ผลของภาวะพหุสัณฐานของ ซิป2ดี6 และ ซิป2ซี19 ต่อเภสัชจลนศาสตร์ของยาฟลูอ็อกซีทีนและภาวะพหุสัณฐานของตัวนำส่งเซโรโทนิน ต่อผลทางคลินิกในผู้ป่วยซึมเศร้าชาวไทย

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
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INFLUENCES OF CYP2D6, CYP2C19 POLYMORPHISMS ON FLUOXETINE PHARMACOKINETICS AND SEROTONIN TRANSPORTER POLYMORPHISM ON CLINICAL OUTCOMES IN THAI PATIENTS WITH DEPRESSIVE DISORDER

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LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
CDR	=	Concentration dose ratio
CI	=	Confident interval
CL	=	Clearance
CYPs	=	Cytochrome P450s
EDTA	=	Ethylenediaminetetraacetic acid
FLX	=	Fluoxetine
HPLC	=	High performance liquid chromatography
HRSD	=	Hamilton Rating Scale for Depression
HT	=	Heterozygous genotype
MDD	=	Major depressive disorder
MT	=	Mutant (Homozygous) genotype
NFLX	=	Norfluoxetine
PCR	=	Polymerase chain reaction
PD	=	Pharmacodynamics
PK	=	Pharmacokinetics
RFLP	=	Restriction fragment length polymorphism
SE	=	Side effect
SERT	=	Serotonin transporter
WT	=	Wild type (Homozygous) genotype
5-HT	=	Serotonin

CHAPTER I

INTRODUCTION

1.1 Rationale and Background

Fluoxetine is the most commonly prescribed drug among the new generation of antidepressants. This drug demonstrates highly protein binding. Cytochrome P450 (CYP) isoenzymes play a major role in phase I metabolism. Its active metabolite is known as norfluoxetine. CYP2D6 and CYP2C19 are the major subfamily to metabolize fluoxetine to norfluoxetine. The volume of distribution for fluoxetine and norfluoxetine ranges from 20 to 45 L/kg. Half-life of this drug and its metabolite are 1-4 days and 7-15 days, respectively. There are many factors involved in fluoxetine pharmacokinetics such as age, weight, lean body mass, drug interaction, treatment length and polymorphism of isoenzymes.[1]

For CYP2D6 genotyping, poor metabolizer (PM) was reported to be about 7% in Caucasian and 1-3% in Asian population. For CYP2C19 genotyping, PM was reported to be about 3-6% in Caucasian and 15-30% in Asian population. Characteristics of allele frequencies of Thai population are not only different from Caucasian, but also different among Asian population. Nakmahachalasint had studied genotype-phenotype of CYP2D6 in 60 Thai participants and had reported that the most frequently found allele of CYP2D6 was CYP2D6*10 (69.49%). Allele frequencies of CYP2D6*5 was 7.63%. [2] Allele frequencies of CYP2C19*1, CYP2C19*2 and CYP2C19*3 in Thai population studied by Tassaneeyakul et al were 0.68, 0.29 and 0.03, respectively. The prevalence of PM estimated from genotype data of Thai population was 9.2%. [3]

Genetic factors play a substantial role in psychopharmacological studies and practice. The depression treatment recommended for Caucasian population study might not be appropriate for Asian

population including Thai depressive patients. Therefore, this study is intended to evaluate the influence of CYP2D6 and CYP2C19 polymorphisms on fluoxetine steady-state plasma concentrations in Thai depressive patients and to determine the association of these polymorphisms and pharmacokinetic parameters. Moreover, the influence of age, weight, polymorphism of CYP2D6, CYP2C19 and serotonin transporter on clinical outcomes and adverse effects of fluoxetine treatment should be investigated. Thus, proper dosage of fluoxetine for individual Thai patients could be determined.

1.2 Hypothesis

- Ho: There is no difference of average of fluoxetine and norfluoxetine plasma concentrations/clearance in CYP2D6 variants in Thai patients with depressive disorder.
- Ha: There is a difference of average of fluoxetine and norfluoxetine plasma concentrations/clearance in CYP2D6 variants in Thai patients with depressive disorder.
- Ho: There is no difference of average of fluoxetine and norfluoxetine plasma concentrations/clearance in CYP2C19 variants in Thai patients with depressive disorder.
- Ha: There is a difference of average of fluoxetine and norfluoxetine plasma concentrations/clearance in CYP2C19 variants in Thai patients with depressive disorder.
- Ho: No association between the serotonin transporter polymorphisms and clinical outcome of fluoxetine.
- Ha: There is an association between the serotonin transporter polymorphisms and clinical outcome of fluoxetine.

1.3 Objectives

1.3.1 To study the influence of CYP2D6 and CYP2C19 polymorphisms on fluoxetine and norfluoxetine plasma concentrations and clearance in Thai patients with depressive disorder.

1.3.2 To determine the association of the serotonin transporter polymorphisms and clinical outcomes (efficacy and adverse effects) of fluoxetine.

1.3.3 To determine the influence of age, weight, polymorphism of CYP2D6, CYP2C19 on clearance of fluoxetine.

1.3.4 To establish the equation to predict clearance of fluoxetine.

1.4 Expected Outcomes

1.4.1 The influence of CYP2D6, CYP2C19, and serotonin transporter polymorphisms on dosage regimen of fluoxetine could be used as the preliminary data to adjust dose of fluoxetine in order to reduce or prevent adverse effects and improve prescribing efficacy for patients with different genotypes.

1.4.2 The equation for prediction clearance of fluoxetine may be useful for depression treatment in clinical practice.

CHAPTER II

LITERATURE REVIEWS

2.1 Fluoxetine

2.1.1 Pharmacodynamics and mechanism of action

Fluoxetine (FLX) is the most commonly prescribed drug among the new generation of antidepressants. The pharmacological activity of fluoxetine is due to specific 5-HT reuptake inhibition at the presynaptic serotonergic nerve terminal. In different to the tricyclic antidepressants, it is essentially lacking any significant affinity to the muscarinic, cholinergic, H1-histaminergic, α1-adrenergic, and 5-HT1 or 5HT2 receptor subtypes. This means a significant reduction in side effects produced by receptor blockade when compared to the older antidepressants. It is also devoid of affinity for cardiac sodium channels and thus demonstrates a superior safety profile with regard to cardiac toxicity. Fluoxetine has no effect on monoamine oxidase activity. [4-5]

2.1.2 Pharmacokinetics

Fluoxetine is well absorbed by oral route, with 72 to 90 percent systemic availability. Maximum plasma concentration could be achieved within 6 to 8 hours after ingestion of a single 40-mg dose. Peak plasma concentration is not affected by food, but absorption may be delayed 1 to 2 hours. This drug are highly protein binding. It is approximately 95 percent bound to serum protein (albumin and α1-acid-glycoprotein). Cytochrome P450 (CYP) isoenzymes play a major role in phase I metabolism. The identified active metabolite is known as norfluoxetine. CYP2D6 and CYP2C19 are the major subfamily to metabolize fluoxetine to norfluoxetine. The volume of distribution for fluoxetine

and norfluoxetine ranges from 20 to 45 L/kg. The rates of plasma clearance are 20 L per hour and 9 L per hour for fluoxetine and its biologically active metabolite (norfluoxetine) respectively. The elimination half-life of this drug and its metabolite are 1-4 days and 7-15 days, respectively. Fluoxetine is widely distributed, including excretion into breast milk. The kidneys excrete inactive metabolites produced by hepatic metabolism. There are many factors involved in fluoxetine pharmacokinetics such as age, weight, lean body mass, drug interaction, treatment length and polymorphism of isoenzymes. [1, 6-8]

Fluoxetine is a racemic mixture of R-fluoxetine and S-fluoxetine in equal proportions. Both are approximately equipotent in 5-HT reuptake inhibition activity, although the S-fluoxetine enantiomer is more slowly eliminated and is, the predominant form in plasma at steady state. The S-enantiomer of norfluoxetine is essentially equivalent to R- or S-fluoxetine, but R-norfluoxetine has significantly less activity. [7]

2.1.3 Indications and dose

Fluoxetine has been approved for many indications as shown in table 2.1

Table 2.1 Indication and oral dose [4]

Indications	Dose
Major depressive disorder	Adult; 20-40 mg/day, maximum: 80 mg/day Children; 8-18 yrs: 10-20 mg/day Elderly; require titration with low dose
Bulimia nervosa	Adult; 60-80 mg/day
Obsessive-compulsive disorder	Adult; 40-80 mg/day Children; 7-18 yrs: initial: 10 mg/day Range: 10-60 mg/day
Premenstrual dysphoric disorder (PMDD)	Adult; 20 mg/day
Panic disorder	Adult; initial: 10 mg/day, slowly increase to 60 mg/day

2.1.4 Drug interactions

Drugs that could be caused drug-drug interaction with fluoxetine treatment were shown in table 2.2

Table 2.2 Drug interactions [4]

Effect	Management
Increased effect/ toxicity	
- nonselective MAO inhibitors (Phenelzine) or other drug with MAO inhibition (linezolid)	Wait 5 weeks after stopping FLX before starting MAOI, 2 weeks after stopping MAOI before starting FLX
- Thioridazine or mesoridazine	Wait at least 5 weeks after discontinuing FLX prior to starting thioridazine
- minophylline, Phenytoin Amphetamine, selected beta-blockers, Citalopram, diazepam Dextromethorphan, Paroxetine, Fluvoxamine, Propanolol, Risper-ridone, Ritonavir Theophylline, Tricyclic antidepressant, Lidocain	FLX may increase the level/ effect of these drugs
- Lithium	Concurrent use may increase risk of neurotoxicity: monitor the symtoms
- Warfarin	FLX may increase the hypo-prothrombinemic response: monitor
- NSAIDs, aspirin, other drug affecting coagulation	Concurrent use: monitor risk of bleeding
Decreased effect	
- Carbamazepine, Phenobarbital, Phenytoin, Rifampin	Levels/effect of FLX may be deceased by these drugs
Ethanol/nutrition/herb interactions	
- Ethanol	Avoid ethanol (may increase CNS depression)
- Herb/Nutraceutical	Avoid Valerian, St John's wort, kava kava, gotu kola (may increase CNS Depression)

2.1.5 Clinical Implementation

Adverse reactions

Predominant effects are central nervous system and gastrointestinal side effects as shown in table 2.3.

Table 2.3 Adverse reactions [4]

Adverse reaction Percentages listed for adverse effect as reported in placebo-controlled trials
> 10% Central nervous system: Headache, Nervousness, Insomnia, Somnolence, Anxiety Gastrointestinal: Nausea, Diarrhea Neuromuscular: Weakness Cardiovascular: Vasodilation, Palpitation, Hypertension
1% to 10% Cardiovascular: Vasodilation, Palpitation, Hypertension Central nervous system: Dizziness, Agitation, Amnesia, Confusion Dermatologic: Rash, Urticaria, Pruritus Endocrine: Metabolic: Ejaculation abnormal, Impotence Gastrointestinal: Dyspepsia, Constipation, Flatulence, Vomiting Weight loss, Appetite decreased Genitourinary: increase urinary frequency Miscellaneous: Flu-like syndrome
< 1% (limited to important or life-threatening) Allergies, Allopecia, Cholestatic jaundice, Anaphylactoid reaction, Angina, Arrhythmia, Asthma, Dyskinesia, Dysphagia, Exfoliative dermatitis, Extrapyrimidal symptom (rare), Gout, Hallucinations, Hepatic Failure/nacrosis, Hemorrhage, Neuroleptic malignant syndrome, Pancreatitis, Photosensitivity reaction, Postural hypotension, QT prolongation, Renal failure, Serotonin syndrome, Stevens-Johnson syndrome, Syncope, Thrombocytopenia, Ventricular tachycardia (including Torsade de pointes)

Efficacy for depression treatment

In contrast to the tricyclic antidepressants (TCAs), there appears to be no significant relationship between blood levels of fluoxetine and subsequent therapeutic response in major depression. [7] Generally, fluoxetine is regarded as a safe antidepressant medication. It is well established as an effective treatment and has safety profile for major depression. [9-13]

2.2 Influence of CYP2D6 and CYP2C19 polymorphisms on fluoxetine pharmacokinetics

For CYP2D6 genotyping, poor metabolizer (PM) was reported to be about 7% in Caucasian and 1-3% in Asian population. For CYP2C19 genotyping, PM was reported to be about 3-6% in Caucasian and 15-30% in Asian population. Characteristics of allele frequencies of Thai population are not only different from Caucasian, but also different among Asian population. Nakmahachalasint had studied genotype-phenotype of CYP2D6 in 60 Thai participants and had reported that the most frequently found allele of CYP2D6 was CYP2D6*10 (69.49%). Allele frequencies of CYP2D6*5 was 7.63 %. [2] Allele frequencies of CYP2C19*1, CYP2C19*2 and CYP2C19*3 in Thai population studied by Tassaneeyakul et al were 0.68, 0.29 and 0.03 respectively. The prevalence of PM estimated from genotype data of Thai population was 9.2%.[3]

From the literatures, the influence of polymorphisms of CYP2D6 and CYP2C19 on the steady-state plasma fluoxetine and norfluoxetine concentration reported the conflicting of results among Caucasian and Asian population. Lerena et al have evaluated the effect of CYP2D6 and CYP2C9 polymorphisms on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions in 64 depressive patients.[14] The fluoxetine/norfluoxetine ratio also correlated with the number of CYP2D6 active genes ($p < 0.01$, $r = -0.39$). The dose-corrected plasma concentrations of fluoxetine and active moiety

(fluoxetine+norfluoxetine) were significantly higher in the CYP2C9*1/*2 and CYP2C9*1/*3 genotype groups than the wild type group ($p < 0.05$). Scordo et al have investigated the influence of CYP2D6, CYP2C9 and CYP2C19 genetic polymorphisms on the steady-state plasma concentrations of the enantiomers of fluoxetine and norfluoxetine in 78 patients.[15] The plasma concentrations of S-norfluoxetine was very low in the CYP2D6 PMs ($p<0.05$). Furthermore, the median S-norfluoxetine/S-fluoxetine ratio were higher in the homozygous than in the heterozygous extensive metabolizers (EMs) ($p<0.05$). Among homozygous EMs for CYP2D6, patients with homozygous for CYP2C9*1 had lower dose-normalized R-fluoxetine concentrations and lower active moiety levels compared with those carrying detrimental CYP2C9 alleles ($p<0.05$). No statistically significant relationship was identified in CYP2C19 genotypes and the dose-normalized plasma concentrations of any of the enantiomers or the active moiety.

In Asian population, Liu et al have investigated the CYP2C19 oxidation polymorphism on fluoxetine metabolism in Chinese healthy subjects who have already known CYP2C19 genotyping.[16] The results indicated that CYP2C19 appear to play a major role in the metabolism of fluoxetine. PMs showed a mean 46% increase in fluoxetine peak plasma concentrations ($p<0.001$), 128% increase in area under the concentration vs time curve ($p<0.001$), 113% increase in elimination half-life ($p<0.001$), and 55% decrease in clearance ($p<0.001$) when compared with EMs.

2.3 Influence of serotonin transporter polymorphisms on pharmacodynamics

The most widely investigated gene is that of the brain serotonin (5-HT) transporter (5HTT). Allelic variation in 5HTT function may lead to both increased susceptibility to anxious or depressive features and less favorable antidepressant responses in patient affected by mood disorders. For interindividual

differences in drug response, two polymorphisms have been observed. The deletion polymorphism in the promoter region of 5HTT was known as short (s) variant, whereas the long (l) variant was the insertion polymorphism. Many studies have tested the association between variation genotyping and clinical response (efficacy and side effect) to SSRIs[17-22]. These findings were inconsistent to conclude a comparison group to determine whether the insertion allele influenced response to any antidepressant.

