ปัจจัยที่มีผลต่ออัตราส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาลาโมทริจีน ในผู้ป่วยชาวไทย

นางสาวนภเกตน์ สิงห์คำ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรมคลินิก ภาควิชาเภสัชกรรมปฏิบัติ
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FACTORS INFLUENCING CONCENTRATION-TO-DOSE RATIO OF LAMOTRIGINE IN THAI PATIENTS

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ภูมิหลัง ความผันแปรทางพันธุกรรมเป็นปัจจัยหนึ่งที่มีผลต่อความผันแปรทางเภสัชจลนศาสตร์ของยา เนื่องจากยาลาโมทริจีนถูกเมแทบอไลซ์ผ่านเอนไซม์ UGT 1A4 เป็นหลัก ถ้ามีการกลายพันธุ์ของยีนที่ควบคุมเอนไซม์ดังกล่าวอาจทำให้ประสิทธิภาพการทำงานของเอนไซม์เปลี่ยนแปลง ส่งผลให้เกิดความผันแปรทางเภสัชจลนศาสตร์ของยาได้

วัตถุประสงค์ เพื่อศึกษาผลของปัจจัยทางพันธุกรรม (ภาวะพหุสัณฐานของยีน *UGT1A4*) และปัจจัยที่ไม่เกี่ยวข้องกับพันธุกรรม (อายุ เพศ น้ำหนัก และยาที่ใช้ร่วม) ต่ออัตราส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาลาโมทริจีน (lamotrigine concentration-to-dose ratio; LTG-CDR) ในผู้ป่วยชาวไทย

วิธีทำการศึกษา เป็นการศึกษาเชิงวิเคราะห์แบบไปข้างหน้าในผู้ป่วย 73 ราย ณ สถาบันประสาทวิทยา ซึ่งได้รับยาลาโมทริจีนในขนาดคงที่เป็นเวลาอย่างน้อย 2 สัปดาห์ โดยทำการวัดระดับยาลาโมทริจีนในเลือดด้วยเทคนิค HPLC และตรวจภาวะพหุสัณฐานของยีน *UGT1A4* ด้วยวิธี Taqman allelic discrimination ใช้สถิติ ANOVA เพื่อวิเคราะห์ความแตกต่างของ LTG-CDR ระหว่างกลุ่มผู้ป่วยที่มีภาวะพหุสัณฐานของยีน *UGT1A4* ที่แตกต่างกันและใช้การวิเคราะห์ถดถอยพหุคูณเชิงเส้นเพื่อหาความสัมพันธ์ระหว่างปัจจัยทางพันธุกรรมและปัจจัยที่ไม่เกี่ยวข้องกับพันธุกรรมที่มีผลต่อค่า LTG-CDR

ผลการศึกษา ในผู้ป่วยชาวไทยพบว่ามีความถี่ของอัลลีล *UGT1A4* 142T>G ร้อยละ 27 แต่ไม่พบภาวะพหุสัณฐานของยีน *UGT1A4* 70 C>T ในกลุ่มผู้ป่วยที่ใช้ยาลาโมทริจีนเดี่ยวๆ หรือใช้ยาลาโมทริจีนร่วมกับยาที่ยับยั้งเอนไซม์และยาที่เหนี่ยวนำเอนไซม์ ซึ่งมียีน *UGT1A4* 142T>G ผิดปกติอย่างน้อย 1 อัลลีล (T/G หรือ G/G) พบว่ามีค่า LTG-CDR ต่ำกว่าผู้ป่วยที่มีลักษณะยีนปกติ (T/T) อย่างมีนัยสำคัญทางสถิติ (*p=0.019*) จากผลการวิเคราะห์ถดถอยพหุคูณเชิงเส้นพบว่า อายุ การใช้ยาที่ยับยั้งเอนไซม์และยาที่เหนี่ยวนำเอนไซม์ร่วมด้วย โดยสมการสามารถอธิบายความผันแปรของค่า LTG-CDR ได้ร้อยละ 20.40

สรุปผล ภาวะพหุสัณฐานของยีน *UGT1A4* อาจมีผลต่อความผันแปรของค่า LTG-CDR ในผู้ป่วยชาวไทย อย่างไรก็ตามเมื่อนำปัจจัยทางพันธุกรรมมาพิจารณาร่วมกับปัจจัยที่ไม่เกี่ยวข้องกับพันธุกรรม ได้แก่ อายุ และยาที่ใช้ร่วม พบว่าภาวะพหุสัณฐานของยีน *UGT1A4* ไม่มีผลต่อความผันแปรทางเภสัชจลนศาสตร์ของยาลาโมทริจีน

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

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

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NOPPAKET SINGKHAM : FACTORS INFLUENCING CONCENTRATION-TO-DOSE RATIO OF LAMOTRIGINE IN THAI PATIENTS. ADVISOR : BARALEE PUNYAWUDHO, Ph.D., CO-ADVISOR : SOMCHAI TOWANABUT, M.D., 100 pp.

Background Genetic variation is one of factors that contribute to the interindividual variability of pharmacokinetic. UGT1A4 is the major enzyme responsible for lamotrigine metabolism. Therefore, *UGT1A4* polymorphisms could lead to the variability of glucuronidation enzyme activity and may contribute to the difference of lamotrigine pharmacokinetics among ethnicities.

Objectives To investigate the effect of genetic (*UGT1A4* polymorphisms) and non-genetic factors (age, gender, body weight, and co-medications) on lamotrigine concentration-to-dose ratio (LTG-CDR) in Thai patients.

Methods A prospective analysis study in 73 patients from Prasat Neurological Institute, who had stable lamotrigine dose for at least 2 weeks. Lamotrigine plasma concentration was determined using HPLC method. Genotyping of *UGT1A4* was carried out by Taqman allelic discrimination assays. ANOVA was used to compare LTG-CDR among groups of different polymorphism. Multiple regression analysis was performed to investigate an association of all factors and LTG-CDR.

Results The allele frequency of *UGT1A4* 142 T>G in Thai patients was 27%. However, the variant of *UGT1A4* 70 C>T was not found. The LTG-CDR of patients having at least 1 variant allele (T/G or G/G) was significantly lower than patients having wild type allele (T/T) for patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducers ($p=0.019$). The stepwise regression model showed that age, the use of enzyme inducers, and enzyme inhibitor influence LTG-CDR. This model could explain 20.40% of the variance of LTG-CDR.

Conclusion *UGT1A4* polymorphism may contribute to the variability of LTG-CDR in Thai population. However, after accounting for age and co-medications, the influence of *UGT1A4* polymorphism was not found.

Department : Pharmacy Practice Student's Signature .....

Field of Study : Clinical Pharmacy Advisor's Signature .....

Academic Year : 2011 Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

ALT	=	Alanine aminotransferase (SGPT)
AST	=	Aspartate aminotransferase (SGOT)
AUC	=	Area under the curve
CL/F	=	Apparent oral clearance
Cmax	=	The maximum concentration
CrCl	=	Creatinine clearance
dL	=	Deciliter
DNA	=	Deoxyribonucleic acid
EIAED	=	Enzyme inducing antiepileptic drugs
GFR	=	Glomerular filtration rate
HPLC	=	High-performance liquid chromatography
HWE	=	Hardy Weinberg equilibrium
kg	=	Kilogram
L	=	Litters
LTG	=	Lamotrigine
LTG-CDR	=	Lamotrigine concentration-to-dose ratio
mg	=	Milligram
min	=	Minute
mL	=	Milliliter
MLR	=	Multiple linear regression
mm	=	Millimeter
mM	=	Millimolar
nm	=	Nanometer
OD	=	Optical density
PCR	=	Polymerase chain reaction
rs number	=	Reference single nuclecotide polymorphisms number
Scr	=	Serum creatinine

SNPs	=	Single nuclecotide polymorphisms
Tmax	=	Time to peak concentrations
UDP	=	Uridine 5'-diphosphate
UDPGA	=	Uridine 5'-diphosphoglucuronic acid
UGTs	=	Uridine-diphosphate glucuronosyltransferases
UV	=	Ultraviolet
VIF	=	Variance inflation factor
V/F	=	Apparent volume of distribution
VPA	=	Valproic acid
μm	=	Micrometer

CHAPTER I
INTRODUCTION

Background and Rationale

Lamotrigine is the new-generation antiepileptic drug that has an indication for several types of seizures. It can be used as a monotherapy or adjunctive therapy. In addition, this drug was approved for using as a mood stabilizer for the treatment of bipolar disorder.[1-3]

Lamotrigine shows linear pharmacokinetics.[2, 4] It is rapidly absorbed with high bioavailability and about 55% of the drug is bound to plasma proteins.[2, 4] Lamotrigine is metabolized via glucuronidation by uridine-diphosphate glucuronosyltransferases (UGTs) enzyme.[5] The half-life of lamotrigine is approximately 22.80-37.40 hours when used as monotherapy, but it can be prolonged to 60 hours when co-administered with valproic acid and shortened to 15 hours when co-administered with enzyme inducers such as carbamazepine, phenytoin, and phenobarbital.[2, 6-7] In general, the therapeutic range of lamotrigine is found to be 1-4 mg/L.[6, 8] However many patients may require concentrations higher than the established therapeutic range.[9-10]

Lamotrigine exhibits high interindividual variability of the pharmacokinetics. Interindividual variation of lamotrigine pharmacokinetics is influenced by several factors such as age, pregnancy, diseases and drug–drug interactions.[7-8, 11] Therapeutic drug monitoring of lamotrigine is important to individualize patient's therapy. It is recommended to monitor lamotrigine concentrations especially in patients suspected of treatment failure due to drug interactions and noncompliance, patients with sign of clinical drug intoxication, patients with a change of physiological state that may alter lamotrigine pharmacokinetics such as pregnancy. Furthermore, it can be used as a reference concentration for dose adjustment in each individual patient.[6, 8, 12-13]

There are evidences of the difference of lamotrigine pharmacokinetics among ethnicities. Hussian and Posner[14] reported that lamotrigine apparent oral clearance (CL/F) was 28.70% lower in Asian compared to Caucasian. Moreover, Grasela et al.[15] found that CL/F of lamotrigine was 25% lower in non-Caucasian compared with

Caucasian patients. The difference of lamotrigine pharmacokinetics among races is probably related to genetic variations in the metabolism of lamotrigine.[14-15]

UGT1A4 is the major enzyme responsible for lamotrigine metabolism.[16] However, other UGTs such as UGT1A3 and UGT2B7 may also play a role in the glucuronidation of lamotrigine.[17-19] The polymorphisms of *UGT1A4* could lead to the variability of glucuronidation enzyme activity and may contribute to the difference of lamotrigine pharmacokinetics among races.[16, 20]

Recent studies have discovered a numerous variations of *UGT1A4* among ethnicities.[21-27] *UGT1A4* 142T>G (L48V) and *UGT1A4* 70C>T (P24T) were first detected in German population, with the frequencies of 9% and 8%, respectively.[22] In Turkish population, the frequencies of *UGT1A4* 142T> G (L48V) and *UGT1A4* 70C>A (P24T) were 12.80% and 1.90%, respectively.[25] In Japanese population, the frequencies of *UGT1A4* 142T>G (L48V) and *UGT1A4* 31C>T (R11W) were 16.50% and 1.20%, respectively.[23, 26] Moreover, the frequencies of *UGT1A4* 142T>G (L48V) and *UGT1A4* 31C>T (R11W) in Korean population were found to be similar to previously reported in Japanese population.[27] Interestingly, the polymorphism of *UGT1A4* 70C>T (P24T) was not found in Asian population.[23, 26-27] The effect of *UGT1A4* polymorphisms on glucuronidation activity was dependent upon a substrate. Previous studies found that an enzyme activity was reduced for β-naphthylamine, benzidine, trans-androsterone and dihydrotestosterone, while it was increased for clozapine glucuronidation.[22, 26]

Several studies have documented the effect of *UGT1A4* polymorphisms on the pharmacokinetics of several drug substrates.[28-29] However, there is only one study investigating an impact of *UGT1A4* polymorphisms on the pharmacokinetics of lamotrigine. The results from this study suggested that *UGT1A4* polymorphisms were associated with the decrease of lamotrigine concentration in Turkish patients using lamotrigine as a monotherapy or polytherapy.[30] However, there are no data available regarding the association of *UGT1A4* polymorphisms and pharmacokinetic of lamotrigine, in Asian population.

Therefore, the purpose of this study was to determine the influence of *UGT1A4* polymorphisms and other non-genetic factors on lamotrigine concentration-to-dose ratio (LTG-CDR) in Thai population. The results from this study can be used for facilitating lamotrigine dose adjustment in clinical practice, specifically in Asian patients.

Hypothesis

UGT1A4 polymorphisms and other non-genetic factors influence LTG-CDR in Thai patients.

Objective

To investigate the effect of *UGT1A4* polymorphisms and other non-genetic factors on LTG-CDR in Thai patients.

