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*CYP3A4*18* and *CYP3A5*3* Polymorphisms in Modulating Susceptibility Risk in Malaysian Chronic Myeloid Leukaemia Patients

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ABSTRACT

CYP3A4 and *CYP3A5* are metabolizing enzymes abundantly expressed in liver and involved in the metabolism of xenobiotics as well as clinically used drugs. Genetic polymorphisms in *CYP3A4* and *CYP3A5* may alter the metabolic ability of individuals. Thus, *CYP3A4* and *CYP3A5* might play an important role in the aetiology of chronic myeloid leukaemia (CML) and as modulators of cancer therapy response. In this study, the impact of two single nucleotide polymorphisms (SNPs) *CYP3A4*18* (878T>C) and *CYP3A5*3* (6986A>G) on CML susceptibility risk was investigated. This case-control study involved a total of 520 study subjects comprising 270 CML patients and 250 normal healthy controls. Genotyping of *CYP3A4*18* and *CYP3A5*3* was performed by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique. The association between allelic variants and CML susceptibility risk was assessed by logistic regression analysis, deriving odds ratio (OR) with 95% confident intervals. The results showed that heterozygous (**1/*1*8*) genotype of *CYP3A4*18* was significantly associated with CML susceptibility risk (OR 3.387; 95% CI: 1.433–8.007, p = 0.005). No homozygous variant (**18/*18*) genotype was detected in this study. On the contrary, homozygous variant *(*3/*3*) and heterozygous (**1/*3*) genotypes of *CYP3A5*3* were associated with significantly lower risk for CML susceptibility (OR 0.140; 95% CI: 0.079–0.246' p < 0.001 and OR 0.310; 95% CI: 0.180–0.535, p < 0.001, respectively). The results prompt us to conclude that genetic variation in *CYP3A4*18* may contribute to a higher risk whereas *CYP3A5*3* polymorphism confers a lower susceptibility risk in Malaysian CML patients.

Keywords: *CYP3A4*18; CYP3A5*3; polymorphisms; chronic myeloid leukaemia; susceptibility risk*

INTRODUCTION

Chronic myeloid leukaemia (CML), a myeloproliferative neoplasm, is characterised by the Philadelphia (Ph) chromosome, resulting from a reciprocal translocation between chromosomes 9 and 22 t(9:22)(q34;q11) (Al-Achkar *et al.*, 2013). Philadelphia translocation t(9;22) (q34;q11) is detected in 95% of CML cases (Zheng *et al.*, 2009). This reciprocal t(9:22) translocation transfers the Abelson (ABL) proto-oncogene on chromosome 9 to the breakpoint cluster region (BCR) of chromosome 22, resulting in the formation of a *BCR-ABL* fusion gene (Al-Achkar *et al.*, 2013), which functions as an oncogene. *BCR-ABL* fusion gene encodes for a 210 kDa protein with increased tyrosine kinase activity. The dysregulated tyrosine kinase activity of *BCR-ABL* fusion gene is responsible for the pathogenesis of CML. CML comprises 15%–20% of all adult leukaemias, with a median age of diagnosis of 50 years (Rumjanek *et al.*, 2013), and rarely occurs in children. Among childhood leukaemias, CML is a rare entity with an annual incidence of one case per million children (Nikumbh *et al.*, 2012).

The risk factors for CML are still unclear. Exposures to radiation or radiation therapy, previous chemotherapy for other types of cancer, long term exposure to high levels of environmental carcinogens such as benzene 1–3 butadiene, dioxin, and metals have all been implicated as risk factors for CML. However, not all individuals exposed to these risk factors do develop CML. Furthermore, a few earlier studies (Biernaux *et al.*, 1995; Bose *et al.*, 1998) had demonstrated the presence of very low levels of *BCR-ABL* fusion gene in the blood of healthy people, but who never developed CML. All the above facts reiterate that *BCR-ABL* fusion gene is essential, but not sufficient for the development of CML. Additional predisposing factors, such as host's inherent susceptibility factors might also be important in the development of CML.

