RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2022; 6(2): 63-9
DOI: 10.21705/mcbs.v6i2.232

Association of CYP2A6 Genetic Polymorphism and Lung Cancer in Female Never Smokers

R.A Henny Anggriani[1], Noni Novisari Soeroso[2], Setia Putra Tarigan[3], Putri Chairani Eyanoer[4], Hidayat[5]

[1]*Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara/Adam Malik General Hospital, Medan, Indonesia*
[2]*Division of Thoracic Oncology, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara/Universitas Sumatera Utara Hospital, Medan, Indonesia*
[3]*Division of Thoracic Oncology, Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara/Adam Malik General Hospital, Medan, Indonesia*
[4]*Department of Public Health, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*
[5]*Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*

Background: The major significant factor that affected lung cancer development among female passive smokers is environmental tobacco smoke. Nicotine can be found in a never smoker population, such as a child whose father is a smoker. Lung carcinogenesis in never smoker populations is affected by nicotine metabolism by CYP2A6 gene, which encodes the main nicotine metabolizing-enzyme. The aim of this study was to assess the genetic polymorphism of CYP2A6 and its association with secondhand smokers among females who have suffered from lung cancer in North Sumatra population.
Materials and methods: This study was a case-control study, composed of 53 case subjects and 46 control subjects that were involved through a purposive sampling technique from two hospitals in Medan. PCR-RFLP was used for the examination of CYP2A6 gene to determine the genotype. The data were analyzed with conditional logistic regression test using Epi Info 7.0 software.
Results: The most common genotype of CYP2A6 detected in this study was *1B/*1B (40.4%), while *1B allele had the highest prevalence (55.5%). There was no significant association between CYP2A6 genotype (*p*-value=0.61) or alleles (*p*-value=0.25) and the incidence of lung cancer.
Conclusion: There was no association between CYP2A6 polymorphism and the incidence of lung cancer in secondhand smoker females.

Keywords*: CYP2A6, PCR-RFLP, female secondhand smokers, lung cancer*

Introduction

Recent global data shows that high incidence of lung cancer in both males and females is closely related to smoking status. Both active and environmental tobacco smokers increase the risk of lung cancer.[1] The United States has reported that 15% of men and 53% of women with lung cancer were those who have never smoked before.[2] Unfortunately, the

Date of submission: December 30, 2021
Last Revised: April 7, 2022
Accepted for publication: April 19, 2022

Corresponding Author:
Noni Novisari Soeroso
Division of Thoracic Oncology
Department of Pulmonology and Respiratory Medicine
Faculty of Medicine Universitas Sumatera Utara/Universitas Sumatera Utara Hospital
Jl. Dr. Mansyur No. 66, Medan, Indonesia
e-mail: noni@usu.ac.id

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v6i2.232

prevalence of lung cancer among never smoker populations in Indonesia has not been accounted yet. The risk factors for lung cancer in non-smokers have been widely studied in all lung cancer patients. A review by the International Agency for Research on Cancer (IARC) concluded that cigarette smoke contains many carcinogenic substances for humans. Cigarette smoke increases lung cancer risk 20% in women and 30% in men due to the passive smoking of their smoker partners. Based on an epidemiological analysis study, non-smokers with smoker spouses had 1.2 times higher risk for having lung cancer than those who live with non-smoker spouses. Moreover, this number may increase in line with the increased cigarette consumption and duration of smoking.[3]

There are several reasons why lung cancer in female never smokers is an interesting topic. Firstly, geographic variations and/or differences in biological susceptibility are more detectable in the never-smoking population, particularly in females, which is the most common risk group in the never-smoking population. Secondly, the number of cancer survivors among people who have never smoked surprisingly increased in developed countries. Thirdly, several studies have shown that lung tumors in people who have never smoked had different molecular profiles and types than smoker-patients. Lastly, passive smokers diagnosed with lung cancer have better responses to targeted therapy than patients who are active smokers.[4]