2.4 Influence of CYP2D6, CYP2C19 and 5HTT polymorphisms on clinical outcomes

Many studies were concentrated to those providing data on effects of genetic polymorphism on pharmacokinetics and pharmacodynamics, although, the studies concerning for the relevant of genetic polymorphism on clinical outcomes and adverse effects were limited. Rau et al developed a pilot study concerning influence of the CYP 2D6 polymorphism on adverse effects and non-response during antidepressant treatment in German white population.[23] They reported that CYP2D6 polymorphism was one of the important factors besides gender, age, weight, and ethnicity in paroxetine population pharmacokinetic parameters study in late-life depression. There were 29% (8 patients of 28 patients) with adverse event during treatment, were homozygous alleles (PMs). This is a 4-fold increases as comparing with the German population ($P<0.001$). Amplification of fully functional alleles was found in 3 of the 16 non-responders (19%). This is approximately 5-fold higher in non-responders than in the population ($P=0.0012$). The different result could be detected by randomized 6 week trial of fluoxetine and nortriptyline developed by Roberts. [24] They suggested about an accurate assessment of the true rate of antidepressant-induced adverse effects in CYP2D6 PMs. Monique et al developed the cohort study to examine the influence of CYP2D6*4 polymorphism on dose, switching and

discontinuation of various antidepressants. [25] The results demonstrated the significant risk of switching to another antidepressant in PMS*4/*4 of tricyclic antidepressant users, whereas, there was no significant difference in SSRIs users. The mean dose could be found lower in PMs than EMs in both tricyclic antidepressants users and SSRIs users. The researchers suggested that another CYP2D6 polymorphism should be evaluated to optimize the outcome of the study. Study of Susuki et al investigated and reported that polymorphisms of serotonin receptor and CYP2D6 synergistically predicted fluvoxamine-induced side effects in Japanese depressed patients.[26] Serotonin transporter polymorphisms and adverse effects studied by Perlis et al reported that the short variant might identify patients at risk for developing insomnia or agitation with fluoxetine treatment in major depressive patients.[27] Moreover, the conflicting results of prediction for response and side effects of various SSRIs have been reported for the 5HTT polymorphism.[28-30]

2.5 Influence of other factors on dosage regimen of fluoxetine

Therapeutic blood level of fluoxetine (100-300 μg/L) and norfluoxetine (100-200 μg/L) varied for interindividuals by many factors. .[31] The study of fluoxetine pharmacokinetics in young and elderly volunteers reported the half-life of fluoxetine to be 25% longer and the half-life of norfluoxetine to be 33% longer in elderly group. The norfluoxetine predose level (C_0) was 22% longer in elderly subjects ($P<0.05$), with comparable decreases in 24-hour area under the concentration-time curve (AUC_{0-24}) and maximum concentration (C_{max}).[32] The study comparing of Fluoxetine pharmacokinetics in pediatric and adolescent patients was conducted by Wilens et al by using sparse blood samples.[33] Age, gender and body weight were evaluated as covariates in the simulation for the model parameter determining. Fluoxetine was 2–fold higher and norfluoxetine was 1.7–fold higher in children relative

to adolescents. Sinha et al developed the fluoxetine population pharmacokinetics to explain the between subject variation by using a general linear model. [34] CL/F and V/F of fluoxetine were 13.0 L/hr and 3,420 L. Lower clearance of fluoxetine could be observed in preadolescent patients. There were some studies about the impact of genetic variations on therapeutic outcomes, incidence and severity of adverse drug reactions and dosing of fluoxetine. Genotype-based dose adjustment might be necessary for fluoxetine. [35]

Since many factors involved in fluoxetine pharmacokinetics such as age, weight, lean body mass, drug interaction, treatment length and polymorphism of isoenzymes. The depression treatment recommended for Caucasian population study might not be appropriate for Asian population including Thai depressive patients. Therefore, this study is intended to evaluate the influence of CYP2D6 and CYP2C19 polymorphisms on fluoxetine steady-state plasma concentrations in Thai depressive patients and to determine the association of these polymorphisms and pharmacokinetic parameters. Moreover, the influence of age, weight, polymorphism of CYP2D6, CYP2C19 and serotonin transporter on clinical outcomes and adverse effects of fluoxetine treatment should be investigated. Thus, proper dosage of fluoxetine for individual Thai patients could be determined.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Study Participants

Definition

Depressive patients: The patients who are diagnosed by using DSM-IV diagnostic criteria as unipolar mood disorder.

Target populations: Thai patients with depressive disorder who are prescribed with fluoxetine for the treatment.

Samples: Outpatients who been prescribed with fluoxetine for depressive disorder either new case or currently use patient.

All collecting data are planned to obtain from outpatient department at Srithanya hospital, Mental Health Department.

Inclusion criteria

1. Adult outpatients (age more than 18 years old) who are diagnosed as depressive patients by DSM-IV criteria and have been prescribed with fluoxetine for more than 8 weeks.
2. The patients who have given the written consent form.
3. The patients who have the good history of drug compliance

Exclusion criteria

1. The patients who have abnormal liver function.
2. Thai patients with depressive disorder who are diagnosed with psychosis features.
3. Thai patients with depressive disorder who have been prescribed the other medications (for chronic diseases) that are the significantly potent inhibitors of CYP2D6 or CYP2C19. The medications are ritonavir, rifampin, carbamazepine, phenobarbital, phenytoin, cimetidine, clarithromycin omeprazole and topiramate.
4. The patients who can not follow up within two months

Sample size:

$$N = [(Z_\alpha+Z_\beta) / Z(r)]^2 + 3$$

$$Z(r) = \tfrac{1}{2} \ln [(1+r) / (1-r)]$$

Significance level (α) = 0.05, $Z_\alpha = 1.96$, $Z_\beta = 1.28$
r from the study of Lerena, 2004[14] = 0.39

$$Z(r) = \tfrac{1}{2} \ln [(1+0.39) / (1-0.39)]$$
$$= 0.4118$$
$$N = [(1.96+1.28) / 0.4118)]^2 + 3$$
$$= 64$$

Sample size will be at least 64 patients.

The influences of five variables (age, weight, polymorphism of CYP2D6, CYP2C19 and 5-HTT) on clinical outcomes and adverse effects of fluoxetine treatment were investigated. It should be about 10 to 12 participants for each variable then the sample size should be 60 patients.

3.2 Study design and method

Study design

Study design has performed the cross-sectional and prospective study. All collecting data were obtained from outpatient department at Srithanya Hospital, Mental Health Department.

Method

Recruitment of participants and data collecting

After the approval from both Ethical Committee of Srithanya hospital and Department of Mental Health, the target participants were recruited into study according to inclusion and exclusion criteria and informed consent were obtained from all participants. Demographic data, clinical data, dosage regimen of

fluoxetine and concomitant drugs will be recorded. For new cases, Baseline scales of symptoms and side effects were assessed by the 17-item Hamilton depression rating scale (Thai HRS-D 17) and side effects collecting form by interviewing. Blood samples were taken to screen hepatic function of all participants.

Blood sample collecting

Sixty-nine patients with depressive disorder were enrolled to the study. Efficacy and side effects of depression treatment with fluoxetine were recorded in collecting data form.

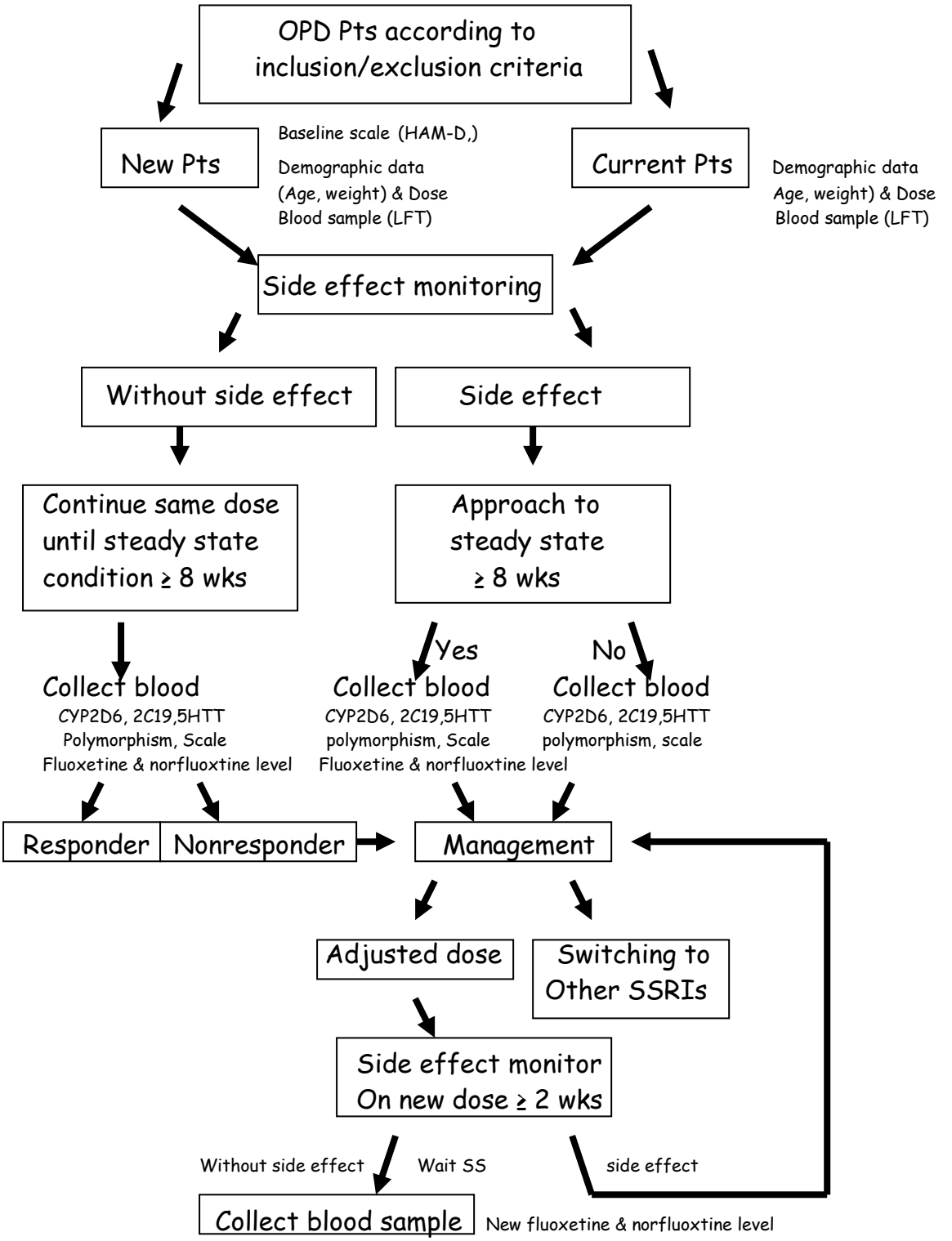
Participants' blood sample (20 ml) were collected to determine the fluoxetine and norfluoxetine plasma concentration (10 ml) and to investigate for CYP2D6 and CYP2C19 genotyping and serotonin transporter polymorphism (10 ml). The plasma samples for drug assay were stored at -20ºC until measurement. The blood samples for genotyping were collected at the same time and stored with EDTA at -40ºC.

For patients without side effects, the same dose of fluoxetine for depression treatment should be continued until steady-state condition (more than eight weeks). Symptoms and side effects were assessed by the Thai HRS-D 17 and side effects collecting form. Currently use cases were observed for symptoms in their medical record and the Thai HRS-D 17 scores of each patient were recorded. For non-responder group, treatment was considered by psychiatrist to adjust fluoxetine dosing or switch to other antidepressants. Switching to other antidepressants was determined for final monitoring. The monitoring process for patients treated with new dosage regimen will be ongoing until achieving for the proper dose of fluoxetine treatment (steady-state condition). Participants' blood sample (10 ml) was collected to determine the fluoxetine and norfluoxetine plasma concentration of the new regimen.

For patients with side effects, participants' blood sample were also collected to determine the fluoxetine and norfluoxetine plasma concentration and to investigate for CYP genotyping and

serotonin transporter polymorphism determination. Treatment was considered by psychiatrist to adjust fluoxetine dosing or switch to other antidepressants. Switching to other antidepressant was determined for final monitoring. The monitoring process for patients treated with new dosage regimen was ongoing until achieving for the proper dose of fluoxetine treatment (steady-state condition). Participants' blood sample (10 ml) was collected to determine the fluoxetine and norfluoxetine plasma concentration of the new regimen. Method for follow-up and clinical outcomes measurement was demonstrated by figure 1.

Figure 1 Experimental Design



The collecting data forms of pharmacist's patient profiles, the Thai HRS-D 17 (Thai version), side effect collecting form, the participant information form and consent form (Thai version) are shown in the appendices.

3.3 Analytical assay for fluoxetine and norfluoxetine

Analytical assay for fluoxetine and norfluoxetine by HPLC (UV detector) method published by Lerena, 2003 was used in this stedy. [36] In brief, the assay involved liquid-liquid extraction into heptane-isoamyl alcohol (97:3 v/v) and re-extraction into acetic acid. After extraction, compounds were separated in a reversed-phase column and assayed by ultraviolet absorption at 226 nm. The extraction recoveries were 93% and 87% for norfluoxetine and fluoxetine, respectively. The mobile phase was a mixture of acetonitrile (30%), water (67%) and acetate buffer (3%). The limit of quantitation under the described conditions was 14 nmol/l for both compounds. Within-day and between-day CV% were less than 10% for both compounds.

Instruments

- HPLC instrument: LC-10AD SHIMADZU, CBM-10A (system controller), SPD-10V UV-Vis detector
- HPLC syringe: HAMILTON, USA
- Centrifuge apparatus (Heraeus Labofuge 200): Thermo Scientific, USA
- Electronic analytical balance: ADAM AFA-210LC, UK
- pH meter (Model 720A): Boston, USA
- Ultrasonic bath: J.P.SELECTA, Spain
- Vortex mixer (VTX-3000L): Uzusio, Japan
- Micropipette: Thermo Scientific, Finland

3.4 Bioanalysis

DNA extraction and genotyping

DNA extraction

DNA will be prepared from leukocytes (buffy coat) of 15 ml. whole blood with an Illustra blood genomicPrep Mini Spin Kit according to the manufacturer's instruction.

Materials

Chemical and reagents

- Proteinase K
- Lysis buffer type 10
- Wash buffer type 6
- Elution buffer type 5

Instruments

- Illustra^{TM} blood mini column, UK
- Collection tube
- Centrifuge Hettick, Germany
- Microcentrifuge Hettick, Germany
- Water bath
- Vortex mixer Labnet, USA
- Micropipettes Eppendorf, Germany
- UV-Vis spectrophotometer Bio-Rad, USA

Extraction methods

1. Equilibrate samples and reagents to room temperature.
2. Heat a water bath to 70ºC. Pre-heat elution buffer before use.
3. For cell lysis preparation, add 20 µl of Proteinase K into the collection tube.

4. Add 200-300 µl of whole blood or its cell fractions into the collection tube (If sample volume less than 200 µl then make up to 200 µl with phosphate buffer solution).
5. Pipette 400 µl lysis buffer type 10, mix by vortex.
6. Incubate at room temperature for 10 minutes.
7. For genomic DNA binding, load the preparation from step 6 into Assembled column and collection tube.
8. Centrifuge at 11,000 rpm for one minutl and discard the solution phase.
9. Pipette 500 µl lysis buffer type 10 into the same tube.
10. Centrifuge at 11,000 rpm for one minute and discard the solution phase.
11. Pipette 500 µl lysis buffer type 6 into the same tube.
12. Centrifuge at 11,000 rpm for 3 minutes and discard the solution phase.
13. For DNA elution, insert column from step 12 into a clean DNase-free microcentrifuge tube.
14. Pipette 200 µl of pre-heated elution buffer into the tube.
15. Incubate at room temperature for one minute.
16. Centrifuge at 11,000 rpm for one minute.
17. Collect the eluate phase.
18. For long term storage, keep purified DNA at-20ºC.
19. Determine OD at 260 mm by UV-Vis. Spectrophotometer and calculate DNA concentration from following equation

DNA concentration (ng/µl) = OD. X 50 x dilution factor

(OD 1.0 is equivalent to approximately 50 ng/µl of double strand DNA)

Genotyping

For genotyping, PCR RFLP modified method to determine the CYP2D6 and CYP2C19 genetic polymorphisms were developed by Steen et al., 1995[37], Johansson et al., 1994[38], Morais et al., 1994.[39] and Scarlett et al, 2000. [40] For CYP2D6 polymorphisms, CYP2D6*5, CYP2D6*10 variants were

analyzed. For CYP2C19 polymorphisms, CYP2C19*2 variants and CYP2C19*3 variants were analyzed.

Genotyping for CYP2D6*5

Materials

Chemical and reagents

- Ultra pure DNase-free water
- Buffer (Quigen)
- dNTPs (Biolabs)
- Forward primer (Proligo)
- Reverse primer (Proligo)
- Hotstar Tag (Quigen)
- DNA

Instruments

- Collection tube with filter tip
- PCR tube
- Microcentrifuge Hettick, Germany
- PCR cabinet Bio-Rad, USA
- Vortex mixer Labnet, USA
- Micropipettes Eppendorf, Germany
- Thermal cycler (Bio-Rad) PTC200, USA

Analytical methods

To prepare the reaction components for one reaction refer to the table below. Final volume was 25 μL.