Cytochrome P450 (CYP) plays an important role in the bio-activation and inactivation of carcinogens and participates in the activation and inactivation of anticancer drugs. Thus, CYP may play an important role, both in the aetiology of cancer and in the modulation of cancer therapy response. CYP3A subfamily is the most abundantly expressed P450 protein in the human liver and intestine and is the predominant subfamily involved in the metabolism of clinically used drugs as well as environmental carcinogens. The two major *CYP3A* enzymes expressed in the liver are *CYP3A4* and *CYP3A5*.

Common allelic variation in the form of single nucleotide polymorphisms of *CYP3A4* are known to affect the catalytic activity. A common *CYP3A4*18* polymorphism located in the exon 10 of *CYP3A4*, involves nucleotide change from tyrosine (T) to cytosine (C) transition at position 878 and results in amino acid change leucine to proline at codon 293 (Leu293Pro) (Hu *et al.*, 2005; Seong *et al.*, 2013). This polymorphism *CYP3A4*18* (878 T > C) leads to altered enzymatic activity. *CYP3A5* which is located on chromosome 7q21.1, exhibits inter-individual variations in expression levels. A polymorphism of *CYP3A5*3* located in the intron 3 of *CYP3A5* involves nucleotide change from adenine (A) to guanine (G) transition at position 6986. Polymorphism *CYP3A5*3* (6986 A > G) produces a cryptic splice site and encodes for an abnormally spliced mRNA with a premature stop codon. *CYP3A5*3* allele can reduce the expression of *CYP3A5* which leads to drug toxicity and subsequent DNA damage.

Since *CYP3A4* and *CYP3A5* are mostly involved in the metabolism of tyrosine kinase inhibitor drug imatinib mesylate, the gold standard drug in the treatment of CML, our group previously investigated the impact of these two single nucleotide polymorphisms (SNPs) in modulating response to imatinib treatment in Malaysian CML patients (Maddin *et al.*, 2016). SNPs are genetic

variations that also modulate (increase or decrease) the risk of certain diseases including cancer. Individual variations in metabolism of carcinogens account for the differences in susceptibility to cancer and could be an attributable risk factor. Case-control study can detect differences in SNPs pattern in two groups (cases and controls) and thereby indicate which pattern is most likely associated with higher or lower disease-causing risk. So, as further extension of our previous study, it was of interest to investigate whether these two SNPs modulate the susceptibility risk to CML. Hence, this case-control study (involving CML patients as cases and normal healthy individuals as controls) was designed to investigate the contribution of *CYP3A4*18* and *CYP3A5*3* polymorphism in modulating susceptibility risk in Malaysian CML patients.

MATERIALS AND METHODS

Subjects and DNA Extraction

This case-control study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USMKK/PPP/JEPeM [244.3.(4)]) and Ministry of Health, Malaysia (KKM/NHSEC/08/0804/P12-687) which complies with the declaration of Helsinki. The cases were Ph chromosome positive CML patients. The normal controls were healthy volunteers without any signs and symptoms of CML or any other cancer and biologically unrelated to the cases. Subjects were recruited from various hospitals in Malaysia including Hospital Universiti Sains Malaysia, Hospital Raja Perempuan Zainab II, Hospital Pulau Pinang, Hospital Raja Permaisuri Bainun, Universiti Kebangsaan Malaysia Medical Center, Sime Darby Medical Centre and Hospital Umum Sarawak. In this study, a total of 520 study subjects comprising 270 CML patients and 250 normal healthy controls were enrolled.

Peripheral blood (3 ml) was collected after obtaining written informed consents from all the study subjects. Genomic DNA was extracted using a DNA extraction kit, QIAGEN QIAamp® DNA Blood Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Genotyping was conducted at Human Genome Centre, Universiti Sains Malaysia.

Genotyping of *CYP3A4*18* and *CYP3A5*3* Polymorphisms

Genotyping of *CYP3A4*18* and *CYP3A5*3* polymorphisms were performed by using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique. Amplification of *CYP3A4*18* was performed by using forward (5′-CACATCAGAATGAAACCACC3′-) and reverse (5′-AGAGCCTTCCTACATA GAGTCA-3′) primers. For *CYP3A5**3, the primers used were 5′-GGTCCAAACAGGG AAGAAATA-3′- (forward) and 5′-CATGA CTTAGTAGACAGATGAC-3′ (reverse). For both *CYP3A4*18* and *CYP3A5*3* polymorphisms, PCR reactions were carried out in a 25 µl volume of 1X PCR Buffer, 2.0 µM of magnesium chloride ($MgCl_2$), 0.5 µM dNTPs, 0.4 µM of each primer and 1.0 U of AmpliTaq Gold Polymerase. Denaturation was at 95°C for 2 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension step at 72°C for 5 min. The 450 bp PCR products of *CYP3A4*18* and the 293 bp PCR product of *CYP3A5*3* were electrophoresed on a 2% agarose gel at 100 V for 30 min.

