

## RESEARCH ARTICLE

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## Correlation between Genetic Polymorphism of CYP2A13 Genotype and Lung Cancer in Female Passive Smokers

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**Background:** Nicotine is metabolized to cotinine by cytochrome P450 enzyme, and this enzyme is involved in the activation of toxic and carcinogenic substances. The aim of this research was to assess the relationship between genetic polymorphism of CYP2A13 and lung cancer incidence in female passive smokers.

**Materials and methods:** This research was a case-control study that involved 104 research subjects. Subjects were recruited through purposive sampling technique from 2 hospitals in Medan, North Sumatra, Indonesia. The case population consisted of female passive smokers with lung cancer and the control population consisted of female passive smokers without lung cancer. All research subjects underwent blood sampling for genomics DNA extraction and CYP2A13 genotyping by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Data was analyzed by conditional logistic regression by Epi Info 7.0 software.

**Results:** Among 104 subjects, 26 (25%) individuals were heterozygous, 76 (73%) individuals were wild type, and 2 (2%) were mutant for the 257Cys allele. There was a significant correlation between CYP2A13 genotype and lung cancer incidence ( $p$ -value<0.05). Female passive smokers with CT genotype had 2.7 greater risk of developing lung cancer than those with CC genotype (wild type). The C allele had more frequency and 1.6 times higher risk of lung cancer compared to T allele with a wide confidence range (0.73–3.52).

**Conclusion:** There was a significant correlation between CYP2A13 polymorphism and lung cancer incidence in female passive smokers.

**Keywords:** polymorphism, CYP2A13, PCR-RFLP, female passive smoker, lung cancer

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## Introduction

Lung cancer is a cancer disease with a high mortality rate, around 1.38 million deaths worldwide each year.<sup>1</sup> The most recent study showed an increase in lung cancer incidence in women who never smoked or passive smokers.<sup>2</sup> Exposure to environmental tobacco smoke (ETS), biomass, radon, asbestos, and heavy metals, as well as infections and genetic factors have also been reported as lung cancer risk factors other than smoking.<sup>3</sup> Based on GLOBOCAN, the mortality rates of lung cancer in men and women who never smoked were 17.1% and 14.7%, respectively per 100,000 people per year in 182 countries.<sup>4</sup> The incidence of lung cancer in women continues to increase every year. According to the American Cancer Society (ACS) in 2020, the death rate from lung cancer reached 135,720 people per year. The ACS also estimated that new cases of lung cancer in women will be around 112,520 with a death rate of 63,220.<sup>5</sup> Lung cancer in women ranks second after breast cancer as the leading cause of death due to its malignancy.<sup>6</sup>

Although most lung cancer cases were related to tobacco smoke, 25% lung cancers worldwide are found in patients who never smoked, especially in Asian countries. Most lung cancer cases in non-smoking patients are found in East Asian patients and have adenocarcinoma histopathology.<sup>7-9</sup>

Regardless of exposure site, ETS is considered to explain only approximately 20% of never smoker females and 10% of never smoker males. Therefore, the main causes of lung cancer in never smokers are unknown. Other risk factors that cause lung cancer in never smoker patients are rarely reported. Various risk factors have been proposed to explain lung cancer incidence in never smokers. Several of these factors, such as polycyclic aromatic hydrocarbons (PAHs), exposure to radon and its decay products, occupational exposure to asbestos, as well as residential and workplace exposure to ETS has been proven to cause lung cancer in never smokers. The frequency of such exposures in never smoker patients with lung cancer is rarely reported in men. Less than 10% of men and 1% of women had been occupationally exposed to specific International Agency for Research on Cancer (IARC)-identified pulmonary carcinogens. Previous studies often reported that there was an excess relative risk of 20% related to ETS in women.<sup>10</sup>

Cigarette smoke can cause harmful health effects in both active and passive smokers. Active smokers primarily

inhale mainstream smoke (MS) from burning tobacco columns and filters that are expelled through the mouthpiece of a cigarette, while passive smokers mostly inhale sidestream smoke (SS), which is emitted from the end of the smoking cigarette into the surrounding air. Sidestream smoke is the main source of ETS.<sup>11</sup>

The components of cigarette smoke release into the free air. If other people, especially non-smokers inhale these components, they will be passive or involuntary smokers.<sup>12</sup> The IARC have evaluated 55 carcinogens in cigarette smoke, including PAHs, N-nitrosamine, inorganic compounds, and miscellaneous organic compounds.<sup>13</sup> Nicotine is the main compound in tobacco that causes dependence on cigarettes.