Allelic Discrimination PCR method

Reagents	Stock concentration (SC)	Final conc(FC)	Volume (μL)	Master mix (MMx..)
Water			9.75	
Buffer	10x	1x	2.5	
Q-solution	5x	1x	5	
dNTPs	10mM	0.2mM	0.5	
Forward primer	10μM	1μM	2.5	
Reverse primer	10μM	1μM	2.5	
Taq	5unit/μL	1.25unit	0.25	

Thermal Cycle conditions

step	Temp (º C)	time
1	95	15 min
2	95	1 min
3	63	45 sec
4	72	5 min
5 (go to 2,12 times)		
6	95	1 min
7	63	45 sec
8	72	5 min+20sec/cycle
9 (go to 6,22 times)		
10	72	10 min
11	25	forever

Genotyping for CYP2D6*10

Materials

Chemical and reagents

- Ultra pure DNase-free water
- TagMan™ universal PCR MasterMix
- TagMan™ drug metabolism genotyping assay mix
- DNA

Instruments

- Collection tube with filter tip
- PCR tube
- Microcentrifuge Hettick, Germany
- PCR cabinet Bio-Rad, USA
- Vortex mixer Labnet, USA
- Micropipettes Eppendorf, Germany
- The StepOnePlus™ real time PCR system (Applied Biosystem Inc., USA)

Analytical methods

To prepare the reaction components for one reaction refer to the table below. The reaction mix contained TagMan drug metabolism genotyping assay mix, TagMan universal PCR master mix, AmpErase UNG, and DNase-free water. The final reaction volume per well was 20 μL in a 96-well plate as follow table below.

Allelic Discrimination PCR method

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 mataboilsm Genotyping Assay Mix	1 μL	1 x
Genomic DNA (10 ng/μL)	2 μL	-
dH2o	7 μL	-
Total	20μL	-

Thermal cycle conditions

Times and Temperatures		
Initial Steps	Denature	Anneal/Extend
HOLD	40CYCLES	
10min 95 º C	15sec 92 º C	90sec 60 º C

Genotyping for CYP2C19*2 and *3

Cytochrome P450 2C19 genotypes were analyzed by Real time PCR, LightCycler® which is based on the principle of Fluoresce Resoanance Energy Transfer (FRET)

For genotyping identification, the melting curve chart shows fluorescence (Y-axis) and temperature (X-axis) while the melting peak chart plots the first negative derivative of the fluaorescence (-d/dT) versus temperature, and shows the melting

temperature of each sample as a peak Therefore, sample in different genotype will shows in different peak.

Single reaction (15 μL) consisted of 4 μL of primer and probes, 2 μL of FastStart DNA mix and 9 μL of water (PCR grade) in per-cooled glass capillaries tube. Add 5 μL of genomic DNA (20-25 ng/μL) to each capillary given 20 μL of final reaction volume. Control DNA (supplied with kit) as positive control and water (supplied with kit) as negative control was analyzed with patient DNA sample in each batch. The genotypes were identified by melting curve in different melting points (Tm). Fluorescence was acquired once per cycle at the end of the annealing in the cycling program. Melting curves were analyzed by slowly increasing temperature (0.2ºC per second) from 40ºC to 85ºC. During melting program, fluorescence emissions were acquired continuously.

CYP2C19*2 was detected with SimpleProbe in channel 530 and CYP2C19*3 was detected with LightCycler Red 640 labeled hybridization probes in channel 640. For CYP2C19*2, Tm of wild type and mutant were 54.1ºC and 47.8ºC, respectively. For CYP2C19*3, Tm of wild type and mutant were 52.9ºC and 60.5ºC, respectively.

Materials

Chemical and reagents

- Ultra pure DNase-free water
- Lyophilized mix of primers and probes
- Lightmix™ – color compensation 530/640
- LightCycler™ FastStart DNA master hybridization probes (Biogenomed co., ltd.)
- HighPure PCR template kit
- DNA

Instruments

- LightCycler™ capillaries 20μl

- PCR Collection tube
- Microcentrifuge Hettick, Germany
- PCR cabinet Bio-Rad, USA
- Vortex mixer Labnet, USA
- Micropipettes Eppendorf, Germany
- The LightCycler™ real time PCR system (Roche co., ltd.)

Analytical methods

Preparation of the LightCycler mix

For use with the Roche FastStart kit

Single reaction	Component
7.4 µL	Water,PCR-grade(colorless cap,provided with the Roche FastStart kit)
1.6 µL	Mg solution25mM(blue cap,provided with the Roche FastStart kit)
4.0 µL	reagent mix (parameter specific reagent containing primers and probes)
2.0µL	FastStart mix(vial1(redcap),combined from vials 1a and 1b,see Roche manual)
15.0 µL	

Programming

Program	Denaturation	Cycling			Meiting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Meiting Curves mode			None
Cycles	1	45			1			1
Segment	1	1	2	3	1	2	3	1
Target [ºc]	95	95	60	72	95	40	85	40
Hold[hh:mm:ss]	00:10:00	00:00:05	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [ºc]	20	20	20	20	20	20	0.2	20
Ramp Rate[ºc] 96	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
Ramp Rate[ºc] 384	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
Acquisition Mode	None	None	Single	None	None	None	Continu.	None
Acquisition [per ºc]							1	

Genotyping for 5-HTTLPR

The association of Serotonin transporter polymorphisms and clinical outcomes (efficacy and side effects) of fluoxetine will be determined. Short and long variants of serotonin transporter will be the investigated polymorphisms. For genotyping, fragments of serotonin transporter gene (serotonin gene-linked polymorphic region: 5-HTTLPR) were amplified by PCR using primers as described by Heils et al, 1996. [41]

Fragments of 5-HTTLPR were amplified by polymerase chain reaction (PCR) using the primers as described with 5-HTTLPR-3: ATGCCAGCACCTAACCCCTAATG plus 5-HTTLPR-2: GAGGGACTGAGCTGGACAACCAC.

Polymorphisms of 5-HTTLPR were determined according to the size which was determined from agarose-gel electrophoresis. The sizes of the *s* and *l* 5-HTTLPR alleles were 469-470 bp and 511-513 bp, respectively.

Materials

Chemical and reagents

- Ultra pure DNase-free water
- Buffer (Quigen)
- Q-solution (Quigen)
- dNTPs (Biolabs)
- Forward primer (Proligo)
- Reverse primer (Proligo)
- Hotstar Tag (Quigen)
- DNA

Instruments

- Collection tube with filter tip
- PCR tube
- Microcentrifuge Hettick, Germany
- PCR cabinet Bio-Rad, USA
- Vortex mixer Labnet, USA

- Micropipettes Eppendorf, Germany
- Thermal cycler (Bio-Rad) PTC200

Analytical methods

Allelic Discrimination PCR method

Reagents	Stock concentration (SC)	Final conc(FC)	Volume (µL)	Master mix (MMx..)
Water			6.125	
Buffer	10x	1x	1.25	
Q-solution	5x	1x	2.5	
dNTPs	10mM	0.2mM	0.25	
Forward primer	20µM	0.2µM	0.125	
Reverse primer	20µM	0.2µM	0.125	
Taq	5unit/µL	0.625unit	0.125	
DNA			2	

Thermal cycle conditions

step	Temp (º C)	time
1	95	15 min
2	95	30 sec
3	61	30 sec
4	72	1min
5	Repeat 2 - 4	39 cycle
6	72	10 min
7	4	Until use

Gel Electrophoresis

Materials

Chemical and reagents

- Distilled water
- Agarose (Molecular Biology Grade)
- Tris-borate solution
- Loading dye
- 100 base-paired DNA reference
- 1kb DNA reference
- Targeted PCR product

Instruments

- Volumetric flask
- Volumetric tube
- Micropipettes Eppendorf, Germany

- Filter tip
- Microwave
- Gel Electrophoresis Instrument with computer programe (Syngene)

3.5 Fluoxetine Pharmacokinetics parameter calculation

Pharmacokinetic parameter

Clearance of fluoxetine was calculated as PK parameters by using the following equation.

$$Cl = (Dose)/ (Css)*(Interval)$$

Css = average concentration at steady state

3.6 Statistical analysis

Data analysis for descriptive statistics and inferential statistics will be generated by program SPSS. All statistical significant level (α) was set at 0.05.

Results from this study were obtained and analyzed as followings:

1. All patients'clinical data and demographic data were presented by descriptive statistics; frequency, percentage mean with standard deviation or median.
2. Fluoxetine and norfluoxetine steady-state plasma concentrations and fluoxetine clearance in participants were presented by descriptive statistics; mean with standard deviation or median.
3. The influence of CYP2D6 and CYP2C19 polymorphisms (wild type, heterozygous and mutant genotypes) on pharmacokinetic (fluoxetine and norfluoxetine normalized steady-state plasma concentrations and fluoxetine clearance) in Thai depressive patients were analyzed by inferential non-parametric statistics (Kruskal-Wallis) or parametric statistics (analysis of variance, ANOVA or t-test).
4. Association of serotonin transporter polymorphism and clinical outcomes (Thai HRS-D score, psychiatrist evaluation and side effects) were investigated by Chi-square test.

5. Influence of variables (age, weight, polymorphism of CYP2D6 and CYP2C19) on fluoxetine clearance were analyzed by multiple regressions.

3.7 Ethical consideration

After providing the patient information, all participants will give written informed consent. Patient's medical data will be protected confidentially. Results of the study will be published in scientific journals in summary. Participants will not be personally identified. Research proposal will be approved by both the Ethical Committee of Srithanya hospital and Department of Mental Health.

CHAPTER IV

RESULTS

4.1 Study population

There are sixty nine of Thai patients with depressive disorder prescribed with fluoxetine and met the inclusion criteria for recruiting to this study.

4.1.1 Demographic data

Of the 69 participants recruited, the demographic data was shown in table 4.1. It consisted of gender, age, weight, fluoxetine dose per day, number of episodes, blood group, underlying diseases, co-medications, side effects and duration of taking fluoxetine.

Table 4.1 Demographic data of participants of this study

Characteristics	Frequency,(mean ±SD)	%, (range)
Total participants	69	100
Gender Female	43	62.3
Male	26	37.7
Age (years)	(38.6 ± 10.3)	(20-62)
20-30	18	26.1
31-40	27	39.1
41-50	14	20.3
51-60	7	10.1
≥61	3	4.3
Weight (kg)	(63.8 ± 14.6)	(36-114)
Episode		
First episode	54	78.3
Second episode	15	21.7
Duration of taking drug (month)	(11.3 ± 10.4)	(2-24)

Table 4.1 (Cont.) Demographic data of participants of this study

Characteristics	Frequency,(mean ±SD)	%, (range)
Dose (mg/day)	(28.3 ± 15.1)	(10-80)
10 mg/day	4	6.3
20 mg/day	37	57.8
30 mg/day	3	4.7
40 mg/day	15	23.4
60 mg/day	3	4.7
80 mg/day	2	3.1
Blood group		
Group A	**6**	**8.7**
Group B	**26**	**37.7**
Group AB	**6**	**8.7**
Group O	**31**	**44.9**
Underlying diseases		
Hypertension	5	23.8
Peptic ulcer	5	23.8
DM	3	14.3
Hyperlipidemia	3	14.3
Hyperthyroidism	3	14.3
Asthma	2	9.5
Co-medications		
Lorazepam	16	26.7
Alprazolam	12	20.0
Diazepam	5	8.3
Amitriptyline	16	26.7
Nortriptyline	5	8.3
Sodium valproate	2	3.3
Propanolol	3	5.0
Side effects (N=24)		
Total symptoms = 29		
Headache	7	24.2
Drawsiness	6	20.7
Nausea	3	10.3
Restless of hands	3	10.3
Palpitation	2	6.9
Loss appetite	2	6.9
Dry mouth	2	6.9
Others	4	13.8

4.1.2 Fluoxetine and norfluoxetine concentrations at steady-state condition

Mean steady-state concentration of fluoxetine (FLX), norfluoxetine (NFLX) and active moiety (FLX + NFLX) were shown in table 4.2. It has not been reported the therapeutic range of fluoxetine and its active metabolite in the literature. Kootstra-Ros J, 2006 presented the recommended therapeutic range for fluoxetine (100-300 µg/L) and norfluoxetine (100-200 µg/L).[31] Most of the concentrations reported in the literatures after taking the standard dose of fluoxetine were much higher than this study .[14-15]

Table 4.2 Steady-state concentrations of drug and metabolite

Drug	FLX Conc. (µmol/L)	NFLX Conc. (µmol/L)	Active moiety (µmol/L)
Average (N=59)$^{\Delta}$	0.15 ± 0.19 (0.01 – 0.88)	0.29 ± 0.34 (0.01 – 1.78)	0.44 ± 0.52 (0.02 – 2.55)

Δ (5 pts: concentration can not detectable, 5 pts: switch to other drugs)

4.2 Influence of CYP2D6 and CYP2C19 polymorphisms on fluoxetine pharmacokinetics

For the prevalence of CYP2D6*5 genotypes and CYP2D6*10 genotypes in this study, the CYP2D6*5 genotype of all participants was identified to be wild type; none was identified as heterozygous or homozygous mutant variants. Whereas, allele frequency of CYP2D6*5 in Thai population was 7.63%. [2] The frequency distributions of CYP2D6*10 were about 20.3%, 33.3% and 46.4% for homozygous wild type, heterozygous and homozygous mutant variants, respectively. Allele frequency of CYP2D6*10 was 65.94%, while allele frequency of CYP2D6*10 in Thai healthy population was 69.49%. [2]

For the prevalence of CYP2C19, the frequency distributions of CYP2C19*2 were about 40.6%, 55.1% and 4.3% for homozygous wild type, heterozygous and homozygous mutant variants,

respectively. CYP2C19*3 were about 87.0% and 13.0% for homozygous wild type and heterozygous variants. In this study, Allele frequency of CYP2C19*1, CYP2C19*2 and CYP2C19*3 were 82.97%, 15.94% and 1.09%, respectively. While allele frequencies in Thai population studied by Tassaneeyakul et al, (2006) were 68.0%, 29.0% and 3.0% respectively. [3]

Demographic data before studying the influence of CYP450 polymorphism on fluoxetine pharmacokinetics was shown in table 4.3. There was no significantly difference in gender, age and weight variables for each genotype.

Table 4.3 Demographic data before studying the influence of CYP450 polymorphism on fluoxetine pharmacokinetics

Data	Genotypes			
CYP2D6*10	Wild type (N = 10)	Heterozygous (N = 27)	Homozygous (N = 32)	**P-value**
Gender				
Male (N=26)	6 (60.0%)	9 (33.3%)	11 (34.4%)	**0.294**
Female (N=43)	4 (40.0%)	18 (66.7%)	21 (65.6%)	
Age (years)	41.7 ± 11.2	39.2 ± 11.0	37.5 ± 9.5	**0.465**
Weight (kg)	65.6 ± 11.3	60.2 ± 14.3	66.4 ± 15.6	**0.250**
CYP2C19*2	Wild type (N = 28)		Not Wild type (N = 41)	**P-value**
Gender				
Male (N=26)	8 (28.6%)		18 (43.9%)	**0.125**
Female (N=43)	20 (71.4%)		23 (56.1%)	
Age (years)	38.2 ± **10.6**		38.9 ± 10.3	**0.781**
Weight (kg)	64.8 ± 16.4		63.2 ± 13.5	**0.669**
CYP2C19*3	Wild type (N = 60)		Not Wild type (N = 9)	**P-value**
Gender				
Male (N=43)	24 (40.0%)		2 (22.2%)	**0.308**
Female (N=26)	36 (60.0%)		7 (77.8%)	
Age (years)	38.1 ± 10.2		42.0 ± 11.0	**0.293**
Weight (kg)	64.8 ± 15.2		57.7 ± 9.4	**0.177**

Influence of individual CYP2D6*10 polymorphisms, CYP2C19*2 and CYP2C19*3 polymorphisms on steady-state normalized PK parameters of fluoxetine was shown in table 4.4a and 4.4b. Influence of CYP2C19*2 and CYP2C19*3 polymorphisms in each CYP2D6*10 variant group on steady-state normalized PK parameters of fluoxetine was shown in table 4.5.