Scope of this study

This study investigated the influence of *UGT1A4* polymorphisms and other non-genetic factors on LTG-CDR in Thai patients. The population of this study is outpatients with epilepsy or psychiatric disorders receiving lamotrigine as a monotherapy or polytherapy at Prasat Neurological Institute. The dependent variable is LTG-CDR. The independent variables are genetic (*UGT1A4* polymorphisms) and non-genetic factors (age, gender, body weight and co-medications).

Significance of the study

The influence of *UGT1A4* polymorphisms and other non-genetic factors on lamotrigine pharmacokinetics will be identified and quantified. By providing an equation useful for predicting lamotrigine plasma concentrations, the results from this study can be used to design lamotrigine dosage regimens in clinical practice.

Conceptual framework

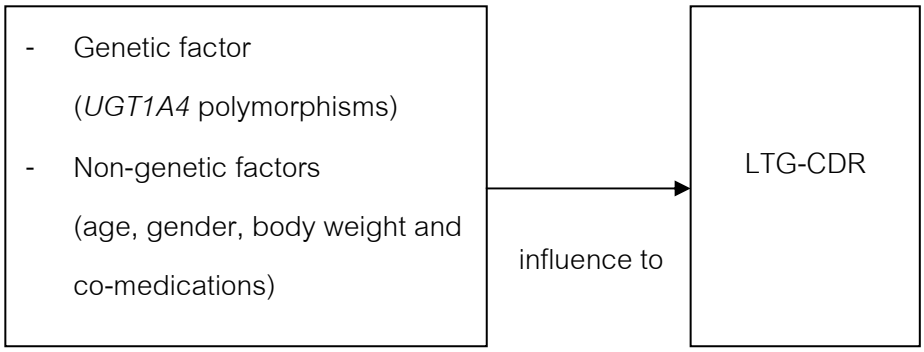


Figure 1 Conceptual framework

Limitation of this study

An application of the results obtained from this study could be limited to the patients having similar characteristics with the patients participating in this study. An extrapolation of the results to other groups of patients should be cautiously performed.

Operational definition

1. Genetic factor was defined as genetic polymorphisms of the UGT 1A4 enzyme that are single-nucleotide polymorphisms (SNPs).
    1.1 *UGT1A4* 142T>G polymorphism is detected at codon 48, with a T to G transversion at position 142 leading to amino acid change,leucine to valine (L48V, submitted to Gen-Bank as UGT1A4*3, rs2011425).[22]
    1.2 *UGT1A4* 70C>T polymorphism is detected at codon 24, with C to A transversion at position 70 leading to amino acid change, proline to threonine, (P24T, submitted to GenBank as UGT1A4*2, rs6755571).[22]
2. Non-genetic factors were defined as patient characteristics including age, gender, body weight and co-medications.
3. Lamotrigine concentration-to-dose ratio (LTG-CDR) was defined as a ratio of the trough concentration of lamotrigine (milligram per liters; mg/L) to the total daily dose of lamotrigine (milligram per kilogram per day; mg/kg/day).

CHAPTER II
LITERATURE REVIEWS

Lamotrigine

Lamotrigine is one of the new-generation antiepileptic drugs. It was approved to be used as an adjunctive therapy for partial seizures, primary and secondary tonic-clonic seizures, and generalized seizures of Lennox-Gastaut syndrome in adult and pediatric patients (≥2 years of age). In addition, it is approved as a monotherapy in adult patients with partial seizures.[1-2, 31]

In 2003, lamotrigine was approved by the U.S. Food and Drug Administration (FDA) for the treatment of bipolar disorder. It is effective when used as a mood stabilizer for maintenance treatment of bipolar disorder in patients with depression.[3, 32] Furthermore, lamotrigine has been used off-label in cyclothymia, resistant unipolar depression, schizoaffective disorder, borderline personality disorder and trigeminal neuralgia.[32-33]

Lamotrigine is phenyltriazine derivative [3, 5-diamino-6-(2, 3-dichlorophenyl)-1, 2, 4-triazine] and it is chemically unrelated to other antiepileptic drugs.[2, 31] The chemical structure of lamotrigine is presented in Figure 2. The pharmacological profile of lamotrigine is similar to phenytoin and carbamazepine.[2] Lamotrigine is available in tablet (25, 50, 100 and 200 mg) and chewable dispersible tablet dosage forms (2, 5 and 25 mg).[1, 31] However, only tablet dosage form is available in Thailand.

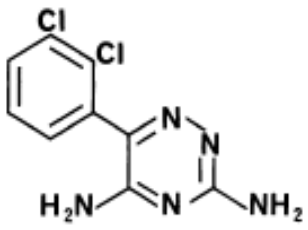


Figure 2 The structure of lamotrigine[34]

Mechanism of action

Lamotrigine affects the voltage-sensitive sodium channels. It acts by stabilizing neuronal membranes and inhibiting the release of excitatory amino acid neurotransmitters (such as glutamate and aspartate) that play a role in epileptic seizures.[2, 35]

The mechanism of action of lamotrigine in patients with bipolar disorder is unclear. However, lamotrigine may be related to the inhibition of sodium and calcium channels in presynaptic neurons which subsequently leads to a stabilization of the neuronal membrane. Additionally, its activity as a mood stabilizing agents is exhibited by the neuroprotective and antiglutamatergic effects.[3, 32]

Pharmacokinetics

Lamotrigine exhibits a linear relationship between doses and drug concentrations. The pharmacokinetics of lamotrigine is similar in both healthy volunteers and patients with epilepsy. Its pharmacokinetics can be sufficiently described by a one-compartment model with a first-order absorption and elimination.[2, 4, 34, 36-37]

1. Absorption

Lamotrigine is rapidly absorbed from gastrointestinal tract with high absolute bioavailability (approximately 98%). Time to peak concentrations (Tmax) is achieved within 1-3 hours after oral administration. The absorption is not influenced by food and there is no first-pass metabolism.[2, 4, 37]

2. Distribution

The apparent volume of distribution (V/F) of lamotrigine in healthy volunteers and patients with epilepsy are approximately 1.20 and 1.36 L/kg, respectively.[34, 36] Plasma proteins binding of lamotrigine is approximately 55%; therefore, it is not likely to participate in protein-binding displacement interactions.[37]

3. Metabolism and excretion

Lamotrigine is mainly metabolized via glucuronidation pathway in the liver by UGT enzymes.[5, 29] UGT1A4 is the major enzyme responsible for lamotrigine metabolism, however other UGTs such 1A3 and UGT2B7 may also play a role in the glucuronidation of lamotrigine.[17-19]

Lamotrigine is metabolized at position 2 of the triazine ring to form a quaternary ammonium glucuronide. The major inactive metabolite of lamotrigine is 2-N-glucuronide (80-90% of the administered dose), whereas 5-N-glucuronide is a minor metabolite (10% of the administered dose). All the inactive metabolites are excreted in the urine.[2, 4, 35] Figure 3 presents the metabolism pathway of lamotrigine by UGT1A4 and UGT1A3 enzyme.

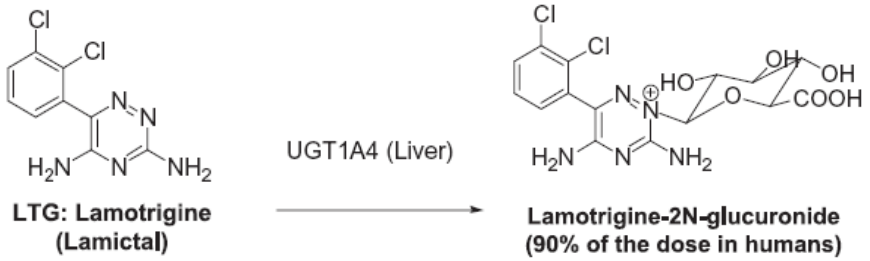


Figure 3 The metabolism of lamotrigine by UGT1A4 enzyme[18]

The mean elimination half-life of lamotrigine is approximately 22.80-37.40 hours when used as a monotherapy in healthy volunteers. The half-life may be altered when co-administered with other enzyme inhibitors or inducers.[2, 34]

The autoinduction of lamotrigine has been postulated. It was found to be completed within 2 weeks after the initiation of therapy and decrease lamotrigine concentration by 17%.[4, 10] However, the conclusion about an autoinduction of lamotrigine is still controversial.

4. Therapeutic drug monitoring of lamotrigine

In general, the therapeutic range of lamotrigine is 1-4 mg/L. This range is based on studies from preclinical and clinical data.[6, 8] The relationship between lamotrigine concentration and pharmacological response is unclear. There is an overlaping of lamotrigne concentrations in patients with or without improved seizure control, as well as in patients with and without adverse effects.[38]

Many patients may require concentrations higher than established therapeutic range (1-4 mg/L).[6, 10] A retrospective survey by Morris et al.[9] suggested that higher plasma concentrations of lamotrigine (3-14 mg/L) were appropriated for the treatment of epileptic patients in clinical practice.

Lamotrigine pharmacokinetics exhibit high interindividual variability due to several factors including age, pregnancy, disease and co-medications.[7-8, 11] Therefore, therapeutic drug monitoring of lamotrigine is important to individualize patient therapy. It is recommended to monitor lamotrigine concentrations especially in patients suspected of treatment failure due to drug interactions and noncompliance; patients with sign of clinical drug intoxication, patients with a change of physiological state that may alter lamotrigine pharmacokinetics such as pregnancy patients. Furthermore, it may be useful for establishing the reference range of concentrations for individual patient when therapy is initiated and after dose adjustments.[6-8, 12-13]

Adverse drug reaction of lamotrigine

The major adverse event of lamotrigine leading to discontinuation of the medication is skin rash. Maculopapular or erythematosus rash is most frequently found (approximately 10% of patients), however serious rash such as Stevens-Johnson syndrome and toxic epidermal necrolysis were also reported (0.13-0.3%).[3, 35]

Typically, skin rash occurs within 4-8 weeks of the initiating treatment. Risk factors associated with skin rash from lamotrigine include a young age, higher initiating dose of lamotrigine, rapid dose titration, gender (with a higher risk in female), and the use of lamotrigine with valproic acid. A reduction of the incidence of skin rash can be

achieved by reducing the starting dose, slow dose titration, and adjusting lamotrigine dose when co-administered with valproic acid. [2, 32, 39-40]

Other common dose-related adverse effects of lamotrigine include headache, nausea, vomiting, dizziness, diplopia, ataxia, blurred vision and tremor.[2, 31-32]

Dosage and administration of lamotrigine

Administration of lamotrigine should be initiated with low dosages and escalated slowly over the first four weeks of the treatment to reduce the risk of skin rash. Additionally, discontinuation of lamotrigine should be performed gradually by tapering the dose over a period of at least 2 weeks to reduce the risk of rebound seizures.[2, 4] Lamotrigine dose recommendations for children and adults patients are presented in Table 1 and 2

Table 1 Recommendation of lamotrigine dose for children [2, 4]

Treatment regimen	Week 1-2	Week 3-4	Maintenance dose
LTG monotherapy	0.50 mg/kg/day	1 mg/kg/day	2-8 mg/kg/day
LTG with EIAED (not taking VPA)	2 mg/kg/day	5 mg/kg/day	5-15 mg/kg/day
LTG with EIAED and VPA	0.20 mg/kg/day	0.5 mg/kg/day	1-5 mg/kg/day

LTG = lamotrigine

VPA = valproic acid

EIAED = enzyme inducing antiepileptic drugs (carbamazepine, phenytoin, and phenobarbital)

Table 2 Recommendation of lamotrigine dose for adults[2, 35]

Treatment regimen	Week 1- 2	Week 3- 4	Maintenance dose
LTG monotherapy	25 mg/day (once a day)	100 mg/day (two divided doses)	100-200 mg/day (one or two divided doses)
LTG with EIAED (not taking VPA)	50 mg/day (once a day)	100 mg/day (two divided doses)	300-500 mg/day (two divided doses) Escalated dose by 100 mg/day every week
LTG with EIAED and VPA	25 mg/day (other day)	25 mg/day (once a day)	100-400 mg/day (one or two divided doses) Escalated dose by 25-50 mg/day every one or two weeks

LTG = lamotrigine VPA = valproic acid

EIAED = enzyme inducing antiepileptic drugs (carbamazepine, phenytoin, and phenobarbital)

Factors associated with lamotrigine pharmacokinetics

The interindividual variation of lamotrigine pharmacokinetics is influenced by several factors such as age, pregnancy, diseases and drug interactions. These factors could be important for lamotrigine dose adjustment.[6, 10]

1. **Age**

Several studies have documented that age is associated with an alteration of lamotrigine elimination. Because lamotrigine is eliminated by conjugation, this pathway is shown to be immature at birth.[41] Recent study reported lamotrigine plasma concentration decreases in newborn. Mikati et al.[42] found that the mean CL/F of lamotrigine in neonates aged <2 months decreases by 50% compared with infants aged 2-12 months (0.12$\pm$0.002 vs 0.22$\pm$0.09 L/h/kg; $p<0.001$).

Previous studies revealed that lamotrigine metabolism rate in children are faster than adults. An average CL/F of lamotrigine in children increases by 35-125% compared with adult.[41] Reimer et al.[43] reported that age is an

important factor with respect to lamotrigine pharmacokinetics. This study showed LTG-CDR decreased approximately 6% per year of age in children and adolescents.