Nicotine is metabolized to cotinine by cytochrome P450 enzymes, such as CYP2A13.<sup>14-15</sup> CYP2A13 is involved in activating toxic and carcinogenic substances, such as hexamethylphosphoramide, N,N-dimethylaniline, 2'-methoxyacetophenone, N-nitrosomethylphenylamine activation, as well as coumarin 7-hydroxylation.<sup>16</sup> CYP2A13 is the most efficient cytochrome P450 enzyme in activation of 4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone (NNK)<sup>17</sup>, oxidation of nicotine to cotinine, and hydroxylation of cotinine to trans-3'-hydroxycotinine and a tobacco-specific lung carcinogen.<sup>18</sup> Gene encoding CYP2A13 was highly expressed in liver and extrahepatic tissues including nasal mucosa, trachea, and lung.<sup>16</sup>

Since CYP2A13 is the most efficient NNK-catalyzing enzyme that expressed in the lung, the present work aimed to investigate the relationship between genetic polymorphisms of CYP2A13 and cytological characteristics (obtained from cytologic/histopathological examination) and their association with exposure to environmental smoke in female passive smokers of lung cancer.

## Materials and methods

### *Study Design and Participants*

This was an analytical observational research, case-control study. The case population in this research consisted of 52 female passive smokers diagnosed with lung cancer based on cytological/histopathological examination in Adam Malik General Hospital and Santa Elizabeth Hospital in Medan. The control population consisted of 52 female passive smokers without lung cancer. The inclusion criteria for all subjects were passive smokers >35 years of age with history of ETS exposure for >10 years. The exclusion criteria were subjects who have consumed dexamethasone, phenobarbital,

coumarin, tryptamine, methoxsalen (8-methoxypsoralen), tranlycypromine, and neo methylthiol. All the subjects had agreed and signed the informed consent. This study has been approved by the Ethics Committee of Faculty of Medicine Universitas Sumatera Utara (No. 543/KEP/USU/2021).

### **Blood Sampling**

Blood samples were collected at Adam Malik General Hospital and Santa Elisabeth Hospital, Medan. Three mL of blood from each research subject was drawn from the median cubital vein into a vacutainer containing EDTA and stored at 4–8°C until DNA isolation. DNA isolation was carried out using standard procedures as soon as possible after blood sampling.

### **CYP2A13 Genetic Polymorphism Examination (PCR-RFLP)**

The CYP2A13 polymorphism rs8192789 c.769C>T p.Arg257Cys, also known as 3375C>T, was examined as previously described<sup>19</sup> with minor modifications. Polymerase chain reaction (PCR) amplification for CYP2A13 was performed using forward (5'-CCTGGACA GATGCCTTTAACTCCG-3') and reverse (5'-TGGCTTTG-CACCTGCCTGCACT-3') primers. The PCR procedure was as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 63°C for 45 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The PCR product (332 bp) was visualized on a 1% agarose gel containing ethidium bromide (0.5 g/mL). Ten µL of PCR product was digested with 10 U fast-digesting restriction enzyme HhaI (Thermo Scientific, Waltham, MA, USA) and appropriate buffer (total volume 20 µL) for 15 minutes at 37°C. The PCR product was undigested for T allele, while the PCR product was digested into 233 and 99 bp fragments for C allele.

### **Data Analysis**

Data was descriptively analyzed to observe frequency distribution based on demographic characteristics of the research subjects. Then, data was analyzed with inferential analysis to assess the relationship between lung cancer incidence and CYP2A13 polymorphism frequency, as well as the histopathological type. Results with *p*-value <0.05 were considered as statistically significant. Data was analyzed with conditional logistic regression using Epi Info 7.0 software.

