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Impact of *CYP3A4* and *CYP3A5* single nucleotide polymorphisms on anastrozole-associated adverse events among Malaysian breast cancer patients

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Abstract. The catalytic activity of the cytochrome P450A (CYP3A4) enzyme is reportedly affected by the presence of single nucleotide polymorphisms (SNPs), leading to inter-individual variability in drug efficacy and adverse reactions. *CYP3A4* polymorphisms can serve as potential biomarkers for predicting the efficacy of many drugs, including those used in breast cancer treatment. This study was conducted on 94 hormone receptor-positive postmenopausal breast cancer patients who received 1 mg of anastrozole per day. Anastrozole-associated adverse events (AAAEs), such as musculoskeletal adverse events (MSAEs), hot flashes, mood disturbance and vaginal dryness/dyspareunia, were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was performed to determine the allelic frequency of *CYP3A4*4, CYP3A4*18A, CYP3A4*18B*, *CYP3A4*22* and *CYP3A5*3*. The frequencies of *CYP3A4*18A* T>C (rs28371759), *CYP3A4*18B* G>A (rs2242480) and *CYP3A5*3* were 0.03, 0.48 and 0.64, respectively. However, no *CYP3A4*4* A>G (rs55951658) or *CYP3A4*22* C>T (rs35599367) alleles were detected. No significant association was observed between the alleles and the development of AAAEs. We have demonstrated for the first time that *CYP3A4*18B* G>A is highly prevalent among Malaysian breast cancer patients. The clinical relevance of *CYP3A4*18B* is currently under investigation by our group.

Keywords: *CYP3A4, CYP3A5*, breast cancer, PCR-RFLP, anastrozole, adverse events

INTRODUCTION

Worldwide, breast cancer is the second most common cancer and by far the most frequent cancer in women, with an estimated 1.7 million cases in 2012. However, in terms of mortality, it ranks 5th, a development believed to be a result of its fairly favourable prognosis (Ferlay *et al.*, 2015). Recent advances in the early detection and treatment of breast cancer have led to a significant increase in the number of survivors, with the 5-year survival rate reaching almost 90% (Siegel *et al.*, 2014). However, a significant proportion of breast cancer survivors suffer several side effects that potentially impair their quality of life, including anastrozole-associated adverse events

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such as musculoskeletal symptoms, hot flashes, vaginal dryness/dyspareunia and mood disturbances (Cella *et al.*, 2006; Mouridsen, 2006; Burstein, 2007; Rocha-Cadman *et al.*, 2012; Kyvernitakis *et al.*, 2014; Stearns *et al.*, 2015).

Anastrozole is a selective third-generation aromatase inhibitor (AI) established as one of the drugs of choice in adjuvant therapy for postmenopausal breast cancer as well as in advanced-stage breast cancer (Ingle and Suman, 2005; Ingle, 2006). Although anastrozole has been shown to be superior and more effective than tamoxifen (Forbes *et al.,* 2008), a significant number of patients still present with large inter-individual variability in tolerability, resulting in serious adverse effects including musculoskeletal complaints and hot flashes, which occasionally leads to patients' withdrawal from treatments (Mouridsen, 2006; Ingle *et al.,* 2010a). This inconsistency has been attributed to inter-individual variability in the pharmacokinetics and/or pharmacodynamics of anastrazole, partly attributable to genetic variations (Abubakar *et al.,* 2014) and other undetermined factors.

Emerging evidence suggests that genetic variation in *CYP3A4* results in functional changes that may alter the activity of the *CYP3A4* enzyme, resulting in inter-patient variability in response to medication (Zanger and Schwab, 2013; Jin *et al.,* 2015). The current database of *CYP3A4* allele nomenclature (http://www.cypalleles.ki.se/cyp3a4.htm) shows that the wild type *CYP3A4*1* allele constitutes 18 subtypes (*CYP3A4*1A-T*). There are also an additional 28 alleles designated as *CYP3A4*2* to *CYP3A4*26* with *CYP3A4*15, CYP3A4*16* and *CYP3A4*18* each having the "B" subtype. Interestingly some of these alleles have been shown to influence enzymatic activity (Werk and Cascorbi, 2014).

