ผลของภาวะพหุสัณฐานของยีน *CYP2D6* ต่อเภสัชจลนศาสตร์ของยาทาม็อกซิเฟนในผู้ป่วยไทยโรคมะเร็งเต้านม

นางสาวภิชญาภา รุ่งวานนท์ชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ปีการศึกษา 2554

EFFECT OF *CYP2D6* POLYMORPHISM ON TAMOXIFEN PHARMACOKINETICS IN THAI BREAST CANCER PATIENTS

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A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Title	EFFECT OF *CYP2D6* POLYMORPHISM ON TAMOXIFEN PHARMACOKINETICS IN THAI BREAST CANCER PATIENTS
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ภิชญาภา รุ่งวานนท์ชัย: ผลของภาวะพหุสัณฐานของยีน *CYP2D6* ต่อเภสัชจลนศาสตร์ของยาทาม็อกซิเฟนในผู้ป่วยไทยโรคมะเร็งเต้านม (EFFECT OF *CYP2D6* POLYMORPHISM ON TAMOXIFEN PHARMACOKINETICS IN THAI BREAST CANCER PATIENTS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.ดวงจิตต์ พนมวัน ณ อยุธยา, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.นพ.นรินทร์ วรวุฒิ, 77 หน้า

งานวิจัยนี้มีวัตถุประสงค์เพื่อเปรียบเทียบระดับยาทาม็อกซิเฟนและเมแทบอไลท์ในผู้ป่วยมะเร็งเต้านมที่มีภาวะพหุสัณฐานของยีน *CYP2D6* ที่มีอัลลีลแบบ *CYP2D6*1* และ *CYP2D6*10* การศึกษาแบบไปข้างหน้านี้เก็บข้อมูล ณ กองตรวจโรคผู้ป่วยนอกศัลยกรรม โรงพยาบาลพระมงกุฎเกล้า กรุงเทพมหานคร มีการตรวจยีน *CYP2D6* ในผู้ป่วยมะเร็งเต้านมจำนวน 67 ราย พบความถี่ของยีน *CYP2D6*10/10* ร้อยละ 29.9, *CYP2D6*1/10* ร้อยละ 37.3 และ *CYP2D6*1/1* ร้อยละ 32.8 ตามลำดับ ระดับยาทาม็อกซิเฟนและเมแทบอไลต์ (ได้แก่ *N*-desmethyltamoxifen และ endoxifen) ในเลือดที่สภาวะคงที่ในผู้ป่วยที่รับประทานยาทาม็อกซิเฟนจำนวน 59 ราย พบว่าทั้ง 3 กลุ่มจีโนไทป์มีค่าระดับยาทาม็อกซิเฟนและเมแทบอไลต์ในพลาสมาแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (P = 0.045) ผู้ป่วยที่มีอัลลีลแบบ *CYP2D6*10/*10* มีระดับ endoxifen ในเลือดต่ำกว่า *CYP2D6 1/*10* และ *CYP2D6 1/*1* อย่างมีนัยสำคัญทางสถิติ 9.62 ng/ml *vs* 15.67 ng/ml และ 21.55 ng/ml ตามลำดับ) นอกจากนี้ระดับ *N*-desmethyltamoxifen และทาม็อกซิเฟนในเลือดในผู้ป่วย ทั้ง 3 จีโนไทป์มีค่าแตกต่างกันอย่างมีนัยสำคัญทางสถิติ (P= 0.020, P= 0.027 ตามลำดับ)

ภาควิชา ... เภสัชกรรมปฏิบัติ .....ลายมือชื่อนิสิต .....

สาขาวิชา..เภสัชกรรมคลินิก.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก…….....

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PICHAYAPA RUNGWANONCHAI: EFFECT OF *CYP2D6* POLYMORPHISM ON TAMOXIFEN PHARMACOKINETICS IN THAI BREAST CANCER PATIENTS.

ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D.,

CO-ADVISOR : ASSOC. PROF. NARIN VORAVUD, M.D., 77 pp.

The purposes of this study were to explore *CYP2D6*10* allele frequency in Thai breast cancer patients and compare tamoxifen and its metabolites plasma concentration between patients with *CYP2D6*1* and *CYP2D6*10* alleles. A prospective clinical study was performed, data was collected at the breast cancer surgery outpatient clinic of Phramongkutklao hospital, Bangkok. Genotyping of *CYP2D6* was analyzed in 67 breast cancer patients. The frequency of *CYP2D6*10/10, CYP2D6*1/10*, and *CYP2D6*1/1* was 29.9 %, 37.3%, and 32.8 %, respectively. Our results showed that the steady-state plasma concentrations of tamoxifen and its metabolites (endoxifen and *N*-desmethyltamoxifen among 59 patients with three *CYP2D6* genotypes were significantly different (P = 0.045). The plasma concentrations of endoxifen in patients carrying *CYP2D6*10/*10* were lower than those with *CYP2D6 1/*10* and *CYP2D6 1/*1* (9.62 ng/ml *vs* 15.67 ng/ml *vs* 21.55 ng/ml, respectively). In addition, the plasma concentrations of *N*-desmethyltamoxifen and tamoxifen among three *CYP2D6* genotypes were also significantly different (P= 0.020, P= 0.027, respectively).

Department : Pharmacy Practice Student's Signature

Field of Study : Clinical Pharmacy Advisor's Signature

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4-OH-Tam	=	4-hydoxy-tamoxifen
100C>T	=	Cytosine is replaced by thymine at position 100
AIs	=	Aromatase inhibitors
ANOVA	=	Analysis of Variance
C188T	=	Cytosine to thymine substitution at nucleotide 188
CC	=	Cytosine/ Cytosine allele
CI	=	Confidence Interval
CT	=	*Cytosine/ thymine* allele
CYP2D6	=	Cytochrome P450, family 2, subfamily D, polypeptide 5
CYP2D7P	=	Cytochrome P450 2D7P gene
CYP2D8P	=	Cytochrome P450 2D8P gene
CYP3A4	=	Cytochrome P450, family 3, subfamily A, polypeptide 4
ddH_2O	=	Double distilled water
DFS	=	Disease free survival
DNA	=	Deoxyribonucleic acid
EDTA	=	Ethylenediaminetetraacetic acid
Endoxifen	=	4-hydroxy-N-desmethyltamoxifen
EM	=	Extensive metabolizer
ER	=	Estrogen receptor
HWE	=	Hardy-Weinberg Equilibrium
HPLC	=	High-performance liquid chromatography
HR	=	Hazard ratio
IM	=	Intermediate metabolizer
mRNA	=	messenger Ribonucleic acid
NDM	=	*N*-desmethyltamoxifen
ng	=	Nanogram
P34S	=	Proline to serine substitution at amino acid 34
PCR	=	Polymerase Chain Reaction
PFS	=	Progression free survival
PM	=	Poor metabolizer
PR	=	Progesterone
Pro	=	Proline

RFLP	=	Restriction fragment length polymorphism
RFS	=	Recurrence-free survival
TTP	=	Time to progression
TT	=	Thymine/thymine allele
Ser	=	Serine
SNP	=	Single Nucleotide Polymorphism
TAM	=	Tamoxifen
UM	=	Ultra-rapid metabolizer
vt/vt	=	variant/variant
wt/wt	=	wild-type/ wild-type

CHAPTER I
INTRODUCTION

Background and Rationale

Cancer in Thailand is becoming an important health problem and leading cause of death with age-standardized incidence rate of 118.6 per 100,000.[1] Breast cancer is the most common cancer in Thai women with age-standardized incidence rate of 20.9 per 100,000.[1] The reduction in mortality has been attributed to both improvements in the adjuvant and metastatic treatment, as well as effects of early diagnosis and screening efforts. The 5-year survival rate was 89% among women diagnosed with early stage of breast cancer. [2]

Estrogen promotes the growth of several types of breast cancer. Women with hormone receptor–positive breast cancer can be given the endocrine therapy that is referred to as hormone therapy to lower estrogen production or to block the effects of estrogen on the growth of breast cancer cells. Tamoxifen competitively binds to estrogen receptors (ER) on tumors and other tissue targets, and results in producing a nuclear complex that decreases deoxyribonucleic acid (DNA) synthesis and inhibits estrogen effects. Tamoxifen has been widely used for the treatment of women with hormone receptor–positive early stage of breast cancer as adjuvant therapy and metastatic breast cancer. Tamoxifen may also be prescribed to women with ductal carcinoma in situ to decrease the risk of breast cancer in women at high risk of developing disease. Tamoxifen is effective in reducing risk of relapse in both premenopausal and postmenopausal breast cancer women.[3, 4] Five years of tamoxifen treatment reduced annual breast-cancer mortality by 30 percent for 15 years, and recurrence rates fell 32 % over the next five years. [5]

Tamoxifen is a prodrug. It needs to be metabolized by human liver enzymes to become an active drug. The major metabolite, *N*-desmethyltamoxifen (NDM) is produced primarily by cytochrome P450 3A4 *(CYP3A4)*, but this metabolite has a low affinity for ER. Two minor metabolites, 4-hydroxytamoxifen (4-OH-Tam) and 4-hydroxy-*N*-

desmethyltamoxifen (endoxifen) are predominantly catalyzed by cytochrome P450 2D6 *(CYP2D6)*. [6] These two metabolites have much greater affinity to ER than tamoxifen and NDM. As compared with the parent drug, 4-OH-Tam and endoxifen have an approximately 100-fold greater affinity for ER and ability to inhibit cell proliferation. [7, 8] Endoxifen has 6-to12-fold higher plasma concentration in women on chronic tamoxifen therapy relative to 4-OH-Tam. [9] Furthermore, endoxifen is associated with equivalent anti-estrogenic potency to 4-OH-Tam, based on the measurement of the effect on mRNA expression of the PR. Consequently, it has been contemplated that endoxifen is the most important active metabolite. [10]

Several factors may cause to reduce the benefits of tamoxifen. It has been increasingly recognized recently that pharmacogenetics may effect on tamoxifen metabolism, efficacy, and safety. The variant *CYP2D6* alleles or use of medications that inhibit the enzyme clearly influence tamoxifen metabolism. In addition, several studies suggest that variants *CYP2D6* alleles may influence long-term outcomes. The studies have shown that women carrying variant *CYP2D6* alleles experiencing more recurrences and shorter relapse-free survival as well as worse event-free survival period compared to patients with normal alleles. [11] More than 100 variant alleles of *CYP2D6* have been identified. These variants are inactive, reduce, or increase *CYP2D6* activity, but others do not change normal activity. The phenotype can generally be grouped into the classifications of extensive, poor, intermediate, or ultra-rapid metabolizers. The extensive metabolizers (EM) have two normal functional alleles and designated as *CYP2D6*1* genotype. Seventy percent of Caucasians and 52% of Asians are classified into this phenotype. The poor metabolizers (PM) have two nonfunctional alleles. In Caucasians, 97% of PM can be identified by four alleles with null activity *(*3, *4, *5, *6).* The *CYP2D6*4* genotype is the most common null allele in Caucasians (5–10%), but in Asian *CYP2D6*5* genotype is more frequent. The Intermediate metabolizers (IM) have two alleles with decreased function, or one allele with decreased function and one nonfunctional allele. The metabolism of Intermediate metabolizers has a slower than extensive metabolizers. The *CYP2D6*10* allele occurs frequently in Asians

(40-50%).[11, 12] The prevalence of *CYP2D6* polymorphism in Thai populations is 51.4% EM (*CYP2D6*1*), 37.8% IM (*CYP2D6*10*), and 5.4% PM (*CYP2D6*5)*. [13]

The purpose of this study was to determine the effect of *CYP2D6* polymorphism on tamoxifen and its metabolites plasma concentration in patients with breast cancer.

Hypothesis

Tamoxifen and its metabolites plasma concentration were different between patients carrying *CYP2D6*1* and *CYP2D6*10* alleles.

Objective

To compare tamoxifen and its metabolites plasma concentration between patients carrying *CYP2D6*1* and *CYP2D6*10* alleles.

Significant of the study

Information about the difference between tamoxifen and its metabolites plasma concentration in patients carrying *CYP2D6*1* and *CYP2D6*10* may be useful as predictors for planning dosage regimen or switching therapy.

Scope of this study

1. Populations of this study were the out patients at Phramongkutklao hospital who were prescribed tamoxifen as an adjuvant therapy.
2. Variables of this study: Dependent variables were tamoxifen and its metabolites plasma concentration. Independent variables were *CYP2D6* polymorphism.

Limitation of this study

Application of this study was limited to specific number of patients that possesses the same characteristics as demonstrated in this study.

Conceptual framework

Conceptual framework is shown in figure 1.

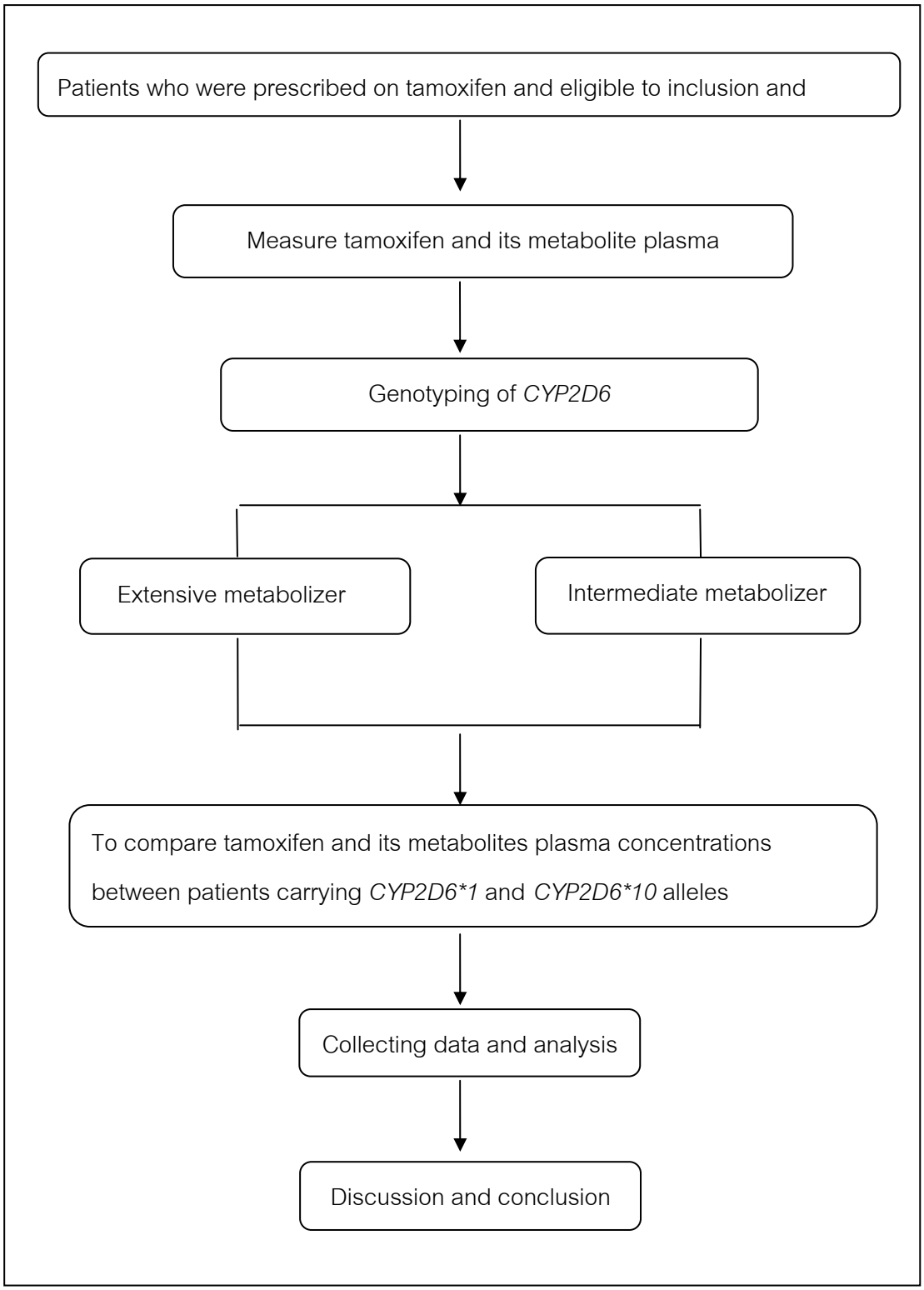

Figure1: Conceptual framework

Operational definition

1. Adjuvant chemotherapy is additional antineoplastic treatment given after the primary treatment to lower the risk that the cancer will come back.
2. *CYP2D6* polymorphism is genotype that control *CYP2D6* enzyme production which has single-nucleotide polymorphism; *CYP2D6*10* is a major variant among Asian population, and is associated with decreased *CYP2D6* enzyme activity resulting in the formation of an unstable enzyme. *CYP2D6*10* allele is substitute amino acid at 100C> T.
3. Extensive metabolizers (EM) have two normal functional alleles. In this study, EM designated as *CYP2D6*1* genotype.
4. Estrogen receptor positive is the cells that have a receptor protein that binds the hormone estrogen. Cancer cells that are estrogen receptor positive may need estrogen to grow, and may stop growing or die when treated with substances that block the binding and actions of estrogen
5. Intermediate metabolizers (IM) have two alleles with decreased0 function or one allele with decreased function and one nonfunctional allele. In this study, IM designated as *CYP2D6*10* genotype.
6. Performance status is a measure of how well a patient is able to perform ordinary tasks and carry out daily activities.
7. Polymorphism is a common variation or mutation in DNA.
8. Tamoxifen and its metabolites plasma concentration measurement is a measurement of tamoxifen, 4-OH-Tam, NDM, and endoxifen that the sampling time after the administration of the next dose in the morning