Table 4.4a Fluoxetine and norfluoxetine pharmacokinetic parameters among CYP2D6*10 variant groups

Genotypes	Steady-state normalized pharmacokinetic parameters			
	Concentration dose ratio		Fluoxetine Clearance	Norfluoxetine/ Fluoxetine ratio
	Fluoxetine μg/ml/mg/kgdose	Norfluoxetine μg/ml/mg/kgdose	**L/kg**	
CYP2D6*10				
Average (Mean±SD)	0.023±0.034	0.045±0.059	0.111±0.200	2.541±2.808
Wild type (N=9)	0.022±0.024	0.043±0.030	0.101±0.145	3.390±2.596*
Heterozygous (N=23)	0.018±0.031	0.045±0.077	0.138±0.221	3.038±3.899
Homozygous (N=32)	0.028±0.038	0.046±0.051	0.094±0.201	1.945±1.657*
p-value	0.604	0.991	0.715	0.228 (* 0.049)

Table 4.4b Fluoxetine and norfluoxetine pharmacokinetic parameters among CYP2C19 variant groups

Genotypes	Steady-state normalized pharmacokinetic parameters			
	Concentration dose ratio		Fluoxetine Clearance L/kg	Norfluoxetine/ Fluoxetine ratio
	Fluoxetine μg/ml/mg/kgdose	Norfluoxetine μg/ml/mg/kgdose		
CYP2C19*2				
Average (Mean±SD)	0.023±0.034	0.045±0.059	0.111±0.200	2.541±2.808
Wild type (N=26)	0.023±0.034	0.038±0.052	0.080±0.094	1.923±1.530
Not wild type (N=38)	0.023±0.034	0.050±0.064	0.132±0.247	2.964±2.376
p-value	0.933	0.933	0.319	0.147
CYP2C19*3				
Average (Mean±SD)	0.023±0.034	0.045±0.059	0.111±0.200	2.540±2.809
Wild type (N=55)	0.022±0.030	0.042±0.056	0.085±0.158	2.290±2.491
Not wild type (N=9)	0.034±0.052	0.060±0.077	0.268±0.339	4.060±4.148
p-value	0.321	0.407	(*0.010)	0.079
CYP2C19*2&*3 (N=64)				
Average (Mean±SD)	0.023 ± 0.034	0.045 ± 0.059	0.111 ± 0.200	2.541 ± 2.809
*2WT & *3WT (N=20)	0.017 ± 0.019	0.028 ± 0.028	0.060 ± 0.047	1.853 ± 1.656
*2WT & *3NWT (N=6)	0.042 ± 0.061	0.073 ± 0.092	0.148 ± 0.168	2.157 ± 1.093
*2NWT & *3WT(N=35)	0.024 ± 0.035	0.051 ± 0.066	0.099 ± 0.194	2.542 ± 2.854
*2NWT&*3NWT (N=3)	0.017 ± 0.026	0.036 ± 0.028	0.132 ± 0.247	7.879 ± 5.752
p-value	0.206	0.189	(*0.023)	(*0.013)

WT = wild type, NWT = not wild type

Table 4.5 Fluoxetine and norfluoxetine pharmacokinetic parameters among CYP2D6*10 combined with CYP2C19*2,*3

Genotypes	Steady-state normalized pharmacokinetic parameters			
	Concentration dose ratio		Fluoxetine Clearance **L/kg**	Norfluoxetine/ Fluoxetine ratio
	Fluoxetine μg/ml/mg/kgdose	Norfluoxetine μg/ml/mg/kgdose		
Influence of CYP2C19*2 and *3 in each CYP2D6 variant groups				
CYP2D6*10				
***10 Wild type (N=9)**				
Average (Mean±SD)	0.022 ± 0.024	0.042 ± 0.030	0.101 ± 0.145	3.390 ± 2.596
*2WT & *3WT (N=4)	0.035 ± 0.032	0.078 ± 0.038	0.042 ± 0.026	2.031 ± 1.301
*2WT & *3NWT (N=1)	0.004	0.017	0.145	4.192
*2NWT & *3WT (N=3)	0.189 ± 0.008	0.054 ± 0.023	0.042 ± 0.023	2.830 ± 0.195
*2NWT & *3NWT (N=1)	0.001	0.013	0.473	9.700
p-value	0.422	0.935	(*0.000)	(*0.034)
***10Heterozygous (N=23)**				
Average (Mean±SD)	0.019 ± 0.031	0.045 ± 0.077	0.138 ± 0.221	3.038 ± 3.899
*2WT & *3WT (N=7)	0.013 ± 0.011	0.023 ± 0.029	0.046 ± 0.028	1.491 ± 1.923
*2WT & *3NWT (N=2)	0.002 ± 0.000	0.003 ± 0.001	0..340 ± 0.139	1.700 ± 0.566
*2NWT & *3WT(N=13)	0.025 ± 0.040	0.065 ± 0.098	0.088 ± 0.078	3.349 ± 4.091
*2NWT & *3NWT (N=1)	0.002	0.027	1.034	13.500
p-value	0.354	0.195	(*0.000)	0.061
***10 Homozygous (N=32)**				
Average (Mean±SD)	0.028 ± 0.038	0.046 ± 0.051	0.094 ± 0.201	1.945 ± 1.657
*2WT & *3WT (N=9)	0.013 ± 0.013	0.023 ± 0.022	0.079 ± 0.061	2.054 ± 1.712
*2WT & *3NWT (N=3)	0.082 ± 0.069	0.137 ± 0.093	0.021 ± 0.017	1.784 ± 0.583
*2NWT & *3WT(N=19)	0.025 ± 0.035	0.041 ± 0.040	0.116 ± 0.257	1.945 ± 1.834
*2NWT & *3NWT (N=1)	0.047	0.068	0.020	1.436
p-value	0.774	0.609	0.885	0.912

WT = wild type, NWT = not wild type

For CYP2D6 groups, there was no statistically significant difference of fluoxetine steady-state normalized plasma concentration, norfluoxetine steady-state normalized plasma concentrations and fluoxetine clearance between CYP2D6*5 variant groups because all of participants were wild type variant. There was no statistically significant difference of the concentration dose ratio (normalized by patient's body weight) of fluoxetine, concentration dose ratio of norfluoxetine and fluoxetine clearance (normalized by patient's body weight) at steady-state condition among CYP2D6*10 variant groups.

There was statistically significant difference of the ratio at steady-state concentration (normalized by dose and weight) of norfluoxetine and fluoxetine when compared homozygous wild type group with homozygous mutant group ($p = 0.049$).

For CYP2C19*2 group, there was no statistically significant difference of the normalized concentration dose ratio (CDR) of fluoxetine, normalized CDR of norfluoxetine and ratio of normalized CDR of norfluoxetine and fluoxetine in all variant groups.

For CYP2C19*3 group, there was no statistically significant difference of the normalized concentration dose ratio (CDR) fluoxetine, normalized of norfluoxetine and ratio of normalized CDR of norfluoxetine and fluoxetine in all variant groups.

For CYP2C19*3 group, average fluoxetine clearance in wild type group and not wild type group were 0.085 L/kg and 0.268 L/kg, respectively. It was statistically significant difference of fluoxetine clearance in this group ($p = 0.010$).

Result for determining the influence of CYP2C19*2 with CYP2C19*3 polymorphisms on steady-state normalized PK parameters, fluoxetine clearance of CYP2C19*2 not wild type group and CYP2C19*3 not wild type group ware approximately 2-fold higher than the CYP2C19*2 wild type group and CYP2C19*3 wild type group. The ratio of normalized CDR of norfluoxetine and fluoxetine of CYP2C19*2 not wild type group

and CYP2C19*3 not wild type group was approximately 4-fold higher than the CYP2C19*2 wild type group and CYP2C19*3 wild type group.

For the influence of CYP2C19*2 and CYP2C19*3 polymorphisms in each CYP2D6*10 variant group on steady-state normalized PK parameters of fluoxetine, it was statistically significant difference of fluoxetine clearance in CYP2D6*10 homozygous wild type group ($p = 0.000$) and CYP2D6*10 heterozygous group ($p = 0.000$).

There was statistically significant difference for the ratio of normalized CDR of norfluoxetine and fluoxetine at steady-state concentration in homozygous wild type group ($p = 0.034$).

4.3 Influence of study factors on clearance of fluoxetine

Influence of study variables (age, weight, polymorphism of CYP2D6 and CYP2C19) on fluoxetine clearance were analyzed by multiple regressions and presented by estimation equation. Multicollinearity of independent factors was also defined. Only CYP2C19*3 polymorphism was selected to the model (model no.1).

The significant model calculated from stepwise linear regression was shown in table 4.6. The best fit model was obtained. The coefficients and p-value of each variable which entered to model by stepwise method were presented in table 4.7. Finally, estimation equation of fluoxetine clearance was determined.

$$\text{Fluoxetine clearance} = 0.085 + 0.183\ \text{CYP2C19*3}$$

Table 4.6 Model summary of stepwise linear regression

Model	Variable enter	R^2	R^2 change	Sig (F change)
1	CYP2C19*3	0.103	0.103	0.010*
2	age, weight CYP2D6*10, CYP2C19*2, CYP2C19*3,	0.159	0.159	0.068

Table 4.7 Coefficients of factors in the best fit equation

Factors	Sig.(p-value)	B	[95%CI]
constant	0.002*	0.085	0.034-0.137
CYP2C19*3	0.010*	0.183	0.046-0.321
Excluded variables			
age	0.553	0.001	-0.003-0.006
weight	0.777	0.000	-0.004-0.003
CYP2C19*2	0.104	0.083	-0.018-0.183
CYP2D6*10	0.756	0.015	-0.082-0.113

4.4 Influence of serotonin transporter polymorphisms on pharmacodynamics

The prevalence of serotonin transporter genotypes in this study, there ware about 60.9%, 24.6% and 14.5% for s/s, s/l and l/l genotype, respectively. Study by Tencomnao et al 2010 in Thai patients with depressive disorder reported that there was about 51.0%, 36.9% and 9.1% for s/s, s/l and l/l genotype, respectively. [(42)]

Demographic data and pharmacokinetic parameters among serotonin transporter genotypes have shown no significant different (table 4.8). Therefore, the factors of demographic data

and dosage regimen did not interfere to the influence of serotonin transporter polymorphism on clinical outcome.

Table 4.8 Demographic data and pharmacokinetic parameters among serotonin transporter genotypes

Data	Average (mean±SD)	Serotonin transporter genotypes s/s (N=42)	s/l (N=17)	l/l (N=10)	P-value
Gender					
Male (N=26)		17(40.5%)	5 (29.4%)	4 (40.0%)	
Female (N=43)		25 (59.5%)	12 (70.6%)	6 (60.0%)	**0.732**
Age (years)	38.6 ± 10.3	39.9(24-62)	36.0(20-61)	37.5(22-54)	**0.414**
Weight (kg)	63.8 ± 14.6	65.5 ± 16.4	58.6 ± 10.4	65.8 ± 11.8	**0.132**
Dose (mg/day)	**28.28 (10-80)**	**28.42 (10-80)**	**26.47 (10-60)**	**31.11 (20-80)**	**0.759**
Fluoxetine concentration (μmol/L)	1.39 ± 1.94	1.20 ± 1.93	1.78 ± 1.89	1.45 ± 2.19	**0.605**
Norfluoxetine concentration (μmol/L)	2.62 ± 3.13	2.20 ± 2.64	3.50 ± 4.38	2.76 ± 1.98	**0.365**
SUM (μmol/L)	4.01 ± 4.89	3.40 ± 4.47	5.27 ± 6.16	4.21 ± 3.83	**0.426**
Concentration Dose ratio μg/ml/mg/kg dose	**0.023 ± 0.033**	**0.020 ± 0.032**	**0.032 ± 0.037**	**0.022 ± 0.033**	**0.503**

Analysis of the results by chi-square test was revealed that patients with l/l genotype had a significantly better response to fluoxetine treatment when compared with s allele carriers as evaluation by the Thai HRS-D scores or psychiatrist efficacy evaluation (table 4.9). Among patients with different serotonin transporter polymorphisms, carriers with s allele had significantly higher rate of various side effects than the l/l genotype group as shown in table 4.10. Five participants had to switch from fluoxetine to another drug regimen for depression treatment. There were four cases with s/s genotype, whereas, one case with l/l genotype.

Influence of serotonin transporter polymorphisms on pharmacodynamics or clinical outcome of fluoxetine was shown in table 4.9 and table 4.10.

Table 4.9 Comparison of drug efficacy in different genotypes

Drug efficacy	Serotonin transporter genotypes		
	s/s (N=42)	s/l (N=17)	l/l (N=10)
Method I : Thai HRS-D 17			
≤ 7	29 (69.0%)	14 (82.4%)	8 (80.0%)
> 7	9 (21.4%)	3 (17.6%)	1 (10.0%)
χ^2 = 13.76 P = 0.001			
Method II : Psychiatrist Efficacy evaluation			
Improve without side effect	22 (52.4%)	11 (64.7%)	7 (70.0%)
Currently use with side effect	16 (38.1%)	6 (35.3%)	2 (20.0%)
χ^2 = 9.05 P = 0.011			

Table 4.10 Comparison of side effects in different genotypes

Side effects	Serotonin transporter genotypes		
	s/s (N=42)	s/l (N=17)	l/l (N=10)
Headache (N=7)	4 (9.5%)	2 (11.7%)	1 (10.0%)
Drawsiness and fatique (N=6)	4 (9.5%)	1 (5.9%)	1 (10.0%)
Other symptoms (N=11)	8 (19.1%)	3 (17.6%)	0 (0.0%)
All adverse events (N=24)	16 (38.1%)	6 (35.3%)	2 (20.0%)
χ^2 = 13.00 P = 0.002			

CHAPTER V

DISCUSSION

This research studied the influence of CYP2D6 and CYP2C19 polymorphisms on steady-state normalized pharmacokinetic parameters of fluoxetine and its active metabolite in Thai patients with depressive disorder. The PK parameters were concentration dose ratio (CDR) of drug, concentration dose ratio (CDR) of its metabolite, fluoxetine clearance and ratio of CDR for metabolite and drug. The influence of serotonin transporter polymorphisms was also determined on pharmacodynamics or clinical outcome for depression treatment.

For CYP2D6 genotyping, the difference of pharmacokinetic parameter among CYP2D6*5 variant groups could not be determined because all of participants were wild type variant. There was no statistically significant difference of the CDR of fluoxetine, CDR of norfluoxetine and normalized fluoxetine clearance among CYP2D6*10 variant groups. However, CDR ratio of norfluoxetine and fluoxetine were higher in homozygous wild type group when compared with homozygous mutant group ($p = 0.049$).

The result was consistent with the study by Lerena et al, 2004. They have evaluated the effect of CYP2D6 and CYP2C9 polymorphisms on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions in 64 Caucasian depressive patients.[14] The fluoxetine /norfluoxetine ratio also correlated with the number of CYP2D6 active genes ($p < 0.01$, $r = -0.39$). The dose-corrected plasma concentrations of fluoxetine and active moiety (fluoxetine+norfluoxetine) were significantly higher in the CYP2C9*1/*2 and CYP2C9*1/*3 genotype groups than the wild type group ($p < 0.05$).

Scordo et al, 2005 have also investigated the influence of CYP2D6, CYP2C9 and CYP2C19 genetic polymorphisms on the steady-state plasma concentrations of the enantiomers of

fluoxetine and norfluoxetine in 78 Caucasian patients.[15] The plasma concentrations of S-norfluoxetine was very low in the CYP2D6 PMs ($p<0.05$). Furthermore, the median S-norfluoxetine/S-fluoxetine ratio were higher in the homozygous than in the heterozygous extensive metabolizers (EMs) ($p<0.05$). Among homozygous EMs for CYP2D6, patients with homozygous for CYP2C9*1 had lower dose-normalized R-fluoxetine concentrations and lower active moiety levels compared with those carrying detrimental CYP2C9 alleles ($p<0.05$). No statistically significant relationship was identified in CYP2C19 genotypes and the dose-normalized plasma concentrations of any of the enantiomers or the active moiety.

For this study, there was no statistically significant difference of CDR of fluoxetine, CDR of norfluoxetine and CDR ratio of norfluoxetine and fluoxetine in all variant groups of CYP2C19*2 and CYP2C19*3 genotypes.

For CYP2C19*3 group, average fluoxetine clearance in wild type group and not wild type group were 0.085 L/kg and 0.268 L/kg, respectively. It was statistically significant difference for fluoxetine clearance in this group ($p = 0.010$).

Results for determining the influence of both CYP2C19*2 and CYP2C19*3 polymorphisms, fluoxetine clearance of CYP2C19*2 not wild type group and CYP2C19*3 not wild type group was approximately 2-fold higher than the CYP2C19*2 wild type group and CYP2C19*3 wild type group ($p = 0.023$). The ratio of normalized CDR of norfluoxetine and fluoxetine of CYP2C19*2 not wild type group and CYP2C19*3 not wild type group was approximately 4-fold higher than the CYP2C19*2 wild type group and CYP2C19*3 wild type group ($p = 0.013$).