In the elderly, the glucuronidation of lamotrigine may be reduced.[41] However the influence of age on lamotrigine pharmacokinetics is still controversial. Even though, previous population pharmacokinetic studies showed that lamotrigine clearance did not depend on age.[14-15, 44-45] Some studies reported the influence of age on lamotrigine pharmacokinetics. The study by Arif et al.[46] found that the median of lamotrigine clearance in older patients (age 55-92 years) was 20% lower than in younger patients (age 16-36 years) (28.80 vs 35.50 ml/hr/kg; $p<0.001$).

2. Gender

Even though, several pharmacokinetic studies found that there is no significant gender difference in lamotrigine pharmacokinetics.[14, 43, 45, 47] A population pharmacokinetic study showed that the volume of distribution of lamotrigine in female was 27% lower than male.[15] Furthermore, previous study suggested that UGTs activity of female could be lower than male, however, the results may limit to some isozymes of UGT and some drug substrates.[48]

3. Body weight

Several population pharmacokinetic studies found the influence of body weight on lamotrigine clearance. In these studies, body weight was included in the final regression model for predicting CL/F of lamotrigine.[15, 44-45]

4. Liver function

Lamotrigine is extensively metabolized in the liver. The clearance of lamotrigine is altered in patients with hepatic impairment and correlated with the severity of hepatic disease.[7, 49] Marcellin et al.[50] found that, in patients with severe cirrhosis (Child-Pugh grade B or C), lamotrigine clearance is decreased

approximately 60% resulting in an increased half-life. Therefore, lamotrigine doses should be reduced 50 to 75%, when it was used in severe cirrhosis patients with Child-Pugh grade B or C.

5. Renal function

A previous study comparing the pharmacokinetics of lamotrigine between patients with chronic renal failure (creatinine clearance; CrCl < 30 ml/min/1.73 mm) and healthy volunteers found the decrease of lamotrigine clearance by 61% and a 53% increase of lamotrigine half-life. However, the difference was not statistically significant.[51] Lamotrigine should be used with caution, especially in patients with glomerular filtration rate (GFR) less than 15 ml/min, as lamotrigine half-life may be prolonged.[52]

6. Pregnancy

Previous studies have documented an altered lamotrigine pharmacokinetics during pregnancy due to physiological alterations such as hepatic enzyme activities and endogenous steroid.[7] A study by Pennell et al.[53] found that lamotrigine clearance increases during pregnancy (up to 330% from baseline) until 32 weeks of gestational age and returns to baseline in the postpartum period.

7. Disease

The impaired UGTs activity in Gilbert's syndrome patients causes an unconjugated hyperbillirubinemia disorder. In these patients, lamotrigine clearance is decreased leading to a prolongation of lamotrigine half-life. When compared with healthy control, lamotrigine clearance was decreased by 32% in Gilbert's syndrome patients. However, the change of lamotrigine pharmacokinetic was not clinically relevant.[4]

8. Drug interaction

Lamotrigine is primarily metabolized by UGT enzymes. Co-administration with other drugs that are metabolized by glucuronidation may be associated with drug interactions. Drugs that are hepatic enzyme inducers can affect the pharmacokinetics of lamotrigine.[54-56]

8.1 Effect of other antiepileptic drugs on lamotrigine pharmacokinetics

Enzyme-inducing antiepileptic drugs including carbamazepine, phenytoin, and phenobarbital can increase UGTs' activity and enhance the metabolism of lamotrigine. Co-administration of these drugs decreases lamotrigine half-life to approximately 15 hours and reduce lamotrigine concentration by 34-52%. Therefore, lamotrigine doses are needed to be increased if any of these enzyme inducing drugs is co-administered.[2, 54, 57]

Oxcarbazepine is a weak enzyme inducing agent that can induce UGT enzymes, resulting in a decrease of lamotrigine concentration by 15 to 75%.[56-57] However, the study by Theis et al.[58] showed that AUC and Cmax of lamotrigine at steady state were not significantly affected by oxcarbazepine.

Although previous studies suggested that co-administration of topiramate can reduce lamotrigine concentration by 40 to 50%,[54, 57] the study by Berry et al. and Doose et al.[59-60] reported a minimal effect of topiramate on lamotrigine concentrations.

Methsuximide is found to be able to reduce lamotrigine concentration by 53%. This drug appears to increase lamotrigine clearance, and decrease lamotrigine concentration leading to uncontrolled seizure. The dose of lamotrigine may need to be increased if methsuximide is given.[54, 57]

On the other hand, valproic acid, a strong enzyme inhibitor, reduces the rate of lamotrigine glucuronidation. The half-life of lamotrigine can be prolonged up to 60 hours and plasma concentration can increase by 200% when co-administration with valproic acid. This interaction is associated with the risk of lamotrigine toxicities, especially rash. However, the incidence of the rash can be

minimized by initiating with low dose and slow titration of lamotrigine dose when valproic acid is co-administered.[2, 54, 57]

Other epileptic drugs, including felbamate and levetiracetam, are not found to affect lamotrigine concentration.[54]

8.2 Effect of psychotropic drugs on lamotrigine

An in vitro study of lamotrigine indicated that the metabolism of lamotrigine was not significantly affected by clozapine, fluoxetine, phenelzine, risperidone, sertraline and trazodone. In addition, the effect of amitriptyline, bupropion, clonazepam, haloperidol, and lorazepam on the metabolism of lamotrigine was minimal.[3, 61]

A study investigated drug interaction between lamotrigine and psychoactive drugs from routine serum concentrations of 829 patients. The results showed that lithium and fluoxetine may associate with a reduction of lamotrigine concentrations. However, the mechanism of this interaction is unknown and required further study. In addition, it was found that LTG-CDR did not alter by other psychotropic drugs such as sertraline, olanzapine and benzodiazepines.[62]

However, previous case report of the patient who used lamotrigine concomitantly with sertraline found that lamotrigine blood level was increased after the addition of sertraline leading to lamotrigine toxicities such as confusion and cognitive impairment. Therefore, sertraline may be able to inhibit lamotrigine glucuronidation.[54, 57]

A study by Sidhu et al.[63] found that, in healthy volunteers using lamotrigine concomitantly with olanzapine, AUC and Cmax of lamotrigine were reduced by 24% and 20%, respectively. However, this interaction was not considered to be clinically significant.

8.3 Effect of oral contraceptives on lamotrigine

The effect of oral contraceptives on lamotrigine pharmacokinetics was documented in several studies. Saber et al.[64] reported that lamotrigine plasma level was reduced by more than 50% when co-administered with oral contraceptives. Reimers et al.[65] found that estrogen-containing oral contraceptives significantly decrease lamotrigine concentration, whereas progestogen-only pills did not alter lamotrigine concentration.

The possible mechanism could be a stimulation of UGT activity by steroid hormones in oral contraceptives, resulting in an increase of lamotrigine metabolism. As lamotrigine concentration decreases, a reduction of seizure control may be observed in some women. Therefore, lamotrigine concentration should be closely monitored and dose adjustment may be necessary when contraceptives are initiated or withdrawn during lamotrigine therapy.[7, 56-57, 66]

8.4 Effect of other drugs on lamotrigine

Acetaminophen is approximately 55% eliminated by glucuronide conjugation. Co-administration of acetaminophen enhances elimination of lamotrigine, therefore it may reduce lamotrigine AUC and half-life by 20% and 15%, respectively. However, this interaction is deemed to be not clinically significant.[54, 57, 66]

A study by Ebert et al. [67] found that rifampicin increases lamotrigine clearance by 97% and decreases lamotrigine half-life by 41% due to an induction of hepatic glucuronidation enzymes. Therefore, rifampicin may reduce lamotrigine efficacy and lamotrigine dose adjustment is required.[57, 66]

Ritonavir may decrease lamotrigine concentration by an induction of glucuronidation. Therefore, lamotrigine efficacy should be monitored in patients taking ritonavir or any ritonavir-boosted antiretroviral regimen and lamotrigine dose may need to be increased.[54, 57, 66]

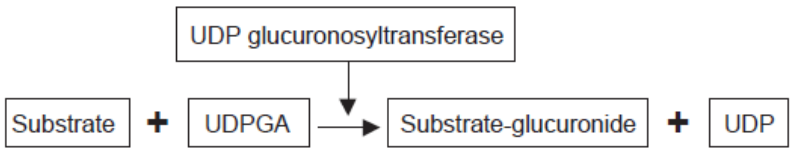
The summary of clinically drug interactions the can alter lamotrigine concentrations are presented in Table 3.

Table 3 Clinically important drug interactions that alter lamotrigine concentrations [54-57, 66]

Increase lamotrigine concentrations	Decrease lamotrigine concentrations
Valproic acid	Phenytoin
Methsuximide	Phenobarbital
	Carbamazepine
	Oral contraceptive
	Rifampicin
	Ritonavir

Uridine 5'-Diphosphate Glucuronosyltransferases (UGTs)

UGTs are a group of phase II enzymes. UGT enzymes play an important role for the metabolism of xenobiotics and endobiotics by the addition of glucuronide from uridine 5'-diphosphoglucuronic acid leading to the formation of water soluble substances which can be excreted via bile and/or urine as presented in Figure 4.[68]



(UDP = uridine 5'-diphosphate, UDPGA = uridine 5'-diphosphoglucuronic acid)

Figure 4 Mechanism of glucuronidation [68]

In human, UGT enzymes have been classified into two families (UGT1 and UGT2) according to amino acid sequences.[21, 24, 28]

1. UGT1 subfamily is encoded by a gene located on chromosome 2 (locus 2q37) and consists of 13 different exon 1 and common exons 2 to 5. There are 13 isoforms of UGT1A; of which 9 isoforms (UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9 and UGT1A10) are functional and the others are pseudogenes.

2. UGT2 subfamily is subdivided into UGT2A and UGT2B and encoded by gene located on chromosome 4 (locus 4q13 and 4q28). All genes of UGT2 subfamily consists of 6 different exons. There are one UGT2A isoform (UGT2A1) and seven UGT2B isoforms (UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17 and 2B28).

Glucuronidation is normally associated with more than one isoforms of UGT enzymes. Each isoforms are overlapping functions and specific to different substrates.[21, 24, 68] Current studies have documented several genetic variations of UGT1A enzymes such as *UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8,* and *UGT1A10* as presented in Figure 5.[28]

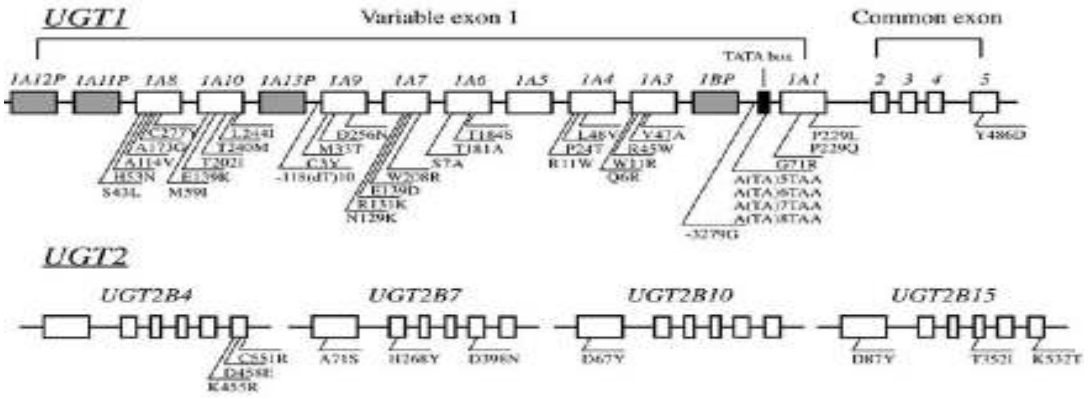


Figure 5 Functional variants in UGT1s and UGT2s [28]

UGT1A4 polymorphisms

UGT1A4 is an important human UGT isoform that catalyzes primary, secondary, tertiary amines, carcinogenic aromatic amines (β-naphthylamine, 4-aminobiphenyl, and benzidine), androgens, progestins, and plant steroids (hecogenin, diosgenin, and tigogenin).[28] Furthermore, many therapeutic agents are substrates of UGT1A4 as shown in Table 4. In human, UGT1A4 enzyme is found abundantly in the liver, followed by colon, small intestine, and bile ducts. [24, 28]

Table 4 Examples of drug substrates metabolized by UGT1A4 enzyme [5, 28-29, 69]

Therapeutic agents	Drug substrates
Tricyclic antidepressants	amitryptyline, imipramine, doxepin
Antipsychotic agents	chlorpromazine, clozapine, olanzapine, trifluoperazine
Anticonvulsants	lamotrigine, retigabine
Antihistaminics	cyproheptadine, diphenhydramine
Anticancer agents	tamoxifen

To date, there are 21 variants of *UGT1A4*, 19 SNPs and 2 frameshift mutations. Among these 21 mutations, 8 mutations lead to an amino acid change, 5 mutations are silent mutation, and the others are in non-coding regions of gene.[21] Table 5 presents summary of genetic variants of the UGT *1A4* gene.

