UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERISATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN CYTOCHROME P450 2D6 GENE IN MALAYSIAN BREAST CANCER PATIENTS

CHIN FEE WAI

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By

CHIN FEE WAI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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CHIN FEE WAI

December 2012

Chairman: Professor Rozita Rosli, PhD

Faculty: Medicine and Health Sciences

Worldwide as well as Malaysia, breast cancer remains the most common type of malignancy and also the major cause of cancer related deaths among women. Tamoxifen is extensively used as adjuvant hormonal therapy for estrogen receptor-positive breast cancer patients to reduce risk of recurrence and mortality. However, variability in response to tamoxifen is observed among breast cancer patients. This may be due to genetic polymorphisms of cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6) gene that influence the CYP2D6 enzyme activity in tamoxifen metabolism. To date, no study has been conducted to investigate single nucleotide polymorphism (SNP) profiles of CYP2D6 gene specific to Malaysian breast cancer patients. Hence, this study aimed to identify SNPs in the coding regions of the CYP2D6 gene in Malaysian breast cancer patients and also to predict the functional effects of the identified non-synonymous SNPs.

The identification of SNPs was successfully achieved through utilisation of high resolution melting (HRM) analysis and confirmatory DNA sequencing. A total of 51 SNPs of the CYP2D6 gene consisting of 40 known SNPs and 11 novel SNPs were identified in this study. Complete genotype concordance was observed in both tumour DNA (carcinoma tissues) and germline DNA (adjacent normal tissues and blood) of 16 breast cancer patients for all the 51 SNPs. Results from logistic regression analysis showed that metastasis status, hormonal receptors status, ethnicity and SNP c.100C>T are the predictors that contribute significantly in predicting survival of breast cancer patients. Apart from that, nine out of 24 non-synonymous SNPs consisting of c.100C>T, c.271C>A, c.545T>C, c.800C>G, c.1316G>A, c.1318C>T, c.1322G>A, c.1405C>G and c.1444G>A are predicted to be deleterious SNPs by *in silico* analysis using bioinformatics software.

CYP2D6*10/*10 was found to be the most prevalent genotype in both Malay and Chinese breast cancer patients with genotype frequency of 28.9% and 57.1%, respectively. On the contrary, Indian breast cancer patients had a high prevalence of CYP2D6*4/*10 genotype with genotype frequency of 42.8%. It has been suggested by previous studies that CYP2D6 phenotypes determined based on CYP2D6 genotypes can be used for predicting the efficacy of tamoxifen. In relation to CYP2D6 phenotype, 61.5% of Malay patients were shown to have genotypes that categorised them as extensive metabolisers, hence, it is expected that the majority of them would benefit from tamoxifen therapy. On the contrary, 57.1% of Chinese as well as Indian patients were categorised as intermediate metabolisers, which suggest that they might experience reduced efficacy of tamoxifen therapy due to

the reduction of CYP2D6 enzyme activity towards tamoxifen metabolism. This indicates that future clinical CYP2D6 genotyping may need to be considered prior to the selection of hormonal therapy for breast cancer patients.

In conclusion, this study represents the first effort to identify CYP2D6 SNP profiles specific to Malaysian breast cancer patients. Additionally, this study also provides information on metaboliser status of the patients, which may potentially aid clinicians towards better treatment management of breast cancer in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENALPASTIAN DAN PENCIRIAN POLIMORFISMA NUKLEOTIDA TUNGGAL DALAM GEN SITOKROM P450 2D6 BAGI PESAKIT-PESAKIT KANSER PAYUDARA MALAYSIA