CHAPTER II
LITERATURE REVIEWS

Tamoxifen monograph

A. Mechanism of action [15]

Tamoxifen is a selective estrogen receptor modulator with potent ant-estrogenic properties. Tamoxifen competitively bind to estrogen receptors (ER) on tumor and other tissue targets, produce a nuclear complex that decreases DNA synthesis. This mechanism causes cells to accumulate in G0 and G1 phases. Tamoxifen may also exert cytotoxic activity by inducing apoptosis independent of ER expression. It is recognized that tamoxifen acts as an estrogen agonist on endometrial, bone and lipid metabolisms, as shown in Figure 2

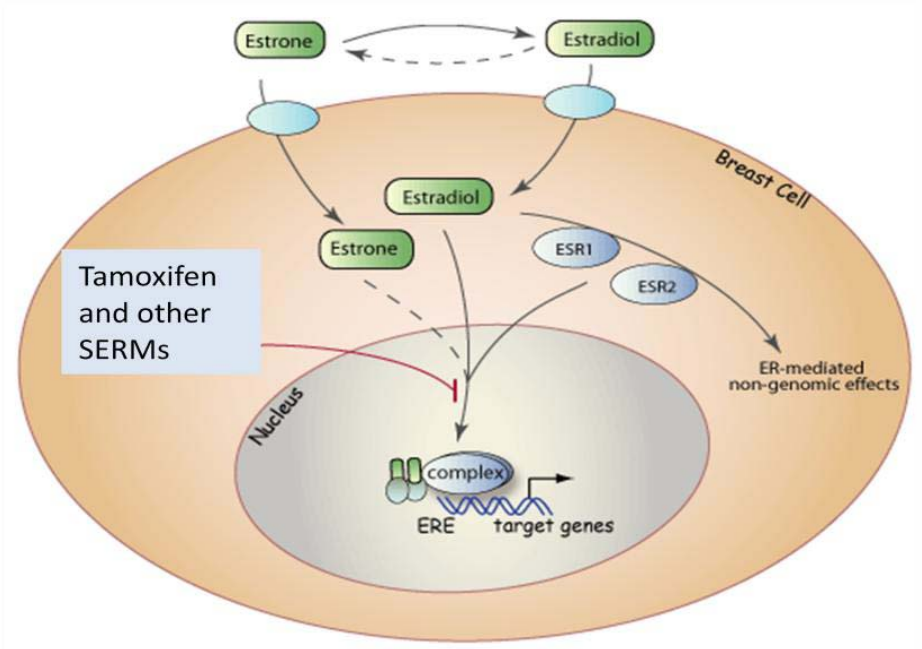

Figure 2. Estradiol binds to the estrogen receptor (ER) and results in changes that bring about cellular proliferation. Tamoxifen is anti-estrogen that competes with estradiol for binding with the ER and blocks the proliferation. Source: modified from www.pharmgkb.org/.

B. Usual dosage [15]

The recommended dose of tamoxifen for breast cancer treatment as adjuvant therapy is 20 mg once daily for two to five years following surgery.

C. Efficacy of Tamoxifen

Tamoxifen has been a standard endocrine therapy for the treatment of ER-positive breast cancer. To date tamoxifen is the only hormonal agent approved by the US Food and Drug Administration (FDA) for the prevention of breast cancer [16], the treatment of ductal carcinoma in situ [17], and the treatment of pre-menopausal breast cancer [18]. Five years of tamoxifen treatment reduced annual breast-cancer mortality by 25% and recurrence rate by 40%. [5] Although tamoxifen is the accepted standard of care for pre-menopausal breast cancer, aromatase inhibitors (AIs) are the drug of choice for post-menopausal breast cancer. For post-menopausal women with breast cancer, there are two treatment algorithms including AIs for 5 years [19. 20] or tamoxifen for 2 to 3 years followed by AIs for 2 to 3 years. [21-23] Five-year AIs treatment reduces the risk of a disease free survival by 18% compared with 5 years of tamoxifen alone, but it does not improve survival rate of patients. [24] In contrast, the sequential of hormonal therapy with tamoxifen for 2 to 3 years followed by AIs reduce the risk of a disease free survival by 40% [21, 22] and improve patient survival rate [21, 25] compared with 5-year treatment of tamoxifen alone. For this reason, tamoxifen commonly used for treatment of post-menopausal breast cancer. [26]

D. Pharmacokinetics of Tamoxifen

1) Absorption [6, 27, 28]

Tamoxifen was absorbed slowly following oral administration. The peak serum concentration after single dose generally occurs 3-6 hours. The extent of absorption in humans has not been determined, but data from animal studies suggest that the drug is well absorbed. The steady-state serum concentrations tamoxifen are attained after 3-4 weeks of continuous dosing, while those of *N*-desmethyltamoxifen are attained after 3-8 weeks of continuous dosing.

2) Distribution [6, 27, 28]

Tamoxifen and its major metabolites distribute to the cytosol of human breast tumor tissue and appear to parallel the relative concentrations in serum, although cytosol concentrations may exhibit even greater interindividual variation than serum concentrations. The high concentrations of tamoxifen are found in uterus and breast tissue. It also distributes to blood brain barrier. The volume of distribution is 20 L/Kg, and the plasma protein binding is 99%.

3) Metabolism [6, 28,]

Tamoxifen is a prodrug, it requires metabolic activation to elicit its pharmacological activity. Tamoxifen undergoes extensive hepatic oxidation by the cytochrome P450; major *CYP3A4, 2C8/9, 2D6*; minor *2A6, 2B6, 2E1* [6], as shown in Figure 3. The major tamoxifen metabolites include *N*-desmethyl-tamoxifen (NDM), 4-hydroxy-tamoxifen (4-OH-Tam), tamoxifen-*N*-oxide, a-hydroxy-tamoxifen, and *N*-didesmethyl-tamoxifen.[6,9,29-31] NDM, resulting from the *CYP3A4/5*-mediated catalysis of tamoxifen, is quantitatively the major primary metabolite of tamoxifen approximately 92% of primary tamoxifen oxidation.[6] Tamoxifen at a dose of 20 mg/day, the steady-state plasma concentrations of tamoxifen and NDM are 362.5 and 654.9 nM, respectively, whereas the steady-state plasma concentrations of 4-OH-Tam are extremely low (9 nM). [32] NDM is an intermediary substrate for secondary metabolism of tamoxifen. [6, 32, 33] It is predominantly biotransformed to a-hydroxy, *N*-desmethyl, *N*-didesmethyl, and 4-hydroxy-N-desmethyl-tamoxifen (endoxifen). The biotransformation of NDM to endoxifen is exclusively catalyzed by *CYP2D6*, whereas other pathways of *N*-desmethyl biotransformation are catalyzed predominantly by the *CYP3A* subfamily. [6] The clinical study has been reported that 4-OH-Tam and endoxifen are the active therapeutic moieties. These two metabolites have 100-fold greater affinity to ER and 30-to100-fold greater potency in suppressing estrogen-dependent cell proliferation, compared with the parent drug. [33-35] Therefore, inter-individual differences in formation of these active metabolites could be one of important factors affecting variability in the response to

tamoxifen. Cytochrome P450 2D6 (*CYP2D6*) is one of the major enzymes metabolism of 4-OH-Tam and endoxifen[6]

4) Excretion [6, 27, 28]

Tamoxifen and its metabolites are mainly excreted by feces (26-65%), and also excreted by urine and bile. The terminal half-life is 5-7 days; (range 3-21 days), and the major metabolites half-life are 9-14 days. The clearance is no information.

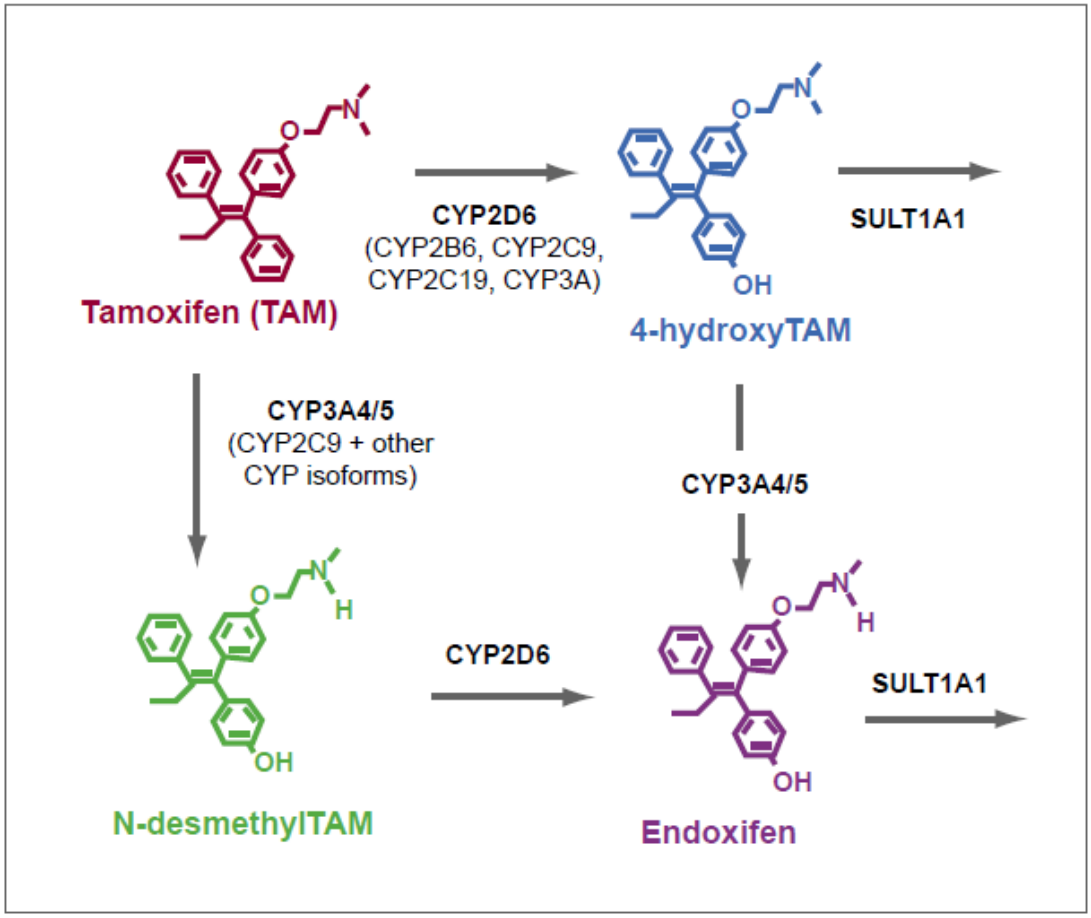

Figure 3. Selected transformation pathways of tamoxifen and the main CYP enzymes involved. The relative contribution of each pathway to the overall oxidation of tamoxifen is shown by the thickness of the arrow, and the principal P450 isoforms responsible are highlighted in larger fonts. Source: Jin et al. *J Natl Cancer Inst.* 2005; 97: 30-39.

E. The standard tamoxifen and its metabolites plasma concentrations [30]

The standard plasma concentrations of tamoxifen and its metabolites are Tamoxifen (15–600 ng/ml), NDM (15–600 ng/ml), 4-OH-Tam (0.25–10 ng/ml), and endoxifen (0.5–20 ng/ml)

Cytochrome P450 2D6 *(CYP2D6)*

The human *CYP2D* locus consists of three highly homologous genes*, CYP2D8P, CYP2D7,* and *CYP2D6. CYP2D6* is Cytochrome P450, family 2, subfamily D, and polypeptide 6. *CYP2D6* gene locates on the long arm of chromosome 22, and composes of nine exons with an open reading frame of 1,491 base pairs coding for 497 amino acids. [36, 37] The locus contains two inactive pseudogenes, *CYP2D7* and *CYP2D8,* [37-39] as shown in Figure 4. *CYP2D7P* carries a T-insertion in exon 1, resulting in disrupting the reading frame, while *CYP2D8P* contains multiple deletions and insertions in its exons. This locus is extremely polymorphic with over 80 variant alleles. The development of human *CYP2D* locus has involved elimination of three genes, and inactivation of two genes (*CYP2D7P* and *CYP2D8P*), and partial inactivation of one gene (*CYP2D6*). [37] *CYP2D6* is one of the most important drug metabolizing enzymes, is involved in the biotransformation of a large number of drugs approximately 25%, [37, 39] as shown in Table 1. The regularly substrates for *CYP2D6* are lipophilic bases with a protonable nitrogen atom, including some antidepressants, antipsychotics, antiarrhythmics, antiemetics, beta-adrenoceptor antagonists (β-blockers) and opioids. The hydroxylation reaction occurs at a distance of 5 or 7A°from the nitrogen atom. The site directed mutagenesis experiments indicate that Asp301 is the negatively charged amino acid accountable for binding to the substrate nitrogen. [40] Furthermore, Ser304, Thr309 and Val370 also involved in substrate binding, although further study is required for confirmation.

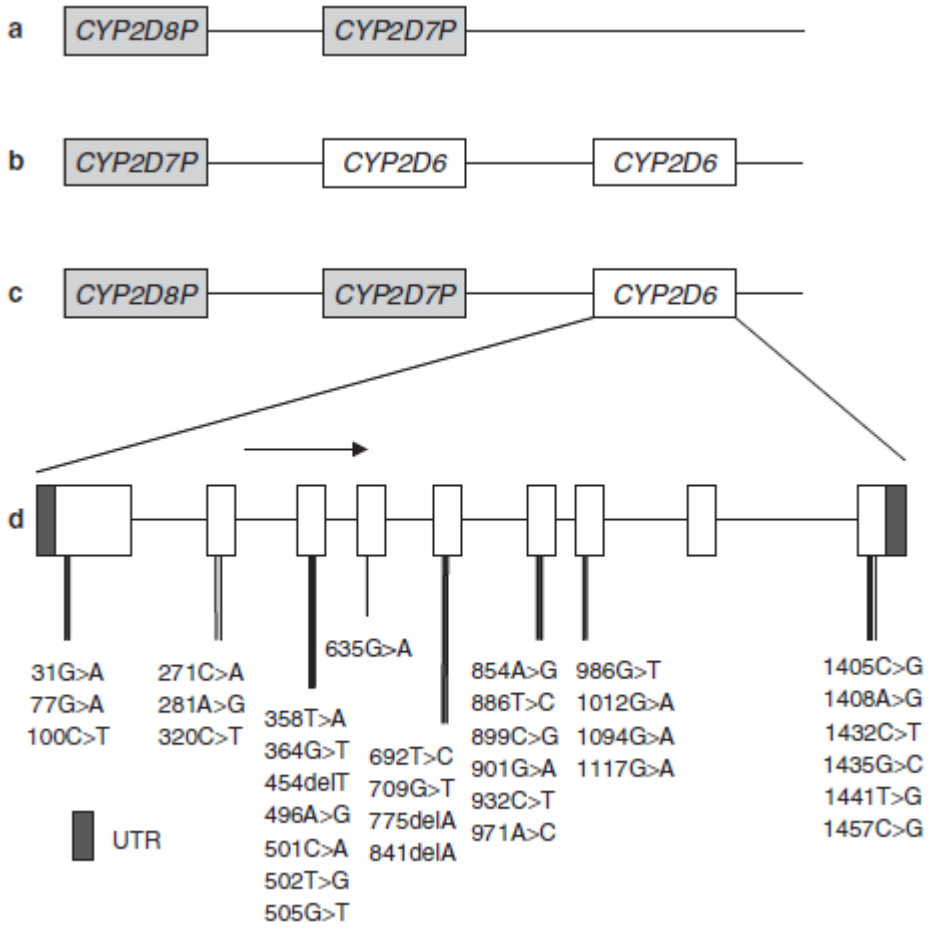

Figure 4. The human *CYP2D* gene cluster and common single nucleotide polymorphisms (SNPs) of *CYP2D6*. *CYP2D6* belongs to a gene cluster of highly homologous inactive pseudogenes *CYP2D7P* and *CYP2D8P*. The *CYP2D6* gene consists of nine exons. (a) The entire *CYP2D6* gene can be deleted (*CYP2D6*5*); (b) *CYP2D6* duplication or multiplication (tandem copy N= 2–12); (c) the *CYP2D* locus; and (d) common SNPs detected in the *CYP2D6* gene. CYP= cytochrome P450; UTR= untranslated region. Source: Shu-Feng Zhou. *Clin Pharmacokinet*. 48 (2009): 689-723.

