RESEARCH ARTICLE

Significant Genotype Difference in the *CYP2E1 PstI* Polymorphism of Indigenous Groups in Sabah, Malaysia with Asian and Non-Asian Populations

Lucky Poh Wah Goh[1], Eric Tzyy Jiann Chong[1], Kek Heng Chua[2], Jitt Aun Chuah[3], Ping-Chin Lee[1]*

Abstract

 ***CYP2E1 PstI* polymorphism G-1259C (rs3813867) genotype distributions vary significantly among different populations and are associated with both diseases, like cancer, and adverse drug effects. To date, there have been limited genotype distributions and allele frequencies of this polymorphism reported in the three major indigenous ethnic groups (KadazanDusun, Bajau, and Rungus) in Sabah, also known as North Borneo. The aim of this study was to investigate the genotype distributions and allele frequencies of the *CYP2E1 PstI* polymorphism G-1259C in these three major indigenous peoples in Sabah. A total of 640 healthy individuals from the three dominant indigenous groups were recruited for this study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) at G-1259C polymorphic site of *CYP2E1* gene was performed using the *Pst* I restriction enzyme. Fragments were analyzed using agarose gel electrophoresis and confirmed by direct sequencing. Overall, the allele frequencies were 90.3% for *c1* allele and 9.7% for *c2* allele. The genotype frequencies for *c1/c1, c1/c2* and *c2/c2* were observed as 80.9%, 18.8%, and 0.3%, respectively. A highly statistical significant difference (p<0.001) was observed in the genotype distributions between indigenous groups in Sabah with all Asian and non-Asian populations. However, among these three indigenous groups, there was no statistical significant difference (p>0.001) in their genotype distributions. The three major indigenous ethnic groups in Sabah show unique genotype distributions when compared with other populations. This finding indicates the importance of establishing the genotype distributions of *CYP2E1 PstI* polymorphism in the indigenous populations.**

Keywords: *CYP2E1 - PstI* polymorphism - indigenous population - Sabah ethnic groups

Introduction

The human *CYP2E1* gene is the only subfamily of human cytochrome P450 E (Zhuge et al., 2003), located on chromosome 10q24.3-qter. *CYP2E1* plays an important role in the metabolic pathway of toxicants, procarcinogens, drugs and produce high levels of reactive oxygen species that lead to formation of cancerous cell (Nakazawa et al., 1996; Wu and Cederbaum, 1996; McCord, 1998). *CYP2E1* gene contains several genetic polymorphisms identified by RFLPs using restriction enzymes such as, *Dra* I, *Rsa* I, *Pst* I, and *Taq* I. These polymorphisms have been associated with various human cancers such as lung cancer, esophageal cancer, breast cancer, colorectal cancer and cigarette smoking-related hepatocarcinogenicity (Uematsu et al., 1992; Raunio et al., 1995; Wu et al., 1997; Lin et al., 1998; Saeed et al., 2013). *CYP2E1* polymorphisms also had an effect towards drug reactions such as the pathogenesis of anti-tuberculosis drug-induced

hepatotoxicity (ATDH) in several populations (An et al., 2012; Gupta et al., 2013). Despite of the various single nucleotide polymorphisms (SNPs) identified, *Pst* I and *Rsa* I polymorphisms are in complete linkage disequilibrium (Maezawa et al., 1994).

CYP2E1 PstI polymorphism (rs3813867) is a 'G' nucleotide to 'C' nucleotide change at 1259 position in the 5'-flanking region of the gene. This polymorphism can affect the transcription level of *CYP2E1* gene *in vitro* and its enzyme activity (Haufroid et al., 2002). Individuals with at least one *c2* allele and exposed to risk factors increase susceptibility to gastric or colorectal cancer (Cai et al., 2001; Kiss et al., 2007; Feng et al., 2012). However, this polymorphism decreases the risk to esophageal cancer (Haufroid et al., 2002) and lung cancer (Wang et al., 2014).

Population differences in the expression of *CYP2E1* gene that lead to malignancy, therapeutic failure and severe toxicity have been connected to its polymorphism. The genotype distribution and allele frequencies of

*[1]Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, Kota Kinabalu, Sabah, [2]Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur, [3]Department of Surgery, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia *For correspondence: leepc@ums.edu.my*

CYP2E1 polymorphism differ significantly across different populations (Bolt et al., 2003; Shahriary et al., 2012; Lakkakula et al., 2013). In Malaysia, there is a high prevalence of nasopharyngeal cancer in the major indigenous groups and high cases of tuberculosis in Sabah (Rundi et al., 2011). Thereby, understanding of population differences in allele and genotype frequencies have the potential to explain some variability to cancer susceptibility, improve clinical practice, and evaluate variability of drug response in these populations.