Following PCR amplification, 4 µl of 450 bp PCR products of *CYP3A4*18* were digested with 1.0 unit of a restriction enzyme (*MspI)* for 1 hour at 37°C. The digested PCR products were analyzed by electrophoresis on a 2% agarose gel at 90 V for 50 min (Figure 1a). The homozygous wild type allele (**1/*1*) was identified by the presence of an undigested band (450 bp), while heterozygous allele (**1/*18*) was confirmed

by the presence of three fragments at 450 bp, 282 bp and 168 bp. The homozygous variant allele (**18/*18*) showed the presence of two fragments at 282 bp and 168 bp.

On completion of PCR amplification of *CYP3A5*3*, 4 µl of PCR products were digested by restriction enzyme *SspI* for 15 min at 37°C (Figure 1b). The digested PCR products were analyzed by electrophoresis on a 3% agarose gel. The homozygous wild type allele (**1/*1* or AA) was identified by the presence of three bands at 148 bp, 125 bp and 20 bp whereas homozygous variant allele (**3/*3* or GG) was confirmed by the presence of two fragments of sizes 168 bp and 125 bp. The heterozygous variant allele (**1/*3* or AG) was identified by the presence of four bands at 168 bp, 148 bp, 125 bp and 20 bp.

DNA Sequencing

Approximately 10% of the undigested PCR products were randomly selected for DNA sequencing to confirm the polymorphic genotypes inferred from RFLP analysis (Figure 2). The PCR products were purified by using a QIAquick PCR purification kit (QIAGEN) and PCR products of sufficiently good quality and quantity were outsourced to First BASE Laboratories Sdn Bhd (Kuala Lumpur, Malaysia) for DNA sequencing.

Statistical Analysis

The frequencies of polymorphic genotypes among CML patients and normal healthy control subjects were compared by using Chi-square test (χ^2). The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a binary logistic regression to investigate the risk association of genotypes with CML susceptibility risk. All statistical tests were two sided, and statistical significance was determined as $p < 0.05$. SPSS v.20.0 (SPSS Inc., Chicago, IL, United States) was utilised. Deviation of the genotypic distribution from Hardy Weinberg equilibrium (HWE) was examined by using a Chi-square goodness of fit test.