Carcinogenic substances from cigarette smoke can affect the central and peripheral airways. There are 20 types of carcinogens found in cigarette smoke which are significantly associated with the development of lung cancer, such as polycyclic aromatic hydrocarbons (PAHs) and nicotine-nitro aminoketone derivatives.[5] Most of the carcinogens in cigarette smoke require metabolic activation, which is generally catalyzed by cytochrome P450 enzymes. Conversion of these carcinogens produces substances that may covalently bind to DNA, thus forming DNA adducts. Subsequently, CYP2A6 is the main enzyme involved in nicotine metabolism that catalyzes the conversion of nicotine to cotinine and cotinine to 3-hydroxy-cotinine (3HC). The increase activity of CYP2A6 will result in increasing 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), the side product of nicotine metabolism that has been shown to correlate with the lung carcinogenesis. Recent studies have been conducted on the genotype and allele variants of CYP2A6 including *4, *7, *9, and *1. Alleles *1A and *1B are the wild types of lung cancer and strongly related to the increased of lung cancer risk.[6,7] The objective of this study was to assess the genetic polymorphism of CYP2A6 and its association with environmental tobacco smoke in female secondhand smokers who suffered from lung cancer among the North Sumatra population.

Materials and methods

Study Design and Participants

This was an analytical observational study conducted within 6 months of the case-control study. The case population of this study were female secondhand smokers with lung cancer, while the control population were females who were not smoking. Inclusion criteria for case group were passive smokers >35 years of age diagnosed with lung cancer based on cytology and histology examination with history of smoke exposure for more than 10 years. Inclusion criteria for control group were passive smokers >35 years of age with history of smoke exposure for more than 10 years. Exclusion criteria for both case and control group were female passive smokers diagnosed with lung cancer who had been prescribed phenobarbital, methoxsalen (8-methoxypsoralen), tranylcypromine, tryptamine, coumarin, and neo methylthiol.

To anticipate errors that may have occurred during the examinations and analysis, we recruited more subjects than the minimum sample numbers. Total 99 subjects were recruited and divided into two groups: 53 subjects in case group and 46 subjects in control group. All the subjects had agreed and signed the informed consent. This study has been approved by the Ethical Committee of the Faculty of Medicine, Universitas Sumatera Utara (No. 542/KEP/USU/2021).

Samples Collection

Blood samples were collected at Adam Malik General Hospital and Santa Elisabeth Hospital, Medan. The blood samples were taken from the median cubital vein as much as 3 ml using vacutainer tube with EDTA. Blood samples were stored in -20°C refrigerator.

DNA Extraction

Blood samples were stored in 4–8°C for DNA extraction. There were two steps in the lysis approach. First, red blood cells were lysed using detergents, such as sodium dodecyl sulfate (SDS) and Triton™ X-100. White blood cells then were lysed to release the cell nucleus, genomic DNA, and RNA. Both steps removed the contaminating RNA, leaving DNA and protein molecules in supernatant.

CYP2A6 Genetic Polymorphism Examination (PCR-RFLP)

2Aex7F (5'-GRCCAAGATGCCCTACATG-3') and 2A6R2 (5'-AAAATGGGCATGAACGCCC-3') primers were used for CYP2A6*1A, CYP2A6*1B, CYP2A6*4A and CYP2A6*4D genotyping. Then, the DNA samples were mixed with a PCR mixture (25 l) that contained 1X PCR buffer (67 mM Tris-HCl buffer (pH 8.8); 16.6 mM $(NH_4)_2SO_4$; 0.45% Triton X-100; 0.02% gelatin), 1.5 mM $MgCl_2$, 0.4 l M of each primer; 250 l M dNTPs, and 1 U Taq DNA polymerase. PCR-RFLP was performed by applying initial denaturation for 1 minute at 95°C, followed by denaturation for 15 seconds at 95°C, annealing for 20 seconds at 60°C and extension for 3 minutes at 72°C for 35 cycles. Then, final extension step for 7 minutes at 72°C was applied. Eco81I, Eco147I and Bsh1236I restriction enzymes (Thermo Scientific, Waltham, MA, USA) were used in this study. The PCR-RFLP product was analyzed with 1.5% agarose gel electrophoresis (Biorad and Scie-plas).