Oleh

CHIN FEE WAI

Disember 2012

Pengerusi: Profesor Rozita Rosli, PhD

Fakulti: Perubatan dan Sains Kesihatan

Di seluruh dunia serta Malaysia, kanser payudara adalah jenis kanser malignan yang paling biasa dan juga punca utama bagi kematian berkaitan kanser di kalangan wanita. Tamoksifen digunakan secara meluas dalam terapi hormon adjuvan bagi pesakit-pesakit kanser payudara reseptor estrogen-positif untuk mengurangkan risiko berulang dan kadar kematian. Walau bagaimanapun, variabiliti respon terhadap tamoksifen dapat diperhatikan di kalangan pesakit-pesakit kanser payudara. Ini mungkin disebabkan oleh polimorfisma genetik bagi gen sitokrom P450, famili 2, subfamili D, polipeptida 6 (CYP2D6) yang mempengaruhi aktiviti enzim CYP2D6 di dalam metabolisma tamoksifen. Setakat kini, tiada kajian dijalankan untuk menyiasat profil polimorfisma nukleotida tunggal (SNP) bagi gen CYP2D6 yang spesifik kepada pesakit-pesakit kanser payudara Malaysia. Oleh itu, kajian ini bertujuan untuk mengenal pasti SNP di kawasan pengekodan gen CYP2D6

bagi pesakit-pesakit kanser payudara Malaysia dan juga meramal kesan fungsian bagi “non-synonymous SNP” yang telah dikenalpasti.

Pengenalpastian SNP telah berjaya dicapaikan melalui penggunaan analisis lebur resolusi tinggi (HRM) dan pengesahan penjujukan DNA. Sebanyak 51 SNP bagi gen CYP2D6 yang terdiri daripada 40 “known SNP” dan 11 “novel SNP” telah dikenalpasti dalam kajian ini. Konkordans genotip lengkap telah diperhatikan dalam kedua-dua DNA tumor (tisu karsinoma) dan DNA titisan germa (tisu normal yang bersebelahan dan darah) bagi 16 pesakit-pesakit kanser payudara untuk kesemua 51 SNP. Keputusan daripada analisis regresi logistik menunjukkan bahawa status metastasis, status reseptor hormon, keetnikan dan SNP c.100C>T adalah peramal yang menyumbang kepada ramalan untuk peluang hidup bagi pesakit-pesakit kanser payudara. Selain daripada itu, sembilan daripada 24 “non-synonymous SNP” yang terdiri daripada c.100C>T, c.271C>A, c.545T>C, c.800C>G, c.1316G>A, c.1318C>T, c.1322G>A, c.1405C>G dan c.1444G>A diramalkan sebagai “deleterious SNP” oleh analisis “*in silico”* dengan menggunakan perisian bioinformatik.

CYP2D6*10/*10 merupakan genotip yang paling lazim di kedua-dua pesakit-pesakit kanser payudara berbangsa Melayu dan Cina dengan frekuensi genotip masing-masing sebanyak 28.9% dan 57.1%. Sebaliknya, pesakit-pesakit kanser payudara berbangsa India mempunyai kelaziman yang tinggi bagi genotip CYP2D6*4/*10 dengan frekuensi genotip 42.8%. Ia telah dicadangkan oleh kajian-kajian dahulu bahawa fenotip CYP2D6 yang ditentukan berdasarkan genotip CYP2D6 boleh digunakan untuk meramalkan keberkesanan terhadap tamoksifen. Sehubungan

dengan fenotip CYP2D6, 61.5% daripada pesakit-pesakit Melayu mempunyai genotip yang dikategorikan sebagai “extensive metaboliser”. oleh itu, ia dijangkakan bahawa majoriti daripada mereka akan bermanfaat daripada terapi tamoksifen. Sebaliknya, 57.1% pesakit-pesakit Cina dan India dikategorikan sebagai “intermediate metaboliser” di mana mereka mungkin mengalami keberkesanan yang berkurangan bagi terapi tamoksifen disebabkan oleh pengurangan aktiviti enzim CYP2D6 terhadap metabolisma tamoksifen. Ini menunjukkan bahawa pengenotipan CYP2D6 secara klinikal mungkin perlu dipertimbangkan pada masa depan sebelum membuat pemilihan terapi hormon bagi pesakit-pesakit kanser payudara.