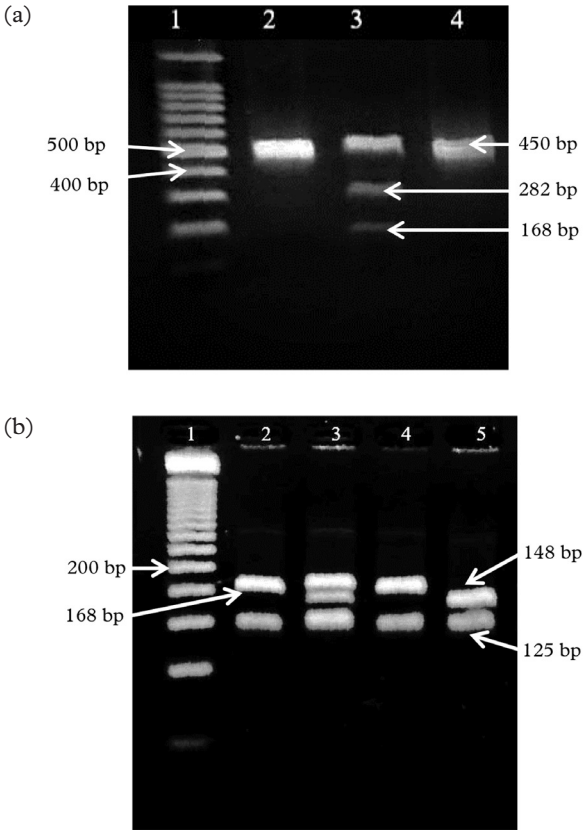

Fig. 1 Gel picture showing different genotype patterns of **a** *CYP3A4*18* (following digestion with *MspI*) and **b** *CYP3A5*3* (following digestion with *SspI*). **a** *Lane 1* shows a 100 bp ladder. *Lanes 2* and *4* shows a homozygous wild type genotype. *Lane 3* indicates a heterozygous genotype. **b** *Lane 1* shows 50 bp ladder. *Lanes 2* and *4* indicates homozygous variant genotype. *Lane 3* indicates heterozygous genotype. *Lane 5* shows homozygous wild type genotype.

RESULTS

The genotypic distribution of the two polymorphisms among the control did not deviate significantly from HWE, $p > 0.05$ (Table 1). All subjects (270 CML patients and 250 normal healthy controls) were successfully genotyped by PCR-RFLP technique. The genotype frequencies and the association of *CYP3A4*18* and *CYP3A5*3* polymorphisms with CML susceptibility risk are shown in Table 2 and Table 3, respectively. In this study, no homozygous variant (**18/*18*) genotype was

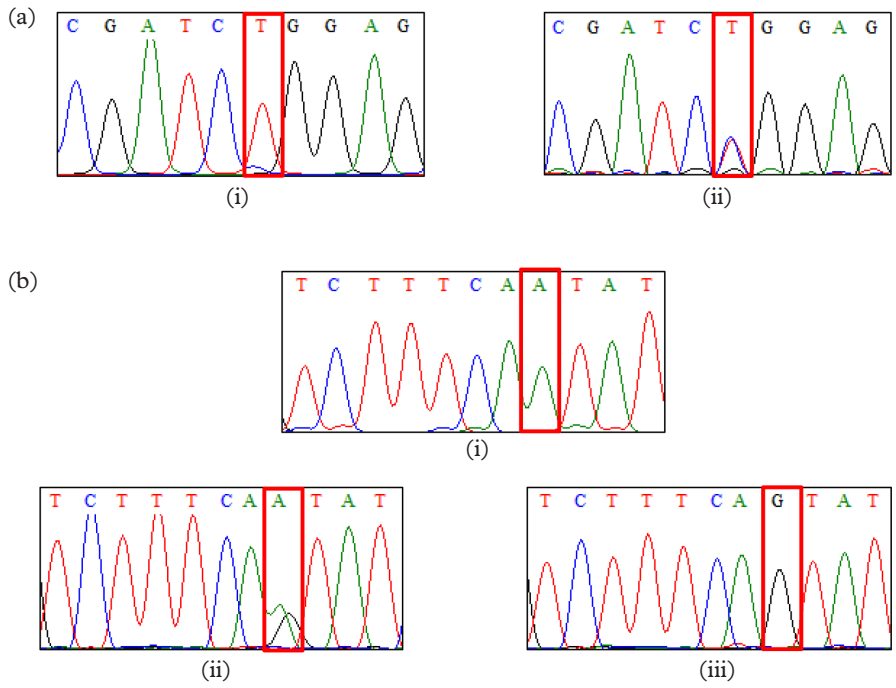

Fig. 2 Sequencing results for **a** *CYP3A4*18* and **b** *CYP3A5**. **a** (i) a homozygous wild type genotype. (ii) a heterozygous genotype. **b** (i) a homozygous wild type genotype. (ii) a heterozygous genotype. (iii) a homozygous variant genotype.

observed for *CYP3A4*18* polymorphism in both CML patients and normal healthy controls. The genotype frequencies of heterozygous (**1/*18*) genotypes were significantly (p = 0.003) higher in CML patients (8.89% in CML patients vs 2.80% in normal healthy controls). However, the frequencies of homozygous wild type (**1/*1*) genotypes were significantly (p = 0.041) higher in normal healthy controls (97.20% in normal healthy controls vs 91.11% in CML patients). When the association of genotype with CML susceptibility risk was assessed, the heterozygous (**1/*18*) genotype of CYP3A4 showed significantly higher risk (OR 3.387; 95% CI: 1.433-8.007, p = 0.005) for CML development.