The corresponding study in Asian population by Liu et al, 2001 have investigated the CYP2C19 oxidation polymorphism on single dose of fluoxetine metabolism in Chinese healthy subjects who have already known CYP2C19 genotyping.[16] The results indicated that CYP2C19 appear to play a major role in the metabolism of fluoxetine. PMs showed a mean 46%

increase in fluoxetine peak plasma concentrations ($p<0.001$), 128% increase in area under the concentration vs time curve ($p<0.001$), 113% increase in elimination half-live ($p<0.001$).

Result for determining the influence of CYP2C19*2 and CYP2C19*3 polymorphisms in each CYP2D6*10 variant group on steady-state normalized PK parameters of fluoxetine, It was statistically significant difference of fluoxetine clearance in CYP2D6*10 homozygous wild type group ($p = 0.000$) and CYP2D6*10 heterozygous group ($p = 0.000$). Moreover, there was statistically significant difference for the ratio of normalized CDR of norfluoxetine and fluoxetine at steady-state concentration in homozygous wild type group ($p = 0.034$).

Grasmader et al investigated the impact of CYP2C9, CYP2C19 and CYP2D6 on plasma concentration of various antidepressants and clinical outcomes in 136 Caucasian depressed inpatients. [(43)]

For CYP2D6 poor metabolizer group and patient taking inhibitors of CYP2D6 had significantly higher mean dose-corrected plasma concentrations than the median concentration for specific antidepressants. Five of the six CYP2D6 poor metabolizers had side effects at their early visits. CYP2C19 extensive metabolizers had lower mean dose-corrected plasma concentrations compared with the median concentrations of antidepressants. Clinical response was not associated with plasma concentrations of antidepressants.

Eap et al investigated the plasma concentrations of the enantiomers of fluoxetine and norfluoxetine after multiple doses in CYP26D-genotyped Caucasian patients. [(44)] Three patients were genotyped as PMs (CYP2D6*4/ CYP2D6*4), two as heterozygous EMs (CYP2D6*1/CYP2D6*4) and six as homozygous EMs (CYP2D6*1/CYP2D6*1). Plasma concentrations were measured at days 7, 14 and 23 of oral administration of 20 mg of drug. Results showed large differences in the mean concentration of (S)-FLX and (S)-NFLX between EMs and PMs on all days examined, but not of (R)-FLX and (R)-NFLX.

CYP2D6 is involved in the demethylation of FLX to NFLX with a stereoselectivity toward the (S)-enantiomer. This study did not determined the stereoselective plasma concentrations, but the results was also confirmed the influence of CYP2D6 on fluoxetine metabolism.

The conflicting results of prediction for response and side effects of various SSRIs have been reported for the serotonin transporter polymorphism. [17-22] From the study, the association of the serotonin transporter polymorphisms and clinical outcome of fluoxetine was determined. The resulted was shown that patients with l/l genotype had a significantly better response to fluoxetine treatment when compared with s allele carriers as evaluation by the Thai HRS-D scores ($p = 0.001$) or psychiatrist efficacy evaluation ($p = 0.011$). Among patients with different serotonin transporter polymorphisms, carriers with s allele had significantly higher rate of various side effects than the l/l genotype group ($p = 0.002$). Among the five participants who had to switch from fluoxetine to another drug regimen for depression treatment, four cases were s/s genotype, whereas, only one case was l/l genotype.

This result was strongly consistent with the study of Susuki et al, 2006. They have investigated and reported that polymorphisms of serotonin receptor and polymorphisms of CYP2D6 synergistically predicted fluvoxamine-induced side effects in Japanese depressed patients.[26] Studied by Perlis et al, 2003 reported that the short variant might identify patients at risk for developing insomnia or agitation with fluoxetine treatment in major depressive patients.[27] The result was consistent with a study in 121 Chinese patients with depressive disorder by Yu et al. [45] Analysis of the results reveals that patients with l/l genotype had a significantly better response to SSRI (fluoxetine) when compared with s allele carriers, as evaluated by Hamilton Depression Rating Scale-score ($p = 0.013$). The corresponding study of 51 Caucasian elderly depressed patients by Rausch et al

confirmed that the l allele variants were associated with better SSRI response ($p < 0.02$). [46]

Conflicting results from another SSRIs has also reported. Takahashi et al investigated the association between serotonergic polymorphism and incidence of nausea in 66 Japanese patients.[30] Results suggested that three polymorphisms in serotonergic system did not affect the development of fluvoxamine-induced nausea, and that incidence of nausea was not a phenomenon that predicts the treatment response to fluvoxamine. Paroxetine studies by Zanardi R et al [47] and Pollock et al [29] revealed that l allele variants were associated with a worse response. Kim et al [48] reported that the s/s subjects showed the better response for fluoxetine or paroxetine in Korean Patients with depressive disorder. Yoshida et al [49] also reported that the s/s Japanese subjects had the better response for fluvoxamine when compared with the l/l variant group.

There are several possible explanations for this discrepancy. Asian study was developed with small sample size. Allele frequency of the long allele variants was different among Caucasian and Asian population. The variation of individual study results could have also been caused by the difference in diagnosis features, duration of treatment, clinical improvement evaluation and type of side effects or adverse effects evaluation. Moreover, there was no structure of adverse drug reaction form in the study. Some serious adverse effects such as agitation or akathesia were difficult to detect. Several confounding factors might not be detected and/or control.

CHAPTER VI

CONCLUSION

This study is to investigate the influence of CYP2D6 and CYP2C19 polymorphisms on steady-state normalized pharmacokinetic parameters of fluoxetine and norfluoxetine in Thai patients with depressive disorder at Srithanya hospital, Mental Health Department. The frequency distributions of CYP2D6*10 were about 20.3%, 33.3% and 46.4% for homozygous wild type, heterozygous and homozygous mutant variants, respectively. Regard to the prevalence of CYP2C19*2 genotypes and CYP2C19*3 genotypes in this study, the frequency distributions of CYP2C19*2 were about 40.6%, 55.1% and 4.3% for homozygous wild type, heterozygous and homozygous mutant variants, respectively. For CYP2C19*3, there were about 87.0% and 13.0% for homozygous wild type and heterozygous variants, respectively.

At steady-state condition, CYP2D6*10, CYP2C19*2 and *3 polymorphisms showed some effect on pharmacokinetic parameters of fluoxetine especially fluoxetine clearance.

Influence of several study variables (age, weight, polymorphism of CYP2D6 and CYP2C19,) on fluoxetine clearance were analyzed by multiple regressions. Only CYP2C19*3 polymorphism was selected to the model. Finally, estimation equation of fluoxetine clearance was determined.

Fluoxetine clearance = 0.085 + 0.183 CYP2C19*3

The influence of the serotonin transporter polymorphisms was determined for fluoxetine pharmacodynamic or clinical outcome. It was found that patients with l/l genotype had a significantly better response to fluoxetine treatment when compared with s allele carriers as evaluation either by the Thai HRS-D scores or psychiatrist efficacy evaluation. Among patients with different serotonin transporter polymorphisms,

carriers with s allele had significantly higher rate of various side effects than the l/l genotype group.

This study provides the preliminary data to reduce or prevent adverse effects and improve prescribing efficacy for depression patients with different genotypes.

The limitation of this study and consideration for further study include the following:

1. Only CYP2D6*5 and CYP2D6*10A was investigated, various other genotypes of CYP2D6 that might significantly affect to pharmacokinetic of drug for depression treatment should be determined.
2. Gathering data was retrospective and/or cross-sectional documents in patient records. Several useful data might not be completed.
3. Serious side effects of fluoxetine were not regarded in this study.
4. Power of the studying can be increased by recruiting more participants, the optimal equation for predicting drug clearance can be obtained.
5. The prospective study for genotype-based drug regimen for depression treatment should be developed to determine the outcomes in clinical setting.
6. Cost for determining the genotypes should be calculated for providing the information to the administration.

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APPENDICES

Appendix A Collecting forms

Date | | | | | | | |

m m d d y y

THAI HRS-D 17
HAMILTON RATING SCALE FOR DEPRESSION (THAI VERSION) 17 items

1. อารมณ์ซึมเศร้า (เศร้าใจ, สิ้นหวัง, หมดหนทาง, ไร้ค่า)

0 ❑ ไม่มี

1 ❑ จะบอกภาวะความรู้สึกนี้ ต่อเมื่อถามเท่านั้น

2 ❑ บอกภาวะความรู้สึกนี้ออกมาเอง

3 ❑ สื่อภาวะความรู้สึกนี้โดยภาษากาย ได้แก่ การแสดงสีหน้า ท่าทาง น้ำเสียง และมักร้องไห้

4 ❑ ผู้ป่วยบอกเพียงความรู้สึกนี้อย่างชัดเจน ทั้งการบอกออกมาเองและภาษากาย

2. ความรู้สึกผิด

0 ❑ ไม่มี

1 ❑ ติเตียนตนเอง รู้สึกตนเองทำให้ผู้อื่นเสียใจ

2 ❑ รู้สึกผิดหรือครุ่นคิดถึงความผิดพลาดหรือการก่อกรรมในอดีต

3 ❑ ความเจ็บป่วยในปัจจุบันเป็นการลงโทษ มีอาการหลงผิดว่าตนผิด

4 ❑ ได้ยินเสียงกล่าวโทษ หรือประนาม และ/หรือเห็นภาพหลอนที่ข่มขู่คุกคาม

3. การฆ่าตัวตาย

0 ❑ ไม่มี

1 ❑ รู้สึกชีวิตไร้ค่า

2 ❑ คิดว่าตนเองน่าจะตาย หรือความคิดใด ๆ เกี่ยวกับการตายที่อาจเกิดขึ้นกับตนเอง

3 ❑ มีความคิดหรือท่าทีจะฆ่าตัวตาย

4 ❑ พยายามที่จะฆ่าตัวตาย (ความพยายามใด ๆ ที่รุนแรง ให้คะแนน 4)

4. การนอนไม่หลับในช่วงต้น

0 ❑ ไม่มีปัญหาเข้านอนแล้วหลับยาก

1 ❑ แจ้งว่านอนหลับยากบางครั้ง ได้แก่ นานกว่า ½ ชั่วโมง

2 ❑ แจ้งว่านอนหลับยากทุกคืน

5. การนอนไม่หลับในช่วงกลาง

0 ❑ ไม่มีปัญหา

1 ❑ ผู้ป่วยแจ้งว่า กระสับกระส่าย นอนหลับไม่สนิทช่วงกลางคืน

2 ❑ ตื่นกลางดึก หากมีลุกจากที่นอน ให้คะแนน 2 (ยกเว้นเพื่อปัสสาวะ)

Date | | | | | | | |

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6. การตื่นตอนเช้ากว่าปกติ

0 ❑ ไม่มีปัญหา

1 ❑ ตื่นแต่เช้ามืด แต่นอนหลับต่อได้

2 ❑ นอนต่อไม่หลับอีก หากลุกจากเตียงไปแล้ว

7. การงานและกิจกรรม

0 ❑ ไม่มีปัญหา

1 ❑ มีความคิดและความรู้สึกว่าตนเองไม่มีสมรรถภาพ อ่อนเปลี้ยหรือหย่อนกำลังที่จะทำกิจกรรมต่าง ๆ : การงาน หรืองานอดิเรก

2 ❑ หมดความสนใจในกิจกรรมต่าง ๆ : งานอดิเรกหรืองานประจำ ไม่ว่าจะทราบโดยตรงจากการบอกเล่าของผู้ป่วย หรือทางอ้อมจากการไม่กระตือรือร้น ลังเลใจและเปลี่ยนใจไปมา (ผู้ป่วยรู้สึกว่าต้องกระตุ้นให้ตนเองทำงานหรือกิจกรรม)

3 ❑ เวลาที่ใช้จริงในการทำกิจกรรมลดลง หรือผลงานลดลง หากอยู่ในโรงพยาบาล, ให้คะแนน 3 ถ้าผู้ป่วยใช้เวลาต่ำกว่า 3 ชั่วโมงต่อวัน ในการทำกิจกรรม (งานของโรงพยาบาลหรืองานอดิเรก) ยกเว้นหน้าที่ประจำวันในโรงพยาบาล

4 ❑ หยุดทำงานเพราะการเจ็บป่วยในปัจจุบัน หากอยู่ในโรงพยาบาล, ให้คะแนน 4 ถ้าผู้ป่วยไม่ทำกิจกรรมอื่นนอกจากหน้าที่ประจำวันในโรงพยาบาล หรือถ้าผู้ป่วยทำหน้าที่ประจำวันไม่ได้ หากไม่มีคนช่วย

8. อาการเชื่องช้า (ความช้าของความคิดและการพูดจา : สมาธิบกพร่อง, การเคลื่อนไหวลดลง)

0 ❑ การพูดจาและความคิดปกติ

1 ❑ มีอาการเชื่องช้าเล็กน้อยขณะสัมภาษณ์

2 ❑ มีอาการเชื่องช้าชัดเจนขณะสัมภาษณ์

3 ❑ สัมภาษณ์ได้อย่างลำบาก

4 ❑ อยู่นิ่งเฉยโดยสิ้นเชิง

9. อาการกระวนกระวายทั้งกายและใจ

0 ❑ ไม่มี

1 ❑ งุ่นง่าน อยู่ไม่สุข

2 ❑ เล่นมือ เล่นผม ฯลฯ

3 ❑ เดินไปมา นั่งไม่ติดที่

4 ❑ บีบมือ กัดเล็บ ดึงผม กัดริมฝีปาก

Date | | | | | | | |

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10. ความวิตกกังวลในจิตใจ

0 ❑ ไม่มีปัญหา

1 ❑ รู้สึกตึงเครียดและหงุดหงิด

2 ❑ กังวลในเรื่องเล็กน้อย

3 ❑ การพูดจาหรือมีสีหน้ามีท่าทีหวั่นกลัว

4 ❑ แสดงความหวาดกลัวโดยไม่ต้องถาม

11. ความวิตกกังวลซึ่งแสดงออกทางร่างกาย

อาการร่วมด้านสรีรวิทยาของความวิตกกังวล เช่น :

ระบบทางเดินอาหาร : ปากแห้ง ลมขึ้น อาหารไม่ย่อย ท้องเสีย ปวดเกร็งท้อง เรอ

ระบบหัวใจและหลอดเลือด : ใจสั่น ปวดศีรษะ

ระบบหายใจ : หายใจหอบเร็ว ถอนหายใจ

ปัสสาวะบ่อย

เหงื่อออก

0 ❑ ไม่มี

1 ❑ เล็กน้อย

2 ❑ ปานกลาง

3 ❑ รุนแรง

4 ❑ เสื่อมสมรรถภาพ

12. อาการทางกาย ระบบทางเดินอาหาร

0 ❑ ไม่มี

1 ❑ เบื่ออาหาร แต่รับประทานโดยผู้อื่นไม่ต้องคอยกระตุ้น
- รู้สึกหน่วงในท้อง

2 ❑ รับประทานอาหารยาก หากไม่มีคนคอยกระตุ้น
- ขอหรือจำต้องได้ยาระบายหรือยาสำหรับอาการของระบบทางเดินอาหาร

13. อาการทางกาย อาการทั่วไป

0 ❑ ไม่มี

1 ❑ ตึงแขนขา หลังหรือปวดศีรษะ ปวดกล้ามเนื้อ หมดแรง และอ่อนเพลีย

2 ❑ มีอาการใด ๆ ที่ชัดเจน ให้คะแนน 2

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14. อาการทางระบบสืบพันธุ์ เช่น : หมดความต้องการทางเพศ ปัญหาด้านประจำเดือน

0 ❑ ไม่มีอาการ

1 ❑ เล็กน้อย

2 ❑ ปานกลาง

15. อาการคิดว่าตนเองป่วยเป็นโรคทางกาย

0 ❑ ไม่มี

1 ❑ หมกมุ่นในตนเอง (ด้านร่างกาย)

2 ❑ หมกมุ่นเรื่องสุขภาพ

3 ❑ แจ้งถึงอาการต่าง ๆ บ่อย ๆ เรียกร้องความช่วยเหลือ

4 ❑ มีอาการหลงผิดว่า ตนป่วยเป็นโรคทางกาย

16. น้ำหนักลด เลือกข้อ ก หรือ ข.

