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Genetic Polymorphism of *CYP2A6* and Its Relationship with Nicotine Metabolism in Male Bataknese Smokers Suffered from Lung Cancer in Indonesia

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Abstract

BACKGROUND: Cytochrome P450 2A6 (CYP2A6) is known as an enzyme which is responsible for the metabolism of chemical compounds.

AIM: This study aimed to analyse the relationship between *CYP2A6* gene polymorphism with nicotine metabolism rates and lung cancer incidence among smokers of Batak ethnic group in Indonesia.

METHODS: This study was a case-control study involving 140 research subjects through a purposive sampling technique from three hospitals in Medan, Indonesia. An examination of nicotine metabolism rates was conducted for all subjects using the 3HC/cotinine ratio parameter with LC-MS/MS technique. The examination of the *CYP2A6* gene was performed with PCR-RFLP. Data were analysed with Conditional Logistic Regression test using Epi Info 7.0 software.

RESULTS: The allele frequencies of *CYP2A6*1A, CYP2A6*1B,* and *CYP2A6*4A* found were 44.3%, 48.9%, and 6.8%, respectively. The *1B allele showed the highest metabolism rate. It is found that slow metabolizer individuals were 5.49 times more likely to develop lung cancer (P = 0.01, 95%CI 1.2-24.8).

CONCLUSION: Among the Bataknese smokers studied, the *CYP2A6*1B* allele was found to be the most common allele and showed the highest rate of nicotine metabolism. However, the results show the insignificant relationship among *CYP2A6* genetic polymorphism, nicotine metabolism, and lung cancer incidence.

Introduction

Lung cancer is the largest case of cancer in the world with an increased number of patient at approximately 1.2 million people/year [1], and it also shows an increase in mortality [2]. One cause of lung cancer is from tobacco smoke because of smoking activity with nicotine and nitrosamine as carcinogenic substances contained in it [3]. Carcinogenic substances in cigarettes require metabolic activation which is carried by enzymes in a human body.

Cytochrome P450 (CYP) is a superfamily of heme-containing monooxygenases involved in the metabolism of drugs, environmental pollutants, and food materials which contain chemical compounds and endogenous compounds [4]. Among the family genes of *CYP2A*, only the *CYP2A6* gene encodes active proteins while the other genes are catalytically defective enzymes [5]. *CYP2A6* gene is also

responsible for the metabolism of carcinogenic substances of tobacco [6] [7].

One way to detect the enzyme capacity of CYP2A6 in metabolising tobacco substances, especially nicotine, is by investigating the nicotine metabolite ratio (NMR) between cotinine (COT) and trans-3'-hydroxycotinine (3HC). As known, nicotine in tobacco is metabolized into cotinine (COT) and then into trans-3'-hydroxycotinin (3HC) by CYP2A6 enzymes [8] [9]. The capacity of CYP2A6 enzyme based on NMR can be used to show the levels of body needs in maintaining the nicotine levels. In this case, there are two types of metabolism which occur, namely fast metabolizer and slow metabolizer. The fast metabolizer signifies a high level of need in maintaining the nicotine levels in the body, so it leads to increased consumption of cigarette and a higher risk of lung cancer whereas the slow metabolizer signifies the opposite [10].

Related to the above explanation, several studies found that the genetic polymorphisms of *CYP2A6* have a relationship with nicotine metabolism. Su *et al.,* [11] found that enzyme activity was detected higher in *CYP2A6*1* which indicated a high rate of nicotine metabolism. Nakajima *et al.,* [12] revealed a large difference in the cotinine ratio of nicotine in each. In their follow-up studies, they concluded that the difference between individuals in the formation of cotinine from nicotine has a strong association with the genetic polymorphism of the *CYP2A6* gene, and this result was found in 92 healthy Japanese. Also, another study also detected that there was a difference in the nicotine metabolism rate between Korean population and the Japanese population [13]. Similarly, Caraballo *et al.* [14] and Peârez-Stable *et al.,* [15] found a difference in nicotine metabolism between smokers from the Black race and Caucasian race.

Besides the relationship between the genetic polymorphism of *CYP2A6* and nicotine metabolism, Fujieda *et al.,* [16] found that there was a clear relationship between the genetic polymorphism of *CYP2A6* and cancer risk in the Japanese population. Tanner *et al.,* [17] also reported the same findings in which they examined the relationship between the genetic polymorphism of *CYP2A6* with nicotine metabolism and the risk of lung cancer in the American Indian population.