## **Results**

Table 1 showed the demographic characteristics of the research subjects. In the case group, the majority of the research subjects were in the 50–59 years old age group (36.5%). Meanwhile, the majority of the research subjects were 40–49 years old in control group (63.5%). In the case group, the majority of research subjects were housewives (71.2%). In the control group, 32.6% of research subjects were nurses who were exposed to cigarette smoke from their husbands. Cigarette smoke came from home environment (61.5% in case population and 65.4% in control population), with 21.2% of subjects in case population were exposed to cigarette smoke for <10 years and 59.6% of subjects in control population were exposed for >10 years. There was also environmental smoke exposure. Most research subjects in the case group were exposed to mosquito coil smoke (23.1%), whereas in control group, most of the subjects (32.7%) were exposed to dust from chalk or lime.

Table 2 showed the distribution of CYP2A13 genotype and allele. CC genotype had the highest frequency in both case and control groups. There was a significant relationship between CYP2A13 genotype and lung cancer incidence (*p*<0.05). People with CT genotype had 2.7 greater risk of developing lung cancer than those with CC genotype. There was no significant relationship between CYP2A13 allele and lung cancer incidence. C allele had higher frequency compared to T allele. Moreover, C allele had 1.6 greater risk of lung cancer than T allele with a wide confidence range (0.73–3.52).

Odds ratio (OR) of environmental exposure variable was 3.03 which means that females who were exposed to environmental smoke will have a 3.03 higher risk of developing lung cancer (Table 3). Table 4 showed the frequency distribution of CYP2A13 genetic polymorphism for both genotypes and histopathological types of lung cancer. CC had the highest genotype frequency. Thirty-two subjects with adenocarcinoma and 2 subjects with squamous cell carcinoma (SCC) had CC genotype. There was no subject with minor genotype variant (TT) diagnosed with adenocarcinoma or SCC. In addition, 18 subjects with adenocarcinoma had CT genotype. No subject diagnosed with SCC had CT genotype. Overall, 96% of subjects were diagnosed with adenocarcinoma, while the other 4% were diagnosed with SCC.

Adenocarcinoma cells were mostly found in subjects with C allele (32 subjects) compared to T allele (18 subjects).

**Table 1. Characteristics of research subjects.**

Variable	CYP2A13				p-value
	Case		Control		
	n	%	n	%	
<b>Age</b>					
<40 years old	0	0	3	5.8	
40-49 years old	10	19.2	33	63.5	
50-59 years old	19	36.5	16	30.8	<0.01
60-69 years old	18	34.6	0	0	
≥70 years	5	9.6	0	0	
<b>Histopathology</b>					
Adenocarcinoma	50	96.2	0	0	N/A
SCC	2	3.8	0	0	
<b>Passive smokers</b>					
In the home environment	32	61.5	34	65.4	0.28
In the work environment	6	11.5	3	5.8	
<b>Cigarette smoke exposure</b>					
<10 years	11	21.2	5	9.6	0.03
≥10 years	10	19.2	31	59.6	
<b>Environmental exposure</b>					
Mosquito coil smoke	12	23.1	7	13.5	
Line dust	11	21.2	17	32.7	
Pesticide	5	9.6	4	7.7	0.37
Stove smoke	11	21.2	7	13.5	
Trash burn	7	13.5	9	17.3	
<b>Ethnicity</b>					
Bataknesse	30	57.7	38	73.1	
Javanese	18	34.6	11	21.2	
Malay	1	1.9	2	3.8	0.049
Chinese	2	3.8	0	0	
Acehnese	1	1.9	0	0	
Minang	0	0	1	1.9	
<b>Total</b>	<b>52</b>	<b>100</b>	<b>52</b>	<b>100</b>	

Meanwhile, SCC was found in 2 subjects with C allele. No subject diagnosed with SCC had T allele (Table 5). PCR-RFLP results of CYP2A13 were shown in Figure 1.