Data Analysis

Data was analyzed using Epi Info 7.0 software. Descriptive analysis was performed to see the frequency distribution, which was based on the demographic characteristics of every sample. After that, the relationship between CYP2A6 polymorphism, the incidence of lung cancer and the type of histopathology was determined with inferential analysis. All results with *p*-value <0.05 were categorized as statistically significant.

Results

In the case group, the majority of the patients were in the 50-59 years age group (37.7%). Meanwhile, in the control group, the majority of the patients were in the 40-49 years age group (60.9%). The source of cigarette smoke mostly came from the home environment (56.6%), since most of the subjects in this study were housewives in both case (75.5%) and control group (32.6%). Mosquito coil smoke (20.8%) and lime dust (30.4%) is the major source of smoke exposure in case and control group, respectively. The majority of the patients in this study were Batak, who accounted for a total of 65 people from control and case group (Table 1).

Genotyping and allele identification of CYP2A6 were carried out using two restriction enzymes. Electrophoresis results of the CYP2A6 PCR-RFLP product from the case and control group samples were shown in Figure 1. There was no significant relationship between the CYP2A6 genotype and the incidence of lung cancer (*p*-value=0.61). After performing multivariate analysis with logistic regression, *1A/*1B and *1B/*1B had a lower lung cancer risk (0.64 times) compared to *1A/*1A genotype but with a wide confidence interval. In addition, there was no significant relationship between the CYP2A6 allele and the incidence of lung cancer (*p*-value=0.25). *1B was the most common allele in this study and had a lower risk (0.75 times) of developing lung cancer with a wide confidence interval. *1B/*1B genotype had the highest genotype frequency and consisted of 20 subjects with adenocarcinoma cancer cells and one subject with squamous cell carcinoma (SCC). For the mutant genotype variant, there was only one subject who had adenocarcinoma cancer cell types. Most of the subjects with *1A allele had adenocarcinoma cancer cell, and two of them had SCC. For the mutant type, we found only one patient with adenocarcinoma cell type and no SCC (Table 2).

Discussion

The relationship between CYP2A6 polymorphisms and the incidence of lung cancer has been reported in several studies. Several studies reported a significant association between CYP2A6 polymorphisms and the incidence of lung cancer. However, other studies reported that there is no association between CYP2A6 polymorphisms and the incidence of lung cancer. There are 20 variants of the CYP2A6 allele that have been identified, and the distribution of these CYP2A6 polymorphisms is different in each population.[8] Asians have a relatively high frequency of CYP2A6*4 variant, whereas CYP2A6*1 is lower. In Caucasians, there was a 1.2% frequency of CYP2A6*4 variant.[9] Another study found CYP2A6*1 and CYP2A6*4 variants in Javanese population.[10]

Theoretically, genetic polymorphisms in CYP2A6 lead to reduced activity of nicotinic and reduced risk of tobacco-induced lung cancer and are associated with lower smoking habits. Mutant type of CYP2A6, *4A has been known to decrease nicotine metabolism resulting in lower accumulations of NNK as the carcinogenic substance of nicotine. Meanwhile, *1A and *1B as wildtype of CYP2A6 increase the nicotine metabolism activity and NNK product.[7]

A previous study found an association between high nicotine dependence in male Batak smokers who had

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v6i2.232

Table 1. General characteristics of subject populations.