Two major *CYP3A5* alleles (*CYP3A5*3* and *CYP3A5*6*) are associated with functional changes in the *CYP3A5* enzyme (Zanger and Schwab, 2013). *CYP3A5*3* (rs776746), with a 6986A>G in intron 3, is the most frequent allele with a reported frequency of 0.12-0.35 in Africans and 0.88-0.97 in Caucasians (Werk and Cascorbi, 2014). Interestingly, its frequency among Malaysians varies based on ethnicities, with frequencies of 0.59 (Malays), 0.72 (Chinese) and 0.50 (Indians) (Hamzah *et al.,* 2014). Recent findings among Malaysians indicate that the presence of the CYP3A5*3 allele can influence the pharmacokinetics of tacrolimus in renal-transplant patients (Hamzah *et al.,* 2014; Mac Guad *et al.,* 2016).

The present study investigates the allelic frequencies of *CYP3A4*4, CYP3A4*18A, CYP3A4*18B, CYP3A4*22* and *CYP3A5*3*. This is the first study to report the presence of the *CYP3A4*18B* allele and its potential impact on anastrozole-associated adverse events in the Malaysian population, in particular in Malaysian breast cancer patients.

MATERIALS AND METHODS

Study population. A total of 94 unrelated breast cancer patients were recruited for this study. The patients attended the Oncology Clinic, Universiti Sains Malaysia, Kelantan, Malaysia between April 2014 and April 2015. The research protocol was approved by the Human Research Ethical Committee of the Universiti Sains Malaysia (USMKK/PPP/JEPeM [260.3.(21)]) and complied with the Declaration of Helsinki. The subjects were postmenopausal women with histologically confirmed hormone receptor-positive stage I, II or III breast cancer based on the American Joint Committee on Cancer (AJCC) staging manual (Sixth Edition). All patients received 1 mg/day of anastrazole. Patients who previously received tamoxifen and were switched to anastrazole (for at least four weeks) at the time of enrolment were also included. However, patients taking hormone replacement therapy (HRT) with an underlying psychiatric illness, chronic liver disease or renal disease were excluded. The study protocols were explained to the patients, and only those who gave informed consent were enrolled.

Patients' demographic data, such as age, marital status, occupational status, educational level, age of menopause, years since menopause, age at time of breast cancer diagnosis, family history of breast cancer and history of contraceptive use, were ascertained. Other variables, such as weight and height, were recorded at the clinic during a routine follow-up visit. Clinical variables, such as cancer stage,

tumour grade, human epidermal growth factor (HER2) status, current anastrazole use, time since anastrazole was administered and the number of comorbidities, were also derived from each patient's case folder and verified by an oncologist for quality control.

Anastrozole-associated adverse events. Patient-reported anastrozole-associated adverse events (AAAEs) were assessed according to the National Cancer Institute's (NCI's) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (CTCAE, 2010). The musculoskeletal adverse events (MSAEs) assessed included joint, muscle and/or bone pain. The case definition for MSAEs was modified from a previous report (Ingle *et al.*, 2010b). Hot flashes and sweating constitute the vasomotor symptoms evaluated in this study, as reported elsewhere (Stearns *et al.*, 2015). Other AAAEs assessed in this study include mood disturbance and vaginal dryness (dyspareunia)

Blood sample collection and DNA isolation. Peripheral blood (1 mL) was collected in EDTA tubes (BD Franklin Lakes, NJ USA) and was stored at -20°C until use. Genomic DNA was extracted from whole blood using a QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The purity of the DNA and its concentration were determined using an Infinite® 200 NanoQuant (Tecan, Switzerland).