## Discussion

Lung cancer in passive smokers is largely found in Asian female population.<sup>20</sup> Besides cigarette smoke, there are

**Table 2. Frequency of CYP2A13 genotypes and alleles between case and control group and their association with lung cancer.**

CYP2A13	Case		Control		p-value	OR	Confidence Interval
	n	%	n	%			
Genotype	CC	34	65.4	42	80.8	1	1
	CT	18	34.6	8	15.4	0.035*	1.07-7.16
	TT	0	0	2	3.8	N/A	N/A
Allele	C	86	82.7	92	88.5	1	1
	T	18	17.3	12	11.5	1.6	0.73-3.52

\*Significant with logistic regression test.

many other risk factors that can cause lung cancer in non-smokers, such as environmental smoke exposure and genetic factors.<sup>4</sup> The other risk factors such as age, inherited genetic susceptibility, exposure to occupational and environmental carcinogens, lack of hormonal balance, pre-existing lung disease, dietary factors and oncogenic viruses, especially HPV infection can also be considered as causes of lung cancer.<sup>22</sup>

CYP2A13 enzyme plays important roles in xenobiotic toxicity and tumorigenesis in the respiratory tract.<sup>16</sup> This means that interindividual variations in CYP2A13 activity is one of the factors that regulates the occurrence of different types of cancers.<sup>15</sup> Several genetic polymorphisms of CYP2A13 have been identified in previous studies, one of them was single nucleotide polymorphism (SNP) C3375T which causes the protein changes (p.Arg257Cys). It has been reported that Arg257Cys polymorphism protects individuals with homozygous Cys257 allele against xenobiotic toxicity.<sup>23</sup>

We found that CYP2A13 genotype was significantly associated with CT genotype and T allele, with 2.7-fold and 1.6-fold higher risk of lung cancer, respectively. These findings were different from previous reports.<sup>24,25</sup> The T allele (p.Arg257Cys) reduces the metabolic turnover of

nicotine, hence serves as protection against xenobiotic toxicity in the respiratory tract.<sup>14</sup>

We found that the majority of age interval in lung cancer patients was 50-59 years. According to a study, most lung cancers were found in the age group above 60 years.<sup>26</sup> Another study reported that the percentages of lung cancer in the age group above 60 years and 51-60 years were 40.8% and 35.3%, respectively.<sup>27</sup> This may be due to an exposure to carcinogenic substances that usually takes a long time to cause an imbalance between the function of oncogenes and tumor suppressor genes, in the process of growth and development of cancer cells.<sup>26</sup> Other lung cancer-related studies have found that the incidence of lung cancer increases with age due to long exposure to high-risk factors and a decrease in the ability of cells to repair.<sup>28</sup>

The most common type of lung cancer in our study was adenocarcinoma. This is in accordance with a previous study which found that the most common type of lung cancer was adenocarcinoma (90.4%), followed by SCC (6.6%).<sup>29</sup> A study in an Indian population reported that adenocarcinoma was the most common lung cancer type with prevalence of 30.9%.<sup>30</sup>

The use of biological materials from both animal and plant, such as wood, plant residues, animal waste, and

**Table 3. OR of environmental exposure variable and their association with lung cancer.**

Environmental Smoke?	Subjects		OR
	Case	Control	
Yes	30	23	
No	16	21	3,03

**Table 4. CYP2A13 genotype frequencies by cancer cell type.**

Genotypes	Adenocarcinoma	SCC
CC	32	2
TT	0	0
CT	18	0

**Table 5. CYP2A13 allele frequencies by cancer cell type.**

Allele	Adenocarcinoma	SCC
C	32	2
T	18	0

charcoal as biomass fuel, may generate polycyclic aromatic hydrocarbons (PAHs) and small particles that are linked to lung cancer.<sup>31,32</sup> Small particulates (particulate matter <10 µm; PM10) could cause prolonged inflammation which will increase the reactive oxygen species (ROS) and directly cause cell damage. On the other hand, PAHs are essential substances that play an important role in DNA adduction and correlate with the expression of several tumor suppressor genes (p53) and oncogenic drivers (KRAS and HRAS).<sup>32</sup> However, the exact timing of biomass exposure on lung cancer incidence remains unknown. In this study, firewood and lime dust had the highest prevalence of biomass exposure, with other biomass remaining as risk factors. The etiology may vary due to social and geographical aspects. A lot of Indonesian women work with pesticides, firewood as cooking fuel, and mosquito repellents. Moreover, since all of the subjects were over 35 years old, they were most likely exposed to chalk (lime dust) during their school days.

