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Brief Communication

The Role of *CYP2A6* Genetic Polymorphism in Nicotine Dependence and Tobacco Consumption among Bataknese Male Smokers

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Abstract

AIM: This research aimed to analyse the relationship between CYP2A6 gene polymorphism with nicotine dependence and its relation to the number of cigarette consumption among Bataknese smokers.

METHOD: This study was a cross-sectional study involving 140 research subjects in Medan, Indonesia.

RESULTS: Nicotine dependence rates were found to be significantly associated with the number of cigarette consumption expressed in the Brinkman Index.

CONCLUSION: The *1A wild-type alleles have a greater risk of high-very high dependence rate compared to the other variants.

Introduction

Cigarettes are one of the causes of public health problems with an estimated mortality rate of 5 million every year [1]. Nicotine is one of the components of cigarettes which has an important role regarding physical dependence mediated through neuronal nicotinic acetylcholine receptors (nAChRs) [2]. This basic mechanism of physical dependence has been known for a long time. However, there are several other factors which have a role in the pathophysiology of physical dependence on nicotine but cannot be explained in detail as it can be influenced by several multifactorial factors. There are two factors which might influence individuals' physical dependence on cigarettes, namely environmental and genetic factors [3].

The *CYP2A6* gene is a gene encoding the P450 2A6 cytochrome which has a role in the physical dependence on nicotine. Furthermore, it is also responsible for 70-90% of nicotine metabolism in the blood into cotinine; thus, it can eliminate or decrease the effect of nicotine to stimulate the brain reward

system [4]. The nicotine dependence will be further assessed using Fagerstrom Tolerance Questionnaire (mFTQ) [5]. On the other hand, the Brinkman Index is used to identify the cumulative number of smoking habits.

In our previous report, the study about the relationship between genetic polymorphism of *CYP2A6* and nicotine metabolism in male Bataknese smokers with lung cancer was explained. Bataknese smokers, which have pure genetic inheritance, used as participants due to their tradition on smoking [6], it can give a proper model for the study related to the genetic factor and the smoking habit.

Thus, this study intends to analyse the relationship between smoking habits of male Bataknese smokers with the Brinkman Index.

Material and Methods

The subjects of this research were 140 Bataknese male with a history of smoking, active smokers, and the age > 20 years. The participants involved were recruited from Haji Adam Malik Hospital, USU Hospital, and Elizabeth Hospital in Medan, North Sumatra, Indonesia. The nicotine dependence was measured using seven items of questionnaire modified according to Fagerstrom Tolerance Questionnaire (mFTQ) with its scoring ratings. The interpretation of this questionnaire was as follows: (1) very low nicotine dependence indicated with a score of <4; (2) low nicotine dependence indicated with a score of 5-7; (3) moderate nicotine dependence indicated with a score of 8-9; (4) high nicotine dependence indicated with a score of 10-14; and (5) very high nicotine dependence indicated with a score of 15-20. Also, the smoking status was documented through interviews. The subject can be categorised as an active smoker if he has a smoking history ≥ 100 cigarettes throughout his life [7]. The severity level of smoking can be assessed using the Brinkman Index. The Brinkman Index value was obtained from the multiplication of the average number of cigarettes smoked a day and multiplied by the duration of smoking (years). The value of Brinkman Index (IB) is mild if 0-199, moderate if 200-599, and severe if > 600 [8].

Genotyping of *CYP2A6* was conducted using the following primer: 2Aex7F (5'-GRCCAAGATGCCCTACATG-3`) and 2A6R2 (5'-AAAATGGGCATGAACGCCC-3`) [9].

The blood sample from the subject (0.5 µg), which obtained by employing Puregene DNA Isolation Kit (Promega), was added with PCR mixtures (25 µl) (It 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). The initial denaturation was then carried out at 95°C (1 minute). After that, the application was applied with denaturation at 95°C (15 seconds), annealing at 60°C (20 seconds), and extension at 72°C (3 minutes for 35 cycles), followed by a final extension at 72°C (7 minutes). The triple-digestion with restriction enzymes, namely Eco81I, AccII, and StuI, was done on the PCR product. The analysis using electrophoresis at 2% of agarose gel was then applied to the product [10]. Data analysis was performed by using Epi Info-7 software.

Results

Based on the data collected, which also was reported in our previous report, there were 106 subjects aged <65 (75.7%) and 34 subjects aged ≥65 (24.3%) involved in the study. The Brinkman Index obtained was 9.3% for mild, 37.9% for moderate, and 52.9% for severe. Therefore, it was discovered that the average age <65 years was the most commonly found with a severe degree of Brinkman Index value. The nicotine dependence was assessed based on the Fagerstrom score using a special questionnaire. Also, the results showed that 91 people (65%) had a very high Fagerstrom score, 31 people (22.1%) had a high Fagerstrom score, and 18 people (12.9%) had low-moderate Fagerstrom score.

Table 1 showed that individuals with the *1A wild-type alleles were 1.13 times more likely to have high-very high nicotine dependence than the variant alleles (*1B and *4A) although this relationship was not statistically significant.

Table 1: The Relationship between *CYP2A6* Genetic Polymorphism and Nicotine Dependence

		High - Very High		Mild - Moderate		*p-value**	OR	95% CI
		n	%	*n*	%			
CYP2A6 allele type	Wild type (*1A)	109	44.7	15	41.7	0.73	1.13	0.55-2.29
	Variant (*1B and *4A)	135	55.3	21	58.3			
	Total	244	100	36	100			

**Logistic Regression Test.*

Table 2 showed that there was a significant relationship between the nicotine dependence level and the number of cigarettes consumed ($p = 0.015$). It can be seen that the higher the level of nicotine dependence, the more the number of cigarettes consumed.

Table 2: The Relationship between Nicotine Dependence and Brinkman Index

		Severe IB		Moderate IB		Mild IB		*p-value**
		n	%	*n*	%	*n*	%	
Nicotine Dependence	High – Very High	66	89.2	48	90.6	8	61.5	0.015
	Low - Moderate	8	10.8	5	9.4	5	38.5	
	Total	74	100	13	100	53	100	

**Chi-Square test.*

Discussion

The results of this study also indicated that there was a significant relationship between *CYP2A6* genotype and the Brinkman Index. However, this study could not determine which allele was associated with the degree of Brinkman Index.

Based on above results, using cigarette smoking as a paradigmatic substance-use problem, these findings suggest that the pathway to dependence is complex. Both genetic and sociocultural factors play a significant aetiological role at the stages of initiation and dependence. For example, social, environmental factors play a major role in the smoking behaviour of Bataknese because smoking becomes an important element in various cultural activities and as a treat that must be provided with food and beverages in each series of customary activities.

In conclusion, the results of this study showed that individuals with the *1A wild-type alleles had 1.13 times greater risk of severe-very severe nicotine dependence compared to the variant alleles (*1B and *4A) although this relationship was not statistically significant. Furthermore, there was a significant relationship found between *CYP2A6* genotype and the Brinkman Index. However, this study could not determine which allele was associated with the degree of the Brinkman Index.

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