Kesimpulannya, kajian ini merupakan usaha yang pertama untuk mengenal pasti profil SNP CYP2D6 yang spesifik kepada pesakit-pesakit kanser payudara Malaysia. Tambahan pula, ia menyediakan informasi terhadap “metaboliser status” bagi pesakit-pesakit di mana ia mungkin berpotensi membantu doktor untuk pengurusan rawatan kanser payudara yang lebih baik pada masa akan datang.

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I am indebted and forever grateful beyond measure to my parents and siblings for their undying love and support in my life as well as my studies. Lastly, I will never forget my lovely dog named Wong Wong for his companionship during my teens even though he is no longer with me anymore.

I certify that a Thesis Examination Committee has met on 5 December 2012 to conduct the final examination of Chin Fee Wai on her thesis entitled "Identification and Characterisation of Single Nucleotide Polymorphisms in Cytochrome P450 2D6 Gene in Malaysian Breast Cancer Patients" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Lye Munn Sann, PhD
Professor (Medical)
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Tan Soon Guan, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Norshariza binti Nordin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Zilfalil bin Alwi, PhD
Professor
School of Medical Sciences
Universiti Sains Malaysia
(External Examiner)

_____

SIEW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 26 February 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Rozita Rosli, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Sabariah Abdul Rahman, MBBS, MPath
Professor
Faculty of Medicine
Universiti Teknologi MARA
(Member)

_____

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

CHIN FEE WAI

Date: 5 December 2012

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

µL	Microlitre
µM	Micromolar
0.5X	0.5 times
10X	10 times
1Mhgvc	1Malaysia Human Genome Variation Consortium
1X	1 time
5X	5 times
6X	6 times
A	Adenine
Å	Angstrom
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1
AC	Doxorubicin and cyclophosphamide
AIC	Akaike information criterion
AJCC	American Joint Committee on Cancer
APS	Ammonium persulphate
ASR	Age-standardised incidence rate
B	Regression coefficient
BIC	Bayesian information criterion
bp	Base pair
BRCA1	Breast cancer 1, early onset
BRCA2	Breast cancer 2, early onset
C	Cytosine
cDNA	Complementary DNA
CI	Confidence interval
cm	Centimeter
CMF	Cyclophosphamide, methotrexate and 5-fluorouracil
CNV	Copy number variation
CYP	Cytochrome P450
CYP2C8	Cytochrome P450, family 2, subfamily C, polypeptide 8
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6
CYP2D7P1	Cytochrome P450, family 2, subfamily D, polypeptide 7 pseudogene 1
CYP2D8P1	Cytochrome P450, family 2, subfamily D, polypeptide 8 pseudogene 1
CYP3A4	Cytochrome P450, family 3, subfamily A, polypeptide 4
CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5

DBH	Dopamine beta-hydroxylase
dbSNP	Short Genetic Variations Database
DCIS	Ductal carcinoma *in situ*
ddATP	Deoxyadenosine triphosphate
ddCTP	Dideoxycytidine triphosphate
ddGTP	Dideoxyguanosine triphosphate
ddNTPs	Dideoxynucleotide triphosphates
ddTTP	Dideoxythymidine triphosphate
DGGE	Denaturing gradient gel electrophoresis
DGV	Database of Genomic Variants
DHPLC	Denaturing high performance liquid chromatography
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
dNTPs	Deoxyribonucleotide triphosphates
DOE	Department of Energy
DRD2	Dopamine receptor D2
Ductal NOS	Ductal not otherwise specified
EDTA	Ethylenediaminetetraacetic acid
EM	Extensive metaboliser
EM algorithm	Expectation-maximization algorithm
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
FAC	5-fluorouracil, doxorubicin or epirubicin, and cyclophosphamide
FDA	Food and Drug Administration
FISH	Fluorescence *in situ* hybridisation
FMN	Flavin mononucleotide
g	Gram
G	Guanine
HER2	Human epidermal growth factor receptor 2
HGNC	HUGO Gene Nomenclature Committee
HGVS	Human Genome Variation Society
HOGG1	8-oxoguanine DNA glycosylase
HRM	High resolution melting
HUGO	Human Genome Organization
HWE	Hardy-Weinberg equilibrium
IDC	Invasive ductal carcinoma
IHC	Immunohistochemistry