There are two important the polymorphisms of *UGT1A4* with known functional effects.[22-23, 25-26]

- *UGT1A4* 142T>G (L48V): A transversion of T to G at nucleotide position 142 (142T>G) leading to a change of amino acid, valine to leucine, at codon 48 (L48V, submitted to Gen-Bank as UGT1A4*3 acn. AF465197).
- *UGT1A4* 70C>A (P24T): A transversion of C to A at nucleotide position 70 (70C>A) leading to a change of amino acid, proline to threonine, at codon 24 (P24T, submitted to GenBank as UGT1A4*2 acn. AF465196).

Table 5 Genetic variants of the UGT *1A4* gene [24]

Allele	Nucleotide change	Amino acid change	Function	Activity *in vivo*	Activity *in vitro*
UGT1A4*1a	Reference sequence		Wild-type	Normal	Normal
UGT1A4*1b	471(C>T)	$C^{157}C$	Silent		
UGT1A4*1c	-219(C>T)/-163(G>A)/ 448(T>C)/804(G>A)/ IVS1+43(C>T)	$L^{150}L/P^{268}P$			
UGT1A4*1d	IVS1+101(G>T)				
UGT1A4*1e	30(G>A)	$P^{10}P$	Silent		
UGT1A4*1f	357(T>C)	$N^{119}N$	Silent		
UGT1A4*1g	-217(T>G)				
UGT1A4*1h	-36(G>A)				
UGT1A4*1i	IVS1+98(A>G)				
UGT1A4*2	70(C>A)	$P^{24}T$			Substrate-dependent
UGT1A4*3a	-219(C>T)/- 163(G>A)/142(T>G)	$L^{48}V$			Reduced transcription Reduced inducibility
UGT1A4*3b	142(T>G)	$L^{48}V$			Low activity
UGT1A4*4	31(C>T)	$R^{11}W$			
UGT1A4*5	127delA/142(T>G)	43fsX22	Frameshift early stop codon		
UGT1A4*6	175delG/325(A>G)	59fsX6	Frameshift-early stop codon		
UGT1A4*7	-219(C>T)/-163(G>A)/ 142(T>G)/271(C>T)/ 448(T>C)/804(G>A)/ IVS1+43(C>T)	$L^{48}V/R^{91}C/L^{150}L/P^{268}P$			
UGT1A4*8	IVS1+1(G>T)		Splicing defect		

Previous studies have shown that the genetic variations of *UGT1A4* are different among the populations. *UGT1A4* 142T> G (L48V) and *UGT1A4* 70C>A (P24T) were first detected in German population with the frequency of 9% and 8%, respectively.[22] In Turkish population, the frequency of *UGT1A4* 142T> G (L48V) and *UGT1A4* 70C>A (P24T) was 12.80% and 1.90%, respectively.[25] In Asian population, the frequency of *UGT1A4* 142T>G (L48V) and *UGT1A4* 31C>T (R11W) in Japanese was found to be 16.50% and 1.20%, respectively. These frequencies are similar to Korean population. However, *UGT1A4* 70C>T was not found in Asian population.[23, 26-27] The summary of the allele frequencies of *UGT1A4* is shown in Table 6.

Table 6 Frequencies of polymorphic variants of the *UGT1A4* in different populations

Nucleotide substitution	Amino acid substitution	% Allele frequency			
		Caucasian		Asian	
		Germany[22]	Turkish[30]	Japanese[23, 26]	Korean[27]
31C>T	R11W	-	-	1.20	1
70C>A	P24T	8	1.90	-	-
142T>G	L48V	9	12.80	16.50	12

The polymorphisms of *UGT1A4* associated with a change of amino acids could lead to the variability of glucuronidation enzyme activity. There are studies indicating that the effect of *UGT1A4* polymorphisms on enzyme activity depends upon substrates.

An in vitro study by Sun et al.[70] determined the effect of *UGT1A4* polymorphisms on glucuronidation activity of tamoxifen and its major active metabolites (trans and cis-4-hydroxytamoxifen). They found that glucuronidation activity of tamoxifen and its metabolites was significantly higher for *UGT1A4* 142T> G (L48V) polymorphism than wild-type. This data indicated that *UGT1A4* 142T> G (L48V) polymorphism may play an important role in clinical response and toxicity in patients using tamoxifen.

In human, a study by Ehmer et al.[22] investigated the polymorphisms of human *UGT1A* gene and described function of these variants and their association with hepatocellular carcinoma (HCC) in 363 German population. They found a high prevalence of SNPs in the human *UGT1A* gene locus, however *UGT1A* SNPs were not associated with HCC. In this study, *UGT1A4* 70 C>T (P24T) and *UGT1A4* 142 T>G (L48V) were detected in 8% and 9% of the population, respectively. Moreover, a comparison of glucuronidation activity between wide-type and these two polymorphisms using amine (β-naphthylamine and benzidine) and steroid (dihydrotestosterone and trans-androsterone) as the substrates found a reduction of an activity of *UGT1A4* 70 C>T (P24T) and *UGT1A4* 142 T>G (L48V). While, *UGT1A4* 142 T>G (L48V) had greater the impact on amine substrate than steroid, *UGT1A4* 70 C>T (P24T) had a higher specific effect on steroid than amine substrates.

Mori et al.[26] identified four SNPs of *UGT1A4*, three in exon 1 (142T>G: L48V, 448T>C: L150L, 804G>A: P268P), and one in intron 1 (867 + 43C>T). This study found that the frequency of *UGT1A4* 142T>G: L48V, 448T>C: L150L, 804G>A: P268P and 867+43C>T was 16.50%, 15.50%, 16.50%, and 15.50%, respectively in Japanese population. However, the polymorphism of *UGT1A4* 70 C>T (P24T) was not found in this study. The results from this study showed that the relative efficiency of *UGT1A4* L48V for clozapine glucuronidation was twice that of wild type. In addition, efficiencies of *UGT1A4* 142 T>G (L48V) in metabolizing trans-androsterone, imipramine, and cyproheptadine were increased, but the efficiency for tigogenin was reduced. Therefore, the glucuronidation activity by *UGT1A4* could be depend upon the substrates.

The results from these two studies showed a differential glucuronidation activity of *UGT1A4* polymorphisms among substrates. In summary, an enzyme activity of *UGT1A4* 142T>G (L48V) was reduced for β-naphthylamine, benzidine, trans-androsterone and dihydrotestosterone, while it was increased for clozapine glucuronidation as presented in Table 7.[22, 26, 28]

Table 7 The comparison of Effects of *UGT1A4* polymorphisms on glucuronidation activity among substrates [22, 26, 28]

Mutations	Substrates (Relative glucuronidation activities compared with wild type)			
	β-naphthylamine	Trans-androsterone	Dihydrotestosterone	Clozapine
UGT1A4 70 C>T (P24T)	30%	62%	66%	-
UGT1A4 142 T>G (L48V)	57%	1.70%	ND*	207%

Moreover, Ghotbi et al.[25] investigated the effects of genetic variants of *UGT1A4*, *CYP1A2*, and *MDR1* on olanzapine plasma levels, in relation to other individual factors (gender, smoking status, body weight, and age) in schizophrenia patients. The results from this study indicated that male gender, *UGT1A4* 142 T>G (L48V) polymorphism, and smoking decreased olanzapine concentration to dose ratio 35, 25, and 21%, respectively. The results from this study showed that male patients who are smokers tend to expose to a lower level of olanzapine, therefore the combination of genetic and environmental factors may increase the risk of therapeutic failure.

Effects of *UGT1A4* polymorphism on lamotrigine pharmacokinetics

UGT1A4 is a primary enzyme responsible for metabolizing lamotrigine, even though the other UGTs such as UGT1A3 and UGT2B7 may also involve.[17-19]

Agikar et al.[18] investigated the glucuronidation of lamotrigine in human liver microsomes. The results from this study showed that UGT1A4 and UGT1A3 involved in the formation of lamotrigine to 2N-glucuronide, whereas UGT2B7 and UGT2B4 did not show any activity. Base on the results from this study, lamotrigine is found to be mainly metabolized by UGT1A4.

Previous studies documented the difference of lamotrigine pharmacokinetics among ethnicities. Hussian and Posner[14] found that lamotrigine clearance was 28.70% lower in Asian compared with Caucasian (1.63 L/hr vs 2.28 L/hr) and the half-life of

lamotrigine was 40% longer in Asian than Caucasian. Similarly, Grasea et al.[15] found that lamotrigine clearance of non-Caucasian patients decrease 25% as compared with Caucasian. These finding revealed the difference of lamotrigine metabolism among races. Even though, the genetic variation could be one of the reasons explaining the difference of lamotrigine metabolism among ethnicities, there are few studies investigating the effect of *UGT1A4* polymorphisms on lamotrigine drug metabolism.

The effect of *UGT1A4* polymorphisms on lamotrigine serum concentration was previously investigated in 129 Turkish patients with epilepsy.[30] In this study the frequency of the heterozygous *UGT1A4* 142T> G (L48V) and *UGT1A4* 70C>A (P24T) was 22.40% and 3.80%, respectively and the homozygous of *UGT1A4* 142T> G (L48V) was 1.55%. The homozygous of *UGT1A4* 70C>A (P24T) was not found in this study. The results showed that *UGT1A4* 142T>G (L48V) is associated with the decrease of lamotrigine concentration in patients receiving lamotrigine as monotherapy (2.40$\pm$1.05 and 3.50$\pm$0.69 mg/L; $p<0.05$ for patients with heterozygous of *UGT1A4* 142T>G and patients with having wild type, respectively). Additionally, in a group of non-smoking patients, it was found that patients with *UGT1A4* 142 T>G polymorphism had lamotrigine concentration 52% lower than patients with wild-type.

In addition to *UGT1A4* polymorphism, UGT2B7 which may involve in the metabolism of lamotrigine was investigated. Sanchez et al.[71] determined the association between *UGT2B7*_-161 C>T and *UGT2B7*_372 A>G polymorphisms and LTG-CDR. In this study, the patients were divided into three subgroups according to lamotrigine co-medications: (1) lamotrigine plus enzyme inducers, (2) lamotrigine plus valproic acid, and (3) lamotrigine plus enzyme inducers and valproic acid or lamotrigine monotherapy. Factors found to be important in explaining the intersubject variability of LTG-CDR include antiepileptic co-medication, patient age, and *UGT2B7*_-161C>T polymorphism. The results found a significant association between *UGT2B7*_-161 C>T polymorphism and LTG-CDR, when age and concomitant antiepileptic drugs were taken into account. However, as lamotrigine was mainly metabolized by UGT1A4, the study investigating the effect of *UGT1A4* polymorphisms should better explain the variability of lamotrigine pharmacokinetics.

To date, there is only one study that investigated an impact of *UGT1A4* polymorphisms on the pharmacokinetics of lamotrigine which was done in Turkish population. However, no data are available regarding the determination of an association of *UGT1A4* polymorphisms and pharmacokinetics of lamotrigine in Asian population.

CHARPTER III
PATIENTS AND METHODS

1. Patients population

The patients were recruited from epilepsy and psychiatric outpatient clinic of Prasat Neurological Institute during 10 January to 30 July 2011. The study protocol was approved by the institutional review board (IRB) of the Prasat Neurological Institute, Bangkok, Thailand. The patients were recruited based on the following inclusion and exclusion criteria.

1.1 Inclusion criteria

(1) Patients aged older than 18 years.

(2) Patients who were being treated with monotherapy or polytherapy of lamotrigine with the same dose for at least two weeks.

(3) Patients who were willing to participate in the study and signed informed consent.

1.2 Exclusion criteria

(1) Pregnancy and lactation patients.

(2) Patients with liver impairment (AST or ALT>3 upper normal limit).

(3) Patients with renal impairment (CrCl < 60 mL/min).

(4) Patients who were treated with phenobarbital (internal standard) as a co-medication

(5) Noncompliance patients by interview the patients or their legal representatives.

(6) Patients with no record of drug history, dose or dosage regimen.

1.3 Sample size determination

The sample size was estimated by

$$N \geq 15\,p$$

Where N refers to the sample size of patients and p refers to the number of tested variables (5 variables; age, gender, body weight, co-medications and polymorphisms of *UGT1A4*).

Therefore

$$N \geq 15 \times 5$$

$$N \geq 75$$

The sample size of at least 75 patients was required in this study.

2. Study protocol

2.1 Patients were enrolled according to inclusion and exclusion criteria.

2.2 Data of patient characteristics were collected from medical record and interviewing the patients. All the information was recorded in the patient data collection form (Appendix D).

2.3 Appointment for blood sample collection was made for the next visit.

2.4 Blood sample was collected from each patient before the next lamotrigine dose (trough) at steady state.

2.5 Blood samples were taken and prepared for measurement of lamotrigine concentration and *UGT 1A4* genotyping.

2.6 Lamotrigine plasma concentrations were measured by high-performance liquid chromatography (HPLC) method.

2.7 After that, LTG-CDR was calculated from the following equation.

$$\text{LTG-CDR} = \frac{\text{Trough concentration of lamotrigine (milligram per liters)}}{\text{Lamotrigine dose (milligram per kilogram per day)}}$$

2.8 Determination of *UGT1A4* genotyping was carried out by Taqman allelic discrimination assays.

2.9 The relationship between genetic and non-genetic factors and LTG-CDR was evaluated by one-way analysis of variance (ANOVA) and multiple linear regression (MLR).