However, several other studies found that there was no apparent relationship between the genetic polymorphism of *CYP2A6* with nicotine metabolism and the risk of developing lung cancer [16] [18] [19] [20] [21]. Based on this, further research on the relationship between the genetic polymorphisms of *CYP2A6* with nicotine metabolism and the risk of lung cancer in only one ethnic group needs to be conducted to obtain more significant results.

Based on the above explanations, this research was aimed to investigate the relationship between genetic polymorphisms of *CYP2A6* in the population of Batak ethnic group with nicotine metabolism and the risk of developing lung cancer. The three allele investigated were *CYP2A6*1A, CYP2A6*1B,* and *CYP2A6*4A.* This based on the study from Oscarson *et al.,* [22] that found those three alleles as the most frequent allele in the Asian population. Also, the Batak ethnic group was chosen due to their tradition to smoke in several traditional ceremonies and their pure genetic inheritance. Thus, this study would give a more significant model of *CYP2A6* genetic polymorphisms associated with nicotine metabolism and the risk of lung cancer.

Methods

This study involved 140 male Bataknese smokers which were recruited from Haji Adam Malik Hospital, USU Hospital, and Elizabeth Hospital in Medan, North Sumatra, Indonesia. The recruited subjects were pure descendants of the Batak ethnicity whose father, mother, and both grandparents were pure Bataknese. All subjects represented 6 sub-ethnics of Batak (i.e. Karo, Pakpak, Toba, Simalungun, Mandailing, and Angkola) [23]. Out of the 140 subjects, 70 smokers who suffered from lung cancer were specified as case samples, and 70 healthy smokers were specified as control samples. All subjects were asked to answer a questionnaire which consisted of structured information about residence, pedigree chart (three breeds of pure Batak ethnicity), occupational history, smoking status, and history of cancer in the family (parents and siblings). Before research, all subjects signed informed consent.

The inclusion criteria for the case sample were lung cancer patients from the Batak ethnic group who have been diagnosed based on cytology or histopathology examination, male lung cancer patients, and smokers. On the other hand, the inclusion criteria for the control sample were individuals who did not have lung cancer matched with the case group according to age, sex, and Batak people who had a smoking history.

The exclusion criteria for the case sample were lung cancer patients who consumed rifampicin, dexamethasone, phenobarbital, methoxsalen (8-methoxypsoralen), tranylcypromine, tryptamine, coumarin, and neonatal thiol, abnormal function of liver and kidney, and were not undergoing chemotherapy while the exclusion criteria for the control sample were healthy individuals who did not consume drugs.

The methods used in this research were purposive sampling and case-control study. The

research was conducted within a period of one year (November 2016 to April 2017), and it has been accepted by the Ethics Committee of the Faculty of Medicine, University of Sumatera Utara.

Nicotine II chewing gum was obtained from Guardian Pharmacia (Singapore). Nicotine, cotinine and 3-hydroxycotinine were purchased from Sigma (St. Louis, Mo). Pure gene deoxyribonucleic acid (DNA) isolation kit was obtained from Promega (Madison, USA). Restriction enzymes were purchased from New England Biolabs (Beverly, Mass). All other chemicals and solvents which had the highest levels were available commercially.

Blood samples from all subjects were collected as much as 2ml, and then stored at -80°C. Genomic DNA was extracted from peripheral lymphocytes using Puregene DNA Isolation Kit (Promega). The *CYP2A6*1A, CYP2A6*1B,* and *CYP2A6*4A* genotypes used the following primer: 2Aex7F (5'-GRCCAAGATGCCCTACATG-3`) and 2A6R2 (5'-AAAATGGGCATGAACGCCC-3`) [24].