ก. เมื่อให้คะแนนโดยอาศัยประวัติ

0 ❑ ไม่มีน้ำหนักลด

1 ❑ อาจมีน้ำหนักลด ซึ่งเกี่ยวเนื่องกับการเจ็บป่วยครั้งนี้

2 ❑ น้ำหนักลดชัดเจน (ตามที่ผู้ป่วยบอก)

3 ❑ ไม่ได้ประเมิน

ข. จากการให้คะแนนประจำสัปดาห์ โดยจิตแพทย์ประจำหอผู้ป่วย

0 ❑ น้ำหนักลดน้อยกว่า 1 ปอนด์ใน 1 สัปดาห์

1 ❑ น้ำหนักลดมากกว่า 1 ปอนด์ใน 1 สัปดาห์

2 ❑ น้ำหนักลดมากกว่า 2 ปอนด์ใน 1 สัปดาห์

3 ❑ ไม่ได้ประเมิน

17. อาการหยั่งเห็นถึงความผิดปกติของตนเอง

0 ❑ ตระหนักว่าตนเองกำลังซึมเศร้า และเจ็บป่วย

1 ❑ ตระหนักว่ากำลังเจ็บป่วย แต่โยงสาเหตุกับอาหารที่ไม่มีคุณค่า ดินฟ้าอากาศ การทำงานหนัก ไวรัส การต้องการพักผ่อน ฯลฯ

2 ❑ ปฏิเสธการเจ็บป่วยโดยสิ้นเชิง

3 ❑ มีการแสดงออกทางพฤติกรรมและอารมณ์ที่รุนแรง

การคิดคะแนน

นำคะแนนทุกข้อมารวมกัน ตามตัวเลขคะแนนหน้าข้อที่เลือก

เทียบความรุนแรงตามตารางข้างล่าง

ระดับ depression	ค่าคะแนน HRSD *
No depression	0-7
Mild depression	8-12
Less than major depression	13-17
Major depression	18-29
Severe major depression	30+

ผลการศึกษาพบว่า HRSD ฉบับภาษาไทย มีค่า standardized Cronbach's coefficient alpha ที่แสดงถึงความสอดคล้องภายในของแบบวัด = 0.738 และค่า Spearman's correlation coefficient ซึ่งบ่งถึงความสัมพันธ์ระหว่างค่าคะแนนที่ได้จากแบบวัด HRSD นี้กับ Global Assessment Scale เท่ากับ -.824

Reference : มาโนช หล่อตระกูล, ปราโมทย์ สุคนิชย์, จักรกฤษณ์ สุขยิ่ง. การพัฒนาแบบวัด Hamilton Rating Scale for Depression ฉบับภาษาไทย. วารสารสมาคมจิตแพทย์แห่งประเทศไทย 2539 ; 41(4) : 235-246.

Download site: แบบวัด http://ramamental.com/topics/thamd.pdf
Download site: วารสาร http://www.dmh.go.th/abstract/pdf/100394104190.pdf

แบบบันทึกอาการข้างเคียงจากการใช้ยารักษาโรคซึมเศร้า

คำชี้แจง อาการข้างเคียง หมายถึงอาการที่ไม่พึงประสงค์ที่เกิดขึ้นกับท่านในระหว่างการเข้าร่วมโครงการวิจัย โดยท่านได้รับประทานยารักษาโรคซึมเศร้าตามที่แพทย์สั่งการรักษา อาการดังกล่าวอาจเกี่ยวข้องหรือไม่เกี่ยวข้องกับฤทธิ์ของยาก็ได้

โปรดกาเครื่องหมาย ✓ ลงในช่องที่ตรงกับอาการข้างเคียงที่เกิดขึ้นในรอบสัปดาห์ที่ผ่านมา

อาการข้างเคียง	ไม่มี	มีเล็กน้อย	มีปานกลาง	มีรุนแรง
1. ปวดศีรษะ				
2. มึนงง				
3. วิงเวียน				
4. ง่วงนอน				
5. หาวบ่อย				
6. คลื่นไส้				
7. อาเจียน				
8. ท้องเดิน				
9. เบื่ออาหาร				
10. ท้องอืด จุกเสียดแน่น				
11. ปากแห้ง				
12. ท้องผูก				
13. อ่อนเพลีย ไม่มีกำลัง				
14. มือสั่น				
15. ตื่นเต้น				
16.วิตกกังวล				
17. กระวนกระวาย				
18. นอนไม่หลับ				
19. น้ำหนักเพิ่ม				
20. สมรรถภาพทางเพศลดลง				
21. ฝันร้าย				
22. ไม่ค่อยมีอารมณ์				
23. ชัก				
24. กล้ามเนื้อเกร็ง				
25. กล้ามเนื้อกระตุก				
26. ปวดเมื่อยกล้ามเนื้อ				
27. เคลื่อนไหวช้าลง				
28. เกล็ดเลือดต่ำ				
29. ปัสสาวะบ่อย				
30. มีน้ำนมหลังออกมา เต้านมคัด				
31. ผื่นแดง คัน				
32. มีไข้สูง				
33. น้ำหนักลด				
34. เหงื่อออกมากตอนกลางคืน				
35. หายใจไม่สะดวก				

Appendix B

Analytical method for Fluoxetine and Norfluoxetine concentration

Plasma concentration of fluoxetine and norfluoxetine was determined by using High Performance Liquid Chromatography with ultraviolet detection.

Calibration curve and linearity

The linearity of calibration curve was determined from five standard plasma concentrations (1, 2, 4, 6, 16 μmol/L) of fluoxetine and norfluoxetine by using doxepine for internal standard as shown in figure 2 and figure 3.

The least square linear regression equation of fluoxetine and its pharmacological metabolite could be obtained. Both drug and active metabolite has been related to the peak area ratio and obtained the optimal R^2.

The equation for fluoxetine was

$$Y = 0.0880\ x - 0.0008\ ,\ R^2 = 0.9932$$

Where; x = plasma fluoxetine concentration
Y = peak area ratio of drug to internal standard
R^2 = coefficient of determination

And the equation for fluoxetine was

$$Y = 0.0336\ x - 0.0028\ ,\ R^2 = 0.9954$$

Where; x = plasma norfluoxetine concentration
Y = peak area ratio of metabolite to internal standard
R^2 = coefficient of determination

Figure 2 Calibration curve of fluoxetine

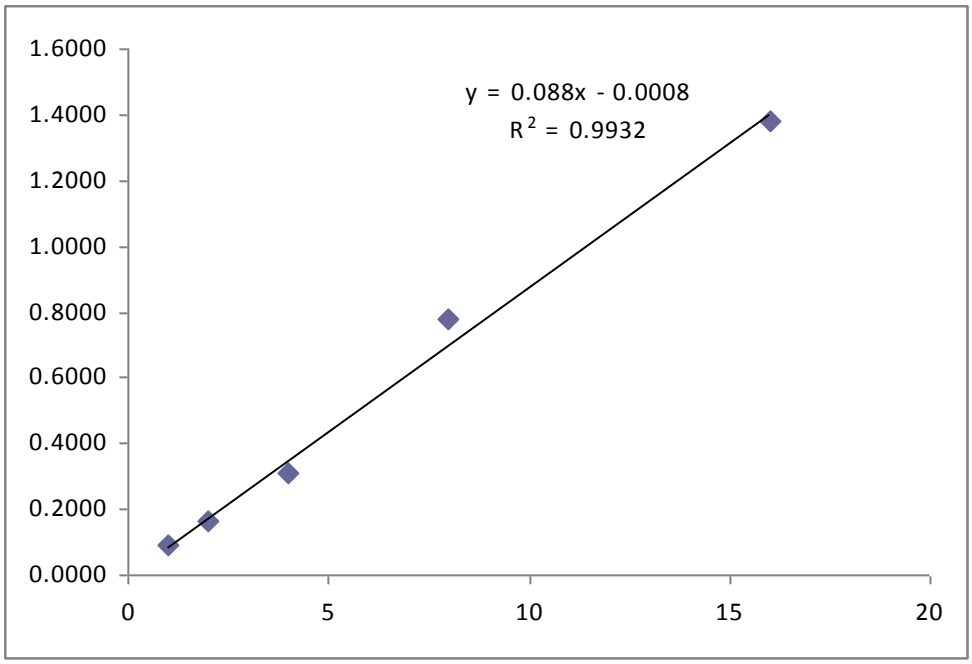
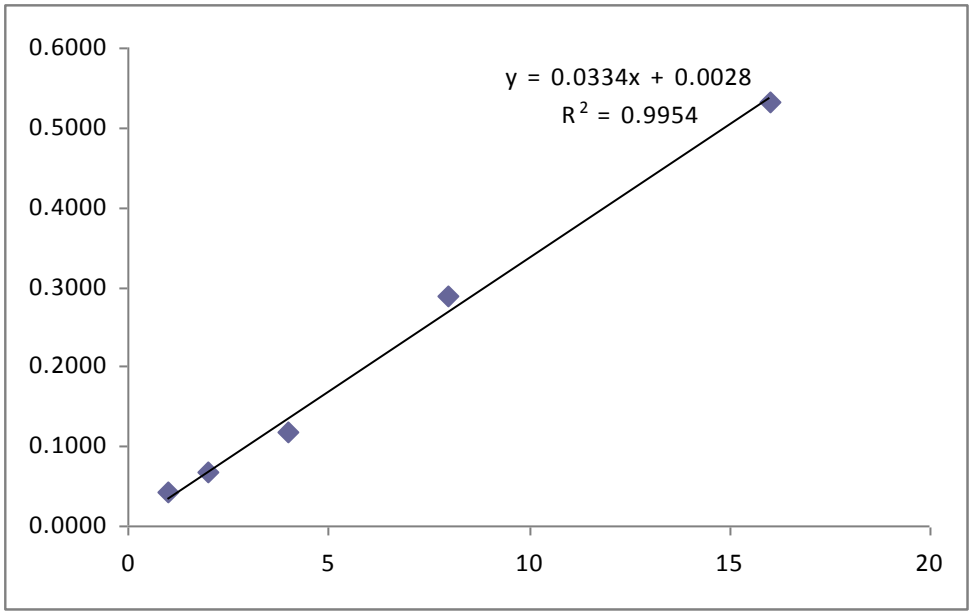
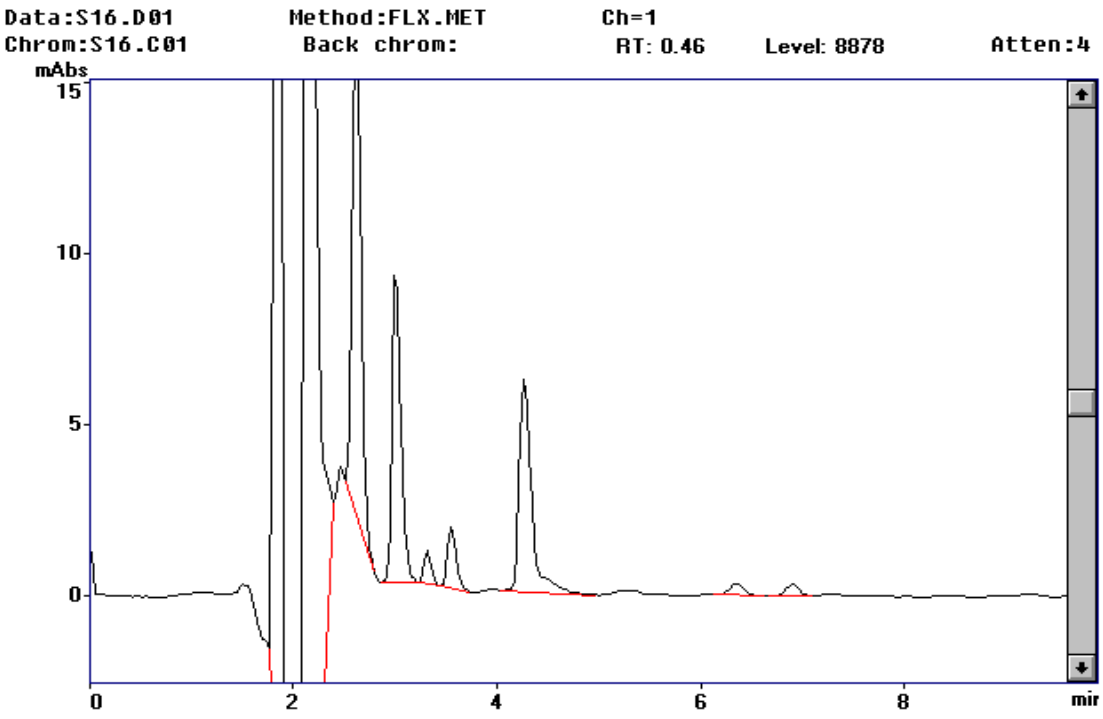
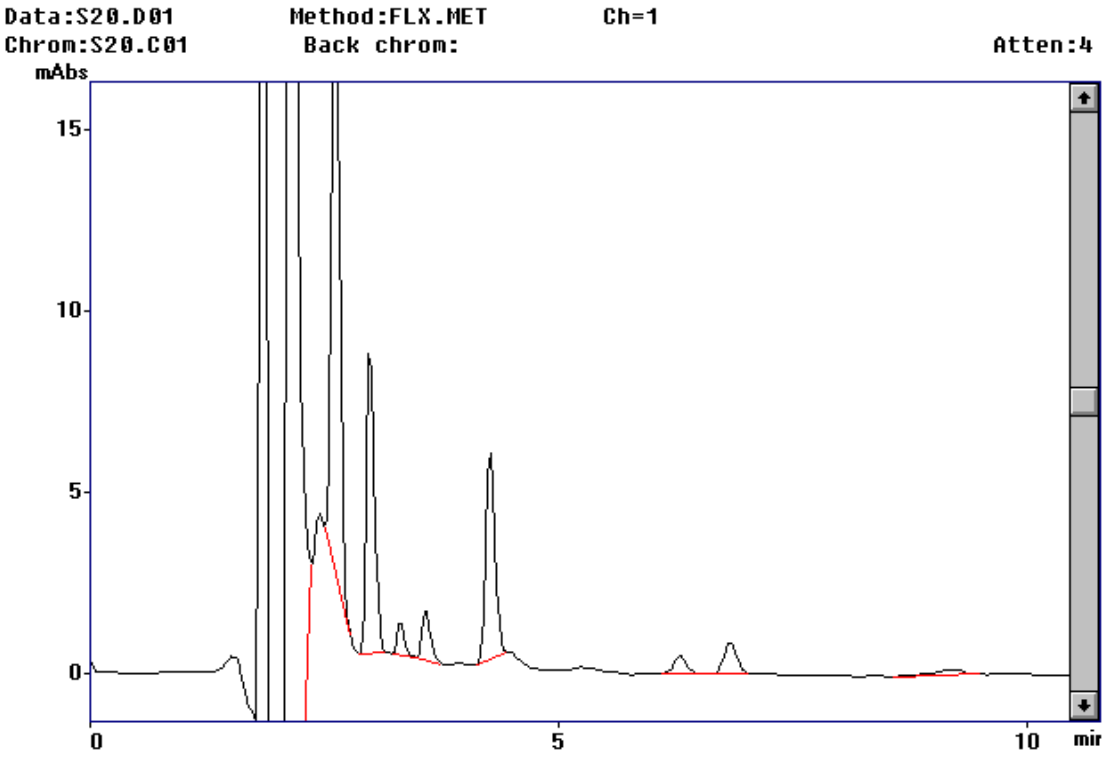


Figure 3 Calibration curve of norfluoxetine



Appendix C
Figure 4 Chromatogram of study results


Data:S16.D01
Method:FLX.MET
Ch=1
Chrom:S16.C01
Back chrom:
RT: 0.46
Level: 8878
Atten:4
mAbs
15
10
5
0
0
2
4
6
8
min


Data:S20.D01
Method:FLX.MET
Ch=1
Chrom:S20.C01
Back chrom:
Atten:4
mAbs
15
10
5
0
0
5
10
min