2.10 Discussions and conclusion.

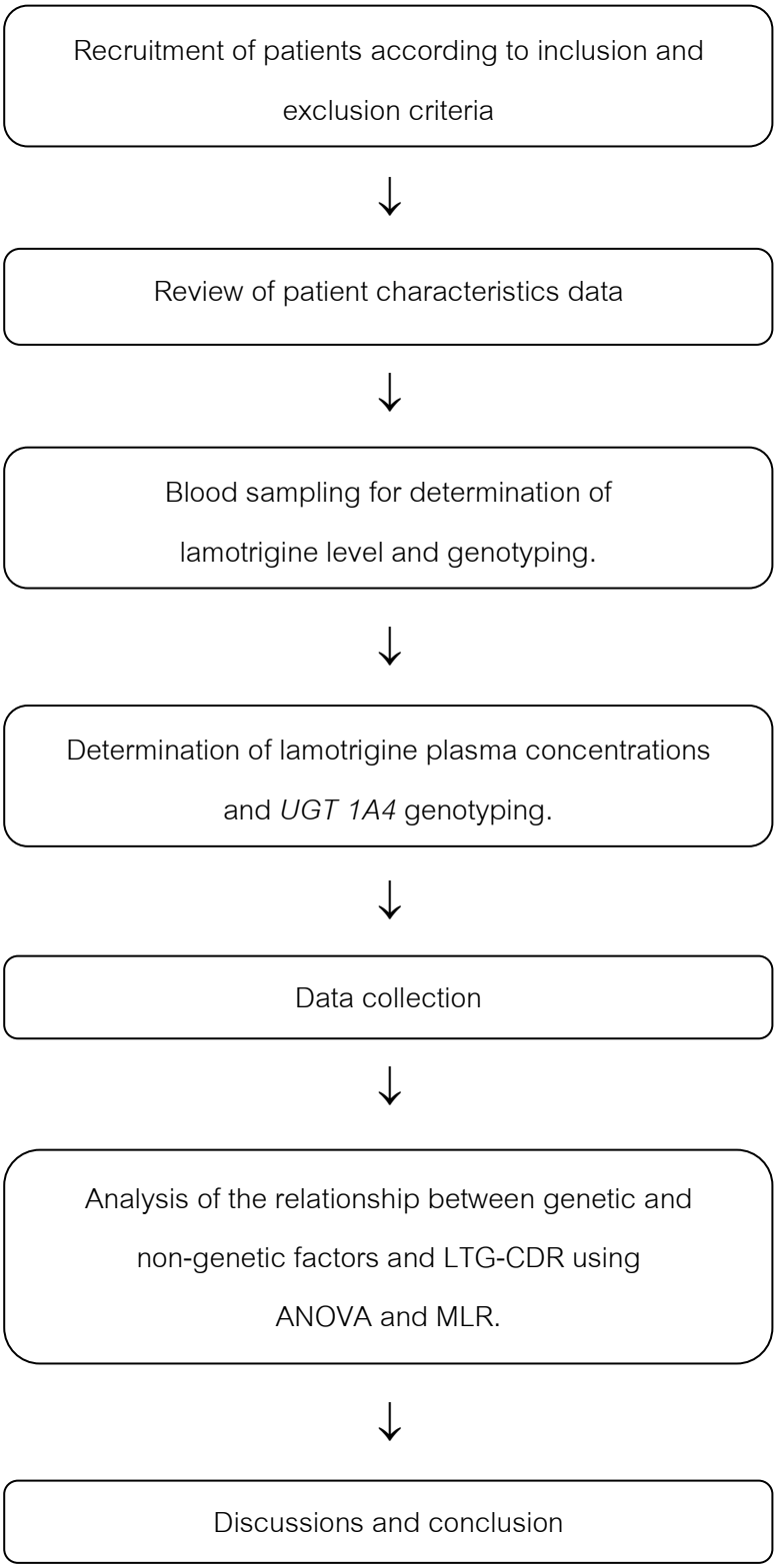


Figure 6 Flow chart of the study protocol

3. Blood collection and preparation

Ten milliliters of whole blood was drawn from the patients before the next lamotrigine dose. Blood samples were collected in two ethylene diamine tetra-acetic acid (EDTA) tubes (5 mL each tube), and were prepared for determination of lamotrigine plasma concentration and *UGT1A4* genotyping as follows:

3.1 Preparation of blood sample for determination of lamotrigine plasma concentration

Whole blood (5 mL) in EDTA tube was centrifuged at 3000 g for 10 minutes at 4^0C, then plasma was removed into 1.50 mL of microcentrifuge tubes and stored at -80 ^{0}C until analysis.

3.2 Preparation of buffy coat for genomic DNA extraction

Whole blood (5 mL) was centrifuged at 2,500 g for 10 minutes at room temperature. After centrifugation, three separate fractions of blood sample were obtained: the upper plasma layer, the interface white blood cell layer (buffy coat), and the lower red blood cell layer. Buffy coat (200 µL) was transferred into 1.50 mL microcentrifuge tube and frozen at -20 ^{0}C until DNA extraction.

4. Bioanalysis

4.1 Lamotrigine plasma concentration

The determination of lamotrigine plasma concentration was performed using HPLC with UV detection method. An analysis of total plasma lamotrigine concentration was performed in the laboratory of Medica Innova Co., Ltd., Bangkok Thailand (Good Laboratory Practice certified by the Departement of Medical Sciences) with a validated method previously described by Angelis-Stoforidisa et al.[72] with slightly modification.

The detailed procedures of an analysis of lamotrigine concentration and method validation were presented in Appendix F. Chromatographic condition for HPLC was presented in Table 8.

Table 8 Chromatographic condition for HPLC

Parameters	Description
Mobile phase	50 mM Potassium dihydrogen phosphate (KH_2PO_4): Acetonitrile:Methanol (72:21:7, v/v), Isocratic
Analytical column	SunFire™ C18, 5 µm, 4.60 x 150 mm
Guard column	Phenomenex® C18, 4 x 3 mm
Autosampler temperature	4°C
Column temperature	40°C
Detector	UV 210 nm
Injection volume	20 µL
Flow rate	1 mL/min
Run time	14 min

4.2 *UGT1A4* genotyping

4.2.1 DNA extraction

The DNA were extracted using QIAamp® DNA Blood Mini kit (Qiagen, Hilden, Germany) by the following procedure as recommended by the manufacturer.[73] The detailed procedures of DNA extraction were presented in Appendix G.

4.2.2 Determination of concentration, yield and purity of DNA

DNA yields were determined from the concentration of DNA in the elution buffer, measured by absorbance at 260 nm. Absorbance readings at 260 nm should be between 0.10 and 1 to be accurate. Purity of DNA was determined by calculating the ratio of absorbance at 260 nm (A260) to absorbance at 280 nm (A280). The absorbance at 260 and 280 nm were measured with a spectrophotometer. Pure DNA should have an A260/A280 ratio of 1.70-1.90.

The quantification and quality of DNA was performed by the optical density measurement (OD) as follows:

1) Dilute a sample of DNA isolation to 1:5, by using DNA 20 µL and deionized water (dH_2O) 80 µL.
2) Prepare dH_2O 100 µL for control.
3) Set spectrophotometer measure OD at 260 and 280 nm.
4) Calculate OD 260/280 ratio to determine purity and estimate the concentration of DNA according to the formula.

DNA concentration (µg/mL or ng/µL) = OD260 x 50 µg/mL x dilution

4.2.3 *UGT1A4* genotyping

Two SNPs including *UGT1A4* 142T>G (L48V) and *UGT1A4* 70C>T (P24T) were investigated. The SNPs detection was carried out by Taqman allelic discrimination assays with fluorogenic probes (Applied Biosystems, California, USA). The probes for all SNPs were designed by Applied Biosystems and were presented in Table 9. All reactions were analyzed by the Applied Biosystems 7500 Real-Time PCR System. The detailed procedures of *UGT1A4* genotyping were presented in Appendix H.

Table 9 Information of the allele probes for the detection of *UGT1A4* polymorphisms

Variant (rs number)	Probes	Sequence of allele probes
UGT1A4 142T>G (rs2011425)	Allele 1	CCCTGGCTCAGCATGCGGGAGGCCGTGCGGGAGCTCCATGCCAGAGGCCA
	Allele 2	CCCTGGCTCAGCATGCGGGAGGCCTTGCGGGAGCTCCATGCCAGAGGCCA
UGT1A4 70C>T (rs6755571)	Allele 1	ACTGCTGCTCCTCCTCAGTGTCCAGACCTGGGCTGAGAGTGGAAAGGTGTT
	Allele 2	ACTGCTGCTCCTCCTCAGTGTCCAGCCCTGGGCTGAGAGTGGAAAGGTGTT

rs number = reference SNP number

5. Data analysis

The data analysis was carried out by the Statistical Package for Social Sciences (SPSS version 17, SPSS Co., Ltd., Bangkok Thailand) software. The significance level of 0.05 was used as criteria for justification of statistical significance. The data were analyzed as follows:

(1) Demographic characteristics were presented as the mean ± standard deviation (SD) for continuous data or percentage and frequency for categorical data.

(2) Prevalence of *UGT 1A4* genotypes was shown as frequency. The distribution of observed genotypes according to Hardy-Weinberg equilibrium was tested by Chi-square. The comparisons of the allele frequencies between different populations were determined by Chi-square test.

(3) The comparisons of LTG-CDR in patients with different *UGT 1A4* genotypes were analyzed by ANOVA or Kruskal-Wallis H test where appropriate. The genotypes were characterized as

Group 1: homozygous wild type (two copies of the common alleles)

Group 2: heterozygous (one copy of the variant allele)

Group 3: homozygous variant (two copies of the variant alleles)

As the number of patients in some genotyping groups can be small, the genotyping group in some analysis will be divided as

Group 1: homozygous wild type

Group 2: at least one variant allele

(4) The influence of genetic (*UGT1A4* polymorphism*s*) and non-genetic factors (age, gender, body weight, and co-medications) on LTG-CDR was investigated using MRL with stepwise method. The multicollinearlity of independent factors were determined. If the variance inflation factor (VIF) between independent variable were greater than 4, only one covariate will be selected to be test in the MRL to avoid the effect of collinearity on the parameter estimates. The criteria for selection were ease of data collection and physiological plausibility.

CHAPTER IV
RESULTS

This study was a prospective study aimed to determine the influence of *UGT1A4* polymorphisms and other non-genetic factors on LTG-CDR in Thai patients treated at Prasat Neurological Institute during 10 January to 30 July 2011. A total of 73 patients were recruited in this analysis.

1. Demographic data of patients

From all 73 patients, 43 were female (58.90%) and 30 were male (41.10%). The mean age (±SD) of the patients was 47.41 (±14.30) years. The mean body weight (±SD) was 62.71 (±12.94) kg.

Most of the patients had normal laboratory values. However, five of the patients had incomplete laboratory data of the liver and renal function. Therefore, the mean values of the population were used for these patients.

Among 73 patients, 43 patients had psychiatric disorder (58.90%), 29 had epilepsy (39.70%) and 1 patient had neuropathic pain (1.40%). From 29 patients with epilepsy, 7 patients had generalized epilepsy (24.14%) and 22 had localization-related epilepsy (75.86%). Most of the patients (65.50%) had no other diseases. The summary of demographic data of 73 patients is presented in Table 10.

Table 10 Summary of the demographic data (N = 73)

Characteristics	Frequency, (mean±SD)	% (range)
Gender		
Female	43	58.90
Male	30	41.10
Age (years)	(47.41±14.30)	(18-82)
Body weight (kg)	(62.71±12.94)	(36-98)
AST (U/L)*	(23.87±7.56)	(12-51)
ALT (U/L)*	(20.96±10.25)	(5-59)
SCr (mg/dL)*	(0.87±0.28)	(0.30-1.90)
Indication of taking lamotrigine		
Epilepsy	29	39.70
Psychiatric disorder	43	58.90
Neuropathic pain	1	1.40
Type of epilepsy		
Generalized epilepsy	7	24.14
localization-related epilepsy	22	75.86
Other diseases		% of total diseases
No other diseases	57	65.50
Dyslipidemia	7	8
Hypertension	8	9.20
Diabetes mellitus	2	2.30
Migraine	2	2.30
Anemia	3	3.40
Thalassemia	1	1.10
Old cerebrovascular accident	4	4.60
Benign prostatic hyperplasia	1	1.10
Dementia	1	1.10
Osteoarthritis	1	1.10

*Data from 68 patients

All patients were treated with lamotrigine as monotherapy or polytherapy at the same dose for at least two weeks. Lamotrigine were administrated once daily or twice daily. Blood samples were collected at steady state and were drawn before the next dose (trough concentration) for determination of lamotrigine concentration.