ILC	Invasive lobular carcinoma
IM	Intermediate metaboliser
INDEL	Insertion/deletion
IUPAC	International Union of Pure and Applied Chemistry
JSNP	Japanese Single Nucleotide Polymorphisms
kb	Kilobase
K_2EDTA	Dipotassium ethylenediaminetetraacetic acid
KCl	Potassium chloride
L	Litre
LCIS	Lobular carcinoma *in situ*
LD	Linkage disequilibrium
LOH	loss of heterozygosity
mg	Milligram
$MgCl_2$	Magnesium chloride
mg/mL	Milligram/millilitre
mL	Millilitre
mL/min	Millilitre/minute
mM	Millimolar
MOSTI	Ministry of Science, Technology and Innovation
mRNA	Messenger RNA
MTHFR	Methylenetetrahydrofolate reductase
Na_2-EDTA	Disodium ethylenediaminetetraacetic acid
NaOAc	Sodium acetate
NBD	National Biotechnology Directorate
NCBI	National Center for Biotechnology Information
NCI	National Cancer Institute
ng	Nanogram
ng/µL	Nanogram/microlitre
NHGRI	National Human Genome Research Institute
NIH	National Institutes of Health
nm	Nanometer
NQO1	NAD(P)H dehydrogenase, quinone 1
^oC	Degree Celsius
OMIM	Online Mendelian Inheritance in Man
OR	Odds ratio
PAGE	Polyacrylamide gel electrophoresis
PanSNPdb	Pan-Asian SNP Genotyping Database

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PM	Poor metaboliser
PolyPhen-2	Polymorphism phenotyping v2
PR	Progesterone receptor
psi	Pound per square inch
RNA	Ribonucleic acid
rpm	Revolutions per minute
rs	Reference SNP
SCF	Standard Chromatogram Format
SE	Standard error
SEER	Surveillance, Epidemiology and End Results
SERM	Selective estrogen receptor modulator
SIFT	Sorting Intolerant From Tolerent
SNP	Single nucleotide polymorphism
ss	Submitted SNP
SSCP	Single-strand conformation polymorphism
T	Thymine
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TEAA	Triethylammonium acetate
TEMED	*N, N, N', N'*-tetramethylethylene diamine
Tris-HCl	Tris (hydroxymethyl) amino methane
UGT1A8	UDP glucuronosyltransferase 1 family, polypeptide A8
UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15
UKM	Universiti Kebangsaan Malaysia
UM	Ultrarapid metaboliser
UPM	Universiti Putra Malaysia
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

THESIS SUMMARY

CHAPTER 1

Problem statement

Breast cancer is the commonest cancer among women worldwide including Malaysia. Close to 75% of invasive breast cancers are estrogen receptor (ER)-positive and tamoxifen is widely used as adjuvant hormonal therapy for this group of patients to reduce the risk of recurrence as well as to improve survival. However, tamoxifen efficacy depends on the metabolism of tamoxifen into its active metabolite endoxifen via cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6). The presence of single nucleotide polymorphism (SNP) in the CYP2D6 gene has leads to individual variations in the catalytic activity of CYP2D6 enzyme, hence contribute to the differences in responsiveness towards tamoxifen therapy among breast cancer patients. Unfortunately, no study has been conducted to investigate CYP2D6 SNPs in Malaysian breast cancer patients in which the polymorphisms may affect response of the patients towards tamoxifen therapy. Therefore, identification of CYP2D6 SNP profiles specific to local breast cancer patients is crucial and necessary.

Research aim

This study aimed to identify SNP profiles of the CYP2D6 gene in Malaysian breast cancer patients and to predict functional effects of the identified non-synonymous SNPs.