Table 1. Drugs of different therapeutic classes known to be metabolized by *CYP2D6* [45]

Therapeutic class	Drug	Pathway catalyzed by CYP2D6
Analgesic	Codeine	O-demethylation
	Dextromethorphan	O-demethylation
	Dihydrocodeine	O-demethylation
	Ethylmorphine	O-deethylation
	Hydrocodone	N-demethylation
	Norcodeine	O-demethylation
	Oxycodone	O-demethylation
Anti-ADHD drug	Atomoxetine	Aromatic hydroxylation
Antiarrhythmics	Aprindine	Aromatic hydroxylation
	Encainide	O-demethylation
	Flecainide	O-dealkylation (?)
	Mexiletine	Aromatic hydroxylation
	N-propylajmaline	Benzylic hydroxylation
	Procainamide	Arylamine N-oxidation
	Propafenone	Aromatic hydroxylation
	Sparteine	Aliphatic hydroxylation
Antidementia drugs	Galanthamine	O-demethylation
	Nicergoline	N-demethylation
Tricyclic antidepressants	Amitriptyline	Benzylic hydroxylation
	Clomipramine	Aromatic hydroxylation
	Desipramine	Aromatic hydroxylation
	Imipramine	Aromatic hydroxylation
	Nortriptyline	Benzylic hydroxylation
Other antidepressants	Citalopram	N-demethylation
	Desmethylcitalopram	N-demethylation
	Fluoxetine	N-demethylation
	Fluvoxamine	unclear
	Maprotiline	unclear
	Mianserin	Aromatic hydroxylation
	Minaprine	Aromatic hydroxylation
	Mirtazapine	Aromatic hydroxylation
	Paroxetine	Demethylenation
	Venlafaxine	O-demethylation
Antidiabetic	Phenformine	Aromatic hydroxylation
Antiestrogen	Tamoxifen	Aromatic hydroxylation

ADHD attention deficit/hyperactivity disorder.

Therapeutic class	Drug	Pathway catalyzed by CYP2D6
Antihypertensives	Debrisoquine	Benzylic hydroxylation
	Guanoxan	Aromatic hydroxylation
	Indoramin	Aromatic hydroxylation
Antiemetics	Dolasetron	Aromatic hydroxylation
	Ondansetron	Aromatic hydroxylation
	Tropisetron	Aromatic hydroxylation
Antihistamines	Mequitazine	Aromatic Hydroxylation
	Promethazine	Aromatic hydroxylation
Antipsychotics	Haloperidol	N-dealkylation
	Perphenazine	N-dealkylation
	Risperidone	Aliphatic hydroxylation
	Thioridazine	Sulfoxidation
	Zuclopenthixol	N-dealkylation
Appetite suppressant	Dexfenfluramine	N-dealkylation
Beta adrenergic blocking agents	Alprenolol	Aromatic hydroxylation
	Bufuralol	Benzylic hydroxylation
	Bunitrolol	Aromatic hydroxylation
	Bupranolol	Aromatic hydroxylation
	Carvedilol	Aromatic hydroxylation
	Metoprolol	Aliphatic hydroxylation
	Propranolol	Aromatic hydroxylation
	Timolol	O-dealkylation
Calcium antagonist	Perhexiline	Aliphatic hydroxylation
MAO-inhibitors	Amiflamine	N-demethylation
	Brofaromine	O-demethylation
Recreational drugs	Methoxyamphetamine	O-demethylation
	MDMA, MDME	Demethylenation
Vasodilatators	Cinnarizine	Aromatic hydroxylation
	Flunarizine	Aromatic hydroxylation

MAO monoamine oxidase, *MDMA* 3, 4 methylenedioxyamphetamine, *MDME* microsomal drug metabolizing enzyme

CYP2D6 polymorphisms

Variability in *CYP* activity may relate to diverse factors. Several drugs are known to interfere with *CYP* enzymes by inhibition or induction, or by using those enzymes in their metabolism. More than 100 variant *CYP2D6* alleles have been described, which manifest in the population in four phenotypes, extensive (normal activity), intermediate (reduced activity), poor (no activity), and ultra-rapid (high activity) metabolizers. [40] Poor metabolizers (PMs) are homozygous for one deficient allele or heterozygous for two different deficient alleles. Intermediate metabolizers (IMs) are heterozygous for one deficient allele or carry two alleles that cause reduced activity. Extensive metabolizers (EMs) are two wild-type alleles. Ultra-rapid metabolizers (UMs) are multiple gene copies. This phenotype classification is based on the bimodal distribution of metabolic ratios (ratio between the urinary recovery of the parent drug and that of its major metabolite in a population [41, 42], as shown in Figure 5. Molecular details of the alleles and protein products for which reliable phenotype information are shown in Figure 6. [42] The PMs and UMs are different from EMs by 5 to 15 fold due to the rates of metabolism or ratios of parent drug to its metabolite concentrations. It is difficult to determine IMs as there is an overlap between IMs and EMs. The UM phenotype has been less characterized than PM phenotype, although it may have significant clinical consequences. [37] The *CYP2D6* alleles are classified as functional alleles (e.g. *CYP2D6*1, *2, *35,* etc.), reduced functional alleles (e.g. *CYP2D6*9, *10, *17, *29, *37, *41*, etc.), and non-functional alleles (e.g. *CYP2D6*3-*8, *11-*16, *18-*20, *36, *38, *40, *42*, etc.). The consequence of *CYP2D6* polymorphisms was shown in Table 2. [36, 43]

Table 2. The consequence of *CYP2D6* polymorphisms. [36, 43]

CYP2D6 allele	Mutation [a]	Consequence of mutation	Enzyme [b]	Metabolizer status
*1	None	None	Normal	EM
*2	4180G>C	Amino acid change	Normal	EM
*17	1023C>T	Amino acid change	Unstable	IM
*41	2988G>A	Splicing defect	Unstable	IM
*10	100C>T	Amino acid change	Unstable	IM
*36	100C>T, gene conversion in exon 9	Amino acid changes	Inactive	PM
*3	A2549del	Frame-shift	Inactive	PM
*4	1846G>A	Splicing defect	Inactive	PM
*5	Gene deletion	No enzyme	No enzyme	PM
*6	T1707del	Frame-shift	Inactive	PM

Note: Some of the frequent *CYP2D6* alleles in the Caucasian, Asian and African populations are represented here. a mutation that is used for genotyping the corresponding CYP2D6 allele; b in vivo enzyme activity (source: www.cypalleles.ki.se/cyp2d6.htm); PM - poor metabolizer; IM - intermediate metabolizer; EM - extensive metabolizer

CYP2D6*1 [37]

*CYP2D6*1* is the second common alleles in Asian population (51.5% alleles frequency). There are no base changes or polymorphisms in CYP2D6*1 allele, which is expected to produce an enzyme with normal activity.

CYP2D6*10

The lower enzyme activity in Asian population is associated with the frequent presence of *CYP2D*10* allele and its variants, *CYP2D*10A* and *CYP2D*10B,* which occur in approximately 50%. These alleles contain a *C188T* (or *C100>T*) mutation that causes a Pro34 Ser amino acid substitution, leading to the formation of an unstable enzyme with lower metabolic activity. [37, 43-44] *CYP2D*10A* previously known as

CPD2D6J, is characterized by the SNPs gene *C188T, G1749C, and G4268C*. *CYP2D*10B* is known as *CPD2DCh1*, composes additional base changes in *CYP2D* gene *C1127T.* Therefore, *C188T* is a major mutation gene in both *CYP2D*10* alleles. Homozygotes of *CYP2D*10* allele are common, and result in the IM phenotype. Individuals with *CYP2D6*1/*10* and *CYP2D6*10/*10* genotypes had 3-fold lower *CYP2D6* protein in their liver microsomes when compared to *CYP2D6*1/*1.* [44] In vitro expression studies have shown that *CYP2D6*10* enzyme had 50-100 fold lower efficiency in metabolizing dextromethorphan and fluoxetine when compared to *CYP2D6*1.* [45]

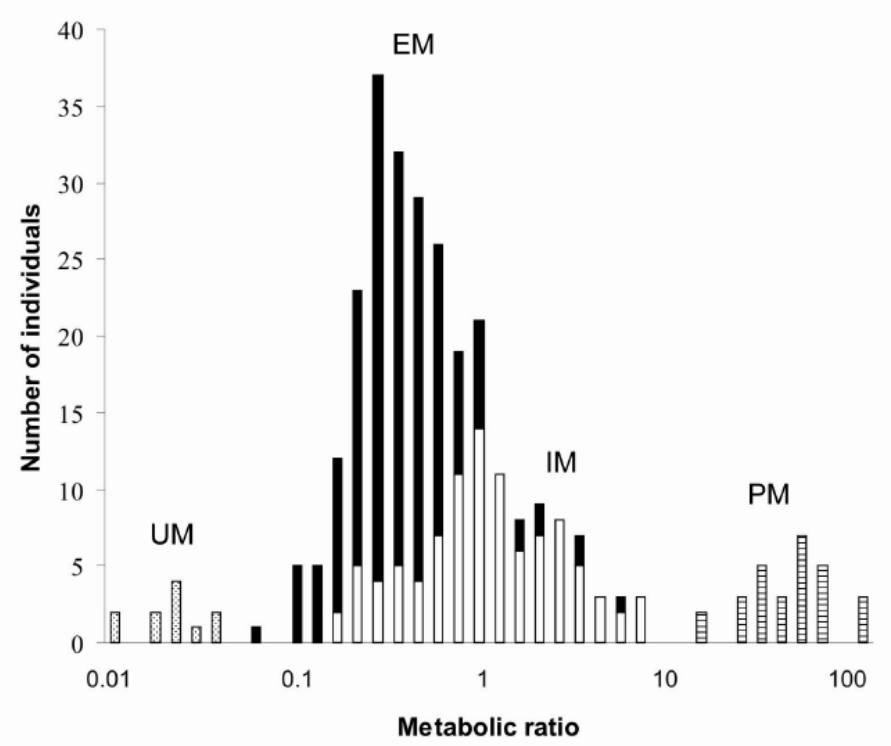

Figure 5. Schematic presentation of the relationship between a parent drug and its major metabolite (metabolic ratio) and the *CYP2D6* genotypes causing altered *CYP2D6* activity. UM: ultrarapid metabolisers; EM: extensive metabolisers; IM: intermediate metabolisers; PM: poor metabolisers. Dotted bars: individuals with two or more gene copies; Filled bars: individuals with two wild-type alleles; Open bars: individuals who are heterozygous for one deficient allele or carry two alleles that cause reduced activity; Striped bars: individuals without any functional allele. Van der Weide *J. Clin Biochem Rev*. 27 (2006): 17–25.

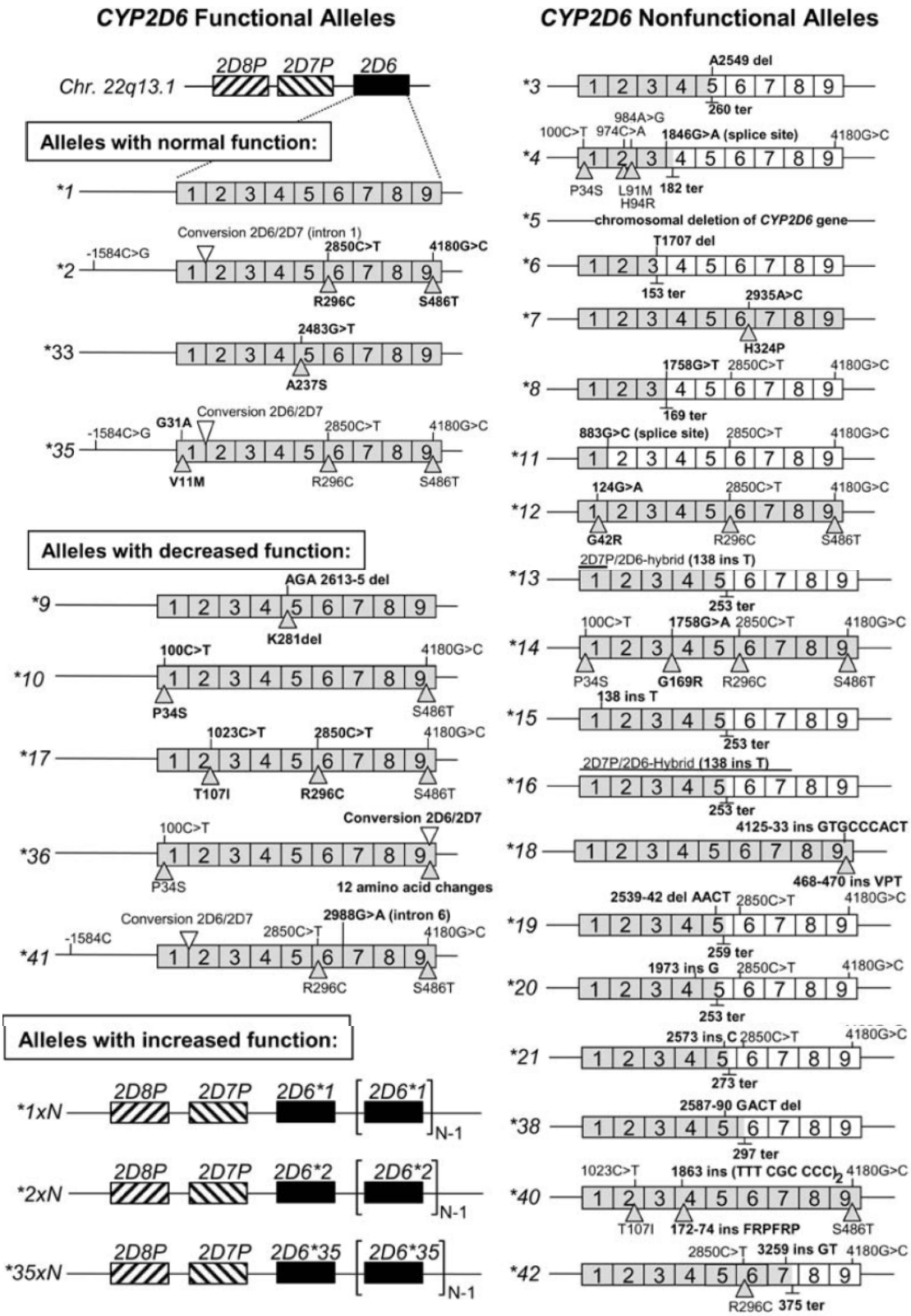

Figure 6. Structure of functional and non-functional *CYP2D6* alleles. Only alleles with available phenotypic information are shown. The 9 exons are indicated by numbered boxes with DNA polymorphisms indicated on top (*del* deletion, *ins*insertion). Predicted amino acid changes and translation termination (*ter*) codons are indicated below. Open reading frames are indicated by *shaded boxes*. Silent mutations and some promoter and intronic polymorphisms as well as alleles with uncertain function are not shown. Zanger UM. . *Naunyn-Schmiedeberg's Arch Pharmacol.* 369 (2004): 23–37.

Prevalence of *CYP2D6* polymorphism

The incidence of single-nucleotide polymorphism (SNP) in *CYP2D6* has been manifested to vary by race and ethnicity [11, 12], as shown in Table 3. The poor metabolizers, which are identified by nonexistence of *CYP2D6* enzyme activity, are found in 7-10% of European and North American Caucasians, and less than 1% of Japanese, Koreans, or Chinese. [39] *CYP2D6*3, CYP2D6*4, CYP2D6*5*, and *CYP2D6*6* cause absence of enzyme activity. [46] *CYP2D6*4* is one of the most important functionally altered null variants in Caucasians (15–21%), but is rare in Asians. [47] The allele frequency of *CYP2D6**5 in Asians is approximately 6%. [42] The alleles associated with reduced enzyme activity resulting from the formation of an unstable enzyme include *CYP2D6*10* (up to 50% in Asians) [47] and *CYP2D6*17* (20–34% in African and African Americans) [48], whereas only 2% Caucasians. [49] The rapid activity spectrum (UM) carry gene duplications and multiplications of functional alleles, which lead to higher *CYP2D6* expression and enzyme activity, with relatively low frequency observed in Caucasians and Asians[47], but commonly observed in Ethiopians (up to 30%).[50-51]

Table 3: Allele frequencies of the *CYP2D6* in Thai population and other ethnic populations.