Appendix D
Genotyping analysis

Figure 5 Allelic plot of CYP2D6*10

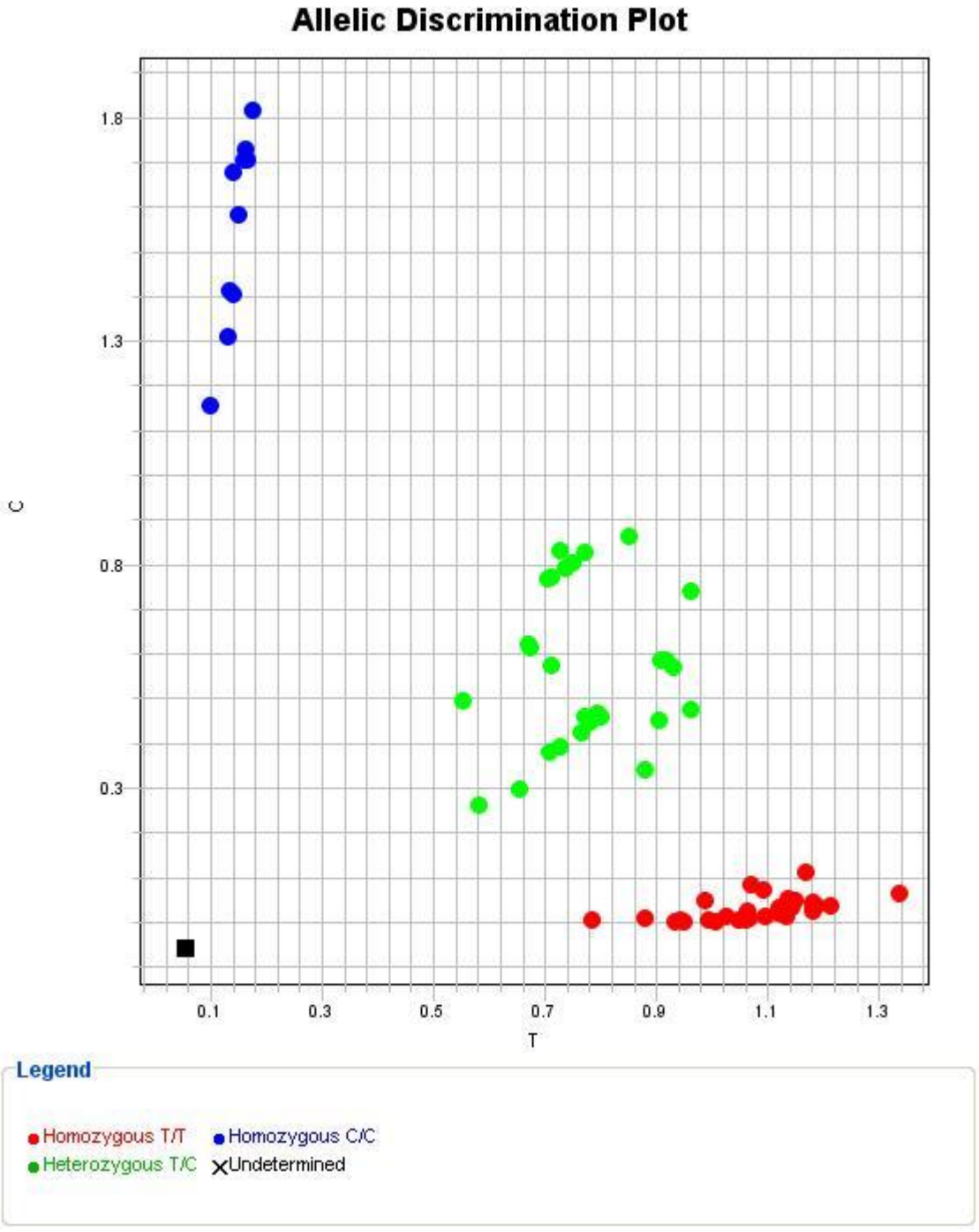


Figure 6 Amplification plot for CYP2D6*10

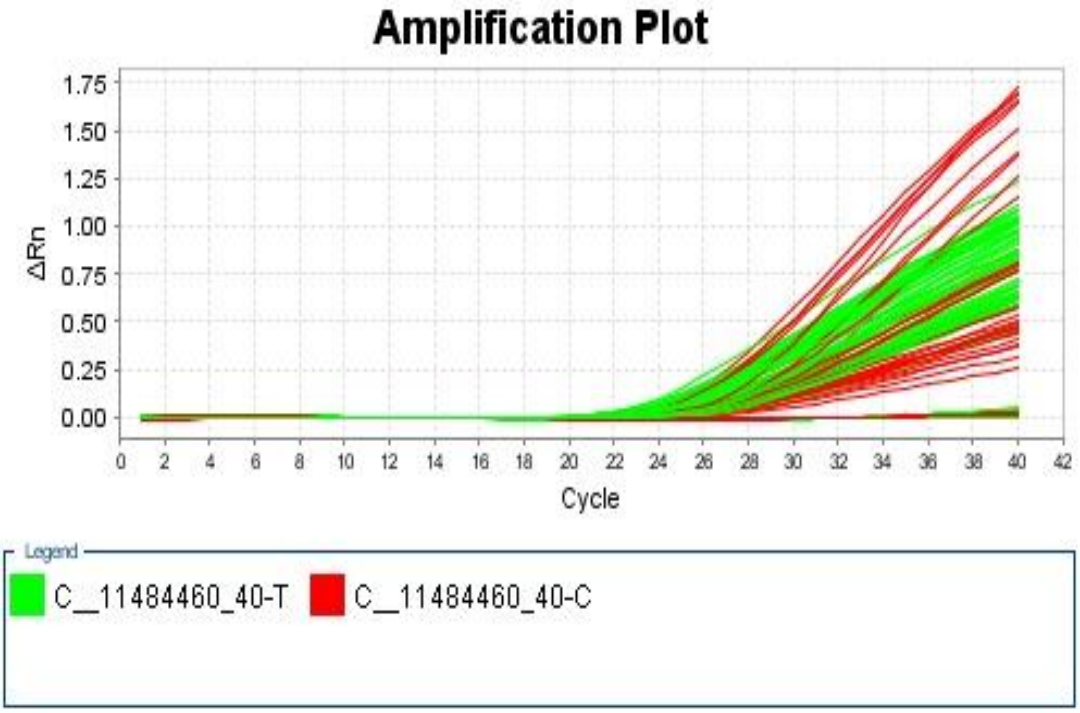
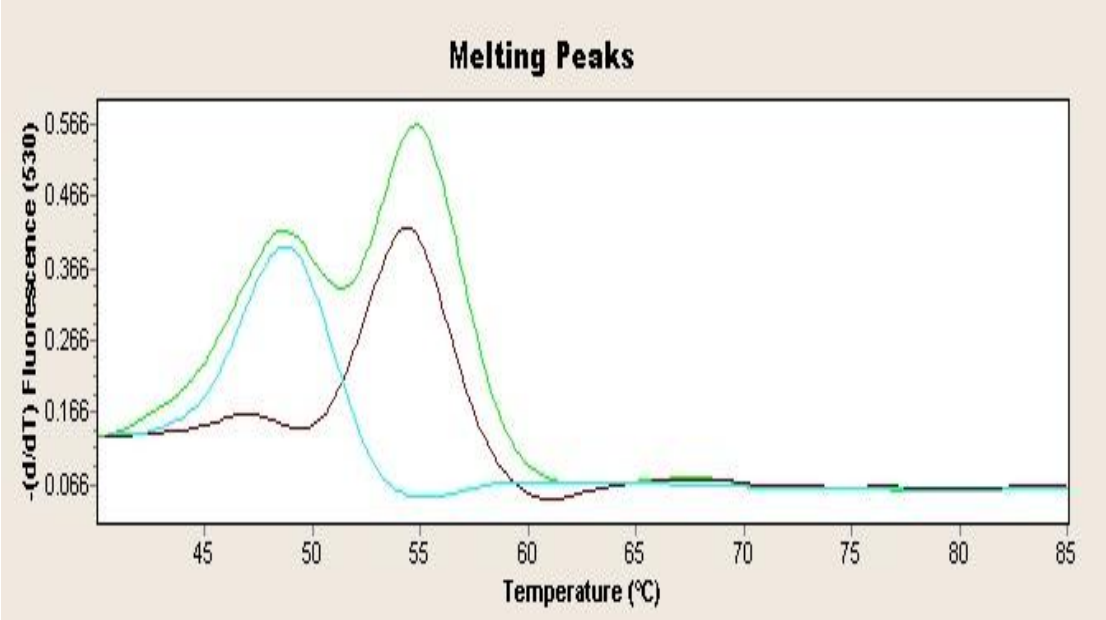


Figure 7 Melting Peaks chart of CYP2C19*2



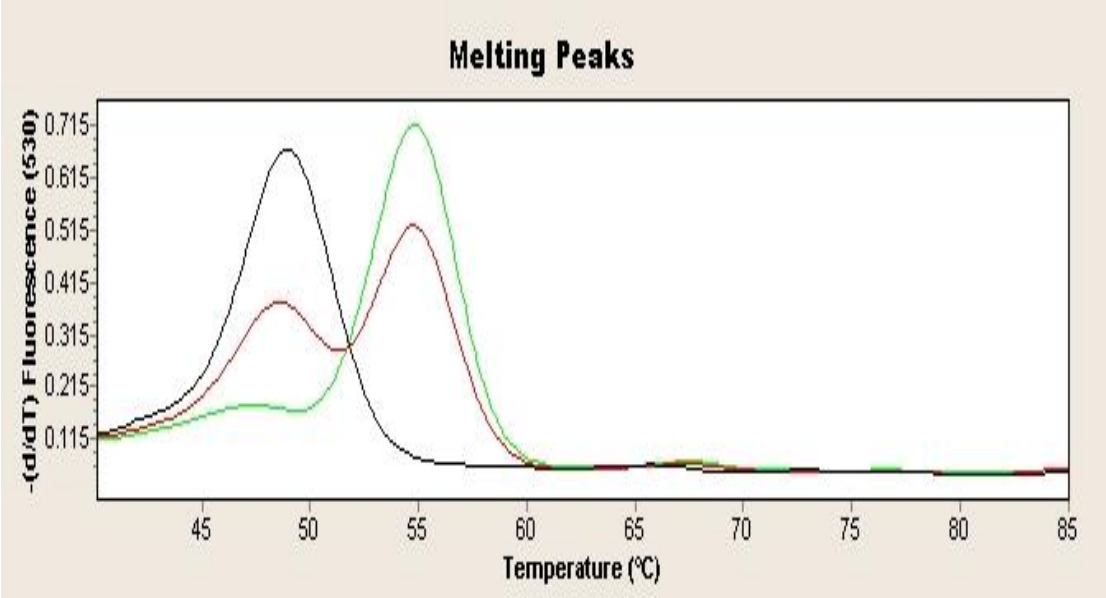
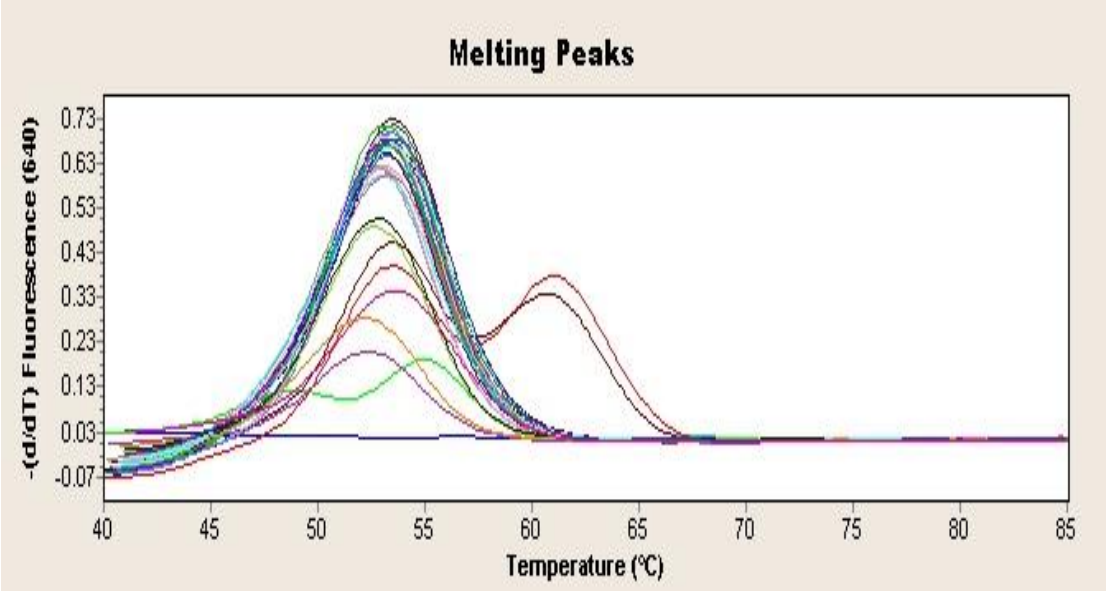


Figure 8 Melting Peaks chart of CYP2C19*3



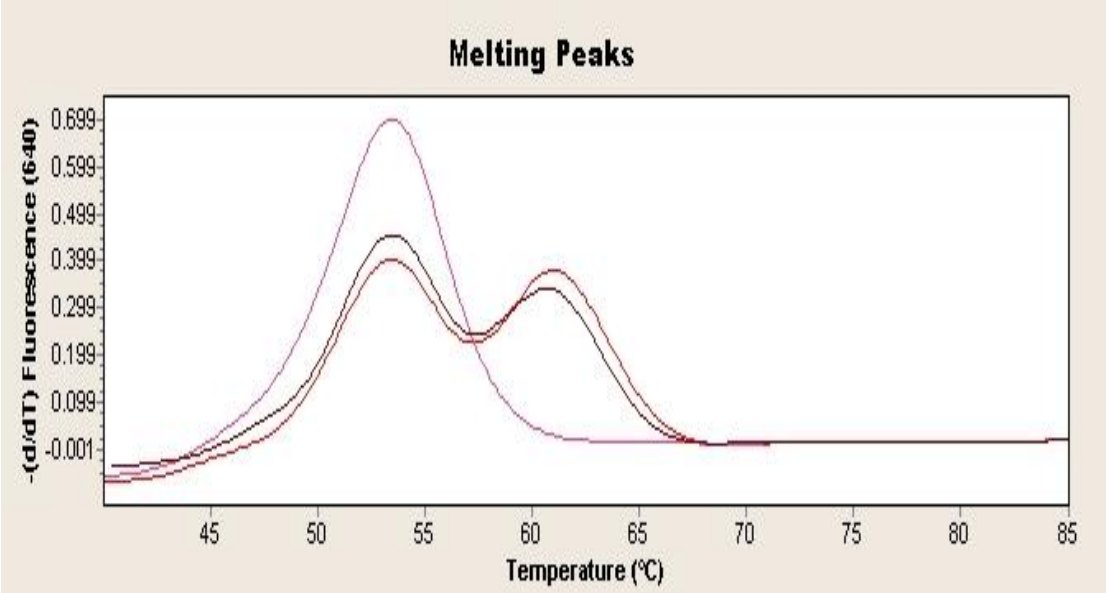
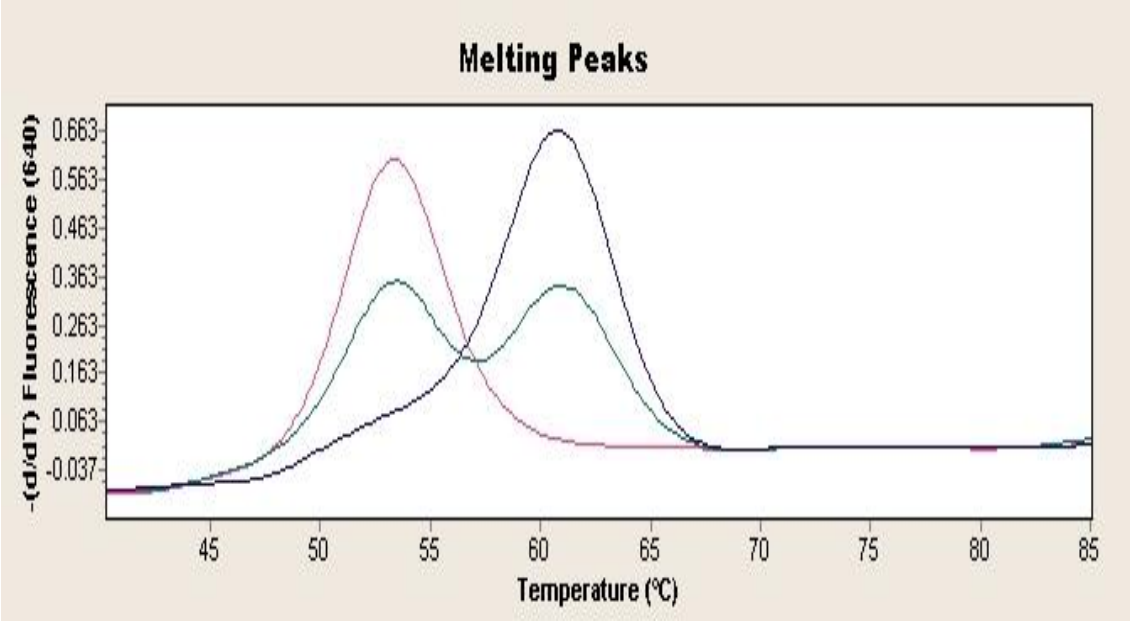