Research objectives

1. To evaluate SNP genotype concordance of CYP2D6 gene assayed from breast carcinoma tissues, adjacent normal tissues and blood.
2. To identify known and novel SNPs as well as SNP haplotypes in the CYP2D6 gene in Malaysian breast cancer patients.
3. To determine association between CYP2D6 SNPs and clinicopathological characteristics of breast cancer patients.
4. To predict functional effects of non-synonymous SNPs of the CYP2D6 gene using bioinformatics analysis.
5. To identify CYP2D6 alleles and to determine frequencies of alleles and genotypes among different ethnic groups of breast cancer patients namely Malay, Chinese and Indian.

CHAPTER 2

Research background

Tamoxifen:

Tamoxifen has been the gold standard for adjuvant hormonal therapy in women with ER-positive breast cancer. The 5-year tamoxifen therapy contributed to the reduction of recurrence risk as well as mortality for breast cancer patients. Tamoxifen acts as an estrogen antagonist, which suppresses the proliferation of estrogen-dependent breast cancer cells. It is predominantly metabolised into its most important active metabolite endoxifen by CYP2D6 enzyme. Several lines of evidence suggested that endoxifen is 30 to 100-fold more potent than tamoxifen in the mediation of antiproliferative effects on breast cancer cells.

CYP2D6:

Many studies have suggested that genetic polymorphisms in CYP2D6 such as SNPs are one of the determinants for endoxifen concentrations in plasma leading to interindividual variability in response to tamoxifen therapy. As such, 30% of tamoxifen treated breast cancer patients experience recurrence and subsequently succumbed to the disease. The CYP2D6*10 (c.100C>T) is a common variant allele, which is widespread in Asian populations and its frequency varies among different ethnicities. It has been reported that the CYP2D6*10/*10 genotype is associated with poorer clinical efficacy of tamoxifen in Asian breast cancer patients.

CHAPTER 3

Methodology

Refer to methodology flow chart for more details on page 55.

CHAPTER 4

Key findings

1. **SNP identification:** a total of 51 SNPs consisting of 40 known SNPs and 11 novel SNPs were identified in 80 Malaysian breast cancer patients.
2. **Genotype concordance study:** complete concordance was observed between tumour (carcinoma tissues) and germline (adjacent normal tissues and blood) genotypes of 16 breast cancer patients.
3. **Logistic regression analysis:** metastasis status, hormonal receptors status, ethnicity and SNP c.100C>T were identified as predictors for survival of breast cancer patients.
4. **Bioinformatics analysis:** nine out of 24 non-synonymous SNPs identified in this study were predicted to be deleterious SNPs. The SNPs consisted of four known SNPs (c.100C>T, c.271C>A, c.1405C>G and c.1444G>A) and five novel SNPs (c.545T>C, c.800C>G, c.1316G>A, c.1318C>T and c.1322G>A).
5. **CYP2D6 allele identification:** CYP2D6*10 was the most common allele in both Malay and Chinese patients with allele frequency of 54.8% and 71.4%, respectively. Indian patients had high allele frequency of 28.6% for the CYP2D6*4 allele.
6. **CYP2D6 genotype identification:** CYP2D6*10/*10 genotype was highly prevalent in Malay and Chinese patients with genotype frequency of 28.9% and 57.1%, respectively. Indian patients had high prevalence for the CYP2D6*4/*10 genotype with a genotype frequency of 42.8%.
7. **CYP2D6 phenotype prediction:** 61.5% of Malay patients were extensive metabolisers (EM) while 57.1% of Chinese as well as Indian patients were intermediate metabolisers (IM).

CHAPTER 5

Conclusion

The SNP detection strategy utilised in this study involved the use of high resolution melting (HRM) analysis for SNP identification and followed by DNA sequencing for confirmation of the identified SNPs. A total of 51 SNPs were identified in 80 samples consisting of breast carcinoma tissues and blood specimens. Results from genotype concordance study substantiated the utilisation of different types of specimens for CYP2D6 SNP genotyping depending on availability of the specimens. In logistic regression analysis, metastasis status, hormonal receptors status, ethnicity and SNP c.100C>T were associated with survival of breast cancer patients, which were in agreement with previous studies. Nine SNPs including c.100C>T were predicted to have deleterious effects on CYP2D6 protein function by bioinformatics analysis. Further elucidation on the deleterious effects through experimental assays is warranted. The prediction of CYP2D6 phenotypes based on genotypes revealed that the majority of the Malay patients were EM and they benefited from tamoxifen therapy. In contrast, most of the Chinese as well as Indian patients were IM with reduced CYP2D6 catalytic activity towards tamoxifen resulting in reduced efficacy of tamoxifen therapy.