Variable	CYP2A6				*p* -value
	Case Group		Control Group		
	n	%	n	%	
Age					
<40 years old	0	0	3	6.5	<0.01
40-49 years old	12	22.6	28	60.9	
50-59 years old	20	37.7	15	32.6	
60-69 years old	17	32.1	0	0	
≥70 years	4	7.5	0	0	
Occupation					
Cleaning service	0	0	7	15.2	<0.01
Teachers	2	3.9	6	13.0	
Housewife	40	75.5	15	32.6	
Nurse	0	0	12	26.1	
Farmer	1	1.9	0	0	
Civil servant	3	5.7	4	8.7	
Entrepreneur	7	13.2	2	4.3	
Source of cigarette smoke					
House environment	30	56.6	30	65.2	0.47
Work environment	7	13.2	4	8.7	
Biomass exposure					
Insect repellant	11	20.8	4	8.7	0.37
Chalk	9	17	14	30.4	
Pesticide	4	7.5	3	6.5	
Trash burnt smoke	6	11.3	9	19.6	
Ethnics					
Batak	29	54.7	34	73.9	0.113
Javanese	19	35.8	9	19.6	
Malay	2	3.7	2	4.3	
Chinese	2	3.7	0	0	
Acehnese	1	1.8	0	0	
Minang	0	0	1	2.1	
Total	**53**	**100**	**46**	**100**	

Tested with Fisher exact test.

the CYP2A6*1A variant (1.13 times) compared to the CYP2A6*4A and CYP2A6*1B variants. However, no association between nicotine metabolism and CYP2A6 polymorphism in Batak smokers were found.[11]

Many risk factors are identified to increase the incidence of lung cancer in non-smokers. Age, environmental tobacco smoke, air pollution both indoors (household smoke) and outdoors (vehicle emissions), inherited genetic susceptibility, and exposure to carcinogens in children were risk factors that correlate with the incidence of lung cancer in people who never smoked. Some other risk factors are occupational and environmental factors, imbalance of hormonal factors, pre-existing lung disease, dietary factors, and oncogenic viruses especially HPV infection.[12]

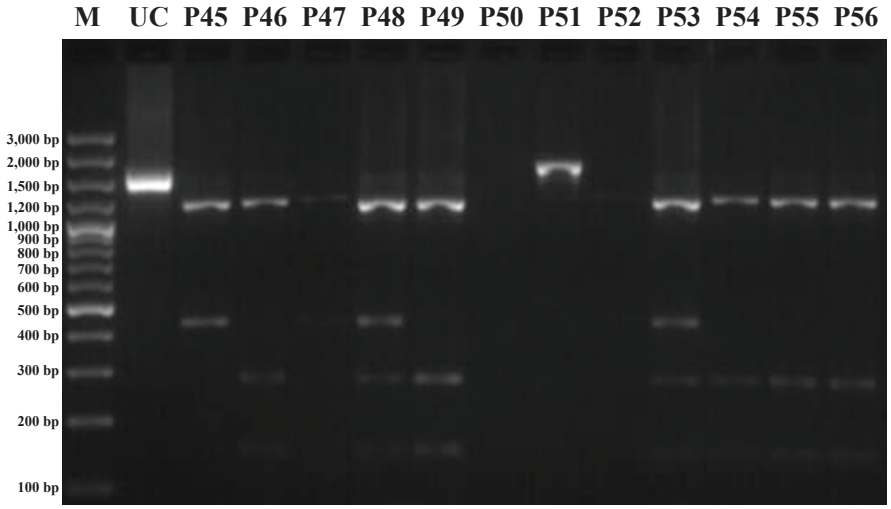

Figure 1. Electrophoresis results of CYP2A6*1A, CYP2A6*1B, and CYP2A6*4A alleles. *1A bands: 1,316, 427, 120, and 104 bp, *1B bands: 1,316, 278, 149, 120, and 104 bp, and *4A bands: 728, 587, 278, 149, 120, and 104 bp. M: Marker (1500bp), UC: Uncut, lane P45: *1A/*1A, lane P46: *1B/*1B, lane P48: *1A/*1B.