PCR-RFLP. PCR to amplify *CYP3A4*4* was performed as reported previously (Ruzilawati *et al.*, 2007) with some modifications. Briefly, the PCR protocol was carried out in a total volume of 25 µl consisting of 1 X buffer, 0.2 mM of dNTP, 2.0 U of Taq polymerase, 0.2 µg of DNA template and 0.2 µM of *4_F and *4_R primers (Table 1). The buffers, dNTPs and Taq polymerase used in all the PCR reactions were supplied by New England Biolabs® Inc., MA, USA. The cycling protocol consisted of an initial denaturation step at 94°C for 30 s, followed by 35 cycles of 30 s at 94°C, 45 s at 65.3°C and 45 s at 68°C. The final extension step was performed at 68°C for 5 min. The PCR product (244 bp) (Figure 1) was digested with 2.0 U of *BsmAI*, followed by incubation for 60 min at 55°C. The PCR reaction to amplify *CYP3A4*18A* was prepared in a total reaction volume of 25 µl that consisted of 1 X PCR buffer, 0.2 mM of dNTP, 2.0 U of Taq polymerase, 0.2 µg of DNA template and 0.2 µM of *18A_F and *18A_R primers (Table 1). The cycling protocol consisted of an initial denaturation step at 94°C for 30 s, followed by 30 cycles of 30 s at 94°C, 45 s at 64.8°C and 45 s at 68°C. The final extension step was performed at 68°C for 5 min. The PCR product (388 bp) (Figure 1) was digested with 8.0 U of *HpaII* at 37°C for 60 min; the enzyme was then inactivated at 80°C for 20 min. The PCR method to amplify *CYP3A4*18B* was modified from a previously described protocol (Hu *et al.*, 2007). The PCR reaction was prepared in a total reaction volume of 25 µl, which consisted of 1 X PCR buffer, 0.2 mM of dNTP, 2.0 U of Taq polymerase, 0.2 µg of DNA template and 0.2 µM of *18B_F and *18B_R primers (Table 1). The cycling protocol consisted of an initial denaturation step at 94°C for 30 s, followed by 35 cycles of 30 s at 94°C, 45 s at 64.8°C and 45 s at 68°C. The final extension step was performed at 68°C for 5 min. The PCR product (331 bp) (Figure 1) was digested with 4.0 U of *RsaI* at 37°C for 60 min. For *CYP3A4*22,* the PCR reaction was prepared in a total reaction volume of 25 µl that consisted of 1 X PCR buffer, 0.2 mM of dNTP, 2.0 U of Taq polymerase, 0.2 µg of DNA template and 0.2 µM of *22_F and *22_R primers (Table 1). The cycling protocol consisted of an initial denaturation at 94°C for 30 s, followed by 35 cycles of 30 s at 94°C, 45 s at 66.8°C and 45 s at 68°C. The final extension was performed at 68°C for 5 min. The PCR product (793 bp) (Figure 1) was digested with 6.0 U of *BseYI* at 37°C for 60 min; the enzyme was then inactivated at 80°C for 20 min. *CYP3A5*3* was genotyped using PCR as previously described by (van Schaik *et al.*, 2002) with slight modifications. Briefly, the PCR reaction was prepared in a total reaction volume of 25 µl consisting of 1 X PCR buffer, 0.2 mM of dNTPs, 2.0 U of Taq polymerase, 0.2 µg of DNA template and 0.2 µM of 5*3_F and 5*3_R primers (Table 1). The cycling protocol was run as follows: denaturation at 94°C for 30 s; 30 cycles of 30 s at 94°C, 45 s at 61.6°C and 45 s at 68°C; and a 5 min final extension at 68°C. The PCR product (293 bp) was digested with 6.0 U of *SspI* at 37°C for 60 min; the enzyme was then inactivated at 65°C for 20 min.

Table 1. Primer sequences used for PCR-RFLP genotyping.

SNPs	Primer	Sequences (5'-3')	PCR product size (bp)	References
CYP3A4*4 A>G	*4_F	CACATTTTCTACAACCATGGAGACC	244	(Ruzilawati *et al.*, 2007)
	*4_R	TACCTGTCCCCACCAGATTCATTCT		(Ruzilawati *et al.*, 2007)
CYP3A4*18A T>C	*18A_F	AATGATTTGCCTTATTCTGGTTCTGT	388	Self-designed
	*18A_R	TTTCACCTCCTCCCTCCTTCTCC		Self-designed
CYP3A4*18B G>A	*18B_F	CCACGAGCAGTGTTCTCTCCTTC	331	Self-designed
	*18B_R	AATAGAAAGCAGATGAACCAGAGCC		(Hu *et al.*, 2007)
CYP3A4*22 C>T	*22_F	GCATAGAGTCTGCAGTCAGGCAAT	793	Self-designed
	*22_R	GATGACAGGGTTTGTGACAGGGG		Self-designed
CYP3A5*3 A>G	5*3_F	CATGACTTAGTAGACAGATGA	293	(Van Schaik *et al.*, 2002)
	5*3_R	GGTCCAAACAGGGAAGAAATA		