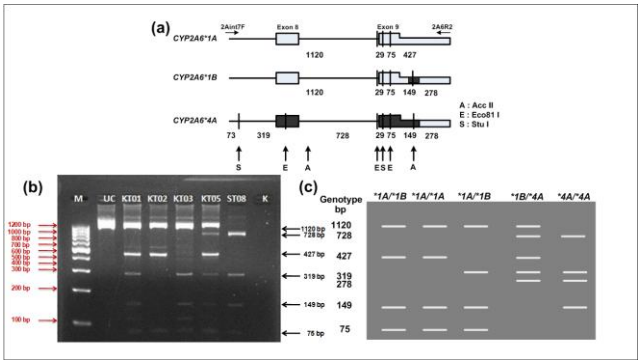

*Figure 1: (a) The CYP2A6*1A, CYP2A6*1B, and CYP2A6*4A allele genotypes by PCR-RFLP. The structure scheme of CYP2A7 and CYP2A6 gene. Dotted boxes and open boxes represented the CYP2A7 and CYP2A6 exon. Lines represented the introns of each gene. The PCR amplification was conducted with a primer pair, shown in horizontal arrows. The amplified DNA was triple-digested by Eco81I, AccII, and StuI. Restriction sites were marked with vertical arrows E, A, and S [33]; (b) Schematic polymerase PCR-RFLP patterns for different of CYP2A6 alleles in the case group 1-5, M = marker, K = control, UC = uncut. The *1A/*1B, 1120, 427, 319, 149, and 75 bp fragments (KT 01). The *1A/*1A, 1120, 427, 149, and 79 bp fragments (KT 02). The *1B/*1B, 1120, 319, 149, and 75 bp fragments (KT 03). The *1A/*4A, 1120, 728, 427, 319, and 278 bp fragments (KT 05). The *4A/*4A, 728, 319, 278, and 129 bp fragments (ST 08). The marker was a 1200 bp ladder marker; (c). Representative photograph of polymerase chain reaction-restriction fragment length polymorphism patterns for different CYP2A6 alleles of CYP2A6*1A, CYP2A6*1B, and CYP2A6*4*

The genomic DNA samples (0.5 µg) was added with PCR mixtures (25 µl) which contained 1 PCR buffer, 1.5 mM $MgCl_2$, 0.4 µM of each primer, 250 µM dNTPs, and 1 U of Taq DNA polymerase. After initial denaturation at a temperature of 95°C for 1 minute, the application was carried out with denaturation at 95°C for 15 seconds, annealing at 60°C for 20 seconds, and extension at 72°C for 3 minutes for 35 cycles, followed by a final extension at 72°C for 7 minutes. The PCR product was triple-digested with restriction enzymes, such as *Eco81I, AccII,* and *StuI*. The product was analysed by electrophoresis at 2% of agarose gel [25]. The *CYP2A6*1A, CYP2A6*1B,* and *CYP2A6*4A* allele genotypes obtained from PCR-RFLP, the schematic polymerase PCR-RFLP patterns for different of *CYP2A6* alleles, and the representative photograph of PCR-RFLP patterns for different *CYP2A6* alleles are shown in Figure 1.

The research subjects should not smoke within 2 weeks to eliminate nicotine and metabolite from cigarettes. On the next day, after a night of fasting, all subjects were asked to chew nicotine gum (Nicotinell gum which contained 4 mg of nicotine) for 30 minutes, and they should chew for 10 per 30 seconds.

Blood samples were collected from the cubital vein just before and two hours after started to chew, and these samples were stored at -20^{o}C before the analysis. Measurement of cotinine and 3'-hydroxycotinine (3HC) was conducted with *Liquid Chromatography Tandem-Mass Spectrometry* (LC-MS/MS). The ratio of 3HC/cotinine was calculated as the nicotine metabolism index. Rubinstein *et al.,* [26] state that the ratio of 3HC/cotinine for slow metabolizers is < 0.5 while the ratio for fast metabolizers is ≥ 0.5. Intraassay and interassay precision tests had been conducted in the LC-MS/MS equipment used.

Data analysis was performed by using Epi Info-7 software. Conditional Logistic Regression test was run to assess the differences between the two groups on demographic factors. Also, conditional logistic regression test and logistic regression test were also run to investigate the relationship between *CYP2A6* genotype and allele with lung cancer incidence and nicotine metabolism rate. The difference between nicotine metabolism rates and *CYP2A6* allele types was tested with the Kruskal Wallis test and Mann Whitney test. Logistic regression test was conducted to assess the effect of the *CYP2A6* allele on nicotine metabolism rate while conditional logistic regression test was conducted to assess the relationship between nicotine metabolism rate with lung cancer incidence. The ratio of nicotine metabolism rate (3HC/cotinine ratio) between the case group and the control group and its relation to *CYP2A6* allele were analysed with the Mann Whitney test. P value < 0.05 was considered significant.