Future research recommendations

1. **Local genetic database:** establishment of a local genetic database for depository of current as well as future genotyping data of CYP2D6 gene.
2. **Pharmacogenetics study:** investigation of possible interrelationships among non-synonymous SNPs as well as novel SNPs, endoxifen plasma concentration and 5-year survival of breast cancer patients.
3. **Functional study:** *in vitro* and *in vivo* studies for investigating the functional effects of deleterious non-synonymous SNPs as predicted by bioinformatics analysis.

CHAPTER 1

INTRODUCTION

Breast cancer is the most common cancer as well as the main cause of cancer-related deaths in women worldwide. Statistics from the World Health Organization (WHO) showed that approximately 460,000 women died from breast cancer in 2008 , which contributed to 6% of the 7.6 million cancer deaths worldwide (GLOBOCAN 2008, 2010). It is projected that cases of breast cancer deaths will continue to increase and reach 737,000 in 2030 (WHO, 2008). In Malaysia, breast cancer is also the commonest cancer in Malaysian women as reported in the third report of the National Cancer Registry, Malaysia 2003-2005 (Lim *et al.*, 2008). A total of 11,952 breast cancer cases were reported in 2003-2005, which accounted for 31.3% of 38,196 new cases of female cancer. The report also revealed that breast cancer is more frequently diagnosed in women as compared to cervical cancer (10.6%), colorectal cancer (9.9%) and ovarian cancer (4.3%).

About 75% of all breast cancer patients have tumours expressing estrogen receptor (ER), which is also known as ER-positive breast cancer (Anderson *et al.*, 2002). Tamoxifen is widely used as adjuvant hormonal therapy for patients with ER-positive breast cancer and five years of tamoxifen therapy reduce the risk of recurrence and mortality rate (Early Breast Cancer Trialists' Collaborative Group, 1998; Early Breast Cancer Trialists' Collaborative Group, 2005a). However, major clinical problems arise in which the efficacy of tamoxifen varies among the patients

due to interindividual variability in response to tamoxifen therapy. Approximately 30% of the patients do not benefit from tamoxifen as they experience recurrence of breast cancer and eventually die from the disease (Early Breast Cancer Trialists' Collaborative Group, 1998).

The key factor leading to differences in the responsiveness of patients towards adjuvant hormonal therapy lies in the metabolism of tamoxifen into its active metabolite 4-hydroxy-N-desmethyltamoxifen (endoxifen). Several studies have shown that endoxifen is 30 to 100 times more potent than pro-drug tamoxifen in inhibiting estrogen-dependent breast cancer cell proliferation (Desta *et al.*, 2004; Johnson *et al.*, 2004). As tamoxifen is metabolised by the human liver enzyme cytochrome P450, family 2, subfamily D, polypeptide 6 (CYP2D6), the metabolic activity of CYP2D6 greatly influences the concentration of endoxifen in the plasma. It has been shown that variability in CYP2D6 metabolic activity is due to the presence of genetic variations such as single nucleotide polymorphism (SNP) in the CYP2D6 gene (Ingelman-Sundberg *et al.*, 2007; Snozek *et al.*, 2009). Hence, individual variations in metabolism of tamoxifen would affect the plasma concentration of endoxifen, which may contribute to interindividual differences in response to tamoxifen and affect clinical outcomes of breast cancer patients.