Mismatch with the *CYP3A5*3* sequence is underlined

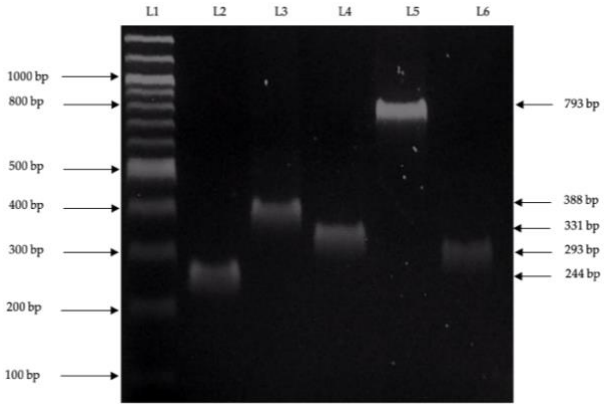

Figure 1. PCR products for *CYP3A4*4* (L2), *CYP3A4*18A* (L3), *CYP3A4*18B* (L4) and *CYP3A4*22* (L5) with band sizes of 244 bp, 388 bp, 331 bp and 793 bp, respectively, on a 2% agarose gel. L1: Quick-Load 100 bp DNA ladder (NEB® Inc, MA, USA).

PCR product purification and DNA sequencing. The PCR products were first purified before being sent for sequencing using Illustra™ ExoProster™ 1-Step Enzymatic and Sequencing Clean-Up (GE HealthCare Life Sciences, UK) according to the manufacturer's instructions. The sequencing results were run through snpBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) and compared against the SNP database (http://www.ensembl.org/index.html). The reference SNP (rs) IDs rs55951658, rs28371759, rs2242480, rs35599367 and rs776746 were identified for *CYP3A4*4, CYP3A4*18A, CYP3A4*18B, CYP3A4*22* and *CYP3A5*3* respectively. At least three representative samples from each genotype were randomly selected for sequencing

Statistical analyses. Data analyses were performed using IBM SPSS Statistics (Version 22.0. Armonk, NY: IBM Corp). A simple logistic regression was used to compare the four outcomes (MSAEs, mood disturbance, hot flashes or sweating and vaginal dryness/ dyspareunia), and all independent variables were assessed. Covariates with a p-value <0.25 or those with a p-value >0.25 that were considered to be clinically significant in simple logistic regression modelling were fitted into the multiple logistic regression models.

RESULTS AND DISCUSSION

A total of 94 patients between the ages of 44 and 83 years, with a mean age of 58.1 (SD, 7.3), were screened for this study (Supplementary data). The digestion of *CYP3A4*4* (244 bp) with *BsmAI* yielded fragments of 15 bp (not shown), 88 bp and 141 bp for the wild type gene (Figure 2). The hypothetical RFLP bands for the homozygous and heterozygous variants are 15 bp, 47 bp, 88 bp and 94 bp or 15 bp, 47 bp, 88 bp, 94 bp and 141 bp, respectively. The digested PCR product of *CYP3A4*18A* had a length of 388 bp for the wild type and 189 bp, 199 bp and 388 bp for the heterozygous variant (Figure 3); the hypothetical RFLP bands for the homozygous variant allele are 188 bp and 200 bp. The digested PCR products of *CYP3A4*18B (*331 bp*)* were 115 bp and 216 bp for the wild type, 331 bp for the homozygous variant and 115 bp, 216 bp and 331 bp for the heterozygous variant (Figure 4). The digested wild type PCR product of *CYP3A4*22* yielded 219 bp and 574 bp fragments (Figure 5). The hypothetical RFLP banding pattern should yield a band of 793 bp for the homozygous variant allele and bands of 219 bp, 574 bp and 793 bp for the heterozygous variant. The digested PCR products of *CYP3A5*3* were as follows: 125 bp and 148 bp fragments for the wild type; 125 bp, 148 bp and 168 bp fragments for the heterozygous variants; and 125 bp and 168 bp fragments for the homozygous variants (Figure 6).