CYP2D6 allele	Mutation	Consequence	% Allele Frequency			
			Caucasians	Africans	Asians	Thais
CYP2D6 *2xn	Gene duplication/ amplification	Increased activity	1-5	10-16	0-2	3.6
*4	Defective splicing	Inactive enzyme	15-21	2	1	1.8
*5	Gene deletion	No enzyme synthesis	2-7	4	6	5.4
*10	P34S, S486T	Decreased activity	1-2	6	51	37.8
*17	T107I, R296C, S486T	Decreased activity	0-2	20-34	0	0.01

Effects of *CYP2D6* polymorphism on tamoxifen and its metabolites plasma concentration

Tamoxifen and its metabolite plasma concentrations are associated with *CYP2D6* polymorphism. Several studies reported the effects of *CYP2D6* polymorphism on pharmacokinetics of *CYP2D6* substrates.[52] There are 3 studies of the effect of *CYP2D6* polymorphism on tamoxifen and its metabolites plasma concentrations in Asian population.

Lim HS.et al. [53] determined the effect of the effect of *CYP2D6* polymorphism *(CYP2D6*2xN, CYP2D6*5, and CYP2D6*10*) on tamoxifen and it metabolites plasma concentrations in 202 women who received tamoxifen 20 mg/day for more than 8 weeks (12 women who were taking tamoxifen for metastatic breast cancer; 190 women who were taking tamoxifen for adjuvant therapy). The steady-state plasma concentrations of both endoxifen and 4-OH-Tam were significantly lower in patients carrying the *CYP2D6*10/*10* genotype than in those with *wt/*10* or *wt/w*t (endoxifen, 7.9 vs 19.9 or 18.1 ng/ml, $P < 0.0001$; 4-OH-Tam, 1.5 vs 2.5 or 2.8 ng/ml, $P < 0.0001$). The patients who were homozygous for **10* or **5 (V/V)* also displayed lower concentrations than those with *w/v* or *w/w* (endoxifen, 8.1 vs 18.0 or 20.7 ng/ml, $P < 0.0001$; 4-OH-Tam, 1.5 vs 2.5 or 2.9 ng/ml, $P < 0.0001$). The mean concentrations of endoxifen and 4-OH-Tam in patients carrying the *CYP2D6*2N* alleles were 18.2 and 2.4 ng/ml, respectively, which were not significantly different compared with the other genotypes for both compounds. In summary, this study suggested that the CYP2D6**10/*10* genotype is a marker, which associated with lower steady-state plasma concentrations of tamoxifen active metabolites.

Xu Y. et al. [54] determined the correlation between *CYP2D6*10* genotype and serum concentrations of tamoxifen and 4-OH-Tam in 37 pre and postmenopausal women who received tamoxifen 20 mg/day as adjuvant therapy. The mean serum concentration of tamoxifen was not significantly different between the genotype groups (*C/C* versus *C/T* or *T/T*: $P = 0.07$ or 0.78). In contrast, the mean serum concentrations of 4-OH-Tam were statistically significantly lower in women carrying *CYP2D6*10* homozygous variant *T/T* genotype than in women carrying homozygous wild-type *C/C*

genotype (*P* = 0.04). The serum concentrations of 4-OH-Tam were not significantly different between women carrying homozygous wild-type *C/C* and heterozygous *C/T* genotype groups (*P* = 0.96). This study showed that the serum concentration of 4-OH-Tam was influenced by *CYP2D6*10* genotype, although recent studies manifested to endoxifen is a new member of active metabolite of tamoxifen, 4-OH-Tam is also well documented to be an important active metabolite of tamoxifen.

Kiyotani K. et al. [55] determined the effects of *CYP2D6* polymorphism and *ABCC2* genotypes on the plasma concentrations of tamoxifen and its metabolites in 98 breast cancer women who received tamoxifen 20 mg/day as adjuvant therapy. All of the decreased and null alleles (**4*, **5*, **10*, **10/*10*, **14*, **21*,**36/*36*, and **41*) were variants alleles (*V),* and **1* and **1/*1* alleles were wild-type allele (*wt)*. The steady-state plasma concentrations of endoxifen among three genotype groups of *wt/wt*, *wt/V*, and *V/V* were significantly different (*P* = 0.0000043). The median plasma concentrations of endoxifen in patients carrying *V/V* (*15.5* ng/ml) and *wt/V* (27.2 ng/ml) were lower than patients carrying *wt/wt* (35.4 ng/ml). The differences in 4-OH-Tam plasma concentrations among three *CYP2D6*10* genotype groups were also statistically significant (*P* = 0.00052). Nevertheless tamoxifen and NDM plasma concentrations were not significantly different among three genotype groups.

Table 4. Summary of clinical studies analyzing impact of *CYP2D6* on the tamoxifen and its metabolite plasma concentrations

Author and Year	Number of Patients	Study design	Patient and Study characteristics	Genotypes	Results
Lim et al. [53] 2007	202	Prospective study	Korean pre-and postmenopausal women with ER or PR-positive, treated with tamoxifen 20 mg/day for 9 months.	*CYP2D6 *2xN,*5,*10*	Mean Endoxifen plasma concentrations (range) *wt/wt* (n=64) 19.9 ng/ml (18.0 to 21.9) *wt/*10* (n=89) 18.1 ng/ml (16.8 to 19.5) **10/*10* (n=49) 7.9 ng/ml (7.1 to 8.8) *P*< 0.0001 4-OH-TAM plasma concentrations *wt/wt* (n=64) 2.8 ng/ml (2.5 t0 3.1) *wt/*10* (n=89) 2.5 ng/ml (2.4 to 2.7) **10/*10* (n=49) 1.5 ng/ml (1.3 to 1.6) *P*< 0.0001
Xu Y. et al. [54] 2008	37	Retrospective study	Chinese women with newly diagnosed breast cancer who were taking tamoxifen (20 mg/day orally) for at least 4 weeks as standard adjuvant therapy.	*CYP2D6 *10*	Tamoxifen plasma concentrations *wt/wt* (n=64) 208.0 ±66.4 ng/ml *wt/*10* (n=89) 272.4 ±100.7 ng/ml *P*= 0.07

CYP2D6=Cytochrome P450 (CYP450) 2D6; ER=estrogen receptor; PR=progesterone receptor; 4-OH-Tam = 4-hydoxy-tamoxifen

Author and Year	Number of Patients	Study design	Patient and Study Characteristics	Genotypes	Results
					**10/*10* (n=49) 215.7 ±81.4 ng/ml *P*= 0.78 4-OH TAM plasma concentrations *wt/wt* (n=18) 5.3 ±1.5 ng/ml *wt/*10* (n=7) 5.2 ±1.8 ng/ml *P*= 0.96 **10/*10* (n=12) 4.1±1.5 ng/ml *P*= 0.04
Kiyotani et al. [55] 2010	98	Retrospective study	Japanese women with ER and/or PR positive invasive breast cancer receiving adjuvant tamoxifen 20 mg/day without any other anticancer treatments for 5 years.	*CYP2D6*1, *10*	Median endoxifen plasma concentrations *wt/wt* = 35.4 ng/ml *wt/*10* = 27.2 ng/ml **10/*10* = 15.5 ng/ml *P*= 0.0000043

CYP2D6=Cytochrome P450 (CYP450) 2D6; ER=estrogen receptor; PR=progesterone receptor; 4-OH-Tam = 4-hydoxy-tamoxifen

Effects of *CYP2D6* polymorphism on clinical outcomes

Several studies reported the correlate *CYP2D6* polymorphism and clinical outcomes of breast cancer women with estrogen receptor positive who received tamoxifen. There are 4 studies of the effect of *CYP2D6* polymorphism on clinical outcomes in Asian population.

Lim HS et al. [53] evaluated the association of *CYP2D6* polymorphisms and clinical outcomes in 21metastatic breast cancer patients (MBC), receiving tamoxifen 20 mg/day. All nine patients (100%) carrying *CYP2D6 wt/wt* or *wt/*10* genotypes, and six of the 12 patients (50%) carrying **10/*10* genotypes had clinical benefit (*P* = 0.0186). The median time to progression (TTP) was decrease in patients carrying *CYP2D6*10/*10*, compared with patients carrying *CYP2D6*1/*1, CYP2D6*1/*10* (5.0 *vs* 21.8 months, *P* = 0.0032). Fifteen of the 21 MBC patients accomplished in clinical benefit (CR, complete response; PR, partial response; SD, stable disease) for 24 weeks, nine MBC patients had progressive disease (PD) or SD with less than 24 weeks, and overall time to progression (TTP) of 8.7 months.

Kiyotani K .et al. [61] evaluated the clinical outcomes of *CYP2D6*10* allele in 67 patients, who had been treated with tamoxifen (20 mg/day) for 5 years as adjuvant monotherapy. Patients carrying *CYP2D6*10* alleles had a significantly higher incidence of 10 years-recurrence after operation (*P* = 0.0057; odds ratio, 16.63; 95% confidence interval (CI), 1.75–158.12), compared with those homozygous for the wild-type *CYP2D6*1* alleles. The result also showed that patients carrying *CYP2D6*10* alleles had a significantly higher incidence of recurrence than the combined group of patients carrying *CYP2D6*1/*1 allele plus *1/10* (*P* = 0.0079; odds ratio, 6.65; 95% CI 1.68–26.38). The increased risk of recurrence might related to the quantity of *CYP2D6*10* alleles (*P* = 0.0031). In Kaplan–Meier analysis, patients carrying *CYP2D6*10/*10* had a shorter significantly recurrence free survival than those with *CYP2D6*1/*1* or those with *CYP2D6*1/*1 + *1/*10* (*P* = 0.0031 or *P* = 0.0010; respectively). After adjustment of prognosis factors, patients carrying *CYP2D6*10/*10* genotype had a significantly shorter recurrence-free survival; RFS (*P* = 0.036; adjusted

hazard ratio, 10.04; 95% CI, 1.17–86.27) compared to patients carrying *CYP2D6*1/*1.* This study indicate that *CYP2D6*10* is one of the most important determinants for clinical outcomes in adjuvant tamoxifen therapy in the Asian population.

Xu Y.et al. [58] evaluated the correlation between *CYP2D6 *10* genotype and clinical outcomes in 152 breast cancer patients who received tamoxifen treatment or those who did not (received chemotherapy). *CYP2D6*10* genotype was significantly associated with 5-year disease-free survival (DFS). Patients carrying homozygous variant *T/T* genotype had a significantly worse DFS than those carrying homozygous wild-type *C/C* or heterozygous *C/T* genotype (89% *vs* 96%, *P* = 0.005). In multivariate analysis, as compared with *C/T* or *C/C* genotype, T/T genotype was an independent prognostic marker of DFS (hazard ratio = 4.7; 95% CI= 1.1–20.0; *P* = 0.04). In subgroup of 125 patients who had ER positive, patients carrying T/T genotype had a worse DFS than those of C/T or C/C genotype *(P* = 0.03). In summary, this study presented that *CYP2D6 *10* genotype influenced on efficacy of tamoxifen treatment in Chinese women.

Kiyotani K .et al. [59] evaluated the clinical outcomes of *CYP2D6*10* allele in 282 invasive breast cancer patients with hormone receptor–positive, receiving tamoxifen monotherapy, including 67 patients in previous study. In Kaplan-Meier analysis, patients carrying one or two variant alleles (wt/vt or vt/vt) had significantly shorter RFS compared with patients carrying homozygous wild-type alleles (wt/wt; log-rank *P* = 0.0002). In Cox proportional hazards analysis, the CYP2D6 genotype was an independent indicator of RFS after adjustment of tumor size and nodal status (*P* = 0.000036). The adjusted hazard ratios (HRs) of patients carrying wt/vt and vt/vt genotypes, relative to patients carrying wt/wt, were 4.44 (95% CI, 1.31-15.00) and 9.52 (95% CI, 2.79-32.45), respectively.

Table 5. Summary of clinical studies analyzing impact of *CYP2D6* on clinical outcomes

Author and Year	Number of Patients	Study design	Patient and Study Characteristics	Genotypes	Results
Lim et al. [53] 2007	21	Retrospective study	Korean women with ER or PR-positive pre-and postmenopausal women, treated with tamoxifen 20 mg/day for 9 months.	*CYP2D6 *2xN,*5,*10*	TTP was shorter in *CYP2D6*10/*10*, compared with other genotypes; **1/*10, *10/*10* (5.0 *vs* 21.8 months, *P = 0.0032*).
Kiyotani et al. [57] 2008	67	Retrospective study	Japanese women with ER and/or PR positive invasive breast cancer with surgical treatment followed by 5 years of tamoxifen 20 mg/day.	*CYP2D6*10*	*CYP2D6*10/*10* had a significantly higher 10 years-recurrence than *CYP2D6*1/*1* (*P*= 0.0057; OR, 16.63; 95% CI, 1.75–158.12). Multivariate analysis: *CYP2D6*10/*10* was associated with shorter RFS (*P*= 0.036; adjusted HR, 10.04; 95% CI, 1.17–86.27).

CI=confidence interval; CYP2D6=Cytochrome P450 (CYP450) 2D6; ER=estrogen receptor; HR=hazard ratio; PR=progesterone receptor; TTP = Time to progression

Author and Year	Number of Patients	Study design	Patient and Study Characteristics	Genotypes	Study design
					Kaplan–Meier analysis: *CYP2D6*10/*10* had a shorter significantly RFS than CYP2D6*1/*1 (*P* = 0.0031) or *CYP2D6*1/*1 + *1/*10* (*P* = 0.0010)
Xu Y. et al.[54] 2008	152	Retrospective study	Chinese women with newly diagnosed breast cancer who were taking tamoxifen (20 mg/day orally) for at least 4 weeks as standard adjuvant therapy.	*CYP2D6 *10*	Homozygous variant *T/T* genotype had a significantly worse DFS than homozygous wild-type *C/C* or heterozygous *C/T* genotype (89% vs 96%, *P* = 0.005).

CYP2D6=Cytochrome P450 (CYP450) 2D6; DFS=disease-free survival; RFS=relapse-free survival.

Author and Year	Number of Patients	Study design	Patient and Study Characteristics	Genotypes	Study design
					Multivariate analysis: T/T genotype was an independent prognostic marker of DFS (hazard ratio = 4.7; 95% CI = 1.1–20.0; *P* = 0.04), compared with *C/T* or *C/C* genotype.
Kiyotani et al. [55] 2010	282	Retrospective study	Japanese women with ER and/or PR positive invasive breast cancer receiving adjuvant tamoxifen 20 mg/day without any other anticancer treatments for 5 years.	*CYP2D6*1, *10*	In Kaplan-Meier analysis, *wt/vt or vt/vt* had significantly shorter RFS than *wt/wt* (log-rank P = 0.0002). The adjusted hazard ratios (HRs) of *wt/V* and *V/V*, relative *to wt/wt*, were 4.44 (95% CI, 1.31 to 15.00) and 9.52 (95% CI, 2.79 to 32.45), respectively.

CI=confidence interval; CYP2D6=Cytochrome P450 (CYP450) 2D6; DFS=disease-free survival; ER=estrogen receptor; HRs=hazard ratios; PR=progesterone receptor.

Tamoxifen and FDA relabeling [59]

FDA Advisory Committee recommended the label of tamoxifen should be changed to include evidence that women with estrogen related breast cancer might be at higher risk for recurrence of the disease in the year 2006. Several studies suggested that the tamoxifen was not as effective in patients carrying poor activity of *CYP2D6* enzyme. That enzyme was responsible for metabolizing tamoxifen into active metabolites, which suppressed cell proliferation. FDA committee considered whether the tamoxifen label should include information about increased risk of recurrence in *CYP2D6* poor metabolizers. However, the committee did not reach a consensus of the recommendation to test the enzyme capabilities. Some experts believed that the genetic test should be recommended, whereas others believed that it should be mentioned in the label as an option for discussion between the physicians and patients.