Lamotrigine dose varied over the range of 25 to 400 mg/day. The mean daily dose per body weight of lamotrigine was 1.82±1.55 mg/kg/day. The mean lamotrigine concentration of patients in this study was 1.93±1.83 µg/mL. Table 11 presents the summary of lamotrigine dose and concentration of the patients in this study.

Table 11 Summary of lamotrigine dose and lamotrigine concentrations (N=73)

Data	Mean±SD	range
Lamotrigine dose (mg/day)	108.73±88.65	25-400
Lamotrigine dose (mg/kg/day)	1.82±1.55	0.27-6.15
Lamotrigine concentration (µg/mL)	1.93±1.83	0.19-8.88
Concentration to dose ratio (kg/L)	1.48±1.58	0.21-12.32

Most of the patients received other co-medications. The major co-medications were vitamin and minerals (54.80%), clonazepam (32.90%), valproic acid (28.80%), and carbamazepine (21.90%). A summary of co-medications of the patients in this study is presented in Table 12. Co-medications categorized according to the possible interaction with UGTs are presented in Table 13.

Table 12 Co-medications data of patients (N = 73)

Co-medications	Frequency	% of total co-medication
1. Antiepileptic drugs		
Carbamazepine	16	21.9
Phenytoin	4	5.5
Valproic acid	21	28.8
Topiramate	10	13.7
Levetiracetam	2	2.7
Pregabalin	1	1.4
2. Mood stabilizing drugs		
Lithium	6	8.2
3. Benzodiazepine		
Alprazolam	4	5.5
Diazepam	11	15.1
Clonazepam	24	32.9
Clobazam	3	4.1
Clorazepate	7	9.6
Lorazepam	5	6.8
4. Antidepressants		
4.1 Selective serotonin reuptake inhibitors (SSRIs)		
Escitalopram	4	5.5
Fluoxetine	4	5.5
Fluvoxamine	2	2.7
Sertraline	7	9.6
4.2 Serotonin-norepinephrine reuptake inhibitors (SNRIs)		
Duloxetine	2	2.7
Venlafaxine	3	4.1
4.3 Noradrenergic and specific serotonergic antidepressants (NaSSAs)		
Mianserin	4	5.5
Mirtazapine	2	2.7
4.4 Tricyclic antidepressants		
Imipramine	3	4.1

Table 12 (Cont.) Co-medications data of patients (N = 73)

Co-medications	Frequency	% of total co-medication
4.5 Selective serotonin reuptake enhancers		
Tianeptine	4	5.5
4.6 Augmenter drugs		
Trazodone	7	9.6
5. Antipsychotics		
5.1 First generation antipsychotics (Typical antipsychoyic)		
Haloperidol	1	1.4
Perphenazine	8	11
Trifluoperazine	2	2.7
5.2 Second generation antipsychotics (Atypical antipsycgotic)		
Risperidone	2	2.7
Quetiapine	8	11
Ziprasidone	3	4.1
Paliperidone	1	1.4
5.3 Third generation antipsychotics		
Aripiprazole	5	6.8
5.4 Combination of two psycho-active agents		
Flupentixol/melitracen(deanxit)	2	2.7
6. Antiparkisonian agent (Antimuscarinic class)		
Trihexyphenidyl	12	16.4
7. Acetylcholinesterase inhibitor		
Galantamine	2	2.7
Rivastigmine	1	1.4
8. β-blockers		
Atenolol	3	4.1
Propranolol	3	4.1
9. Calcium channel blockers		
Felodipine	2	2.7
Manidipine	3	4.1

Table 12 (Cont.) Co-medications data of patients (N = 73)

Co-medications	Frequency	% of total co-medication
10. Angiotensin converting enzyme inhibitors (ACEIs)		
Enalapril	3	4.1
11. Angiotensin receptor blockers (ARBs)		
Lozartan	1	1.4
12. Antithrombotics		
Aspirin	5	6.8
Cilostazol	1	1.4
Warfarin	1	1.4
13. Antidiabetic drugs		
Metformin	2	2.7
Sitagliptin	1	1.4
14. Antihyperlipidaemic agents		
Simvastatin	7	9.6
Atrovastatin	1	1.4
Rosuvastatin	1	1.4
Niacin	1	1.4
Fenofribrate	1	1.4
15. Antiulcer agents		
Omeprazole	1	1.4
Lanzoprozole	1	1.4
Ranitidine	5	6.8
16. Oral contraceptives	2	2.7
17. Vitamin and minerals	40	54.8
18. Other drugs	12	16.6

Table 13 Co-medications categorized by UGTs interaction (N = 73) [56-57, 62, 74]

UGTs inducers	UGT inhibitors	No affect	Unclear	No data
Carbamazepine	Valproic acid	Levetiracetam	Topiramate	Cilostazol
Phenytoin		Pregabalin	Lithium	Warfarin
Oral contraceptives		Aripiprazole	Alprazolam	Metformin
		Mianserin	Diazepam	Sitagliptin
		Mirtazapine	Clobazam	Simvastatin
		Venlafaxine	Clonazepam	Atrovastatin
		Perphenazine	Clorazepate	Rosuvastatin
		Aspirin	Lorazepam	Niacin
		Atenolol	Escitalopram	Fenofribrate
			Clozapine	Omeprazole
			Fluoxetine	Lanzoprozole
			Sertraline	Propranolol
			Risperidone	Felodipine
			Trazodone	Manidipine
			Haloperidol	Enalapril
			Ranitidine	Lozartan
			Quetiapine	Galantamine
			Imipramine	Rivastigmine
				Tianeptine
				Trifluoperazine
				Ziprasidone
				Paliperidone
				Trihexyphenidyl
				Flupentixol
				Melitracen

When co-medications were divided based on drug interaction with lamotrigine, there could be divided into 4 groups: (1) lamotrigine monotherapy (n=36), (2) lamotrigine combination with enzyme inhibitor (n=15), (3) lamotrigine combination with enzyme inducers (n=16), and lamotrigine combination with enzyme inhibitor and enzyme inducers (n=6). Co-medications categorized by drug interaction with lamotrigine are shown in Table 14.

Among 15 patients using lamotrigine combination with enzyme inhibitor, valproic acid is the only drug indentified as an enzyme inhibitor. Among 16 patients using lamotrigine combination with enzyme inducers, 10 patients were using carbamazepine, 4 patients were using phenytoin, and 2 patients were using oral contraceptive.

Table 14 Co-medications categorized by drug interaction with lamotrigine (N = 73)

Co-medication groups	Frequency	% of total co-medication
LTG	36	49.31
LTG + enzyme inhibitor	15	20.55
LTG + enzyme inducers	16	21.92
LTG + enzyme inhibitor + enzyme inducers	6	8.22

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptive

2. Population allelic frequencies

A total of 80 patients were genotyped in this study. Two SNPs including *UGT1A4* 142 T>G (L48V) and *UGT1A4* 70 C>T (P24T) were identified by Taqman allelic discrimination assays using Taqman probe. This study found that the allele frequency of *UGT1A4* 142 T>G (L48V) in Thai patients was 27% and 73% for T and G alleles. However, the variant of *UGT1A4* 70 C>T (P24T) was not found in this study.

Genotyping data from a total of 80 Thai patients are shown in Table 15. When the patients were divided into 3 groups base on *UGT1A4* 142 T>G (L48V) genotyping, 43 patients (54%) were homozygous T/T, 31 patients (39%) were heterozygous T/G, and

6 patients (7%) were homozygous G/G. Allele SNPs were in the Hardy Weinberg equilibrium (HWE) *(P>0.05).*

Table 15 Prevalence of *UGT1A4* 142 T>G (L48V) polymorphism

UGT1A4	(80 patients x 2 alleles)				Genotypes	Observed N=80	%	Predicted (HWE)
	Alleles	N=160	%	95%CI				
142T>G	T	117	73	0.66-0.80	TT	43	54	43
(L48V)					TG	31	39	31
	G	43	27	0.20-0.34	GG	6	7	6
						Chi-square = 0.017, *p = 0.991*		

Allelic frequencies of *UGT1A4* 142 T>G genotypes were in HWE, p =0.991.

The calculation of allelic frequencies follow were as:

The number of the T allele = (43 x 2) + (31 x 1) = 117 alleles

The number of the G allele = (6 x 2) + (31 x 1) = 43 alleles

The frequency of the T allele = p = 117 / (117 + 43) = 0.73

The frequency of the G allele = q = 43 / (117 + 43) = 0.27

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

p^2 = 0.73 x 0.73 = 0.5329

2pq = 2 x 0.73 x 0.27 = 0.3942

q^2 = 0.27 x 0.27 = 0.0729

The total number of patients included to this study was 80

Expected number of TT = 0.5329 x 80 = 42.63

Expected number of TG = 0.3942 x 80 = 31.54

Expected number of GG = 0.0729 x 80 = 5.83

The observed number of TT = 43

The observed number of TG = 31

The observed number of GG = 6

Chi-square = 0.017, p = 0.991

Therefore, we can conclude that the population is in HWE.

3. Comparison of *UGT1A4* allele frequencies among different populations

The allele frequency of *UGT1A4* is shown in Table 16. When compared with other populations, the allele frequency of *UGT1A4* 142 T>G (L48V) in this study was significantly different from Caucasians including German and Swedish populations (*P<0.001* and *P=0.001,* respectively).[22, 25] However, it was similar to the frequency obtained from Turkish population *(P=0.404)*.[30] When compared with other Asian populations, the allelic frequency of *UGT1A4* 142 T>G (L48V) in this study was significantly different from Japanese and Korean populations.[23, 26-27] The results from this study showed no *UGT1A4* 70 C>T (P24T) polymorphisms in Thai populations which was similar to a previous study in Japanese populations.[23, 26]

Table16 Comparison of *UGT1A4* allele frequencies among different populations

Polymorphism	Ethnicity	Number of subjects	% allele frequency		p-value*
			T	G	
UGT1A4 142 T>G	Thai (this study)	80	73	27	
	Japanese[23]	256	87.11	12.89	<0.001
	Japanese[26]	100	83.50	16.50	0.017
	Germany[22]	316	91	9	<0.001
	Turkish[30]	129	76.74	23.26	0.404
	Swedish[25]	112	87.05	12.95	0.001
	Korean[27]	40	85	15	0.049
			C	T	
UGT1A4 70 C>T	Thai (this study)	80	100	0	-
	Japanese[23]	256	100	0	-
	Japanese[26]	100	100	0	-
	German[22]	318	92	8	<0.001
	Turkish[30]	129	96.20	3.80	0.008

*Chi square test

4. Effect of *UGT1A4* 142 T>G polymorphism on LTG-CDR

A total of 73 patients were included in this analysis. The patient demographic data categorized by *UGT1A4* 142 T>G genotypes (T/T, T/G and G/G) were not significantly different among groups except for co-medications as shown in Table 17.

Table 17 Demographic data of patients categorized into 3 groups based on *UGT1A4* 142 T>G genotypes (N=73)

Demographic data	Mean±SD or Median			p-value
	UGT1A4 142 T>G genotypes			
	T/T (n=39)	T/G (n=28)	G/G (n=6)	
Gender (male/female) [a]	19/20	10/18	1/5	0.253
Age (years) [b]	47.87±15.19	46.96±13.76	46.50±12.91	0.956
Body weight (kg) [b]	65.02±13.60	60.32±11.86	58.80±12.47	0.256
LTG dose (mg/day) [c]	50	100	125	0.549
LTG dose (mg/kg/day) [c]	0.85	1.46	2.26	0.259
LTG level (μg/mL) [c]	1.13	1.39	1.58	0.581
LTG-CDR (kg/L) [c]	1.21	1.06	1.06	0.707
Co-medication groups [a]				
LTG	18	15	3	0.005
LTG + inhibitor	7	7	1	0.091
LTG + inducers	12	3	1	0.002
LTG + inhibitor + inducers	2	3	1	0.607

[a] Chi-square test, [b] One-way ANOVA, [c] Kruskal-Wallis H test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

The mean LTG-CDR of 73 patients was 1.48±1.58 kg/L with the range of 0.21-12.32 kg/L. When the median of LTG-CDR were compared among the groups of *UGT1A4* 142T>G genotypes, the median of LTG-CDR were not different among groups (*p=0.707*). The summary of the LTG-CDR for each group of *UGT1A4* 142T>G genotypes was presented in Figure 7.