The allelic and genotypic frequencies of the various SNPs are shown in Table 2. In the present study, no significant association was observed between any of the alleles and the AAAEs (Table 3). However, the patients' ages were associated with the occurrence of hot flashes (adjusted odds ratio, OR = 0.91, 95% confidence interval CI = 0.84-0.97, p = 0.021). In addition, patients receiving anastrozole for more than one year had a greater chance of developing mood disturbances (OR = 3.07, CI = 1.02-9.24, p = 0.046) and vaginal dryness/dyspareunia (OR = 18.00, CI = 3.71-87.40, p = 0.000) compared to patients receiving the drug for less than one year.

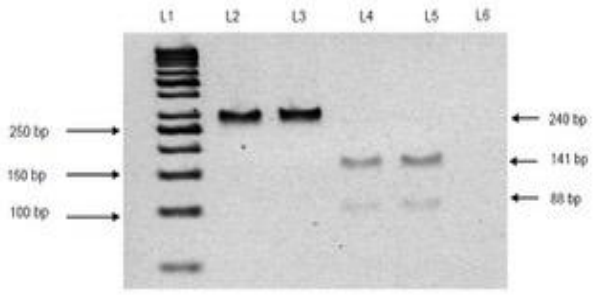

Figure 2. PCR products for *CYP3A4*4* on a 3% agarose gel before (L2 and L3) and after (L4 and L5) digestion with BsmAI. L1: GeneRuler 50 bp DNA ladder (Thermo Fisher Scientific Inc, MA, USA). L2 and L3: undigested *CYP3A4*4* PCR product (244 bp). L4 and L5: digested PCR products for wild type *CYP3A4*4* (141 bp and 88 bp). L6: negative control. Gel picture was taken using the negative mode.

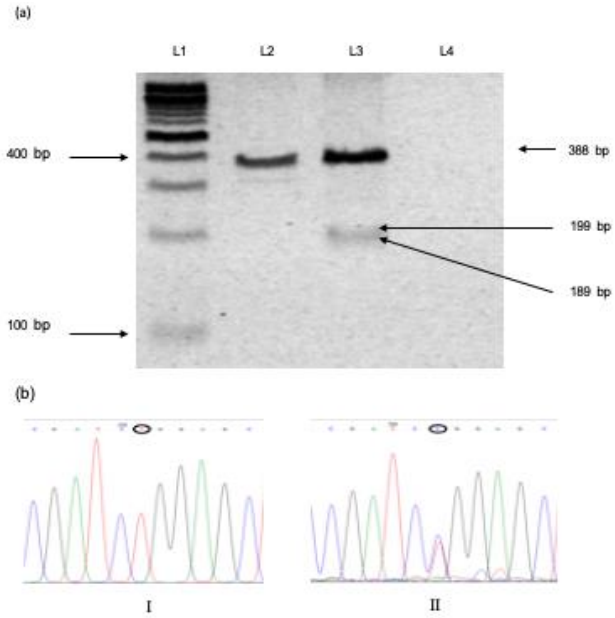

Figure 3. (a) PCR products for *CYP3A4*18A* on a 2% agarose gel before (L2) and after (L3) digestion with *HpaII*. L1: GeneRuler 50 bp DNA ladder (Thermo Fisher Scientific Inc, MA, USA). L2: undigested *CYP3A4*18A* PCR product (388 bp). L3: digested PCR products for heterozygous *CYP3A4*18A* (199 bp and 189 bp). L4: negative control. **(b)** Direct sequencing of the *CYP3A4*18A* allele showing a chromatogram (reverse sequence) of patients with wild type (I) and heterozygous variants (II). The highlighted "A" is adenine, indicating an absence of a *CYP3A4*18A* SNP in this subject, and the highlighted "G" is guanine, indicating the presence of heterozygous *CYP3A4*18A* in this subject.