Sabah is one of the 13 states in Malaysia, is located in Borneo Island and also known as North Borneo has approximately 3.3 million populations, comprising of 32 ethnic groups, out of 28 are indigenous (Wise, 2008). The three major indigenous ethnics in Sabah are KadazanDusun, Bajau, and Rungus (Hans et al., 2008; Leete, 2008).

The *CYP2E1* polymorphism is well studied in other populations, but very limited study was observed for Malaysian populations. Previous findings of the *CYP2E1* polymorphism association study only include Malays, Chinese, and Indians as they are the three major ethnic groups of Malaysia (Kandasami et al., 2003), but did not include the three major indigenous ethnic groups originated from East Malaysia, Sabah. The present study aimed to provide basic information about the genotype distributions and allele frequencies of *CYP2E1 PstI* polymorphism in the three major indigenous populations in Sabah that could give an insight for the variability of drug response and cancer susceptibility in future.

Materials and Methods

Study population and DNA isolation

Blood samples were collected from 640 subjects who are not genetically related and were selected randomly during several blood donation campaigns from February 2011 till January 2013. Informed consent was obtained from subjects prior to venous blood collection for this cohort study. DNA was isolated from 2 mL of whole blood using alkaline lysis method and stored at -20°C. This study was approved by the Department of Health in Sabah and ethical approval for this study was obtained from University Malaya Medical Centre (UMMC) Ethical Board with Ref. 654.1.

Genetic analysis

The *CYP2E1 PstI* polymorphism (rs3813867) was determined by PCR-RFLP using sense (5'-CCAGTCGAGTCTACATTGTCA-3') and antisense (5'-TTCATTCTGTCTTCTAACTGG-3') primers as previously described (Hayashi et al., 1991). A total of 20 μL PCR reaction mixture contained 100 ng of DNA template, 0.2 μM of each primers, 1.5 mM of MgCl$_2$, 0.2 mM of dNTPs, 1X PCR Buffer, and 1 unit of *Go Taq*® Flexi DNA polymerase (Promega, USA). PCR was performed with the following conditions: 5 min at 94°C, 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 min, followed by final cycle at 72°C for 7 min. The PCR products were subjected to digestion using 5 units of *Pst* I (New England BioLabs® Inc., Ipswich,

MA) restriction enzyme per 15 μL reaction mixture and incubated at 37°C for 16 hours. The digested products were analyzed by electrophoresis in a 2.0% EtBr stained agarose gel in 1X TAE buffer. The presence of restriction site yielded fragments of 120 bp and 290 bp corresponding to *c2/c2* genotype (variant); 410 bp, 120 bp, and 290 bp corresponding to *c1/c2* genotype (heterozygous). A 410 bp corresponding to *c1/c1* genotype (wild-type) was observed in the absence of restriction site. PCR-amplified DNA samples were subjected to direct sequencing using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA) to further confirm the *CYP2E1 Pst* I polymorphism.

Statistical analysis

SPSS Software V17.0 (SPSS Inc., Chicago, Illinois) was used in the statistical analysis. The Hardy-Weinberg equilibrium (HWE) for each population was determined using "Chi-Square" (χ^2) test at 5% significant level and degree of freedom =1. The results of this study were compared with other populations by the same test. A $p<0.05$ is considered statistically significant and $p<0.001$ is highly statistical significant.

Results and Discussion

The 640 subjects consists of 361 KadazanDusun (219 males and 142 females), 154 Bajau (122 males and 32 females), and 125 Rungus (65 males and 60 females). The age of the subjects ranged from 13 to 80 years old with the mean age±S.D. of 28.7±15.4 (Table 1). The genotype distributions for KadazanDusun, Bajau, Rungus, and total sum of the subjects in this study did not deviate from HWE ($p > 0.05$). The heterozygosity and homozygosity of this SNP was confirmed by direct sequencing.