CYP2D6 genotyping

The published methods for genotyping *CYP2D6* had relied on gene sequencing or use of mismatched primers to generate restriction sites to enable restriction fragment length polymorphism (RFLP) analysis. Sequencing was expensive, and required specialized equipment. RFLP might be an option, but could be time-consuming. In *CYP2D6* analysis, the amplification, digestion and visualization methods were technically more involved than standard RFLP protocols. This was due to the absence of naturally occurring splice site for known restriction endonucleases. Allelic discrimination assay was an alternative method which was rapid and reliable for genotyping *CYP2D6* polymorphism. In allele specific polymerase chain reaction (PCR) amplification, oligonucleotides specific for hybridizing with the common or variant alleles are used for parallel amplification reaction and then identify for the presence or absence of the appropriate amplified DNA products by real-time fluorescence-based analysis, melt curve analysis or gel electrophoresis.[60-61]

The plasma concentration of tamoxifen and its metabolites analytical methods [63]

The analytical methods of tamoxifen and its metabolites had been documented, including GC–MS [64-65], TLC [66-67], HPLC [68-71], LC–MS [72-73].The procedures with gas chromatography or capillary electrophoresis–electrospray ionization with mass spectrometry are high specificity, but required derivatization of sample and implicate equipment not generally available [29]. Several studies published thin-layer or high-performance liquid chromatographic methods [29, 68-71, 74-75] involved photochemical conversion of tamoxifen and its metabolites to fluorescent phenanthrene derivates, which might be detected by fluorescence detectors with high sensitivity. Golander Y and Sternson LA [74] described the high-performance liquid chromatography method with fluorescent detection (HPLC-FLU) by offline pre-column UV irradiation for tamoxifen and metabolites analysis. Subsequently, several methods displayed the technical problem of assay: broad, irregular peaks and irreproducible, resulting in chromatograph and avoided the problem by using post-column irradiation.[68] The offline pre-column HPLC-FLU method still had some advantages in the sensitivity and convenience, compared to other post-column methods.

CHAPTER III
PATIENTS AND METHOD

This study was conducted from March 2011 to February 2012 at Phramongkutklao Hospital, Bangkok, Thailand.

1. Study design

A prospective clinical study was used. Demographic data and measured drugs plasma concentrations from patients were collected, *CYP2D6* genes were genotyped, and the data were then analyzed.

2. Patients

2.1 Population and samples

2.1.1 Population for *CYP2D6* genotypic analysis is breast cancer patients and population for tamoxifen and its metabolites analysis is breast cancer patients who received tamoxifen 20 mg/day.

2.1.2 Samples are breast cancer patients who were outpatients at Phramongkutklao Hospital during March 2011 to February 2012 and met the inclusion criteria.

2.2 Inclusion criteria

2.2.1 Patients who were diagnosed to breast cancer.

2.2.2 Age over 20 years old.

For the part of tamoxifen and its metabolites analysis, patients would be included if they were in criteria as described below.

2.2.3 Patients received tamoxifen 20 mg/day as adjuvant therapy for at least 8 weeks.

2.2.4 Patients with estrogen or progesterone receptor positive.

2.2.5 Patients with Eastern Cooperation Oncology Group (ECOG) 0-2.

2.2.6 Patients with normal renal function (serum creatinine (SCr) 0.5-1.2 mg/dl).

2.2.7 Patients with normal liver function (AST, ALT 0-38 units/liter).

2.2.8 No history of thromboembolism, and cerebrovascular disease.

2.2.9 All patients consented to enroll in this study.

2.3 Exclusion criteria

2.3.1 Patients were taking concomitant medicines that are known to inhibit *CYP2D6* activity.

2.3.2 Patients with non-compliance of taking tamoxifen therapy.

2.3.3 Patients whose medical records were not complete or whose required data could not be revealed or were missing

2.4 Sample size determination

Sample size calculation was based on probability to random patients in each genotype group. Given probability of patients in the *CYP2D6 *10* was 0.38 according to data from study of Tassaneeyakul et al, sample size were calculated as below

Formula $n = \frac{p(1-p)(Z_{\alpha/2})^2}{E^2}$ ($\alpha = 0.05$, $Z_{\alpha} = 1.96$, E (error) = 0.1)

$$n = \frac{0.38(0.62)(1.96)^2}{(0.1)^2}$$

$$n = 91$$

Sample size should be at least 91 cases to include patients with *CYPD2D6*10* enough for comparison.

Sample size in this study should be not less than 91. However, with limited time and resources we decided to include last patient in January 2012 which 67th patient were included into the study.

3. Study protocol

3.1 Study protocol was approved by Institutional Review Board, Royal Thai Army, Medical Department, (Bangkok, Thailand), IR number Q039H/53.

3.2 Patients were enrolled following inclusion and exclusion criteria.

3.3 The investigator explained the objective and study protocol to the eligible patients or their legal guardians. Patients or their legal guardians signed in the consent form.

3.4 Demographic data were collected from electronic databases and medical records

3.5 Made an appointment for patient to have his/her blood sample collected at the next visit time. Blood samples for tamoxifen and its metabolites plasma concentrations were drawn at steady state (at least 8 weeks of tamoxifen treatment).

3.6 Coordinated the physicians to order blood samples for tamoxifen and its metabolites in order to measure plasma concentrations and *CYP2D6* genotyping.

3.7 Coordinated the medical technologist for blood sample processing to be measured the plasma concentrations of tamoxifen and its metabolites and *CYP2D6* genotyping.

3.8 Measured tamoxifen and its metabolites serum concentrations and analyzed *CYP2D6* genotyping.

3.9 Collected all the required data and analyzed.

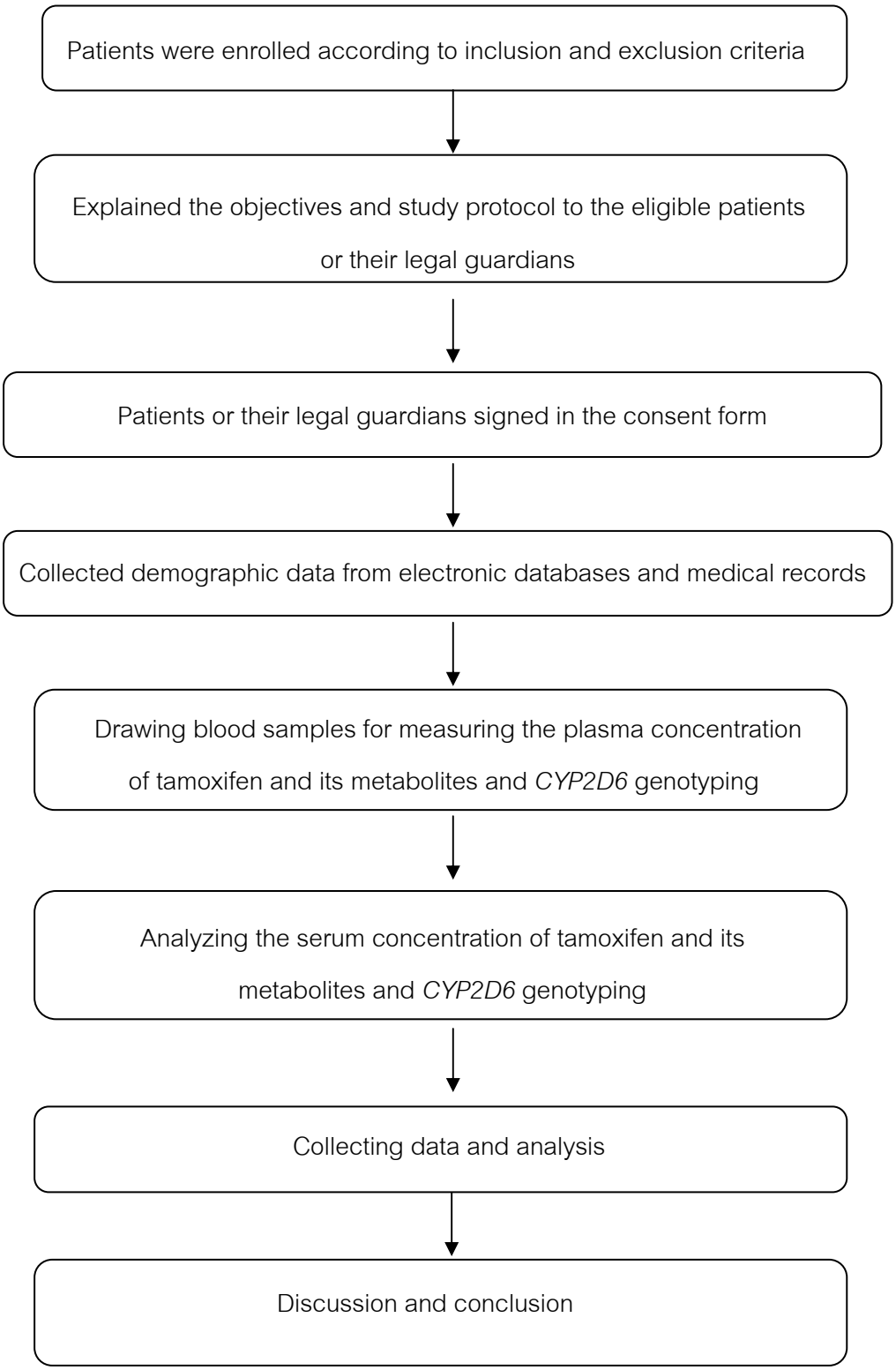

Figure 7: Study protocol

Fifty-eight patients who met the inclusion criteria participated in this study. Blood sampling for tamoxifen and its metabolites plasma concentrations were obtained at the steady-state (at least 8 weeks of tamoxifen treatment). Whole blood was drawn from patients after the administration tamoxifen in the morning. Volume of blood sample was 10 ml, collected in 2 tubes, 5 ml of clot blood tube (red-stopper) for tamoxifen and its metabolites measurement, and 5 ml of Vacutainer® tube (purple-stopper) containing EDTA for *CYP2D6* genotyping analysis.

Whole blood in the EDTA tube was prepared as buffy coat by centrifuge at 2,500 x g for 10 minutes at room temperature. After centrifugation, 3 different fractions were separated, the upper clear layer was plasma; the intermediate layer was buffy coat; containing concentrated leukocytes; and the bottom layer contained concentrated erythrocytes. Two-hundred microliter (µl) of buffy coat was pipetted into microcentrifuge tube size 1.5 ml and stored in a freezer at -20 °C until extracted for DNA.

5. Bio-analysis

5.1 DNA extraction

Buffy coat was used for DNA extraction by utilizing QIAamp® DNA Blood Mini kit.

5.1.1 Materials

All materials were used for DNA extraction, are shown in Table 6, Table 7 and Table 8.

Table 6. Chemical and reagents for DNA extraction

Reagent	Company	Country
Absolute etanol	Carlo erba	Italy
Buffer AL	Qiagen	Germany
Buffer AW1	Qiagen	Germany
Buffer AW2	Qiagen	Germany
Buffer AE	Qiagen	Germany
QIAGEN® protease	Qiagen	Germany
Protease solvent	Qiagen	Germany

Table 7. Apparatus for DNA extraction

Apparartus	Company	Country
Centrifuge (Universal 320)	Hettick	Germany
Vortex mixer (S0100-220)	Labnet	USA
Heating block (Dri-block DB-2D)	Techne	UK
Microcentrifuge (5415R)	Eppendorf	Germany
Spectrophotometer (Smart spec 3000)	Bio-rad ™	USA
Freezer	Sanyo	Japan
StepOnePlus™ Real time PCR Systems	Applied Biosystems Inc.	USA

Table 8. Supplies for DNA extraction

Supplies	Company	Country
Microcentrifuge tube (1.5 ml)	Treff AG.	Switzerland
Pipette tip (Blue and Yellow)	Scientific Plastics	USA
Micropipette 1,000 µl	Eppendorf	Germany
Micropipette 200 µl	Eppendorf	Germany
Micropipette 20 µl	Eppendorf	Germany
QIAamp Mini spin Column	Qiagen	Germany
Collection tube 2 ml	Qiagen	Germany

5.1.2 DNA Extraction method [76]

1. Equilibrate samples and reagents to room temperature.
2. Heat a heating block to 56°C.
3. Pipette 20 µl QIAGEN Protease into a 1.5 ml microcentrifuge tube containing buffy coat 200 µl.
4. Mix by vortex mixer for 15 seconds.
5. Add 200 µl buffer AL to the sample. Mix by vortex mixer for 15 seconds.
6. Incubate at 56°C for 10 minutes.
7. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
8. Add absolute ethanol (96–100%) 200 µl to the sample, and mix again by vortex mixer for 15 seconds. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

9. Carefully apply the mixture to the QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp Mini spin column in a 2 ml clean collection tube, and discard the tube containing the filtrate.
10. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp Mini spin column in a 2 ml clean collection tube, and discard the collection tube containing the filtrate.
11. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes.
12. Place the QIAamp Mini spin column in a 2 ml new collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.
13. Place the QIAamp Mini spin column in a 1.5 ml clean microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 minute, and then centrifuge at 6000 x g (8000 rpm) for 1 minute.
14. For long-term storage of DNA, eluting in Buffer AE and storing at –20°C.

5.2 *CYP2D6* genotyping

The *CYP2D6* genotype was determined by the TaqMan Allelic Discrimination Assay. The primers and probes for this assay are commercial available through Applied Biosystems Inc, USA. (TaqMan Drug Metabolism Genotyping Assay, Assay ID: C_11484460_40 for C>100T, Applied Biosystems Inc.) TaqMan PCR and fluorescence measurements were performed using the StepOnePlus™ Real time PCR Systems (Applied Biosystems Inc., Foster City, CA USA) following the manufacturer's instructions. See methods at Appendix D.

5.3 Measurement of serum concentrations of tamoxifen and its metabolites

Plasma concentrations of tamoxifen and metabolites, N-desmethyl-tamoxifen (NDM), and endoxifen were measured using high-performance liquid chromatography method with fluorescence detection as described by Zhu YB et al. [63], with modifications. Tamoxifen (≥ 99%), NDM (> 98%), (*Z*)-4-OH-tam (≥ 98%), endoxifen (mixture of *E* and *Z* isomer 25:75), and internal standard mexiletine (> 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All these were stored at -20 ˚C until use. All other chemicals and reagents used were of qualified commercial products.

5.3.1 Sample preparation [63]

All plasma samples were taken out from –20°C, and thawed at room temperature. One milliliter plasma was placed into clean centrifuge tube, and then 5 μl internal standard solution were added to each tube and mixed. The mixtures were added to 1.5 ml Acetonitrile, vortex-mixed for 1 minute and centrifuged for 20 minutes. Exactly 1 ml of supernatant was transferred to clear vial and left under UV lamp for 20 minutes before injection of 20 microliters onto the column.

5.3.2 Instruments and chromatography conditions [63]

High-performance liquid chromatography was accomplished by using an Agilent 1200 series liquid chromatography with a binary pump, on-line degasser, autosampler and column heater, a UV detector and a fluorescence detector.

Separation was carried out on an Agilent Extend C_{18} chromatography column (150 mm x 4.6 mm, 5 μm, Agilent, USA) set at 35°C, and the mobile phase was composed of methanol-1% triethylamine aqueous solution (pH 11: 82:18, v/v), a flow rate of 1.1 mi/min. Fluorescence detector was set as excitation wavelength (λ_{ex}) 260 nm., and emission wavelength (λ_{em}) 375 nm. Peak areas of each compound were generated from computerized software (Agilent, USA).

6. Statistical analysis

Statistical analyses were determined using the Statistical Package for Social Sciences (SPSS Co., Ltd., software version 17.0.) Both descriptive and inferential statistics were determined. The level of significance was set at an $\alpha = 0.05$. Continuous variables was determined for normality of the distribution using Kolmogorov–Smirnov test and determined for homogeneity of variance using Levene's test.

Demographic data were determined and presented as mean ± SD, median, percentage or frequency where appropriate for qualitative or quantitative variables. The differences in the plasma concentrations of tamoxifen and it metabolites among genotypes (*CYP2D6*1 VS CYP2D6*1*/0 *VS CYP2D6*10/10*) were analyzed using a one-way analysis of variance (ANOVA) test, median test or Kruskal-Wallis H test. Statistic tests provided two-sided *P* value, and a significant level $P < 0.05$ was used.

CHAPTER IV
RESULTS

Part 1 Prevalence of *CYP2D6*10*

A prospective data were collected from electronic databases and medical records at breast cancer surgery clinic, Phramongkutklao hospital during March 2011 to February 2012.

Demographic data

For the part of *CYP2D6*10* prevalence, a total of 67 eligible women with diagnosed breast cancer were enrolled from breast cancer surgery clinic at Phramongkutklao hospital. Seventy-one percent (48 of 67) was premenopausal and 28.4% (19 of 67) was postmenopausal women. The mean age was 48.1 ± 8.4 years. The staging was displayed in 3 groups; stage I (25.4%), stage II (59.7%), and stage III (14.9%), Patient's characteristic was shown in Table 9.