Table 1 Hardy-Weinberg Equilibrium test in controls

Polymorphisms	Chi square (χ^2)	*p*-value
*CYP3A4**18	0.050	0.822
*CYP3A5**3	0.034	0.853

For *CYP3A5*3* polymorphism, the frequencies of homozygous variant (**3/*3*) genotypes were significantly higher in normal healthy controls (49.60% in normal healthy controls vs 24.44% in CML patients, p < 0.001). However, the frequencies of homozygous wild type (**1/*1*) genotypes were significantly higher in CML patients (29.63% in CML patients vs 8.40% in normal healthy controls) with p < 0.001. There was no significant difference in the frequencies of heterozygous (**1/*3*) genotype between cases and controls (45.93% in CML patients vs 42.00% in normal healthy controls, p = 0.368). On evaluating the association of genotypes with CML susceptibility risk, the homozygous variant (**3/*3*) and heterozygous (**1/*3*) genotypes of *CYP3A5* showed significantly lower risk (OR 0.140; 95% CI: 0.079–0.246, p < 0.001 and OR 0.310; 95% CI: 0.180–0.535, p < 0.001, respectively).

Table 2 Genotype and allele frequencies of *CYP3A4*18* and *CYP3A5*3* in CML patients and normal healthy controls

SNP (rs number)		Genotype frequencies		
		CML patients n = 270 (%)	Healthy controls n = 250 (%)	*p*-value
*CYP3A4*18* (rs28371759)	Genotype			
	Homozygous wildtype (**1/*1*)	246 (91.11)	243 (97.20)	**0.041***
	Heterozygous (**1/*18*)	24 (8.89)	7 (2.80)	**0.003***
	Homozygous variant (**18/*18*)	-	-	-
	Allele			
	*1	516 (95.56)	493 (98.60)	
	*18	24 (4.44)	7 (1.40)	**0.004***
*CYP3A5*3* (rs776746)	Genotype			
	Homozygous wildtype (**1/*1*)	80 (29.63)	21 (8.40)	**< 0.001***
	Heterozygous (**1/*3*)	124 (45.93)	105 (42.00)	0.594
	Homozygous variant (**3/*3*)	66 (24.44)	124 (49.60)	**< 0.001***
	Allele			
	*1	284 (52.59)	147 (29.40)	
	*3	256 (47.41)	353 (70.60)	**< 0.001***

*$p < 0.05$ is statistically significant

Table 3 Genotype frequencies and association of *CYP3A4*18* and *CYP3A5*3* polymorphisms with CML susceptibility

SNP (rs number)	Genotype	Genotype frequencies		Susceptibility risk	
		CML patients n = 270 (%)	Healthy controls n = 250 (%)	OR (95% CI)	*p*-value
*CYP3A4*18* (rs28371759)	Homozygous wildtype (**1/*1*)	246 (91.11)	243 (97.20)	1.000	-
	Heterozygous (**1/*18*)	24 (8.89)	7 (2.80)	3.387 (1.433–8.007)	**0.005***
	Homozygous variant (**18/*18*)	-	-	-	-
*CYP3A5*3* (rs776746)	Homozygous wildtype (**1/*1*)	80 (29.63)	21 (8.40)	1.000	-
	Heterozygous (**1/*3*)	124 (45.93)	105 (42.00)	0.310 (0.180–0.535)	**< 0.001***
	Homozygous variant (**3/*3*)	66 (24.44)	124 (49.60)	0.140 (0.079–0.246)	**< 0.001***

*$p < 0.05$ is statistically significant

In the present study, we also evaluated the association of the polymorphic genotype-genotype combinations (Table 4) of *CYP3A4*18* and *CYP3A5*3* with CML susceptibility risk. The results showed that the combinations of wild type genotype of *CYP3A4*18* and heterozygous genotype of *CYP3A5*3* as well as combination of wild type genotype of *CYP3A4*18* and homozygous variant genotype of *CYP3A5*3* posed a significantly lower risk for CML development.