The *c1* and *c2* allele frequencies of *CYP2E1 PstI* polymorphism of this study were 90.3% and 9.7%, respectively. The genotype frequencies of this study were 80.9% for *c1/c1*, 18.8% for *c1/c2*, and 0.3% for *c2/c2* genotypes. The genotype frequency of *c1/c1* was highly observed in these populations followed by *c1/c2* and *c2/c2* genotypes (Table 2). Similar genotype distribution was observed in KadazanDusun, Bajau and Rungus ethnic groups (Table 1). Genotype *c2/c2* was not observed in Bajau and Rungus ethnic groups.

CYP2E1 PstI polymorphism shows variability among different populations and ethnicities backgrounds. This plays a significant contribution to the individual differences in the predisposition of disease development and adverse drug reactions (Costa et al., 2012). *CYP2E1* polymorphisms in different populations have shown a different risk factor towards anti-tuberculosis drug-induced hepatotoxicity (Lee et al., 2010). In addition, susceptibility to disease such as cancer for individuals and ethnic groups can be investigated using genetic markers like SNPs (Danko and Chaschin, 2005). Hence, this is the first study to document the genotype distributions and allele frequencies of *CYP2E1 PstI* polymorphism in the indigenous East Malaysia populations with a large sample size. This study provides the first insight to the future prospects of genetic variation associated with *CYP2E1 PstI* polymorphism in the indigenous Sabah populations.

There were no genotype distributions differences (p>0.001) among the KadazanDusun, Bajau, and Rungus in this study (Table 1). This suggests that they are closely related in their genetic composition which was in line with the population studies based on Alu insertions of the same indigenous groups in Sabah (Kee et al., 2012). Therefore, these ethnic groups could share the same level of disease predisposition and drug metabolizing effects.

The study of *CYP2E1 PstI* polymorphism could be clinically important as the prediction of adverse drug reactions by genotyping of drug-metabolizing enzymes has the potential in drug and dosage determination in the beginning of therapy. The *CYP2E1* gene had shown to encode enzyme that is involved in the metabolism of several drugs such as paracetamol, chlorzoxazone and ethanol (Agundez et al., 1997). Investigators reported that Japanese (Kim et al., 1996) had a lower oral clearance of the chlorzoxazone (*in vivo* probe) when compared with Caucasians. Hence, the indifference of genotype distributions in the three major indigenous groups of this study may also suggest that they could share similar drug reactions or metabolism but differ from Japanese or Caucasian.

Studies on SNPs might reveal valuable population data regarding the relationships that share among different populations (Kayaalti and Soylemezoglu, 2010). In a combination analysis of the indigenous populations in Sabah, the *CYP2E1 PstI* genotype distributions were compared with Asians (Chinese, Korean and Taiwanese) and non-Asians (Brazilian, Caucasian, French and Hungarian) populations. As shown in Table 2, there was a highly statistical significant difference (p<0.001) in the genotype distributions of the indigenous ethnics in Sabah when compared with all Asians and non-Asians populations. This implicates that the evolutionary course changes the distributions of autosomal chromosome *CYP2E1 PstI* SNP in human populations even though the three major indigenous ethnic groups in Sabah could be descendants from Southern China (Kee et al., 2012).

The *c1/c1* genotype frequency of this study (80.9%) was relatively similar to Chinese (Feng et al., 2012) (73.3%), but relatively higher than Taiwanese (Yu et al., 1995) (63.3%) and Korean (Park et al., 2003) (64.8%) populations. However, all non-Asian populations had a significantly higher *c1/c1* genotype frequency (>90%) when compared with Asian and ethnic groups of Sabah in the current study. The *c1/c2* genotype frequency of the indigenous populations in Sabah (18.8%) was less prominent when compare with other Asian populations but relatively similar to the Chinese population (Feng et al., 2012) (23.3%). On the contrary, all Asians populations including the indigenous Sabah populations of this study had a significantly higher *c1/c2* genotype frequency when compared with all non-Asian populations (Caucasian, Hungarian, French, and Brazilians). The *c2/c2* genotype frequency of indigenous ethnics in Sabah (0.3%) was the lowest among Asian populations. An exception of Chinese (Guo et al., 2008) population showed a significantly high *c2/c2* genotype frequency (15.6%) when compared with all other Asian and non-Asian populations. These differences of genotype frequencies in a population play an important role in the development of personalized medicine as different genotype might metabolize drug slightly different.