Table 9: Demographic data of patients

Descriptive data	N	%
Number of patients	67	100
Age (year),		
Mean ± SD	48.1 ± 8.4	
Height (cm.)		
Mean ± SD	156.7 ± 4.3	
Weight (kg.)		
Mean ± SD	55.3 ± 8.2	
Menopausal status		
Premenopausal	48	71.6
Postmenopausal	19	28.4
Stage		
I	17	25.4
II	40	59.7
III	10	14.9
Tumor size, cm		
≤ 2	28	41.8
>2	39	58.2
Nodal status		
N0	36	53.7
N1	23	34.3
N2	8	11.9
ER status		
Positive	60	89.6
Negative	7	10.4
PR status		
Positive	56	83.6
Negative	11	16.4

Abbreviations: ER, estrogen receptor; PR; progesterone receptor

The frequency of *CYP2D6*10*

Among the three genotypes group, 32.8% of the patients was *CYP2D6* homozygous variant genotype (*CYP2D6*10/*10*), 37.3% was *CYP2D6* heterozygous genotype (*CYP2D6*1/*10*), and 29.9% was *CYP2D6* homozygous wild type genotype (*CYP2D6*1/*1*). The allele frequency of *CYP2D6*1* was 49% and *CYP2D6*10* was 51%, as shown in Table 10.

Prevalence of *CYP2D6*10* calculation

Allelic frequencies of *CYP2D6* genotypes were in Hardy-Weinberg Equilibrium (HWE), P = 0.088, as shown in Table 10. The calculation if allelic frequencies were in HWE:

The number of the *1 allele = (20 x 2) + (25 x 1) = 65 alleles

The number of the *10 allele = (22 x 2) + (25 x 1) = 69 alleles

The frequency of the *1 allele = p = 65 / (65 +69) = 0.49

The frequency of the *10 allele = q = 69 / (65 + 69) = 0.51

The proportion of expected **1/*1, *1/*10* and **10/*10* genotypes could be predicted from HWE: p+q = 1 and $(p + q)^2 = 1$ or $p^2 + 2pq + q^2 = 1$

p^2 = 0.49x 0.49 = 0.2401

2pq = 2 x 0.49 x 0.51 = 0.4998

q^2 = 0.51 x 0.51 = 0.2601

The total number of patients included to this study was 67

Expected number of **1/*1* = 0.2401 x 67 = 16.09 ≈ 16

Expected number of **1/*10* = 0.4998 x 67 = 33.49 ≈ 34

Expected number of **10/*10* = 0.2601 x 67 = 17.43 ≈ 17

The observed number of **1/*1* = 20

The observed number of **1/*10* = 25

The observed number of **10/*10* = 22

Chi-square = 4.853, *P* = 0.088. Therefore, we would accept the null hypothesis that the observed and expected values are not significantly different, and that our population is indeed in Hardy Weinberg equilibrium.

Table 10: Prevalence of *CYP2D6* genotype

(67 patients x 2 alleles)				Genotypes	Observed N=67	%	Predicted (HWE)
Alleles	N=134	%	95%CI				
1	65	49	45.5-52.5	***1/*1***	20	32.8	16
				1/*10	25	37.3	34
10	69	51	47.5-54.5	***10/*10***	22	29.9	17
Chi-square = 4.853, *P* = 0.088							

***CYP2D6* genotypic distribution among patients who were treated with tamoxifen**

For the part of tamoxifen and its metabolites plasma concentrations analysis, 59 eligible patients would be included. *CYP2D6*10* allele frequencies and distribution of genotypes were shown in Table 11. The frequencies of both *CYP2D10* were within Hardy-Weinberg equilibrium.

Table11. Allele frequency and genotypes of *CYP2D6*

	Allele frequency		Genotype N (%)		
CYP2D6	**1*	**10*	**1/*1*	**1/*10*	**10/*10*
	0.47	0.53	16 (27.1)	23 (39)	20 (33.9)

Part 2 Plasma concentration of tamoxifen and its metabolites

Out of Sixty seven patients who were genotyped, 60 women who received tamoxifen 20 mg/day as adjuvant therapy were included in analysis of Tamoxifen and its metabolites; one was excluded due to noncompliance to tamoxifen treatment. Therefore, tamoxifen and its metabolites plasma concentrations of fifty-nine patients were used in analysis. A prospective data, March 2011 – February 2012, were collected from electronic databases and medical records at the breast cancer surgery clinic, Phramongkutklao hospital. Duration of tamoxifen treatment was in range of 1.5 month to 79 months (median 26 months).

Plasma concentration of tamoxifen and its metabolites

Range, mean±SD and median concentrations of tamoxifen and its metabolite at steady state of all 59 patients were presented in Table 12.

Table 12. Plasma concentration of tamoxifen and its metabolites

	Range (ng/ml)	Mean (±SD) (ng/ml)	Median (ng/ml)
Tamoxifen	28.12-714.56	367.09 ± 146.33	336.51
NDM	87.19-1355.71	532.50 ± 236.63	532.70
Endoxifen	1.88 – 66.15	18.5 ±12.61	15.33

Part 3 Effect of CYP2D6 polymorphism on tamoxifen and its metabolites plasma concentration

Polymorphisms of *CYP2D6* influenced in the differences of tamoxifen and its metabolites. Tamoxifen and NDM in patients with homozygous wild type were lower than patients with heterozygous and homozygous variants *CYP2D6*10*, while the opposite pattern of differences were seen in endoxifen concentrations. Patients with homozygous variant *CYP2D6*10* had endoxifen concentrations lower than the other groups. These results indicated the impacts of *CYP2D6* polymorphism on tamoxifen metabolism.

Average Tamoxifen and its metabolites plasma concentrations in different *CYP2D6* genotypes were shown in Table 13.

Table 13. Tamoxifen and its metabolites plasma concentrations in different *CYP2D6* genotypes

Concentration (ng/ml)	**1/*1*	**1/*10*	**10/*10*	*P-value*
Tamoxifen	323.6±79.8	336.3±151.1	437.3±161.2	0.027*
NDM	458.7±129.8	481.6±213.5	650.1±287.4	0.020*
Endoxifen (mean)	22.4±12.8	17.9±9.8	14.7±14.7	0.191
Endoxifen(median)	21.55	15.67	9.62	0.045*

Note: median was presented for END concentrations because of distribution was not normal

The differences of tamoxifen and its metabolites plasma concentrations were also shown in the box plots in Figure 8 to 10.

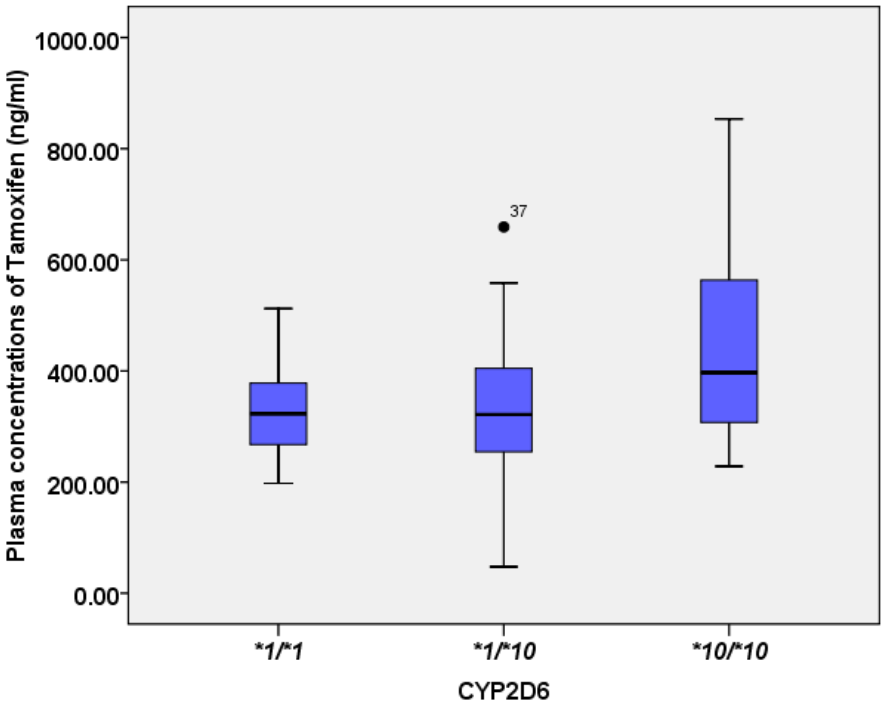

Figure 8. Tamoxifen plasma concentrations in different *CYP2D6* genotypes

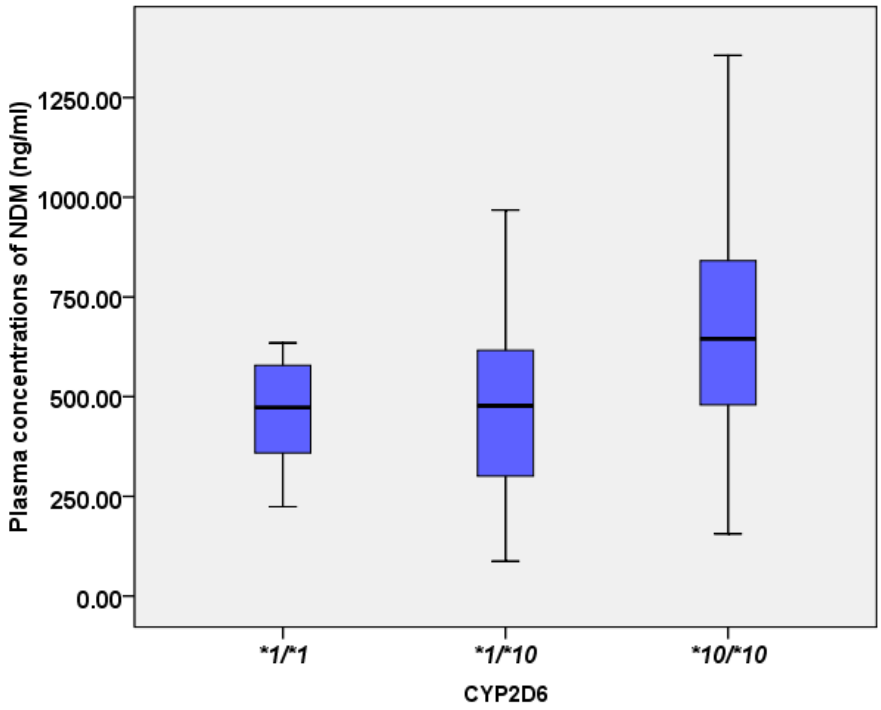

Figure 9. NDM plasma concentrations in different *CYP2D6* genotypes

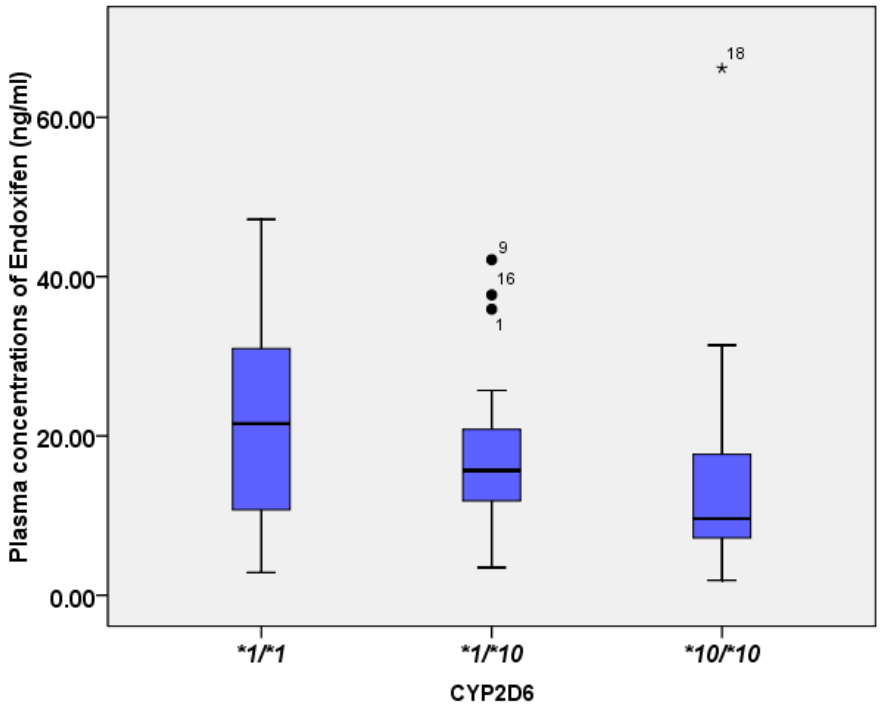

Figure 10. Endoxifen plasma concentrations in different *CYP2D6* genotypes

CHAPTER V
DISCUSSION AND CONCLUSION

Discussion

Part 1 Prevalence of *CYP2D6*10*

*CYP2D6*10* is the most frequently *CYP2D6* alleles found in Asian populations, which has been reported to be approximately 50%, and associated with decreased *CYP2D6* activity. In Thai populations, there was only few studies about *CYP2D6*10* frequency. Our results showed the frequency of *CYP2D6*10* to be 51%, which was nearly to previous studies. [12, 61] Nakmahachalasint P. [61] reported the frequency of *CYP2D6*10* in Thai subjects to be 69.5%. Tassaneeyakul W. [12] reported the frequency of *CYP2D6*10* in Thai subjects to be 37.8%. In addition, the prevalence of *CYP2D6*10* in Thai women was much higher than in Caucasians, which was reported to be only 1-2%. [12]

Part 2 Concentration of tamoxifen and its metabolites

This study is the first study to determine the steady state concentration of tamoxifen and its metabolites in Thai breast cancer women. In our results, the mean plasma concentration of tamoxifen (367.09 $\pm$ 146.33 ng/ml), NDM (532.50 $\pm$ 236.63 ng/ml), and endoxifen (18.5 $\pm$12.61 ng/ml) were similar to reported in the literatures [29, 63]. We analyzed tamoxifen and its metabolites plasma concentrations by Zhu et al [63] modification method. Even though our result could not detect 4-OH-Tam plasma concentration which is one of active metabolites due to its low plasma concentration, we could determine the endoxifen plasma concentration which is another active metabolites and 5- to 10-fold higher concentrations than 4-OH-Tam [9, 31].

Part 3 Effect *of CYP2D6*10* on tamoxifen and its metabolites plasma concentrations

*CYP2D6*10* is the most common polymorphism in Asian populations that reduces enzymatic activity. Our study hypothesized that invasive breast cancer patient carrying *CYP2D6*10*, who received adjuvant tamoxifen, might not produce a sufficient concentration of pharmacologically active tamoxifen metabolites. Endoxifen is highly active tamoxifen metabolites with100-fold greater affinity to ER and 30-to-100-fold greater potency in suppressing estrogen-dependent cell proliferation, thus endoxifen is a major metabolite to predict the therapeutic outcomes. There was no prior study of the endoxifen plasma concentration in Thailand. This study is the first study to determine the association between *CYP2D6*10* and the plasma concentration of tamoxifen and its metabolites in Thai breast cancer women.

Results in this study were consistent with the previous studies of Lim et al. [53] and Kiyotani K et al.[55] which showed that the plasma concentration of endoxifen was significantly lower in women with variant *CYP2D6* homozygous genotypes (*CYP2D6*10/*10*) than those with heterogenous genotypes (*CYP2D6*1/*10*) or homozygous wild type genotypes (*CYP2D6*1/*1*). Lim et al. [53] reported that the steady-state plasma concentrations of endoxifen were significantly lower in patients with the *CYP2D6*10/*10* genotype than those with *CYP2D6*1/*10* or *CYP2D6*1/*1* (endoxifen, 7.9 ng/ml *vs* 19.9 ng/ml *vs* 18.1 ng/ml, $P < 0.0001$). Kiyotani K et al. [55] reported that the steady-state plasma concentrations of endoxifen among three genotype groups of *CYP2D6*1/*1*, **1/*10*, and **10/*10* were significantly different ($P = 0.0000043$). Median plasma concentrations of endoxifen in patients carrying *CYP2D6*10/*10* and *CYP2D6*1/*10* were 15.5 ng/ml and 27.2 ng/ml, respectively, which were only 43.8% and 76.8% of the concentration detected in patients with *CYP2D6*1/*1* (35.4 ng/ml). In this study, the patients carrying *CYP2D6*10/*10* had the lowest median plasma concentrations of endoxifen (9.62 ng/ml) compared to *CYP2D6*1/*10* and *CYP2D6*1/*1* (15.67 ng/ml and 21.55 ng/ml, respectively). However, our data demonstrated the significant different of mean NDM and Tamoxifen plasma concentrations among three

genotypes which was not similar to Lim et al [53] that showed there were no significant association between *CYP2D6* genotype and tamoxifen and NDM plasma concentrations.

Conclusion

In summary, this study indicated that *CYP2D6*10* gene was important factors that influenced the plasma concentrations of tamoxifen and its metabolites especially endoxifen. The *CYP2D6* polymorphic information of each patient should be considered in the selection of adjuvant hormonal treatment.

Limitation

Although the endoxifen is a potent active metabolite, 4-OH-Tam is also another important active metabolite, which is 5-to-10-fold lower concentration than endoxifen. Our HPLC analysis method cannot detect 4-OH-Tam plasma concentration, Therefore, the HPLC method should be improve for measurement of this active metabolite.