Results

This study involved 70 lung cancer patients in the case group and 70 healthy people in the control group. The demographic characteristics of all subjects, such as 5 sub-ethnics of Batak (i.e. Toba,

Karo, Simalungun, Mandailing, and Pak Pak), types of cigarette, Brinkman Index (multiplication of smoking duration with number of cigarette per day), types of cancer, and Body Mass Index (BMI) can be seen in Table 1.

Table 1 shows that there was no significant difference in age, sub-ethnics of Batak, and the amount of cigarette consumption expressed in the Brinkman Index between the case group and the control group which means that these factors were not potentially biased in this study. The demographic factors which showed significant differences between the two groups were BMI and the types of cigarette.

Table 1: Demographic characteristics of subjects

		Total	Cases (n = 70)	Control (n = 70)	*P-value*
		N (%)	n (%)	n (%)	
Age	<65	106 (75.7)	52 (74.3)	54 (77.1)	0.69
	≥ 65	34 (24.3)	18 (25.7)	16 (22.9)	
Bataknese Sub ethnic	Toba	94 (67.1)	49 (70)	45 (64.3)	0.09
	Karo	34 (24.3)	13 (18.6)	21 (30)	
	Simalungun	4 (2.9)	1 (1.4)	3 (4.3)	
	Mandailing	7 (5)	6 (8.6)	1 (1.4)	
	Pakpak	1 (0.7)	1 (1.4)	0 (0.0)	
BMI	Underweight	16 (11.4)	6 (8.6)	10 (14.3)	0.01[a]
	Normoweight	59 (42.2)	44 (62.9)	15 (21.4)	
	Overweight	29 (20.7)	10 (14.2)	19 (27.1)	
	Obersity	26 (18.6)	10 (14.2)	26 (37.2)	
Type of cigarette	Mild	29 (20.7)	10 (14.3)	19 (27.2)	< 0.001[a]
	Kretek	66 (47.1)	26 (37.1)	40 (57.1)	
	Mix	45 (32.2)	34 (48.6)	11 (15.7)	
Brinkman Index	Mild	13 (9.3)	6 (8.6)	7 (10)	0.63
	Moderate	53 (37.8)	24 (34.3)	29 (41.4)	
	Severe	74 (52.9)	40 (57.1)	34 (48.6)	
Cytology/ histopathology subtype	Squamous cell ca	5 (7.1)	5 (7.1)	0	NA
	Adenocarcinoma	65 (92.9)	65 (92.9)	0	

[a] significant with Conditional Logistic Regression test (matched based on gender and Batak ethnic group); BMI (body mass index), NA (not available).

After the PCR-RFLP was performed on all research subjects, data of *CYP2A6* genotypes and alleles were obtained as shown in Table 2. The frequency of *CYP2A6* genotypes in both groups did not show significant deviations from Hardy-Weinberg Equilibrium ($p > 0.05$ for the case group and the control group). Among 140 male Batak smokers, the frequencies of *CYP2A6*1A, CYP2A6*1B,* and *CYP2A6*4A* alleles were 44.3%, 48.9%, and 6.8%, respectively.

This is quite surprising because *1A allele is assumed as the wildtype in most populations and other ethnic groups. In contrast, this study found that the highest number of allele found was *1B. The highest number of genotypes found was *CYP2A6 *1A/*1B* with 37.1%, and it was followed by *CYP2A6*1A/*1A* with 24.3%.

There was a significant difference in nicotine metabolism rates among the types of the *CYP2A6* allele. Although the average metabolic rate of all allele groups was classified into the slow metabolizer (ratio of 3HC/cotinine ≤ 0.5), the *1B allele showed the highest metabolic rate compared to other alleles, which was 3 times higher than the *4A allele and 1.5 times higher than the *1A allele.