DISCUSSION

Majority of cancers develop as a result of interaction between environmental factors and hosts's inherent genetic susceptibility. Although *BCR-ABL* fusion is an essential mechanism needed, other predisposing cellular or molecular events are also required for chronic myeloid leukaemogenesis. CYP450 enzymes are involved in activation of a number of exogenous pro-carcinogens into highly reactive electrophilic carcinogenic

Table 4 Association of genotype combinations of *CYP3A4*18* and *CYP3A5*3* with CML susceptibility risk

Polymorphisms combinations		CML patients n = 270 (%)	Normal healthy controls n = 250 (%)	OR (95% CI)	*p*-value
CYP3A4*18	***CYP3A5*3***				
Wild type (**1/*1*)	Wild type (**1/*1*)	74 (27.41)	20 (8.00)	1.000	-
Wild type (**1/*1*)	Heterozygous (**1/*3*)	109 (40.37)	102 (40.80)	0.289 (0.164–0.507)	**< 0.001***
Wild type (**1/*1*)	Variant (**3/*3*)	61 (22.59)	121 (48.40)	0.136 (0.076–0.244)	**< 0.001***
Heterozygous (**1/*18*)	Wild type (**1/*1*)	6 (2.22)	1 (0.40)	1.622 (0.184–14.258)	0.663
Heterozygous (**1/*18*)	Heterozygous (**1/*3*)	14 (5.19)	3 (1.20)	1.261 (0.330–4.823)	0.743
Heterozygous (**1/*18*)	Variant (**3/*3*)	6 (2.22)	3 (1.20)	0.541 (0.124–2.354)	0.412
Variant (**18/*18*)	Wild type (**1/*1*)	-	-	-	-
Variant (**18/*18*)	Heterozygous (**1/*3*)	-	-	-	-
Variant (**18/*18*)	Variant (**3/*3*)	-	-	-	-

*$p < 0.05$ is statistically significant

molecules (Indulski and Lutz, 2000). These electrophiles can bind to DNA and form adducts leading to mutations in proto-oncogenes and tumor suppressor genes, and initiate carcinogenesis if not repaired by the DNA repair system. Genetic polymorphisms have emerged in recent years as important determinants of disease susceptibility and severity. Genetic variations in genes encoding Phase I and Phase II xenobiotic enzymes have been linked with the variation in susceptibility of different individuals/ethnic groups/populations towards leukaemia (Jamil and Reddy, 2007). *CYP3A* family has been found to be abundantly expressed in the human liver and is the predominant subfamily involved in the metabolism of clinically used drugs which participate in the metabolic activation and metabolism of several carcinogens. Interindividual variation in *CYP3A* activity might have a major impact on pharmacokinetics and metabolism of majority of xenobiotics. *CYP3A4* and *CYP3A5* acts as synergistic defense mechanism against the intrusion of xenobiotics. To the best of available knowledge, this is the first study investigating the contribution of *CYP3A4*18* and *CYP3A5*3* polymorphisms in modulating the susceptibility risk in Malaysian CML patients.

CYP3A4 enzymes are involved in the detoxification of foreign chemicals (such as carcinogens) and the metabolism of drugs. In earlier studies, the variant allele *CYP3A4*18* was detected at a frequency of 1.7% in healthy Korean population (Lee *et al.*, 2007), 2% in healthy Chinese population (Dai *et al.*, 2001), 1.3% in healthy Japanese population (Yamamoto *et al.*, 2003) and 2.07% among Malaysian diabetics (Ruzilawati *et al.*, 2007). In the present study, the frequency of *CYP3A4*18* variant allele was detected at 4.44% in Malaysian CML patients and 1.40% in normal healthy controls. As for *CYP3A5*3*, the variant allele was detected at frequency of 70% in Singaporean breast cancer (Lim *et al.*, 2011), 67% in healthy Vietnamese subjects (Veiga *et al.*, 2009), 47.41% in Malaysian CML patients and 70.60% in normal healthy controls. The allele frequencies of *CYP3A4*18* and *CYP3A5*3* were checked and found to be in HWE (Table 1).