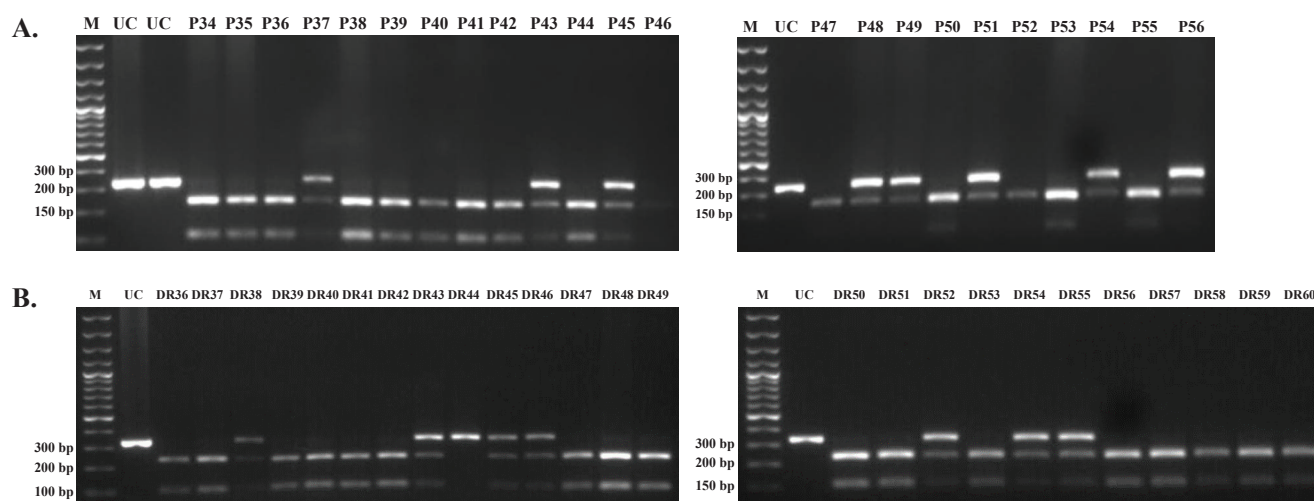
This research had a small sample size and short research duration. RT-PCR with further sequencing analysis can be used to analyze whether the polymorphisms are related to the type of CYP2A13 mutations in certain geographic areas, especially in Indonesia. Analysis of geographical factors as well as a larger scale of study are required to assess the effect of CYP2A13 polymorphism towards lung cancer incidence.

## Conclusion

There is a significant relationship between CYP2A13 genotype and lung cancer incidence. Female passive smokers with CT genotype have 2.7 higher risk of developing lung cancer compared to those with CC genotype (wild type). Meanwhile, there was no significant relationship between the CYP2A13 allele and the incidence of lung cancer. The most common allele in this study was the C allele, which tends to be more protected from lung cancer than the T allele.

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**Figure 1. PCR-RFLP results of CYP2A13 gene.** A: Case group. Heterozygous genotype (332, 233, 99 bp) was found in P34-39, P41-43, P45-46, and P51. Wild type genotype (233, 99 bp) was found in P40, P44, P47-50, P52-56. Mutant genotype (332 bp) was not found in the case group. B: Control group. Wild type genotype (233, 99 bp) was found in DR36-37, DR39-42, DR47-49. Heterozygous genotype (332, 233, 99 bp) was found in DR38, DR43, DR45-46. Mutant genotype (332 bp) was found in DR44. M: marker, UC: uncut.

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