Table 2: Frequency of CYP2A6 genotype and allele and its relation to nicotine metabolism rate

Genotype	Subjects*	
	n	%
*CYP2A6 *1A/*1A*	34	24.3
*CYP2A6 *1A/*1B*	52	37.1
*CYP2A6 *1B/*1B*	42	30
*CYP2A6 *1A/*4A*	4	2.9
*CYP2A6 *4A/*4A*	8	5.7

Allele	Subjects*		*3HC/cotinine Ratio*[d]	
	n	%		
*CYP2A6 *1A*	124	44.3	0.14 ± 0.31	b
*CYP2A6 *1B*	137	48.9	0.21 ± 0,37	a
*CYP2A6 *4A*	19	6.8	0.07 ± 0.21	c

*All frequencies of *CYP2A6* genotypes were in Hardy-Weinberg equilibrium; [a] $p < 0.001$ with Kruskal Wallis test; [b]$p = 0.02$ with Mann Whitney U test; [c] $p < 0.001$ with Mann Whitney U test; [d] data are expressed in mean ± standard deviation.

To investigate the association between *CYP2A6* genotype and allele with lung cancer incidence and nicotine metabolism rate, a cross-tabulation was conducted as shown in Table 3. Nicotine metabolism rate was measured using a 3HC/cotinine ratio parameter in serum, and it was measured 2 hours after the administration of nicotine gum. A ratio value of 3HC/COT < 0.5 was classified as slow metabolizer while a value ≥ 0.5 was classified as fast metabolizer.

Table 3 shows that there was no significant relationship between *CYP2A6* genotype and an allele with lung cancer incidence and nicotine metabolism rate ($P > 0.05$). One interesting point was that almost all subjects were classified as slow metabolizers, and only 13 people (18.6%) were classified as fast metabolizers. Based on Table 3, individuals with *CYP2A6*1B* allele were 2.07 times more likely to be fast metabolizer if compared with the *1A allele (wildtype). In contrast, individuals with *4A allele were more likely to be slow metabolizer although this relationship was not statistically significant. These findings are in line with the data shown in Table 4 which states that the *1B allele has the highest 3HC/cotinine ratio compared to the other alleles.

Table 3: Association of *CYP2A6* genotype and allele with lung cancer incidence and nicotine metabolism rate

		Lung Cancer		*P-value*	Nicotine Metabolism		*P-value*	OR	95% CI
		Case	Control		Fast	Slow			
CYP2A6 Genotype	**1A/*1A*	15 (21.4)	19 (27.1)	0.374[a]	0(0)	34 (26.5)	0.142[b]	NA[c]	NA
	**1A/*1B*	30 (42.8)	22 (31.4)		7(53.8)	45 (35.1)			
	**1B/*1B*	21 (30)	21 (30)		5(38.5)	37 (28.9)			
	**1A/*4A*	2 (2.9)	2 (2.9)		1(7.7)	3(2.3)			
	**4A/*4A*	2 (2.9)	6 (8.6)		0 (0)	8 (6.2)			
	Total	70 (100)	70 (100)		13 (100)	127 (100)			
CYP2A6 Allele	**1A*	62 (44.3)	62 (44.3)	0.187[a]	8(30.8)	116(45.7)	0.19[b]	1[d]	1
	**1B*	72 (51.4)	65 (46.4)		17(65.4)	120(47.2)		2.07	0.8-4.9
	**4A*	6 (4.3)	13 (9.3)		1 (3.8)	18 (7.1)		0.76	0.09-6.45
	Total	140 (100)	140 (100)		26 (100)	254 (100)			

[a] Conditional Logistic Regression test with matched gender and Batak ethnic group; [b] Logistic Regression test; [c] OR not available; [d] Reference value.

Theoretically, individuals who are fast metabolizers will inhale more nicotine in cigarettes, and thereby increase the risk of lung cancer incidence. Surprisingly, this study found the opposite. Individuals who were slow metabolizers were 5.49 times more likely to develop lung cancer than fast metabolizers (95% CI 1.2 – 24.8; $P = 0.01$), as shown in Table 4. The difference in nicotine metabolism rate between the case group and the control group and its

relationship with *CYP2A6* allele can be seen in Figure 2.

Table 4: Association of nicotine metabolism rate with lung cancer incidence

Nicotine Metabolism	Cancer n (%)	Control n (%)	*P - value*	OR
Fast metabolizer	2 (2.9)	11 (15.7)	0.01[a]	1[b]
Slow Metabolizer	68 (97.1)	59 (84.3)		5.49 (1.2-24.8)
Total	70 (100)	70 (100)		

[a] Significant with Conditional Logistic Regression test; [b] Reference value.