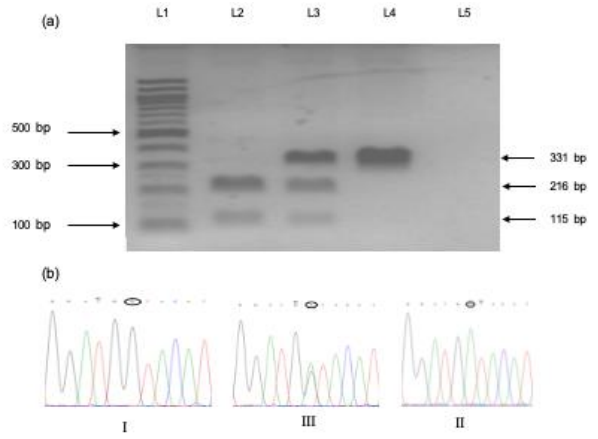

Figure 4. (a) PCR products for *CYP3A4*18B* on a 2% agarose gel following digestion with *RsaI*. L1: Quick-Load 100 bp DNA ladder (NEB® inc, MA, USA). L2: digested wild type *CYP3A4*18B* PCR product (115 bp and 216 bp). L3: digested PCR products for heterozygous *CYP3A4*18B* (115 bp, 216 bp and 331 bp). L4: undigested product for homozygous *CYP3A4*18B* (331 bp). L5: negative control. **(b)** Direct sequencing results for the *CYP3A4*18A* allele showing a chromatogram of patients with wild type (I), heterozygous (II) and homozygous (III) variants. The highlighted "G" in (I) is guanine, indicating the presence of the wild type *CYP3A4*18B*, and the highlighted "G" in (II) is guanine, detected in addition to the "A" allele, indicating the presence of heterozygous *CYP3A4*18B*. The highlighted "A" in (III) indicates the presence of a homozygous variant.

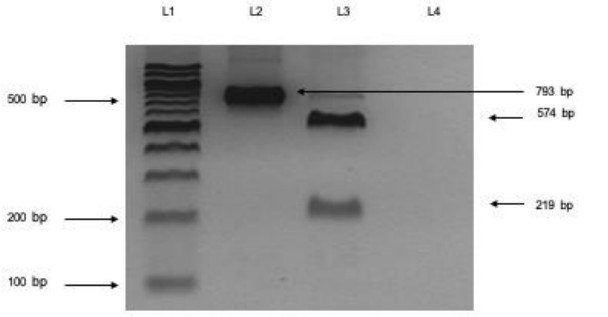

Figure 5. PCR products for *CYP3A4*22* on a 2% agarose gel before (L2) and after (L3) digestion with *BseYI*. L1: Quick-Load 100 bp DNA ladder (NEB® inc, MA, USA). L2: undigested *CYP3A4*22* PCR product (793 bp). L3: digested wild type *CYP3A4*22* PCR product (219 bp and 574 bp). L4: negative control.