Further study

Further studies should determine the impact of polymorphisms on the endoxifen plasma concentrations, which effected by other enzymes in the metabolic pathway of tamoxifen. The association of *CYP2D6*10* and clinical outcomes should be also evaluated.

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APPENDICES

APPENDIX A

แบบบันทึกข้อมูลผู้ป่วยมะเร็งเต้านม

ส่วนที่ 1: ข้อมูลทั่วไปของผู้ป่วย

1. HN..... วันที่……

ที่อยู่……..โทรศัพท์……...

2. วัน เดือน ปี เกิด ……/……/……อายุ.....ปี 3. ECOG score ……..……

4. ส่วนสูง.....เซนติเมตร 5. น้ำหนัก.....กิโลกรัม

6. ประวัติการแพ้ยา ❒ ไม่มี ❒ มี ระบุ.....

7. ประวัติโรคประจำตัว ❒ ไม่มีโรคประจำตัว ❒ มีโรคประจำตัวระบุ…….....

8. ยารักษาโรคประจำตัว มีจำนวน.....ชนิด ได้แก่

.....

.....

ตอนที่ 2: ข้อมูลเกี่ยวกับโรคและการรักษา

1. ระยะโรคมะเร็งเต้านม

❒ ระยะที่ 1 ❒ ระยะที่ 2 ❒ ระยะที่ 3

2. ขนาดก้อนมะเร็ง ❒ ≤ 2 เซนติเมตร ❒ > 2 เซนติเมตร

3. การกระจายไปที่ต่อมน้ำเหลือง

❒ N0 ❒ N1 ❒ N2 ❒ N3

4. การกลับเป็นซ้ำของมะเร็งเต้านม ❒ ไม่มี ❒ มี

5. ระยะเวลาที่ใช้ยาทาม็อกซิเฟน.....ปี.....เดือน

6. ผลข้างเคียงจากการใช้ยา ❒ ไม่มี ❒ มี ระบุ.....

7. การรักษาอื่นๆที่ไม่ใช่ยา ❒ ไม่มี ❒ มี ระบุ.....

ตอนที่ 3: ข้อมูลการตรวจวัดระดับยาในเลือด

วันที่	**เวลา รับประทานยา**	**เวลา เจาะเลือด**	**ระดับยาในเลือด** (ng/mL)			
			Tamoxifen	NDM*	4-OH-tam*	Endoxifen*

*NDM = N-desmethyl tamoxifen, 4-OH-tam = 4-hydroxytamoxifen, Endoxifen = 4-hydroxy-N-desmethyltamoxifen

ตอนที่ 4: **ข้อมูลการตรวจยีน *CYP2D6***

1. ภาวะพหุสัณฐานของยีน *CYP2D6*

❒ **1* ❒ **10*

2. ลักษณะของอัลลีล:

❒ **1/*1*

❒ **1/*10*

❒ **10/*10*

APPENDIX B

เอกสารชี้แจงข้อมูล/คำแนะนำแก่ผู้เข้าร่วมการวิจัย

(Patient/Participant Information Sheet)

ชื่อโครงการ ผลของภาวะพหุสัณฐานของยีน *CYP2D6* ต่อเภสัชจลนศาสตร์ของยาทาม็อกซิเฟนในผู้ป่วยไทยโรคมะเร็งเต้านม

ชื่อผู้วิจัย เภสัชกรหญิงภิชญาภา รุ่งวานนท์ชัย นิสิตระดับปริญญาโท ภาควิชาเภสัชกรรมปฏิบัติ สาขาเภสัชกรรมคลินิก คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สถานที่วิจัย โรงพยาบาลพระมงกุฎเกล้า

บุคคลและวิธีการติดต่อเมื่อมีเหตุฉุกเฉินหรือความผิดปกติที่เกี่ยวข้องกับการวิจัย

1. เภสัชกรหญิงภิชญาภา รุ่งวานนท์ชัย

ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ สาขาเภสัชกรรมคลินิก
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ติดตามตัว 08-1721-1567

2. พันเอก นพ.สุขไชย สาทถาพร

ที่อยู่ กองศัลยกรรม
โรงพยาบาลพระมงกุฎเกล้า

โทรศัพท์ติดตามตัว 08-1372-2929

3. นายแพทย์นรินทร์ วรวุฒิ

ที่อยู่ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์
จุฬาลงกรณ์มหาวิทยาลัย
โรงพยาบาลจุฬาลงกรณ์

โทรศัพท์ที่ทำงาน 0-2256-4533

ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยนี้เนื่องจากท่านเป็นโรคมะเร็งเต้านมและได้รับการรักษาด้วยยาทาม็อกซิเฟน โดยท่านจะได้อ่านข้อมูลก่อน (หรือทีมแพทย์ผู้ศึกษาวิจัยอ่านให้ท่านรับทราบ) ถ้าท่านมีข้อข้องใจสงสัยใดๆ เกี่ยวกับการศึกษาวิจัยนี้ สามารถซักถามผู้ทำการศึกษาวิจัยหรือแพทย์ที่ทำการศึกษาวิจัยได้ หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัย ท่านจะได้รับสำเนาใบยินยอมที่ท่านเซ็นชื่อกำกับเก็บไว้ 1 ฉบับ

ความเป็นมาของโครงการ

ทาม็อกซิเฟนเป็นยาที่ใช้สำหรับป้องกันมะเร็งเต้านม การรักษาเสริมหลังผ่าตัด และการรักษาในระยะแพร่กระจายทั้งในผู้ป่วยมะเร็งเต้านมวัยก่อนหมดประจำเดือนและหลังหมดประจำเดือน ทาม็อกซิเฟนเป็นสารประกอบที่ไม่ออกฤทธิ์จะต้องถูกเปลี่ยนแปลงยาที่ตับผ่านเอนไซม์ *CYP450* ได้แก่ *CYP2D6* *CYP3A4*,

CYP2C9, CYP2C19 และ *CYP2B6* ได้สารประกอบที่ออกฤทธิ์ โดย *CYP2D6* จะเป็นเอนไซม์หลักที่สำคัญในกระบวนการเปลี่ยนแปลงยา จากความก้าวหน้าทางเภสัชพันธุศาสตร์พบว่าความผิดปกติของยีน *CYP2D6* มีผลต่อกระบวนการเปลี่ยนแปลงยาทาม็อกซิเมนและประสิทธิผลของการรักษา โดยพบการกลับเป็นซ้ำของมะเร็งเต้านมสูง

ในประเทศไทยยังไม่เคยมีการศึกษาผลของความผิดปกติของยีน*CYP2D6* ต่อระดับทาม็อกซิเฟนและเมแทบอไลท์ในเลือด ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของความผิดปกติของยีน *CYP2D6* ต่อระดับทาม็อกซิเฟนและเมแทบอไลท์ในเลือดและหาความสัมพันธ์ระหว่างความผิดปกติของยีน *CYP2D6* ต่อผลการรักษาทางคลินิก เพื่อใช้เป็นแนวทางในการรักษาและเกิดประโยชน์สูงสุดต่อผู้ป่วยแต่ละราย

วัตถุประสงค์

1. เปรียบเทียบระดับทาม็อกซิเฟนและเมแทบอไลท์ในเลือดในผู้ป่วยโรคมะเร็งเต้านมที่มีความผิดปกติของยีน *CYP2D6* กับผู้ป่วยที่มียีน *CYP2D6* ปกติ
2. เพื่อศึกษาความผิดปกติของยีน *CYP2D6* ต่อผลการรักษาทางคลินิก
3. เพื่อศึกษาระดับทาม็อกซิเฟนและเมแทบอไลท์ในเลือดกับผลการรักษาทางคลินิก

รายละเอียดที่จะปฏิบัติต่อผู้เข้าร่วมการวิจัย

หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัยนี้กรุณาเซ็นชื่อลงในใบยินยอม ท่านจะได้รับการตรวจดังต่อไปนี้

เมื่อท่านมาพบแพทย์ตามนัดท่านจะได้รับการชั่งน้ำหนัก วัดส่วนสูง และได้รับการเจาะเลือดดังต่อไปนี้ ท่านจะได้รับการเจาะเลือดปริมาณ 10-15 มิลลิลิตร (2-3 ช้อนชา) เพื่อตรวจหา

1. ระดับทาม็อกซิเฟนและเมแทบอไลท์ในเลือด
2. ลักษณะของยีน *CYP2D6*

และท่านจะได้รับการสอบถามข้อมูลพื้นฐานทั่วไปโดยใช้แบบสอบถาม

หมายเหตุ ในการนัดเจาะเลือดจะทำในวันที่ท่านต้องมาพบแพทย์อยู่แล้ว และท่านไม่ต้องเสียค่าใช้จ่ายใดๆ ที่นอกเหนือไปจากค่ารักษาพยาบาลของท่านตามปกติ

ประโยชน์ที่จะเกิดแก่ผู้เข้าร่วมการวิจัยและประโยชน์ในทางวิชาการต่อส่วนรวม

1. ได้ทราบลักษณะของยีน *CYP2D6* ของตัวท่านเอง ซึ่งเกี่ยวข้องกับระดับยาในเลือดและผลการรักษา
2. ได้ข้อมูลระดับยาทาม็อกซิเฟนของท่านเมื่อได้รับขนาดยาในปัจจุบันและสามารถใช้เป็นค่าอ้างอิงต่อไป
3. ข้อมูลการศึกษาที่ได้สามารถช่วยให้บุคลากรทางการแพทย์นำมาพิจารณาวางแผนการรักษา เพื่อนำไปสู่การการเลือกยาและคำนวณขนาดยาที่เหมาะสมให้กับผู้ป่วยโดยเกิดประโยชน์สูงสุด
4. ข้อมูลการศึกษาที่ได้สามารถลดค่าใช้จ่ายในการรักษาในกรณีที่ผู้ป่วยมียีน *CYP2D6* ผิดปกติ โดยใช้กำหนดขนาดยาที่เหมาะสมแทนการเปลี่ยนไปใช้ยากลุ่ม Aromatase inhitors เนื่องจากยากลุ่ม Aromatase inhitors เป็นยาที่มีราคาแพงกว่ายาทาม็อกซิเฟนมาก

ความเสี่ยงจากการเข้าร่วมการวิจัย

ความเสี่ยงในการเจาะเลือดคือ อาจมีอาการปวด หรือมีจ้ำเลือดบริเวณที่เจาะ แต่มีความเสี่ยงน้อยมากที่จะเกิดการติดเชื้อ

หากท่านไม่ต้องการเข้าร่วมการศึกษาวิจัย หรือเปลี่ยนใจระหว่างร่วมศึกษาวิจัย

ท่านไม่จำเป็นต้องเข้าร่วมการศึกษาวิจัยนี้หากท่านไม่สมัครใจ หลังจากตัดสินใจเข้าร่วมการศึกษาแล้ว ท่านสามารถถอนตัวได้ตลอดเวลา การตัดสินใจของท่านจะไม่มีผลต่อการรักษาในอนาคตหรือการดูแลอื่นใด หากท่านไม่ต้องการเข้าร่วมการศึกษาหรือต้องการหยุดการศึกษา ณ เวลาใดก็ตาม

การเก็บข้อมูลเป็นความลับ

ข้อมูลของท่านที่ถูกบันทึกไว้ระหว่างการศึกษาจะถูกเก็บไว้เป็นความลับตลอดเวลาเช่นเดียวกับข้อมูลที่เกี่ยวข้องจากแฟ้มเวชระเบียนของโรงพยาบาล คณะกรรมการจริยธรรมการวิจัยและพนักงานหรือผู้วิจัยสามารถที่จะขอตรวจสอบข้อมูลเหล่านี้ได้ โดยข้อมูลเหล่านี้จะยังเก็บรักษาไว้เป็นเรื่องลับเฉพาะ

ข้อมูลส่วนตัวที่ท่านไม่ต้องการเปิดเผยจะถูกเก็บรวบรวมไว้ในฐานข้อมูล และนำมาใช้เพื่อวัตถุประสงค์ทางการวิจัยทางการแพทย์เฉพาะในส่วนที่เกี่ยวข้องกับการศึกษา โดยจะมีการกำหนดสิทธิการเข้าถึงการใช้งานเฉพาะแพทย์ผู้ศึกษาวิจัยและบุคคลที่แพทย์ผู้ศึกษาวิจัยอนุญาตเท่านั้นที่จะมีรหัสผ่านในการเข้าถึงข้อมูล ทั้งนี้เพื่อวัตถุประสงค์ทางการศึกษาวิจัยทางการแพทย์ **โดยไม่มีการอ้างถึงชื่อและเลขประจำตัวผู้ป่วยของท่าน ทางโรงพยาบาลพระมงกุฎเกล้าจะทำทุกวิถีทางเพื่อให้เกิดความมั่นใจว่าข้อมูลส่วนตัวของท่านจะถูกปกป้องไว้**

APPENDIX C

หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย

(Consent Form)

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย รวมทั้งประโยชน์ที่เกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยเมื่อใดก็ได้ โดยการบอกเลิกการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการรักษาโรคและการรับบริการต่างๆที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความสมัครใจต่อหน้าพยาน เพื่อเป็นหลักฐานสำคัญ

ลงชื่อ.....ผู้เข้าร่วมโครงการวิจัย / ผู้แทนโดยชอบธรรม

(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....ผู้ดำเนินการโครงการวิจัย

(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน

(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน

(..... ชื่อ-นามสกุล ตัวบรรจง)

ในกรณีที่ผู้เข้าร่วมโครงการวิจัยไม่สามารถลงลายมือชื่อด้วยตนเองได้ ให้ผู้แทนโดยชอบตามกฎหมายซึ่งมีส่วนเกี่ยวข้องเป็น.....ของผู้เข้าร่วมโครงการวิจัยเป็นผู้ลงนามแทน

วันที่ลงนาม.....

ใบแสดงเจตนายินยอมให้เก็บตัวอย่างเพื่อการตรวจทางเวชพันธุศาสตร์

วันที่.....เดือน.....พ.ศ. 2554

ข้าพเจ้า.....อายุ.....ปี.....

อนุญาตให้นายแพทย์/แพทย์หญิง.....เก็บตัวอย่างตรวจคือ เลือด จากข้าพเจ้า เพื่อประโยชน์ในการศึกษาวิจัยเรื่อง “ผลของภาวะพหุสัณฐานของยีน *CYP2D6* ต่อเภสัชจลนศาสตร์ของยาทาม็อกซิเฟนในผู้ป่วยไทยโรคมะเร็งเต้านม” ที่ข้าพเจ้าเข้าร่วมในการวิจัย

ข้าพเจ้าได้รับทราบข้อมูลเกี่ยวกับการวิจัยดังกล่าวดังนี้

1. วัตถุประสงค์ในการวิจัย
2. ประโยชน์ที่คาดว่าจะได้รับ
3. การตรวจดังกล่าวจะกระทำโดยไม่เปิดเผยข้อมูลส่วนตัวของข้าพเจ้าแก่บุคคลอื่น ที่ไม่เกี่ยวข้องกับการวิจัย
4. การเก็บตัวอย่างตรวจนี้กระทำโดยการเจาะเลือดดำ ซึ่งมีผลข้างเคียงคือ ความเจ็บปวด เลือดซึม หรือการติดเชื้อ ซึ่งเกิดได้น้อยมาก และถ้าหากเกิดขึ้น ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยแพทย์ผู้ทำหัตถการหรือแพทย์และบุคลากรทางการแพทย์คนอื่นที่ได้รับมอบหมาย
5. การตรวจดีเอ็นเอจะตรวจเฉพาะยีน *CYP2D6* เลือดหรือสารสกัดดีเอ็นเอที่เหลือจากการทำวิจัยจะไม่มีการเก็บไว้

ข้าพเจ้าได้รับทราบข้อมูลในเอกสารให้ความยินยอมนี้ และได้มีโอกาสซักถามแพทย์จนเข้าใจดี ข้าพเจ้าจึงลงนามไว้ข้างท้ายนี้เพื่อเป็นหลักฐาน

ลงชื่อ.....ผู้ยินยอม

( ) หรือผู้แทนโดยชอบธรรม (ระบุความเกี่ยวข้อง)

ลงชื่อ.....พยาน

( )

ลงชื่อ.....พยาน

( )

ใบส่งเจาะเลือดเพื่อตรวจวัดระดับยาและเก็บเลือดไว้ตรวจยีน *CYP2D6*

ชื่อโครงการวิจัย ผลของภาวะพหุสัณฐานของยีน *CYP2D6* ต่อเภสัชจลนศาสตร์ของยาทาม็อกซิเฟนในผู้ป่วยไทยโรคมะเร็งเต้านม

ชื่อ-สกุลผู้ป่วย.....HN.....