Figure 2 showed that there was no significant difference in the level of nicotine, cotinine, and 3HC (OH-cotinine) in plasma based on *CYP2A6* allele types in both groups (the cancer group and the control group). It indicates that the influence of *CYP2A6* allele lies in the nicotine metabolism rate of the subjects (3HC/cotinine ratio), but it does not affect the rate of nicotine absorption in the body.

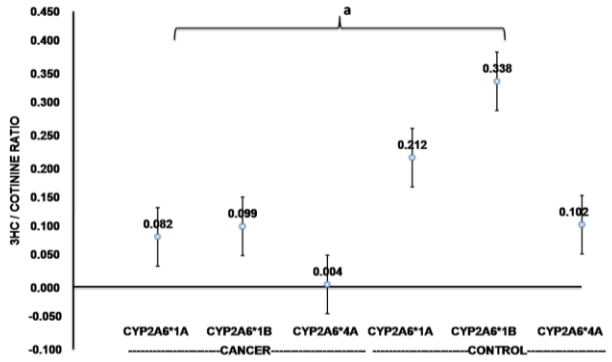

Figure 2: Comparison of nicotine metabolism rate (3HC/cotinine ratio) between the case group and the control group and its relationship with CYP2A6[a] allele. There was a significant difference in the level of 3HC/cotinine ratio between the case group and the control group (P < 0.001 with Mann Whitney test)

Discussion

In this study, the age range of lung cancer patients as the research subjects was 40-64 years. This based on the research conducted by Mong *et al.,* [27] that found that the age of most lung cancer patients was over 60 years (± 51.4%) because exposure to carcinogenic substances took a long time to cause an imbalance between oncogene function and tumor suppressor gene in the growth process of cancer cells, whereas lung cancer found at a young age or below the age of 50 years was associated with genetic factor or started smoking at a young age [28].

The ethnic group studied was Batak as the population of research subjects. In addition to the pure genetic inheritance in Batak, Batak society in North Sumatra has been accustomed to cigarettes, including mild cigarettes, *kretek*, and white cigarettes. Therefore, social environment is highly influential on the smoking behaviour of Batak ethnicity because smoking has an important element in various processes of customary activities. Furthermore, cigarettes must be provided along with food and beverages in every series of traditional event. After the traditional event finishes, cigarettes which are not consumed will be taken back by the event owner. Teenagers and children will usually hide 1-2 cigarettes, and then the cigarettes will be consumed alone furtively or together with their friends. This causes Batak children to have been accustomed to consuming cigarettes since young age [29].

Regarding smoking and different types of cigarette, this study also found that the most common lung cancer found, adenocarcinoma is considered to have a relation to the type of cigarette consumed. This based on the study that mentions that previously the most common type of cytology/histopathology of lung cancer was the squamous cell carcinoma; however, in recent decades, adenocarcinoma has been the most prevalent type of lung cancer. This might occur because of the interest change in the type of cigarette consumed [30]. Thus, in the case of Batak population, *kretek*, which was discovered to be more popular than mild cigarettes contains cloves which make smokers inhale more deeply, so the cigarette smoke will pass through small respiratory tracts which will make them exposed to more carcinogens [31]. Besides, Thun *et al.* [32] state that higher levels of nitrate contained in the tobacco mixture are also associated with the development of adenocarcinoma type of cancer cells. Stellmann *et al.,* [33] also state that extremely high levels of nitrosamines in the peripheral respiratory tract would trigger the occurrence of cell changes into adenocarcinoma. Thus, those results are also by the fact that tobacco-specific N-nitrosamines (TSNAs) induce adenocarcinoma type of lung cancer [34].