The CYP2D6 gene is highly polymorphic in nature with a total of 74 alleles having been reported to date in the CYP2D6 Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2d6.htm). It is noted that the frequency of CYP2D6 alleles vary widely among different populations. In Asian populations,

approximately 50% of the CYP2D6 alleles are functional alleles associated with normal CYP2D6 metabolic activity while reduced function alleles associated with lower CYP2D6 metabolic activity account for 40% of the population (Bradford, 2002). CYP2D6*10 allele is the most common reduced function allele in Asians with allele frequency of 37.8%, 45.6% and 52.5% in Japanese (Man *et al.*, 2010), Korean (Lee *et al.*, 2009) and Chinese (Qin *et al.*, 2008) populations, respectively.

The CYP2D6*10 allele involves a single nucleotide substitution of cytosine (C) to thymine (T) at position 100 (c.100C>T) in CYP2D6 exon 1 resulting in proline (Pro) being changed to serine (Ser) at amino acid 34 (p.Pro34Ser), which encodes for an unstable enzyme with reduced activity (Nakamura *et al.*, 2002). The CYP2D6*10 allele seems to be clinically important in tamoxifen treated Asian breast cancer patients in which it is associated with lower plasma concentrations of 4-hydroxytamoxifen and endoxifen, and higher risk of recurrence (Lim *et al.*, 2007; Kiyotani *et al.*, 2008; Xu *et al.*, 2008). Therefore, it is suggested that CYP2D6 genotyping be considered before the prescription of tamoxifen for selecting an optimal hormonal therapy for breast cancer patients.

Identification of genetic polymorphisms in CYP2D6 has been extensively carried out in Caucasian as well as Asian populations. However, it is not surprising that novel variants of CYP2D6 are continuously reported due to the high polymorphic nature of the CYP2D6 gene as mentioned previously. In recent years, numerous novel SNPs have been reported in Asian populations. Qin *et al*. (2008) reported a total of 12 novel SNPs being found in the Chinese population and another 14 novel SNPs were

reported in a subsequent study by Zhou *et al.* (2009). On the other hand, one novel SNP was found in the Korean population (Lee *et al.*, 2009) and another novel SNP was found in the Japanese population (Matsunaga *et al.*, 2009). All these point towards the necessity of continuous studies to be carried out for identifying novel SNPs in the CYP2D6 gene as it may potentially affect the efficacy of tamoxifen metabolism.

In Malaysia, studies involving CYP2D6 genotyping were only carried out in healthy subjects (Ismail and Teh, 2001; Teh *et al.*, 2001; Ismail *et al.*, 2003) and patients with cardiovascular diseases (Teh *et al.*, 2004). None of the studies involved breast cancer patients, although the CYP2D6 gene plays an important role in tamoxifen metabolism. Furthermore, these studies only focused on several commonly reported CYP2D6 alleles of the Asian populations. So far, no study has been conducted on surveying the CYP2D6 gene for SNPs that are unique to local breast cancer patients. In view of the lack of information in this area, this study aimed to identify SNP profiles of the CYP2D6 gene in Malaysian breast cancer patients and to predict functional effects of the identified non-synonymous SNPs. The objectives of this study are as follows:

1. To evaluate SNP genotype concordance of CYP2D6 gene assayed from breast carcinoma tissues, adjacent normal tissues and blood.
2. To identify known and novel SNPs as well as SNP haplotypes in the CYP2D6 gene in Malaysian breast cancer patients.

3. To determine association between CYP2D6 SNPs and clinicopathological characteristics of breast cancer patients.
4. To predict functional effects of non-synonymous SNPs of the CYP2D6 gene using bioinformatics analysis.
5. To identify CYP2D6 alleles and to determine frequencies of alleles and genotypes among different ethnic groups of breast cancer patients namely Malay, Chinese and Indian.

It is expected that the results obtained in this study will contribute to an understanding of the CYP2D6 genetic polymorphisms in Malaysian breast cancer patients. In addition, the SNP data from the CYP2D6 gene as well as the CYP2D6 phenotypes that are predicted from the genotypes will potentially be useful for future applications of pharmacogenetics in the local breast cancer population.

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