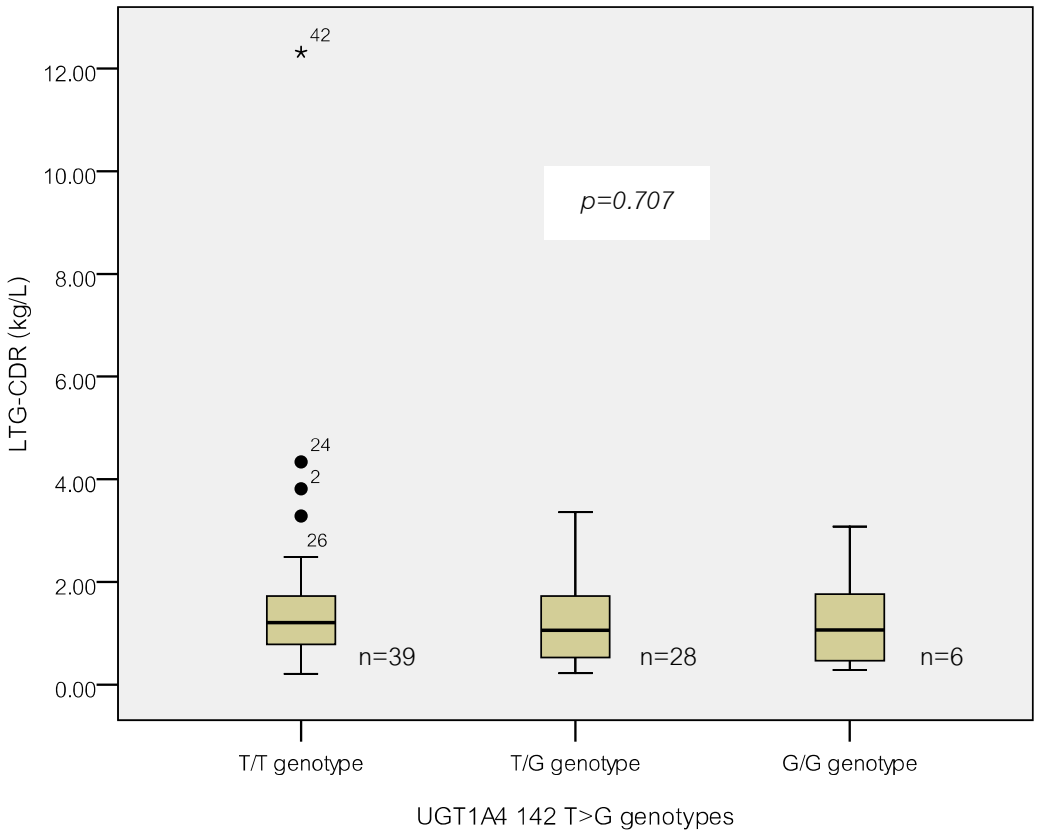


Figure 7 Boxplot of LTG-CDR for the different *UGT1A4* 142 T>G genotypes (3 groups)

Among 73 patients, one patient had lamotrigine concentration of 4.39 µg/mL, corresponding to LTG-CDR 12.32 kg/L, which was extremely high than others. Therefore, further analysis was performed by excluding this patient in the analysis. The results showed that the median of LTG-CDR were not significantly different among these 3 groups (*p=0.763*).

When the data were categorized into 2 groups: patients with homozygous wild type alleles (T/T) and patients with at least 1 variant allele (T/G or GG), the demographic data of patients were not significantly different between groups (Table 18). However, the numbers of patients using lamotrigine + enzyme inducers were significantly different among these 2 groups

Table 18 Demographic data of patients categorized into 2 groups based on *UGT1A4* 142 T>G genotypes (N=73)

Demographic data	Mean±SD or Median		p-value
	UGT1A4 142 T>G genotypes		
	T/T (n=39)	T/G or G/G (n=34)	
Gender (male/female) [a]	19/20	11/23	2.010
Age (years) [b]	47.87±15.19	46.88±13.42	0.770
Body weight (kg) [b]	65.02±13.60	60.05±11.79	0.102
LTG daily dose (mg/day) [c]	50	100	0.309
LTG dose (mg/kg/day) [c]	0.85	1.46	0.120
LTG level (μg/mL) [c]	1.13	1.50	0.420
LTG-CDR (kg/L) [c]	1.21	1.06	0.407
Co-medication groups [a]			
LTG	18	18	1.000
LTG + enzyme inhibitor	7	8	0.796
LTG + enzyme inducers	12	4	0.046
LTG + enzyme inhibitor + enzyme inducer	2	4	0.414

[a] Chi-square test, [b] independent t-test, [c] Mann-Whitney U test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

The LTG-CDR data were presented in Figure 8. The median of LTG-CDR in patients having at least 1 variant allele of *UGT1A4* 142T>G was lower than those with homozygous wild type. However, the difference of the median of LTG-CDR was not statistically significant (*p=0.407*).

Again, one patient with LTG-CDR of 12.32 kg/L was considered to be an outlier (patient number 42). When this patient was excluded from the data, the median of LTG-CDR was not significantly different between groups (*p=0.470*).

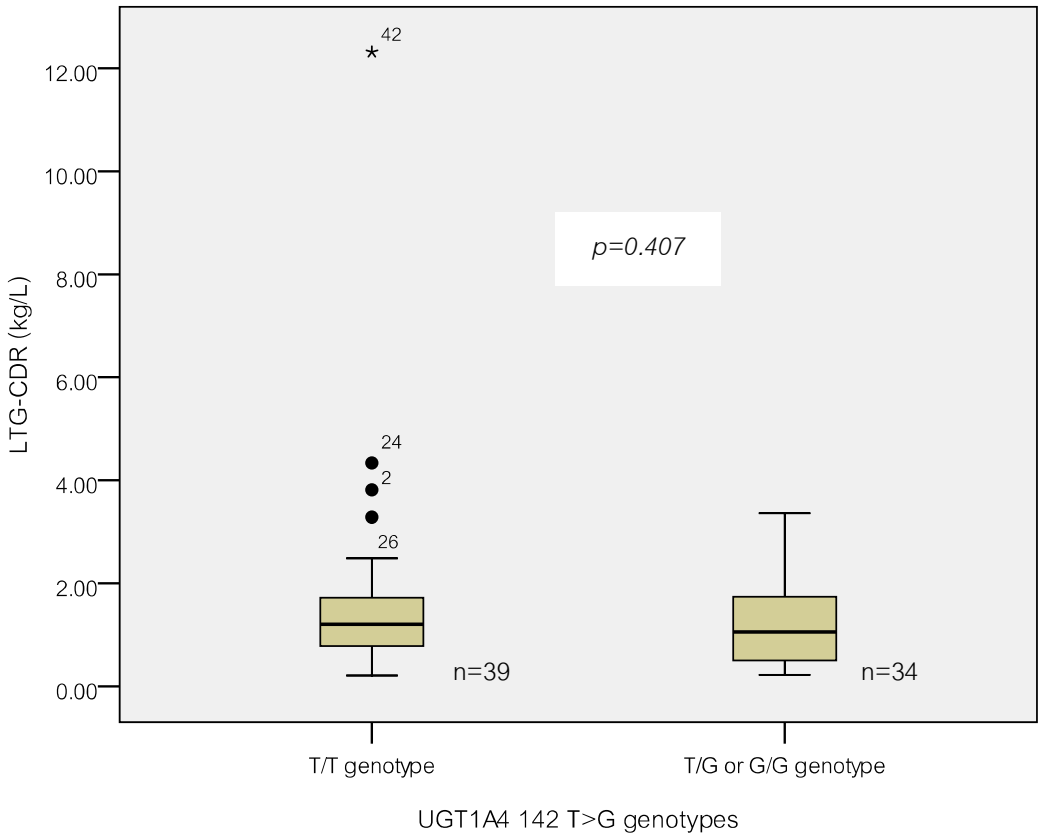


Figure 8 Boxplot of LTG-CDR for the different *UGT1A4* 142 T>G genotypes (2 groups)

5. Effect of co-medications on LTG-CDR

As co-medications may interfere the analysis of LTG-CDR among the genotyping groups. The subgroup analysis of the LTG-CDR taking into account the co-medications was performed. The patients were divided into 4 groups based on co-medications: (1) lamotrigine monotherapy, (2) lamotrigine combination with enzyme inhibitor, (3) lamotrigine combination with enzyme inducers, and (4) lamotrigine combination with enzyme inhibitor and enzyme inducers.

Table 19 shows the comparisons of patient's characteristics among groups. Gender and body weight were not significantly different among these 4 groups. However, age, lamotrigine daily dose (mg/day), lamotrigine dose (mg/kg/day), lamotrigine level and LTG-CDR were significantly different among these 4 groups.

Table 19 Comparisons of patient's characteristics among difference co-medications groups (N=73)

Patient's characteristics	Mean ± SD or Median				p-value
	LTG (n=36)	LTG + inhibitor (n=15)	LTG + inducers (n=16)	LTG + inhibitor + inducers (n=6)	
Gender (male/female) [a]	11/25	10/5	6/10	3/3	0.112
Age (years) [b]	53.33±13.78	44.47±14.69	39.44±11.24	40.50±9.69	0.003
Body weight (kg) [c]	63.35	69.80	55.00	55.25	0.112
LTG daily dose (mg/day)[c]	50	50	200	200	<0.001
LTG dose (mg/kg/day) [c]	0.77	0.78	3.27	3.62	0.001
LTG level (µg/mL) [c]	1.03	2.40	1.22	2.97	0.001
LTG-CDR (kg/L) [c]	1.25	2.62	0.52	1.04	<0.001

[a] Chi-square test, [b] One-way ANOVA, [c] Kruskal-Wallis H test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

The median of LTG-CDR of patients using lamotrigine monotherapy, patients using lamotrigine + enzyme inhibitor, patients using lamotrigine + enzyme inducers, and patients using lamotrigine + enzyme inhibitor + enzyme inducers were 1.25, 2.62, 0.52, and 1.04 kg/L, respectively. The summary of LTG-CDR among different co-medication groups was present in Figure 9.

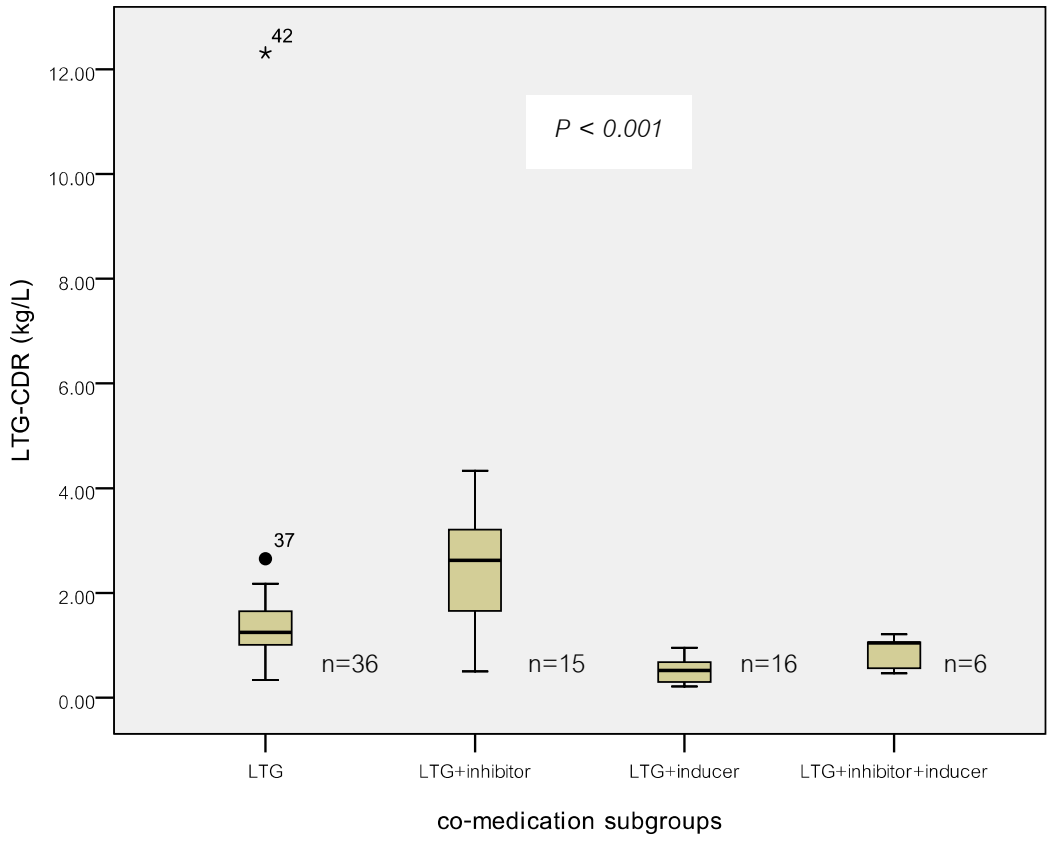


Figure 9 Boxplot of LTG-CDR versus co-medication 4 groups

The multiple comparisons of the median of LTG-CDR among different co-medication groups was summarized in Table 20. The median of LTG-CDR of patients using lamotrigine + enzyme inhibitor was significantly higher than lamotrigine monotherapy, lamotrigine + enzyme inducers, and lamotrigine + enzyme inhibitor + enzyme inducers ($p<0.001$).

The patients using lamotrigine + enzyme inducers had a significantly lower the median of LTG-CDR than those using lamotrigine monotherapy, lamotrigine + enzyme

inhibitor, and lamotrigine + enzyme inhibitor + enzyme inducers (*p<0.001*). Moreover, this study found that the median of LTG-CDR were not significantly different between the patients using lamotrigine monotherapy and lamotrigine + enzyme inhibitor + enzyme inducers (*p=0.052*).