CYP2E1 polymorphism had been shown to be associated with disease susceptibility and several types of chemical-induced diseases (Wu et al., 1998; Liu et al., 2001; Deng et al., 2014). The *c2/c2* genotype was associated to the increased risk of gastric cancer in Korean (Park et al., 2003) and Chinese (Feng et al.,

Table 1. Distribution of Selected Variables in this Study

	KadazanDusun[a] (N=361), N (%)	Bajau[b] (N=154), N (%)	Rungus[b] (N=125), N (%)	Total (N=640), N (%)
Age				
<50	311 (86.2)	133 (86.4)	112 (89.6)	556 (86.9)
≥50	50 (13.8)	21 (13.6)	13 (10.4)	84 (13.1)
Mean±SD	29.2±15.7	30.9±13.5	24.4±15.7	28.7±15.4
Range	16-80	17-76	13-71	13-80
Gender				
Male	219 (60.7)	122 (79.2)	65 (52.0)	406 (63.4)
Female	142 (39.3)	32 (20.8)	60 (48.0)	234 (36.6)
Allele				
c1	654 (90.6)	285 (92.5)	217 (86.8)	1156 (90.3)
c2	68 (9.4)	23 (7.5)	33 (13.2)	124 (9.7)
Genotype				
c1/c1	295 (81.7)	131 (85.1)	92 (73.6)	518 (80.9)
c1/c2	64 (17.7)	23 (14.9)	33 (26.4)	120 (18.8)
c2/c2	2 (0.6)	0 (0.0)	0 (0.0)	2 (0.3)
HWE (X^2)*	1	0.55	2.89	3.28

*Did not deviate from HWE (X^2<3.84); [a]KadazanDusun as reference; [b]No highly statistical significant difference (p>0.001)

Table 2. *CYP2E1 PstI* Genotype Distributions across Different Populations

	Study	Population	Genotype, N (%)		
			c1/c1	c1/c2	c2/c2
Asian	Present Study	East Malaysian[a]	518 (80.9)	120 (18.8)	2 (0.3)
	Yu et al. 1995	Taiwanese[b]	95 (63.3)	49 (32.7)	6 (4.0)
	Park et al. 2003	Korean[b]	94 (64.8)	48 (33.1)	3 (2.1)
	Guo et al. 2008	Chinese[b]	225 (46.9)	180 (37.5)	75 (15.6)
	Feng et al. 2012	Chinese[b]	374 (73.3)	119 (23.3)	17 (3.3)
	Lakkakula et al. 2013	South Indian[b]	207 (97.6)	5 (2.4)	0 (0.0)
Non-Asian	van der Logt et al. 2006	Caucasian[b]	389 (94.4)	21 (5.1)	2 (0.5)
	Kiss et al. 2007	Hungarian[b]	456 (91.2)	42 (8.4)	2 (0.4)
	Küry et al. 2007	French[b]	1029 (92.0)	88 (7.9)	1 (0.1)
	Rossini et al. 2007	Brazilians[b]	230 (92.7)	15 (6.1)	3 (1.2)
	Catterchio et al. 2008	Caucasian[b]	1162 (93)	85 (7)	0 (0.0)

[a]Indigenous populations in Sabah (KadazanDusun, Bajau and Rungus) as reference; [b]Highly statistical significant difference (p<0.001)

2012) populations. Additionally, *c2* allele was statistically significant associated with the occurrence of colorectal cancer in Hungarian (Kiss et al., 2007) population. The associations of *CYP2E1 PstI* polymorphism in Northern and Southern China for esophageal cancer differs (Wang et al., 2012). The association study of *CYP2E1 PstI* polymorphism in indigenous groups in Sabah differs from other populations as there was a significant difference in their genotype distributions. Therefore, this finding reflects the importance of taking these differences into account when discussing the genetic markers for diseases and drug response in Sabah ethnics and other populations (Persson et al., 1993).

In summary, no genotype distributions difference was observed among the three major indigenous Sabah populations of this study is also supported by genetic differentiation study using Alu insertions analysis in previous study (Kee et al., 2012). However, the genotype distributions of indigenous ethnics in Sabah differ from other Asian and non-Asian populations. This finding highlighted the significant of ethnic and geographical differences in the prevalence of *CYP2E1 PstI* polymorphism. Hence, it is important to establish the genotype distributions and allele frequencies of the indigenous populations in Sabah. The unique genotype distributions in these indigenous ethnic groups revealed in this study signifies the need to establish its own data set. Studies involving larger groups in future will provide more valuable information of the association between *CYP2E1* polymorphisms to diseases susceptibility and drug effects in the different populations.

Acknowledgements

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