Biomass fuel is a biological fuel synthesized from animals and plants, such as wood, plant residues, animal waste, and charcoal.[13] Although there has been a shift in using traditional fuels to electricity and liquefied petroleum gas, people in rural areas in Southeast Asia, particularly Indonesia, still use them to cook and heat food.[14] PAHs and small particles-generated particulate matter are linked to lung cancer. Small-sized particulates may cause prolonged inflammation which would increase reactive oxygen species (ROS) and directly cause cell damage.

PAHs is an essential substance that plays an important role in DNA adduction and correlates with the expression of several tumor suppressor genes, such as p53 as well as oncogenic activators such as KRAS and HRAS.[15] However,

Table 2. Frequency of genotype and allele of CYP2A6 and their associations with lung cancer.

Characteristics	Case Group		Control Group	*p* -value	Odds Ratio	Confidence Interval
	Adenocarcinoma	SCC				
	n	n	n			
Genotype CYP2A6						
*1A/*1A	16	1	11		1.00	1.00
*1A/*1B	14	1	15		0.64	0.22-1.83
*1B/*1B	20	0	20	0.61	0.64	0.24-1.72
*1A/*4A	0	0	0			
*4A/*4A	1	0	0		N/A	N/A
Allele CYP2A6						
*1A	30	2	37		1.00	1.00
*1B	20	0	55	0.25	0.75	0.42-1.33
*4A	1	0	0		N/A	N/A

Tested with conditional logistic regression test.

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v6i2.232

the exact timing of biomass exposure in the incidence of lung cancer has not been known yet. In this study, firewood and insect repellent had the highest prevalence of biomass exposure. The social and geographical aspects of developing countries can make this etiology differ. In Indonesia, females use firewood as cooking fuel, or work as farmers that use pesticides. Moreover, some local societies use mosquito repellent dust due to the geographical location where the mosquito population is high.

The most common type of cancer found in smokers is SCC. Meanwhile, in recent years researchers have recognized adenocarcinoma as the dominant type of lung cancer in smokers. This shift was reported by researchers as a result of a decrease in the amount of PAHs and an increase in nitrosamines in the smoke inhaled from filter cigarettes. Thus, the increase in adenocarcinoma is thought to be related to nitrosamines. Within the main bronchus, SCC predominates in the central airway. Whilst the adenocarcinoma predominates mostly in the periphery of the lung. Due to the amount of CYP2A6 mRNA expressed in human bronchial epithelial cells, CYP2A6 can activate nitrosamines in tobacco smoke in bronchi. This mechanism may explain the significant association between CYP2A6 polymorphisms and the risk of SCC.

The presence of functional CYP2A6 in pulmonary periphery is controversial. The presence of functional CYP2A6 in pulmonary periphery is controversial because *4/*4 genotype had less pronounced effect in adenocarcinoma compared to small cell or squamous carcinoma. Researchers have confirmed in larger cohort studies that these differences are simple and suggestive. The risk of adenocarcinoma may be more closely related to the activity of CYP2A13 which is reported to have a higher metabolic capacity to activate NNK and is expressed higher in peripheral lung tissue compared to CYP2A6.

This study has several limitations. This study did not show the involvement of CYP2A6 gene in the carcinogenesis process. This work may demonstrate the existence of other factors, such as genetic factors and environmental factors that play a role in the incidence of lung cancer. Therefore, further analysis is needed to determine certain factors related to lung cancer incidence in female never smoker populations. In this study, allele variant *4 was only found in one sample. Thus, more samples should be involved in further research to find more genetic polymorphisms.

Conclusion

There are no associations between CYP2A6 polymorphisms and the incidence of lung cancer influenced by genes or genetic variations. Adenocarcinoma had the highest frequency, although it has not been clearly associated with CYP2A6 genetic polymorphism. Thus, further research is needed to investigate the influence of other genes and biomass exposure on carcinogenesis.

