
Multivariate analysis of risk factors associated with *TP53* mutations

Variable	*P*	Odds ratio estimation	95% CI
1. Pesticide exposure	0.02	6.6	1.2–35.9
2. Wood house	0.04	1.9	0.4–9.5
Cement house		11.1	1.5–84.4
3. Inside kitchen	0.01	7.1	1.4–35.2

The multivariate analysis was adjusted for age, gender, pesticide exposure, history of respiratory disease, family history of cancer, house pattern, location of house, and position of kitchen in house.

There is evidence that distinct mutation patterns in *TP53* are linked with environmental carcinogens [10], but little information on the genetic factors of lung cancer are found in this region. Since *TP53* mutations have been detected in preneoplastic lesions of the lung, and loss of *TP53* gene function allows the progression of a clonal population of cancer cells, it is likely that *TP53* mutations may be an important step in the progression of non–small cell lung cancer [9]. In this study, multivariate logistic regression adjusting for confounding factors showed a statistically significant association between *TP53* mutation and pesticide exposure, cement housing, and use of an indoor kitchen. The IARC has classified insecticides and pesticides as probable human lung carcinogens [31]. Previous reports have suggested that pesticides currently widely used in the United States and elsewhere have been found to be significantly associated with lung cancer risk [32].

The association between *TP53* mutation and house characteristics, to our knowledge, has not yet been reported. In this research, a cement house and an indoor kitchen were shown to be associated with increased risk of *TP53* mutation. Both factors can cause poor ventilation as well as indoor air pollutants, which contain a complex mixture of bio-aerosols and nonbiologic particles. Several studies have reported many risk factors of an indoor environment associated with lung cancer, such as heterocyclic amines generated during high temperature cooking of meat and cooking oil fumes [33–35]. Lui et al. [33] also reported that increased lung cancer risk was found among subjects living in a house without a separate kitchen and with poor air circulation.

Besides cooking fumes, in some places, house characteristics also included indoor radon levels. The World Health Organization has classified radon as a caracinogen. Radon can enter houses through cracks in the floors, walls, or foundations and accumulate indoors. Wiwatanadate et al. [5] reported that the level of indoor radon in houses of lung cancer patients in the Sarapee district in Chiang Mai was higher than that in houses of healthy subjects. In addition, infiltrations of outdoor air pollution can affect indoor air quality. Chunram et al. [36] reported that extracts of indoor PM 2.5 both in residential and commercial buildings in Chiang Mai show mutagenic effects in the Ames test. This particulate matter can penetrate deep into alveolar sacs of the lung and accumulate in the respiratory system, becoming associated with negative health effects, including lung cancer. For our further study, certain indoor carcinogens that cause *TP53* mutations in this patient group should be defined.

Our study showed that the *TP53* somatic mutation did not correlate with age or gender. The result is consistent with earlier lung cancer studies of several patient populations, such as the northern Polish [30] and Japanese [14]. Morrogi et al. [37] examined the *TP53* mutation spectrum in female lung cancer patients in comparison with males who were matched by age, race, and tobacco level. They found similar frequencies and types of somatic *TP53* mutations in females and males.

Both epidemiologic and experimental evidence have demonstrated that tobacco smoking is strongly associated with lung cancer. In this research, nonsmokers were excluded from the calculation because there was no mutation in this group. When examining the association between *TP53* mutation and status as former, current, or passive smoker, the risk of mutation in former and current smokers was similar, but the risk was double in passive smokers (without statistical significance). This result is likely because of the small sample sizes of lung cancer patients in this study. The previous information about passive smoking reported that the aerosol particle size in the side-stream smoke is much smaller than that of the mainstream smoke. These particles can rapidly penetrate into human lungs. The concentration of carcinogen in side-stream smoke is also several times higher than in the mainstream [38].

There were no associations between mutation and burning activity, alcohol consumption, chewing of fermented tea leaves or betel nut, source and preparation of drinking water, history of respiratory disease, or family history of cancer. Thus, there is no evidence to suggest that these factors induced lung carcinogenesis via the *TP53*-dependent pathway in our study patients.

In summary, this research showed that the *TP53* germline mutation was not responsible for lung cancer predisposition in Thai patients of the upper northern part of the country. Types and spectra of *TP53* mutations in this patient group seem to be different from other populations, judging by comparison with the current *TP53* database. This difference may be due to different mutagen origins and/or mechanisms of mutation. In this patient group, *TP53* mutations appear to have been induced by chemical exposures or risk factors other than tobacco smoke. Pesticide exposure and habitation of a poorly ventilated house appear to be the most important factors related to the tumorigenesis of lung cancer via *TP53* mutations in our study group.

Acknowledgments

The authors wish to thank all the patients for providing the detailed information and samples necessary for the study. We are also grateful to Assoc. Prof. Nirush Lertprasertsuke, Assoc. Prof. Phongtape Wiwatanadate, and Assist. Prof. Somchareon Saeteng, the physicians from Faculty of Medicine, Chiang Mai University, for their valuable comments and suggestion. This research project was funded by Thailand Research Fund (grant no. PHD/0048/2546) and the National Research Council of Thailand.

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