Interindividual variation associated with lung cancer risk is determined by uptake variation and metabolism of carcinogenic tobacco substances. The uptake of carcinogenic substances is related to the uptake of nicotine (the addictive component of tobacco). Previous research has found that pulmonary tumorigenesis can be triggered by the anti-apoptotic effect of cotinine through the activation of PI3K/Akt pathway mediated by CYP2A6 [35]. CYP2A6 in smokers is the main enzyme in nicotine metabolism. Nicotine will be metabolized into cotinine (COT), and COT will be metabolized into trans-3-hydroxycotinine (3HC) with the help of CYP2A6 enzymes. If the smoker is a slow metabolizer of nicotine, he only smokes a few cigarettes; thus, the level of exposure to carcinogenic materials will be low. Also, it is also reported that plasma cotinine concentrations, which are the main metabolite of nicotine, play an important role in the development of lung cancer incidence [36]. In the wildtype of *CYP2A6*, the risk of lung cancer incidence will increase. However, if gene deletion *CYP2A6*4* allele is found, it can reduce lung cancer incidence.

The most common *CYP2A6* allele in both groups in this study was *1B. *CYP2A6*1B* gene allele gives a high metabolic index, faster than the effect of *1A allele. Oscarson *et al.,* [22] which discovered the frequency of *CYP2A6*1B* allele was 30.0% in Caucasians (Spaniards) and 40.6% in Asian (Chinese) populations, found that the *CYP2A6* genetic polymorphism might be associated with lung cancer in the American and Asian populations, but it is not the same for the Caucasian population. This suggests that different ethnic will result in a different relationship between the two. In Batak ethnic population, the *1B allele was found about 48.9% higher than some other research population, but no significant relationship was found between *CYP2A6* genotype and allele with lung cancer incidence (P > 0.05). A different result was found by Fujieda *et al.,* [16] which stated that *CYP2A6* is not only one of the major determinants which do affect not only smoking behaviour, but also individual's susceptibility to lung cancer for Japanese Men population.

Several studies have also investigated the relationship between deleted polymorphisms, *CYP2A6*4,* and the risk of lung cancer in different populations. However, the results are inconsistent. One study in Japan reported a 50% reduction in cancer risk related to *CYP2A6*4* statistically while a similar study in China found a twofold increase in lung cancer risk in individuals with *CYP2A6*4.* Several meta-analyses reported a statistically significant 50% lower crude odds ratio in lung cancer with slow metabolizer smokers in the Asian populations. By our results, *CYP2A6*4* was associated with an approximately 30% decreased the risk of lung cancer [18].

The results of this study showed that there was no significant relationship between *CYP2A6* genotype and allele with nicotine metabolism rate (*P* > 0.05). One interesting thing found in this study was that almost all subjects were classified as slow metabolizers, and only 13 people (18.6%) were classified as fast metabolizers. However, there was a significant difference in nicotine metabolism rates among the types of the *CYP2A6* allele. The average metabolic rate of all allele groups was classified as the slow metabolizer (3HC/cotinine ratio ≤ 0.5), but the *1B allele showed the highest metabolic rate compared to other alleles, which was 3 times higher than the *4A allele and 1.5 times higher than the *1A allele. The *1B allele tends to be a fast metabolizer than the *4A allele. Over 20 years ago, individuals who metabolised nicotine poorly would smoke fewer cigarettes a day or less intense per cigarette than smokers who metabolised nicotine more efficiently [18]. This poor metabolism will tend to develop lung cancer because of its lower exposure to carcinogens in nicotine through the smoke. Several studies have reported that smokers who carry a lack of activity or less *CYP2A6* allele would smoke less. However, only in the Asian populations, both Japanese and Chinese, which have a high genetic variant prevalence, have an association between *CYP2A6*, smoking dose, and lung cancer. Theoretically, individuals who are fast metabolizers will inhale more nicotine in cigarettes, and thereby increase the risk of developing lung cancer. Surprisingly, this study found the opposite. Individuals who were slow metabolizers were 5.49 times more likely to develop lung cancer than the fast metabolizers (95% CI 1.2 – 24.8; *P* = 0.01). Based on the above explanation, the relationship between genetic polymorphisms of *CYP2A6* with nicotine metabolism and the risk of developing lung cancer in Batak population was found to have different results from different populations and races. Therefore, further research is important to investigate the differences in other populations and races.

In conclusion, *CYP2A6* activities signified the need for more cigarettes to maintain the levels and the metabolism of nicotine. The most common *CYP2A6* allele in the two groups was *1B. The results showed that no significant relationship was found between *CYP2A6* and nicotine metabolism in lung cancer patients. It was also found that the *1B allele was more likely to be a slow metabolizer than the *1A allele.

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