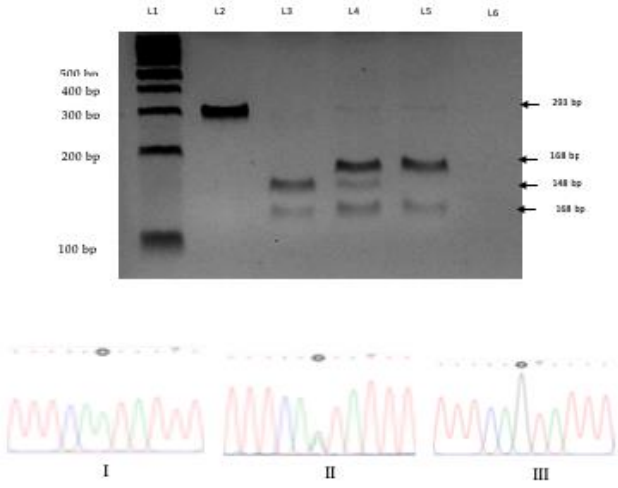

Figure 6. PCR products for *CYP3A5*3* on a 4% agarose gel before and after digestion with *SspI*. L1: Quick-Load 100 bp DNA ladder. L2: undigested PCR product (293 bp). L3: digested wild type PCR products (125 bp and 148 bp). L4: PCR product of the digested heterozygous variant (125 bp, 148 bp and 168 bp). L5: PCR products for the digested homozygous variant (125 bp and 168 bp). L6: negative control. (b) Direct sequencing results for the *CYP3A5*3* allele showing chromatograms from patients with wild type (I), heterozygous (II) and homozygous (III) variants. The highlighted "A" is adenine, indicating the presence of wild type *CYP3A5*3* in this subject. The highlighted "G" in (II) is guanine, detected in addition to "A" (adenine), indicating the presence of a heterozygous variant. The highlighted "G" in (III) is also guanine, indicating the presence of a homozygous variant.

Table 2. Allelic and genotypic frequencies of *CYP3A4*4*, *CYP3A4*18A*, *CYP3A4*18B*, *CYP3A4*22,* and *CYP3A5*3 A>G*.

SNP	Genotypic frequency (%)			Allelic frequency	
CYP3A4*4 A>G	AA	AG	GG	A	T
	100	ND	ND	1.0	ND
CYP3A4*18A T>C	TT	TC	CC	T	C
	95	5	ND	0.97	0.03
CYP3A*18B G>A	GG	GA	AA	G	A
	22	60	18	0.52	0.48
CYP3A4*22 C>T	CC	CT	TT	C	T
	100	ND	ND	1.0	ND
CYP3A5*3 A>G	AA	AG	GG	A	G
	18	35	47	0.36	0.64

ND, Not detected

In the present study, we successfully genotyped *CYP3A4*4*, *CYP3A4*18A*, *CYP3A4*18B, CYP3A4*22 and CYP3A5*3* in breast cancer patients (n=94) using the PCR-RFLP method. This study is the first to report the genotypes of *CYP3A4*18B* and *CYP3A4*22* among Malaysians, in particular Malaysian breast cancer patients.

In this study, five out of 94 subjects had the heterozygous variant *CYP3A4*18A* (frequency of 0.03), while the remaining 89 had the wild type allele. No individual had a homozygous variant for this allele. This was similarly reported in another study in which five out of 121 healthy Malaysian subjects were found to have the heterozygous *CYP3A4*18A* allele (Ruzilawati *et al.*, 2007).

The identification of the recently described intronic *CYP3A4*18B* allele among Malaysians is a novel finding of this study. The variant "A" allele had a frequency of 0.48 with 18 (19.1%) of the subjects having the homozygous "A/A" variant (Table 2). It was reported earlier that the presence of the *CYP3A4*18B* variant among Chinese renal transplant recipients could affect the pharmacokinetics of cyclosporine. Both trough levels and 2 h post-dose concentrations were significantly lower in patients who were homozygous for *CYP3A4*18B* when compared to patients with the wild type allele, suggesting the presence of increased CYP3A4 enzyme activity (Qiu *et al.*, 2008). These findings are further corroborated by additional studies reporting increased CYP3A4 activities among healthy Chinese volunteers treated with cyclosporine and tacrolimus (Hu *et al.*, 2007; Shi *et al.*, 2011; Tao *et al.*, 2015).

The present study also demonstrates that the occurrence of *CYP3A5*3* at an allelic frequency of 0.64, with 47 (50%) of the subjects carrying the homozygous "G/G" variant, is very common among Malaysians. The allelic frequency of *CYP3A5*3* obtained in our study is similar to that reported for kidney transplant Malaysians, with a mean frequency of 0.60 [0.59 (Malaysian), 0.72 (Chinese) and 0.50 (Indians)] (Hamzah *et al.*, 2014). The slight disparity in the frequencies may be attributed to a difference in the proportion of the three ethnicities. In our study, only a single Indian subject was included; a higher number of Indian subjects (n=7) were included in the report by Hamzah *et al.* (2014).