วันนัดเจาะเลือด.....

❒ **เก็บเลือดปริมาณ** 5 ml ใส่ EDTA tube **แล้วแช่ที่อุณหภูมิ** 2-8 **องศาเซลเซียส** เพื่อให้ผู้วิจัยนำไปตรวจยีน *CYP2D6*

❒ **เก็บเลือดปริมาณ** 5 ml ใส่ EDTA tube **แล้วแช่ที่อุณหภูมิ** 2-8 **องศาเซลเซียส** เพื่อให้ผู้วิจัยนำไปตรวจยีน *CYP2D6*

แพทย์ผู้สั่ง.....

( )

ผู้วิจัย.....

(เภสัชกรหญิงภิชญาภา รุ่งวานนท์ชัย)

เบอร์โทรศัพท์ติดต่อ 08-1721-1567

APPENDIX D

TaqMan® Drug Metabolism Genotyping Assays
(TaqMan® MGB probes, FAM™ and VIC® dye-labeled)

Assay ID: C_11484460_40

rs: 1065852

Chemical and reagents

1. TaqMan® Drug Metabolism Genotyping Assays Mix,
   Applied Biosystems, USA
2. TaqMan® Genotyping Master Mix,
   Applied Biosystems, USA

Apparatus

1. MicroAmp Optical 96-well reaction plate
2. MicroAmp Optical Adhesive Film kit
3. Vortex mixer
4. StepOnePlus™ Real time PCR Systems,
   Applied Biosystems Inc., Foster City, CA USA

Supplies

1. Disposable gloves
2. Pipette tip 10 µl (White) Scientific Plastics, USA
3. Micropipette 10 µl Eppendorf, Germany

Overview

TaqMan® Drug Metabolism Genotyping Assays consist of a 20X mix of unlabeled PCR primers and TaqMan® MGB probes (FAM™ and VIC® dye-labeled). These assaysare designed for the allelic discrimination of specific Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (indels).Each assay enables scoring of both alleles of a biallelic polymorphism in a single well. All assays are optimized to work with TaqMan® Universal PCR Master Mix No AmpErase® UNG (P/N 4324018)† and with genomic DNA. These products utilize the modified thermal cycling parameters described below in Table B.

Procedure

To prepare the reaction components for one reaction refer to the table below. The StepOnePlus™ Real time PCR Systems. (Applied Biosystems Inc., Foster City, CA USA). The reaction mix contains TaqMan Drug Metabolism Genotyping Assay Mix, TaqMan Universal PCR Master Mix, No AmpErase UNG, and DNase-free water. The final reaction volume per well is 20 µl in a 96-well plate as follow table below.

Table A. Allelic Discrimination PCR Reaction

Reaction Components	Volume/Well (20 µl volume reaction) *	Final concentration
TaqMan® Universal PCR Master Mix (2 X)	10 µl	1 X
20 X TaqMan® Drug metabolism Genotyping Assay Mix	1 µl	1 X
Genomic DNA (10 ng/µL) **	2 µl	-
dH_2O	7 µl	-
Total	20 µl	-

* If different reaction volumes are used, amounts should be adjusted accordingly.

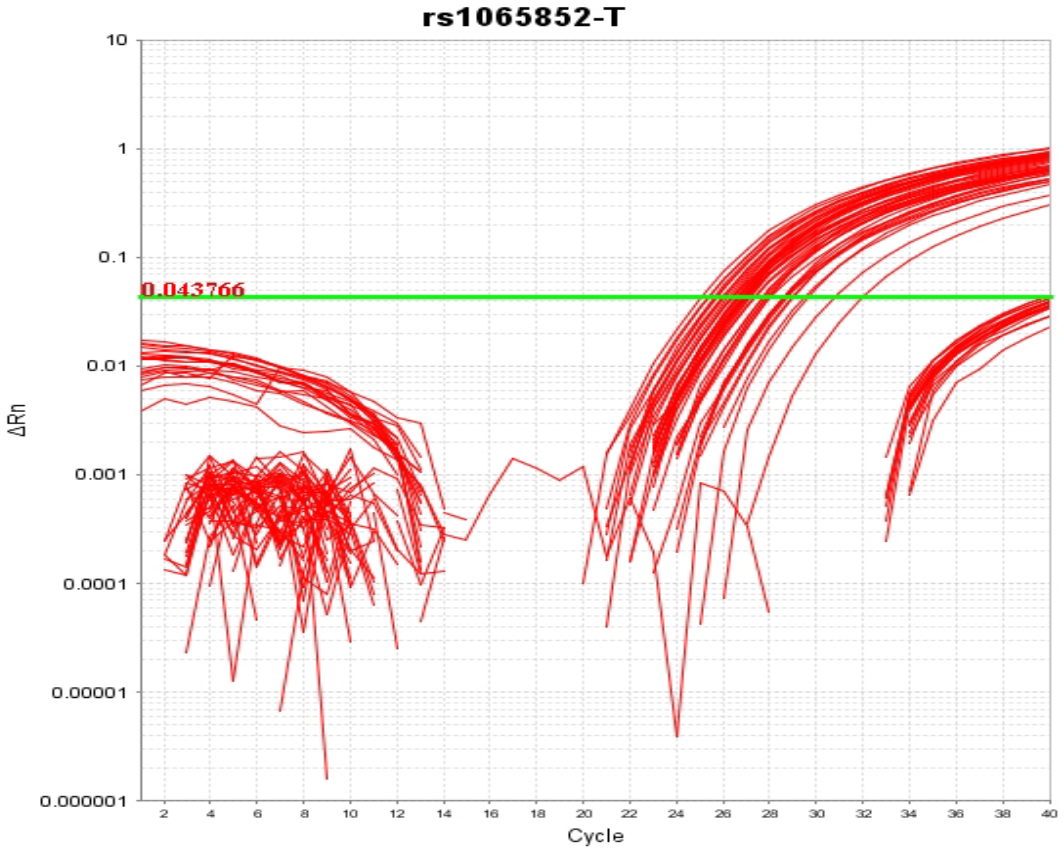
** 3-20 ng of genomic DNA per well. All wells on a plate should have equivalent amounts of genomic DNA.

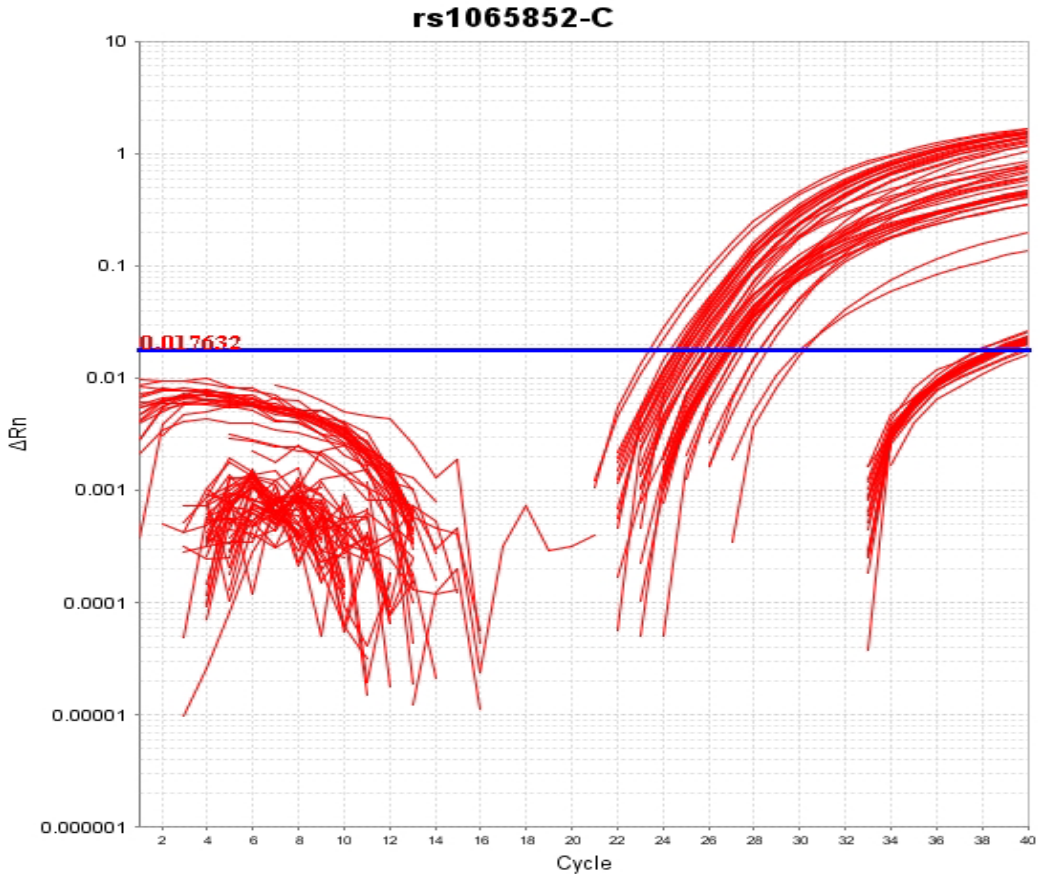
Table B. Thermal Cycler Conditions

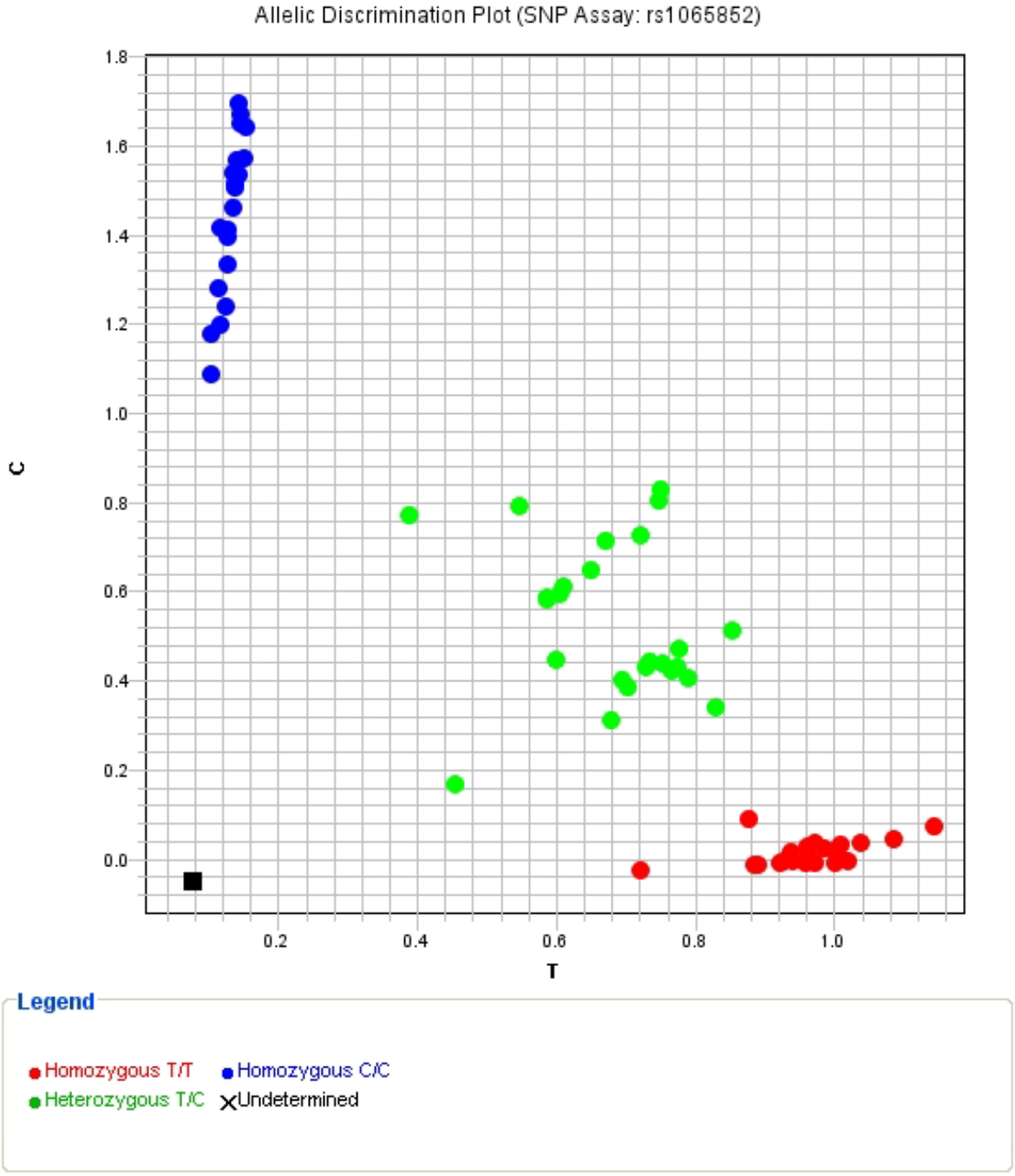
Times and Temperatures		
Initial Steps	Denature	Anneal/Extend
HOLD	40 cycles	
10 min 95 ^{0}C	15 sec 92 ^{0}C	90 sec 60 ^{0}C

Storage

Store between -15°C and -20°C; minimize freeze thaw cycles.


rs1065852-T
0.043766
ΔRn
Cycle


rs1065852-C
0.017632
ΔRn
Cycle


Allelic Discrimination Plot (SNP Assay: rs1065852)
C
T
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.2
0.4
0.6
0.8
1.0
Legend
Homozygous T/T
Homozygous C/C
Heterozygous T/C
Undetermined

APPENDIX E

Plasma concentration of tamoxifen, NDM, and endoxifen from the total patients included into the study.

No.	*CYP2D6*	Endoxifen	NDM	Tamoxifen
1	*1/*10	35.91	87.19	280.26
2	*10/*10	6.95	667.02	487.32
3.	*10/*10	14.12	687.86	562.43
4.	*1/*10	6.27	441.42	272.75
5.	*1/*10	15.67	429.44	47.51
6.	*1/*10	11.95	195.31	144.90
7.	*10/*10	31.36	306.73	314.21
8.	*1/*10	19.76	298.61	340.36
9.	*1/*10	42.12	223.31	289.63
10.	*10/*10	10.85	973.23	609.48
11.	*10/*10	8.20	480.76	251.03
12.	*1/*1	6.97	584.37	409.12
13.	*1/*1	47.18	557.13	512.21
14.	*10/*10	10.02	618.25	454.42
15.	*10/*10	11.53	803.14	574.61
16.	*1/*10	37.71	477.22	133.58
17.	*1/*1	23.20	612.84	384.84
18.	*10/*10	66.15	182.70	279.52
19.	*1/*10	11.23	532.70	231.92
20.	*1/*10	3.47	230.26	149.04
21.	*10/*10	7.86	537.00	258.66
22.	*1/*1	28.74	372.76	264.18
23.	*10/*10	6.52	477.89	315.10
24.	*10/*10	1.88	370.95	300.35
25.	*1/*1	20.38	356.70	318.60
26.	*10/*10	7.70	622.87	398.08
27.	*1/*10	10.70	292.04	300.64

No.	*CYP2D6*	Endoxifen	NDM	Tamoxifen
28.	*10/*10	9.23	675.94	395.74
29.	*1/*1	32.98	406.07	270.68
30.	*10/*10	31.38	878.89	564.62
31.	*1/*1	20.50	609.75	322.28
32.	*10/*10	3.26	1355.71	853.27
33.	*1/*10	11.75	779.85	533.94
34.	*1/*1	9.64	360.68	209.74
35.	*1/*1	40.53	467.02	373.35
36.	*1/*10	16.51	556.42	362.96
37.	*1/*10	25.34	967.67	659.12
38.	*10/*10	22.17	916.03	543.48
39.	*1/*10	16.31	583.26	558.11
40.	*1/*10	12.13	649.47	542.99
41.	*10/*10	18.84	909.22	611.14
42.	*1/*1	21.27	491.59	323.59
43.	*1/*10	15.33	463.36	360.38
44.	*1/*1	28.97	338.88	332.80
45.	*1/*10	13.58	724.14	503.51
46.	*10/*10	2.33	156.22	228.49
47.	*10/*10	7.46	599.74	358.02
48.	*10/*10	16.54	780.90	385.74
49.	*1/*10	21.90	411.48	399.56
50.	*1/*10	9.86	720.43	409.57
51.	*1/*10	17.04	597.46	318.14
52.	*1/*1	21.83	271.06	241.73
53.	*1/*1	11.82	223.82	197.76
54	*1/*10	25.71	478.88	338.81
55	*1/*10	14.90	302.55	236.09
56	*1/*1	35.73	479.13	336.51
57	*1/*1	2.86	634.66	297.24
58	*1/*10	16.36	634.47	321.65
59	*1/*1	6.35	572.89	382.65

VITA

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