Figure 8 Melting Peaks chart of CYP2C19*3



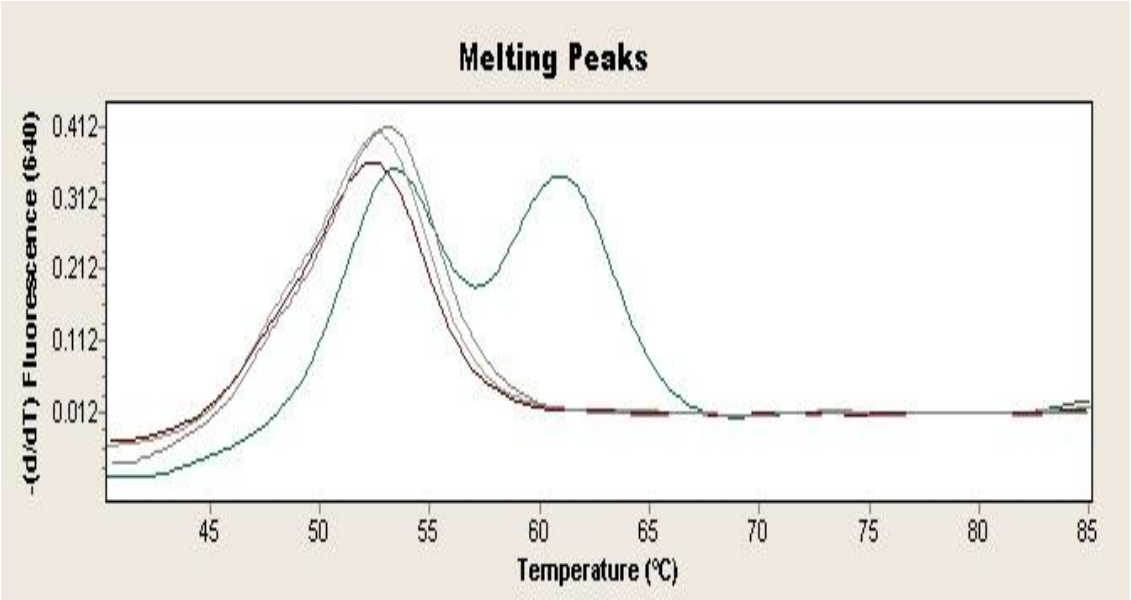


Figure 8 Melting Peaks chart of CYP2C19*3

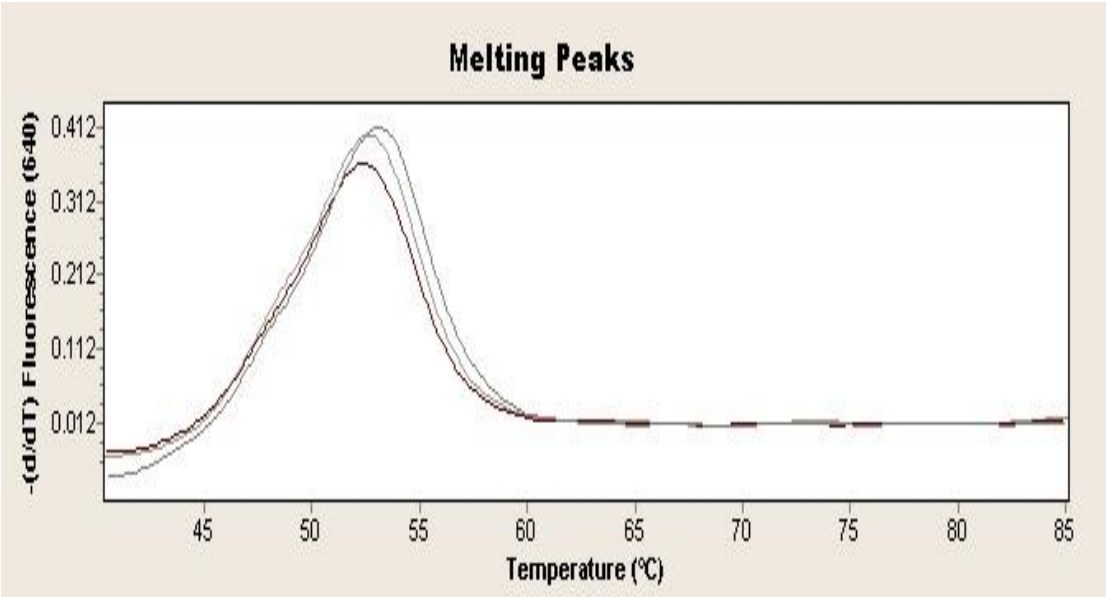
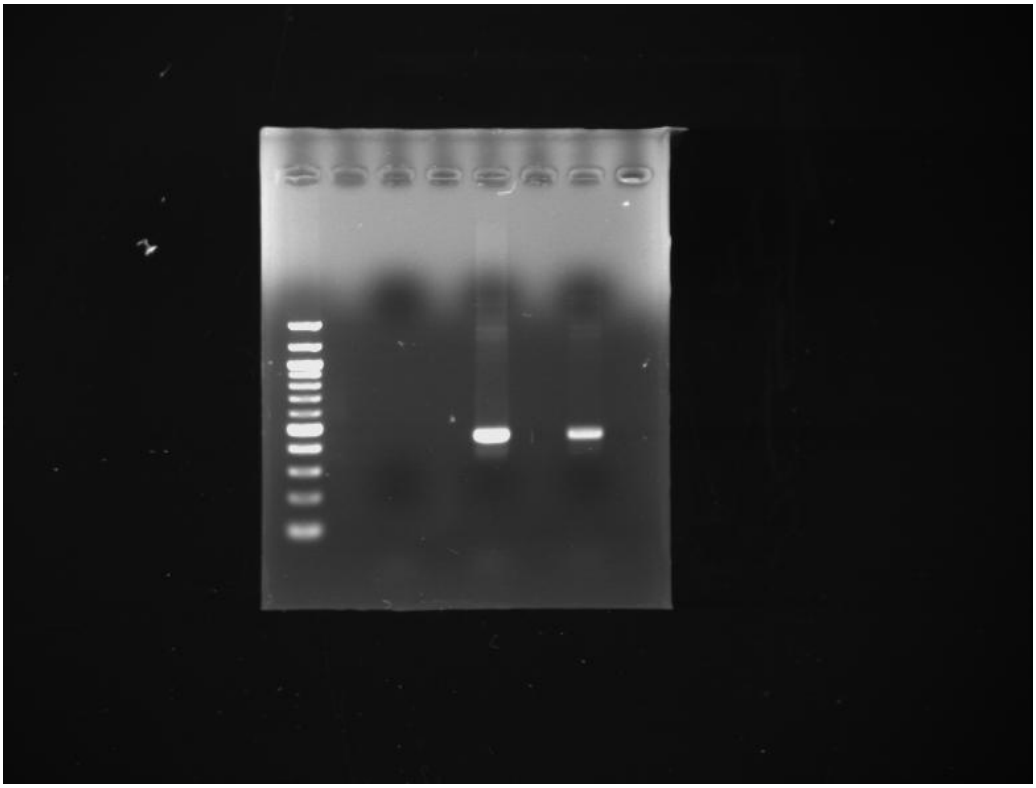


Figure 9 Gel electrophoresis of 5HTTLPR



Appendix E
Data analysis of Pharmacokinetics parameters of Fluoxetine by Non parametric statistics

Fluoxetine and norfluoxetine pharmacokinetic parameters in CYP2D6 variant groups

Genotypes	Steady-state normalized pharmacokinetic parameters			
	Concentration dose ratio		Fluoxetine	Norfluoxetine/
	Fluoxetine μg/ml/mg/kgdose	Norfluoxetine μg/ml/mg/kgdose	Clearance **L/kg**	Fluoxetine ratio
CYP2D6*10				
Median				
Wild type (N=9)	34.44	37.56	33.33	43.00
Heterozygous (N=23)	29.43	29.52	37.35	32.17
Homozygous (N=32)	34.16	33.22	28.78	29.78
p-value	0.614	0.522	0.240	0.169

Fluoxetine and norfluoxetine pharmacokinetic parameters in CYP2C19 variant groups

Genotypes	Steady-state normalized pharmacokinetic parameters			
	Concentration dose ratio		Fluoxetine	Norfluoxetine/
	Fluoxetine μg/ml/mg/kgdose	Norfluoxetine μg/ml/mg/kgdose	Clearance **L/kg**	Fluoxetine ratio
CYP2C19*2				
Median				
Wild type (N=26)	32.35	29.85	31.92	29.81
Not wild type (N=38)	32.61	34.32	32.89	34.34
p-value	0.956	0.345	0.837	0.338
CYP2C19*3				
Median				
Wild type (N=59)	32.60	32.04	31.35	31.45
Not wild type (N=9)	31.89	35.33	39.56	38.89
p-value	0.915	0.622	0.220	0.267

Appendix F
Data of individual participant

ID	Female	Age	Dose/d	Weight	ConcFLX	ConNFLX	SUM	SERT	*10	*2	*3	HRSD	SE
1	female	28	10	53	2.18	3.76	5.94	s/l	MT	WT	WT	9	efficacy with SE
2	female	47	20	63	3.48	8.55	12.03	s/l	MT	WT	HT	7	efficacy
3	female	24	40	52	8.33	12.05	20.38	s/s	MT	WT	HT	6	efficacy
4	female	22	40	51	7.73	17.81	25.54	s/l	HT	MT	WT	5	efficacy
5	male	18	20	55	3.35	5.46	8.81	s/l	WT	WT	WT	7	efficacy with SE
6	female	34	20	50.2	2.36	3.39	5.75	s/s	MT	HT	HT	5	efficacy
7	female	36	20	70.9	2.21	3.46	5.67	l/l	MT	HT	WT	10	efficacy
8	female	42	80	66	7.03	5.77	12.8	l/l	MT	HT	WT	6	efficacy
9	female	36	20	61.6	0.76	4.2	4.96	l/l	HT	WT	WT	6	efficacy with SE
10	female	59	20	63.8	0.26	1.09	1.35	s/s	WT	WT	HT	6	efficacy
11	male	34	20	83.2	0.1	1.56	1.66	s/s	HT	HT	WT	6	efficacy
12	female	43	20	66	0.84	4.61	5.45	s/l	HT	HT	WT	4	efficacy with SE
13	male	54	20	60	1.65	4.74	6.39	s/l	WT	HT	WT	4	efficacy
14	male	33	60	71	8.76	10	18.76	s/s	MT	HT	WT	4	efficacy
15	male	26	40	79	1.96	3.67	5.63	s/s	MT	HT	WT	7	efficacy
16	male	39	20	52	0.68	1.78	2.46	l/l	WT	HT	WT	5	efficacy with SE
17	male	45	20	68	0.59	2.27	2.86	s/s	WT	WT	WT	6	efficacy with SE
18	male	33	99	70	999	999	999	s/s	HT	HT	WT	6	Switch to others
19	female	32	20	54.6	1.11	1.96	3.07	s/s	MT	HT	WT	5	efficacy
20	male	35	40	62	1.92	2.78	4.7	s/s	MT	WT	HT	5	efficacy
21	female	46	20	75	1.47	2.53	4	s/s	MT	WT	WT	5	efficacy
22	male	45	30	56	1.94	3.49	5.43	s/s	HT	WT	WT	13	efficacy with SE
23	female	30	20	64	1.08	3.75	4.83	s/l	MT	HT	WT	5	efficacy
24	female	37	20	36	1.35	6.47	7.82	s/s	HT	HT	WT	12	efficacy with SE
25	female	38	20	90.3	0.72	1.82	2.54	s/s	MT	HT	WT	7	efficacy

ID	Female	Age	Dose/d	Weight	ConcFLX	ConNFLX	SUM	SERT	*10	*2	*3	HRSD	SE
26	female	38	20	44	0.92	2.77	3.69	s/s	MT	HT	WT	10	efficacy
27	female	41	20	54	0.65	3.4	4.05	s/s	HT	HT	WT	10	efficacy
28	female	50	99	67	999	999	999	s/s	HT	MT	WT	9	Switch to others
29	female	30	20	64	0.6	1.92	2.52	s/s	MT	WT	WT	9	efficacy
30	male	22	99	70	999	999	999	l/l	HT	HT	WT	7	Switch to others
31	female	40	20	67	1.08	3.24	4.32	l/l	WT	HT	WT	5	efficacy
32	female	49	40	50	0.6	4.56	5.16	l/l	MT	HT	WT	10	efficacy
33	female	30	20	86.5	0.41	0.77	1.18	s/s	WT	WT	WT	2	efficacy with SE
34	female	52	20	60	0.23	0.41	0.64	s/s	MT	WT	WT	3	efficacy with SE
35	male	33	80	72	2.67	2.53	5.2	s/s	MT	HT	WT	5	efficacy
36	male	33	20	65	0.87	1.83	2.7	s/s	HT	HT	WT	3	efficacy
37	female	26	10	50	0.36	0.1	0.46	s/s	HT	WT	WT	5	efficacy with SE
38	female	35	20	55.3	0.49	0.79	1.28	s/l	HT	WT	WT	4	efficacy
39	male	38	40	91.3	0.99	1.13	2.12	s/l	HT	WT	WT	8	efficacy
40	male	61	10	63	0	0	0	s/s	MT	HT	WT	2	efficacy with SE
41	female	61	40	48	1.3	1.31	2.61	s/l	HT	HT	WT	4	efficacy with SE
42	female	45	40	54	1.45	2.09	3.54	s/s	HT	HT	WT	5	efficacy with SE
43	female	30	20	49.7	0.3	0.1	0.4	s/l	HT	HT	WT	6	efficacy
44	male	38	40	52.3	3.39	2.61	6	s/l	WT	WT	WT	4	efficacy
45	female	48	20	66	1.17	0.1	1.27	s/l	HT	WT	WT	5	efficacy
46	male	37	20	55	0.26	0.54	0.8	s/s	HT	HT	WT	2	efficacy
47	female	28	40	50	0.68	1.46	2.14	s/s	MT	WT	WT	12	efficacy
48	female	29	20	50.3	0.32	1.34	1.66	s/l	MT	HT	WT	9	efficacy
49	male	45	30	76.4	0.1	0.97	1.07	s/s	WT	HT	HT	11	efficacy with SE
50	female	56	40	46.6	0.1	1.25	1.35	s/s	HT	HT	HT	8	efficacy with SE
51	male	27	40	114	0.53	3.06	3.59	s/s	MT	WT	WT	8	efficacy
52	male	26	20	52	0	0	0	s/l	HT	HT	WT	5	efficacy with SE
53	female	30	60	65	1.55	3.3	4.85	s/l	HT	HT	WT	5	efficacy

ID	Female	Age	Dose/d	Weight	ConcFLX	ConNFLX	SUM	SERT	*10	*2	*3	HRSD	SE
54	male	29	20	74	0	0	0	l/l	MT	WT	WT	6	efficacy
55	male	37	20	95	0	0	0	s/s	MT	HT	WT	5	efficacy with SE
56	female	33	30	100	0	0	0	s/s	HT	WT	WT	6	efficacy with SE
57	female	35	20	55	0.38	0.1	0.48	s/l	MT	HT	WT	3	efficacy with SE
58	female	50	40	52.5	0.92	0.1	1.02	s/s	MT	WT	WT	4	efficacy
59	female	39	10	50	0.1	0.21	0.31	s/s	HT	WT	HT	7	efficacy
60	male	39	20	83	0.13	0.1	0.23	s/s	MT	HT	WT	4	efficacy
61	male	28	40	90	0.56	1.65	2.21	l/l	MT	HT	WT	7	efficacy
62	female	52	40	73	0	0	0	s/s	MT	HT	WT	11	efficacy with SE
63	male	57	20	63.9	0.38	0.39	0.77	s/s	HT	HT	WT	4	efficacy
64	female	34	20	65	0.3	0.61	0.91	s/s	MT	WT	WT	10	efficacy with SE
65	female	62	99	43	999	999	999	s/s	HT	WT	WT	7	Switch to others
66	female	47	99	75	999	999	999	s/s	WT	WT	WT	6	Switch to others
67	female	54	20	55.4	0.1	0.22	0.32	l/l	MT	MT	WT	6	efficacy
68	male	35	60	63	3.13	5.84	8.97	s/s	MT	HT	WT	10	efficacy with SE
69	female	39	20	55	0.1	0.13	0.23	s/s	HT	WT	HT	4	efficacy with SE

VITAE

Mrs. Kamolwan Tantipiwattanaskul was born on the 20th of October in 1969 in Bangkok. She graduated a Bachelor degree in Pharmacy and a Master degree of Pharmacy Administration from Faculty of Pharmacy, mahidol University. Her family with three children has been in Bangrak district, Bangkok.