Interestingly, in the present study, no variants were detected for the *CYP3A4*4* and *CYP3A4*22* alleles. The absence of the *CYP3A4*4* variant allele in this study is in agreement with our previous study in which no *CYP3A4*4* variants were detected in 121 healthy Malaysians (Ruzilawati *et al.*, 2007). Generally, this allele has a frequency of 0.015-0.033 in Asians, but it has not been reported in Caucasians or African Americans (Werk and Cascorbi, 2014).

The relatively small sample size makes it difficult to conclude that *CYP3A4*22* is absent in a particular Malaysian population. A larger prospective study should be undertaken to further establish the actual allelic frequency of the newly detected *CYP3A4*18B* allele and the undetected *CYP3A4*22* allele in the Malaysian population.

Table 3. *CYP3A4*18A*, *CYP3A4*18B* and *CYP3A5*3* variants and odds ratios of having anastrozole-associated adverse events (AAAEs).

Variables	Musculoskeletal adverse events	
	OR (95% CI)	***p* value**
CYP3A4*18A		
Wild type (TT)	1	
Heterozygous (TC)	0.37 (0.04, 3.43)	0.380
CYP3A4*18B		
Wild type (GG)	1	
Heterozygous (GA)	0.61 (0.22, 1.69)	0.342
Homozygous (AA)	0.77 (0.21, 2.80)	0.691
CYP3A5*3		
Wild type (AA)	1	
Heterozygous (AG)	1.05 (0.32, 3.45)	0.933
Homozygous (GG)	0.82 (0.26, 2.56)	0.728
	Vasomotor symptoms	
CYP3A4*18A		
Wild type (TT)	1	
Heterozygous (TC)	4.06 (0.64, 25.82)	0.137
CYP3A4*18B		
Wild type (GG)	1	
Heterozygous (GA)	1.00 (0.33, 3.03)	1.000
Homozygous (AA)	1.042 (0.25, 4.26)	0.955
CYP3A5*3		
Wild type (AA)	1	
Heterozygous (AG)	1.04 (0.26, 4.11)	0.955
Homozygous (GG)	1.68 (0.46, 6.06)	0.427
	Mood disturbances	
CYP3A4*18A		
Wild type (TT)	1	
Heterozygous (TC)	1.23 (0.12, 11.82)	0.856
CYP3A4*18B		
Wild type (GG)	1	
Heterozygous (GA)	0.53 (0.15, 1.87)	0.325
Homozygous (AA)	0.67 (0.38, 3.40)	0.644
CYP3A5*3		
Wild type (AA)	1	
Heterozygous (AG)	1.03 (0.17, 6.31)	0.971
Homozygous (GG)	2.21 (0.43, 11.32)	0.343
	Vaginal dryness/dyspareunia	
CYP3A4*18A		
Wild type (TT)	1	
Heterozygous (TC)	1.46 (0.15, 14.13)	0.743
CYP3A4*18B		
Wild type (GG)	1	
Heterozygous (GA)	0.31 (0.80, 1.22)	0.095
Homozygous (AA)	0.98 (0.22, 3.43)	0.984
CYP3A5*3		
Wild type (AA)	1	
Heterozygous (AG)	0.32 (0.64, 1.66)	0.177
Homozygous (GG)	0.61 (0.54, 2.45)	0.490

Simple logistic regression; OR - odds ratio; 95%CI - 95% confidence interval.

The clinical relevance and contribution of these potential biomarkers to inter-individual variability in response to anastrozole should also be evaluated in a larger cohort.

CONCLUSION

Our study reports, for the first time that *CYP3A4*18B* allele has a high frequency in Malaysian postmenopausal breast cancer patients. The study also confirms a similar allelic frequency for *CYP3A4*4* and *CYP3A4*18A* in Malaysians, as previously reported. No *CYP3A4*22* SNPs were found in any of the study subjects. No significant associations were established between the *CYP3A4*18B, CYP3A4*18B* and *CYP3A5*3* variants in terms of the development of AAAEs (MSAEs, hot flashes, mood disturbance and vaginal dryness/dyspareunia). The clinical relevance of the newly detected *CYP3A4*18B* is currently under investigation by our group.

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