The present study showed that the heterozygous (**1/*18*) genotype was significantly associated with higher risk (OR 3.387; 95% CI: 1.433–8.007, $p = 0.005$) for CML development. It is reasonable to suggest that polymorphism of *CYP3A4*18* may act synergistically with the *BCR-ABL* fusion oncogene in causing CML. Earlier, Kang *et al.* (2009) reported that codon 293 is located at the start of the highly conserved helix I. Change of a single amino acid, L293P, at the beginning of helix I influences the overall protein structure and this leads to the modification of the arrangement of substrate recognition sites (SRS) regions, the important sites for substrate recognition, and substrate access to the active site (Kang *et al.*, 2009). The conformational change in *CYP3A4*18* may lead to alteration of metabolic activity, depending on substrate types (Maekawa *et al.*, 2010). So, it is reasonable to suggest that individuals with the heterozygous genotype (**1/*18*) of *CYP3A4* have decreased ability to detoxify carcinogens and have a greater risk of developing CML, compared to the individuals who have homozygous wild type genotype which efficiently detoxifies the carcinogens. A study reported that polymorphism of *CYP3A4*18* was not associated with pediatric tuberculosis risk in Chinese population (Feng *et al.*, 2012).

On the contrary, the homozygous variant (**3/*3*) and heterozygous (**1/*3*) genotypes of *CYP3A5*3* were found to be associated with a significantly lower risk for CML susceptibility with OR 0.140; 95% CI: 0.079–0.246, $p < 0.001$ and OR 0.310; 95% CI: 0.180–0.535, $p < 0.001$, respectively. Since the frequency of variant genotype (**3/*3*) was higher in normal healthy controls (49.60% vs 24.44%), it is suggested that this variant allele may protect an individual from the harmful effects of carcinogens thereby lowering the CML susceptibility risk. Our study is in concordance with a study in Taiwanese population by Liu *et al.* (2002) and in a North Indian population by Bajpai *et al.* (2010) which showed that the polymorphism of *CYP3A5*3* was not associated with the risk of CML development. However, our study is contradictory with another Indian study by Sailaja *et al.* (2010), which found association of *CYP3A5*3* polymorphism with higher risk of CML development. In a different study, homozygous variant (**3/*3* or GG) genotype of *CYP3A5* (6986A > G) was found to be significantly associated with increased susceptibility to ischemic stroke and atherothrombotic events in stroke patients by interacting with *ALOX5AP*-SG12S114A > T (Yi *et al.*, 2015). Other than that, a study by Feng *et al.* (2012) reported that the variants of *CYP3A5* 6986A > G contributed to protection from pediatric tuberculosis in China.

Although some polymorphisms do not show any significant associations when acting individually, risk association may still remain possible when the genotypes and alleles of different polymorphisms are analysed in combination form. When the genotype combinations of *CYP3A4*18* and *CYP3A5*3* were assessed, the combinations of *CYP3A*1/*1* + *CYP3A5*1/*3* and *CYP3A4*1/*1* + *CYP3A5*3/*3* showed significantly lower risk (OR 0.289; 95% CI: 0.164–0.507, $p < 0.001$ and OR 0.136; 95% CI: 0.076–0.244, $p < 0.001$, respectively) for CML susceptibility. Although the heterozygous genotype of *CYP3A4*18* previously showed higher risk for development of CML, no significant association with CML susceptibility risk was detected when the genotype was in combination with *CYP3A5*3*. It can be suggested that individuals who have *CYP3A5*3* polymorphism may have lower risk in development of CML. However, the mechanism on how these combinations can lower CML susceptibility risk is unclear. Hence, further studies may be needed to give a clearer explanation on this aspect.

The results of the present study are in agreement as well as in contradiction with few other studies. This could be due to difference in the genetic background of study subjects, variation in sample size and study

design, variation and intensity in exposure to different types of environmental carcinogens and gene-gene interactions. Our study has few limitations. Other risk factors such as exposure to carcinogens or lifestyle habits which might affect the polymorphisms of *CYP3A4*18* and *CYP3A5*3* in association with CML development was not accounted in this study. Other than that, study on correlation of variant allele with expression of *CYP3A4*18* and *CYP3A5*3* gene that is linked to the development of CML also was not investigated. The results derived by taking into consideration of all these limitations, might give a better explanation for CML susceptibility.

CONCLUSION

The results prompt us to suggest that genetic variations of *CYP3A4*18* may contribute a higher risk whereas *CYP3A5*3* polymorphism might contribute to a lower risk for CML susceptibility in Malaysian subjects.

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