References

1. Bhopal A, Peake MD, Gilligan D, Cosford P. Lung cancer in never-smokers: A hidden disease. J R Soc Med. 2019; 112(7): 269-71.
2. Dela Cruz CS, Tanoue LT, Matthay RA. Lung cancer: Epidemiology, etiology, and prevention. Clin Chest Med. 2011; 32(4): 605-44.
3. Kim CH, Lee YC, Hung RJ, McNallan SR, Cote ML, Lim WY, *et al*. Exposure to secondhand tobacco smoke and lung cancer by histological type: A pooled analysis of the International Lung Cancer Consortium (ILCCO). Int J Cancer. 2014; 135(8):1918–30.
4. Toh CK, Gao F, Lim WT, Leong SS, Fong KW, Yap SP, *et al*. Never-smokers with lung cancer: Epidemiologic evidence of a distinct disease entity. J Clin Oncol. 2006; 24(15): 2245–51.
5. Brambilla E, Gazdar A. Pathogenesis of lung cancer signalling pathways: Roadmap for therapies. Eur Respir J. 2009; 33(6): 1485–97.
6. Ezzeldin N, El-Lebedy D, Darwish A, El Bastawisy A, Abd Elaziz SH, Hassan MM, *et al*. Association of genetic polymorphisms CYP2A6*2 rs1801272 and CYP2A6*9 rs28399433 with tobacco-induced lung cancer: Case-control study in an Egyptian population. BMC Cancer. 2018; 18(1): 525. doi: 10.1186/s12885-018-4342-5.
7. Tan W, Chen GF, Xing DY, Song CY, Kadlubar FF, Lin DX. Frequency of CYP2A6 gene deletion and its relation to risk of lung and esophageal cancer in the Chinese population. Int J Cancer. 2001; 95(2): 96–101.
8. Yoshida R, Nakajima M, Watanabe Y, Kwon JT, Yokoi T. Genetic polymorphisms in human CYP2A6 gene causing impaired nicotine metabolism. Br J Clin Pharmacol. 2002; 54(5): 511–7.
9. Rao Y, Hoffmann E, Zia M, Bodin L, Zeman M, Sellers EM, *et al*. Duplications and defects in the CYP2A6 gene: Identification, genotyping, and in vivo effects on smoking. Mol Pharmacol. 2000; 58(4): 747–55.
10. Patramurti C, Sugiyanto, Nurrochmad A, Martono S. Polymorphism of cytochrome P450 2A6 (CYP2A6*1 and CYP2A6*4) among Javanese Indonesian smoker and non smoker. Indonesian J Pharm. 2015; 26(1): 11-19.
11. Soeroso NN, Zain-Hamid R, Sinaga BYM, Sadewa AH, Syafiuddin T, Syahruddin E, *et al*. Genetic polymorphism of CYP2A6 and its relationship with nicotine metabolism in male bataknese smokers suffered from lung cancer in Indonesia. Open Access Maced J Med Sci. 2018; 6(7): 1199–205.
12. Soeroso NN, Ananda FR. Lung cancer among never-smoker women: An epidemiological data in North Sumatera, Indonesia. Int J Respir Med. 2019; 1(1): 1-9.
13. Torres-Duque C, Maldonado D, Pérez-Padilla R, Ezzati M, Viegi G. Biomass fuels and respiratory diseases: A review of the evidence. Proc Am Thorac Soc. 2008; 5(5): 577–90.

14. Lim WY, Seow A. Biomass fuels and lung cancer. Respirology. 2012; 17(1): 20–31.
15. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71(3): 209–49.
16. Gasperino J. Gender is a risk factor for lung cancer. Med Hypotheses. 2011; 76(3): 328–31.
17. Couraud S, Souquet PJ, Paris C, Dô P, Doubre H, Pichon E, *et al*. BioCAST/IFCT-1002: Epidemiological and molecular features of lung cancer in never-smokers. Eur Respir J. 2015; 45(5): 1403–14.
18. Mong C, Garon EB, Fuller C, Mahtabifard A, Mirocha J, Mosenifar Z, *et al*. High prevalence of lung cancer in a surgical cohort of lung cancer patients a decade after smoking cessation. J Cardiothorac Surg. 2011; 6: 19. doi: 10.1186/1749-8090-6-19.