Table 20 Multiple comparisons of LTG-CDR among different combination therapy groups (N=73)

Combination therapy groups	LTG	LTG + inhibitor	LTG + inducers	LTG + inhibitor + inducers
LTG				
LTG + inhibitor	<0.001*			
LTG + inducers	<0.001*	<0.001*		
LTG + inhibitor + inducers	0.052	<0.001*	<0.001*	
Median of LTG-CDR (kg/L)	1.25	2.62	0.52	1.04

* Statistically significant differences was calculated using Mann-Whitney U test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

6. Effect of *UGT1A4* 142T>G polymorphism on LTG-CDR in subgroup analysis based on co-medications

The comparison of the LTG-CDR for *UGT1A4* 142T>G genotype groups (T/T, T/G and G/G) when the patients were categorized into 4 groups based on different combination therapies are shown in Table 21.

The results showed that the LTG-CDR of patients having T/T, T/G and G/G genotype was not significantly different among groups. However, the LTG-CDR of patients having at least 1 variant allele of *UGT1A4* 142T>G (T/G and G/G) tends to be lower than the patients having homozygous wild type allele (T/T).

Table 21 Comparison of LTG-CDR in difference *UGT1A4* 142T>G genotypes (3 groups) when categorized patients into 4 groups base on co-medication (N=73)

Co-medication subgroups	Mean ± SD or Median of LTG-CDR (kg/L)			p-value
	UGT1A4 142 T>G genotypes			
	T/T	T/G	GG	
LTG (n=36)	1.33 (n=18)	1.07 (n=15)	1.22 (n=3)	0.175[a]
LTG + enzyme inhibitor (n=15)	2.73±1.12 (n=7)	2.24±1.10 (n=7)	- (n=1)	0.630[b]
LTG + enzyme inducer (n=16)	0.56±0.23 (n=12)	0.39±0.21 (n=3)	- (n=1)	0.309[b]
LTG + enzyme inhibitor + enzyme inducers (n=6)	1.13 (n=2)	1.04 (n=3)	- (n=1)	0.117[a]

[a] Kruskal-Wallis H test, [b] independent t-test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

Due to the small number of patients in some genotyping groups, the patients were divided into 2 groups: (1) patients having at least 1 variant allele (T/G or G/G), and (2) patients having homozygous wild type allele (T/T). The subgroup comparisons of the median of LTG-CDR for *UGT1A4* 142T>G genotype groups (T/G or G/G and T/T) is shown in Table 22.

The results showed that the median of LTG-CDR of patients having at least 1 variant allele and patients having homozygous wild type allele was not significantly different among groups. However, the median of LTG-CDR of patients having at least 1 variant allele of *UGT1A4* 142T>G (T/G and G/G) tends to be lower than the patients having homozygous wild type allele (T/T).

Table 22 Comparison of LTG-CDR in different *UGT1A4* 142T>G genotypes (2 groups) when categorized patients into 4 groups based on co-medication (N=73)

Co-medication subgroups	Mean±SD or Median of LTG-CDR		
	UGT1A4 142 T>G genotypes		
	T/T	T/G or G/G	p-value
LTG (n=36)	1.33 (n=18)	1.13 (n=18)	0.074[a]
LTG + enzyme inhibitor (n=15)	2.73±1.12 (n=7)	2.34±1.06 (n=8)	0.501[b]
LTG + enzyme inducers (n=16)	0.56±0.23 (n=12)	0.36±0.18 (n=4)	0.132[b]
LTG + enzyme inhibitor + enzyme inducers (n=6)	1.13 (n=2)	0.80 (n=4)	0.133[a]

[a] Mann-Whitney U test, [b] independent t-test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

Patients using lamotrigine monotherapy showed a similar the median of LTG-CDR than those using lamotrigine + enzyme inhibitor + enzyme inducers (Table 19). The patients in this study were divided into 3 groups based on co-medication: (1) lamotrigine monotherapy or lamotrigine combination with enzyme inducers and enzyme inhibitor, (2) lamotrigine combination with enzyme inhibitor, and (3) lamotrigine combination with enzyme inducers.

The comparison of the LTG-CDR for *UGT1A4* 142T>G genotype groups (T/T, T/G and G/G) when the patients were categorized into 3 groups based on difference combination therapies were shown in Table 23.

The results showed that the LTG-CDR of patients having T/T, T/G and G/G genotype was not significantly different among groups. However, the LTG-CDR of patients having at least 1 variant allele of *UGT1A4* 142T>G (T/G and G/G) tends to be lower than the patients having homozygous wild type allele (T/T).

Table 23 Comparison of LTG-CDR in difference *UGT1A4* 142T>G genotypes (3 groups) when categorized patients into 3 groups base on co-medication (N=73)

Co-medication subgroups	Mean ± SD or Median of LTG-CDR			p-value
	UGT1A4 142 T>G genotypes			
	T/T	T/G	GG	
LTG or LTG + enzyme inhibitor + enzyme inducers (n=42)	1.26 (n=20)	1.04 (n=18)	1.06 (n=4)	0.063[a]
LTG + enzyme inhibitor (n=15)	2.73±1.12 (n=7)	2.24±1.10 (n=7)	- (n=1)	0.630[b]
LTG + enzyme inducers (n=16)	0.56±0.23 (n=12)	0.39±0.21 (n=3)	- (n=1)	0.309[b]

[a] Kruskal-Wallis H test, [b] independent t-test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

Due to the small number of patients in some genotyping groups. The patients were divided into 2 groups: (1) patients having at least 1 variant allele (T/G or G/G), and (2) patients having homozygous wild type allele (T/T). The subgroup comparisons of the median of LTG-CDR for *UGT1A4* 142T>G genotype groups (T/T and T/G or G/G) when categorized patients into 3 groups based on co-medication was shown in Table 24.

The results showed that the median of LTG-CDR of patients having at least 1 variant allele (T/G or G/G) was significantly lower than patients having homozygous wild type allele (T/T) for patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducer (*p=0.019*). The LTG-CDR data of patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducer was presented in Figure 10.

However, the median of LTG-CDR of patients having a wild type of *UGT1A4* 142 T>G (T/T) was not significantly different from those having at least 1 variant allele (T/G or G/G) for patients using lamotrigine + enzyme inhibitor and lamotrigine + enzyme inducer (*p=0.501* and *p=0.132*, respectively).

Table 24 Comparison of LTG-CDR in different *UGT1A4* 142T>G genotypes (2 groups) when categorized patients into 3 groups based on co-medication (N=73)

Co-medication subgroups	Mean±SD or Median of LTG-CDR		p-value
	UGT1A4 142 T>G genotypes		
	T/T	T/G or G/G	
LTG or LTG + enzyme inhibitor + enzyme inducers (n=42)	1.26 (n=20)	1.04 (n=22)	0.019[a]
LTG + enzyme inhibitor (n=15)	2.73±1.12 (n=7)	2.34±1.06 (n=8)	0.501[b]
LTG + enzyme inducers (n=16)	0.56±0.23 (n=12)	0.36±0.18 (n=4)	0.132[b]

[a] Mann-Whitney U test, [b] independent t-test

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

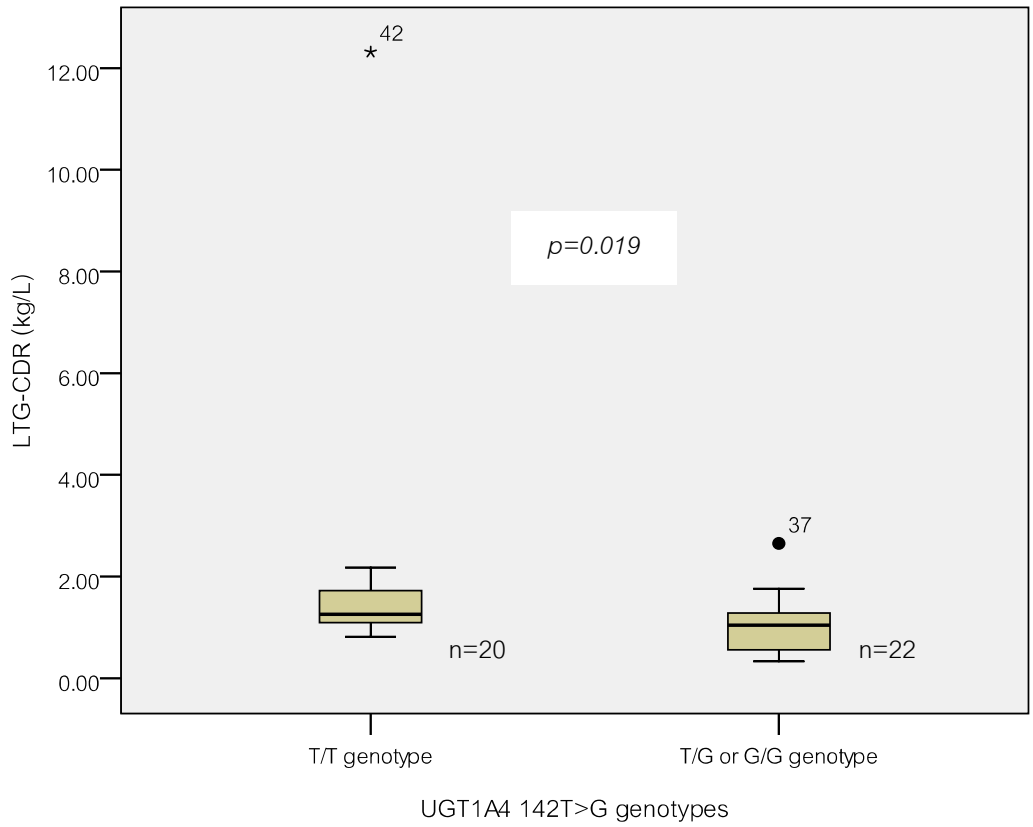


Figure 10 Boxplot of the LTG-CDR for the different *UGT1A4* 142 T>G genotypes for patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducer (n=42)

Among 73 patients, one patient had extremely high lamotrigine concentration than others. Therefore, this patient was excluded from the analysis. The results showed that the median of LTG-CDR of patients having at least 1 variant allele (T/G or G/G) was significantly lower than patients having homozygous wild type allele (T/T) for patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducer (*p=0.030*).

7. Predicting equations of LTG-CDR

The multiple regression analysis was performed to create a model for predicting LTG-CDR (kg/L). Non-genetic factors including age, gender, body weight and co-medications, and genetic factor (*UGT1A4* 142 T>G genotypes) were tested to be included into the model using stepwise method. The results showed that enzyme inducers, enzyme inhibitor, and age were significantly influence LTG-CDR.

Table 25 shows the summary of stepwise linear regression model for LTG-CDR. Based on the regression model, model 3 which included the use of enzyme inducers, enzyme inhibitor, and age into the model was the best fitted model. This model could explain 20.40% of the variance of LTG-CDR (Adjusted R-square=0.204; $p< 0.001$).

Table 25 Model summary of stepwise linear regression for prediction of LTG-CDR

Model	Variable entered	R	R-square	Adjusted R-square	Sig. (F change)	Model Sig. (ANOVA)
1	LTG + enzyme inducers	0.368	0.136	0.124	0.001	0.001
2	LTG + enzyme inducers LTG + enzyme inhibitor	0.429	0.184	0.161	0.046	0.001
3	LTG + enzyme inducers LTG + enzyme inhibitor Age	0.487	0.237	0.204	0.032	< 0.001

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

The coefficients of each variable in the final model and their p-values were presented in Table 26. When the covariates were tested for multicolinearity, all the correlation between two covariates was less than 0.52. Therefore, they were not highly correlated and were all tested in the regression model (data not shown).

Table 26 Coefficients of factors in the regression model for prediction of LTG-CDR

Model	Parameter	Unstandardized Coefficients		t	Sig.	95% CI	
		B	Std. Error			low	high
3	Constant	0.206	0.697	0.296	0.768	-1.184	1.596
	LTG + enzyme inducers	-0.928	0.378	-2.454	0.017	-1.682	-0.173
	LTG + enzyme inhibitor	0.929	0.372	2.496	0.015	0.186	1.672
	Age	0.027	0.013	2.185	0.032	0.002	0.053

LTG = lamotrigine

Enzyme inhibitor = valproic acid

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives

Therefore, the final model can be presented as follows:

LTG-CDR (kg/L) = 0.206 + (-0.928) [LTG + enzyme inducer] +
(0.929) [LTG + enzyme inhibitor] + 0.027 [Age (years)]

LTG = lamotrigine

Enzyme inhibitor = valproic acid (0=unused and 1=used)

Enzyme inducers = carbamazepine, phenytoin, and oral contraceptives (0=unused and 1=used)

CHAPTER V
DISCUSSIONS AND CONCLUSIONS

The purpose of this study was to investigate the effect of genetic and non-genetic factors on LTG-CDR in Thai patients receiving treatment at Prasat Neurological Institute during 10 January to 30 July 2011. All patients were received lamotrigine as monotherapy or polytherapy at the same dose for at least two weeks.

A total of 80 patients were recruited. Among them, 7 patients were excluded; 2 patients had lamotrigine concentration below quantification limit due to non-compliance and 5 patients had an unusually high peak of an internal standard due to the use of phenobarbital (internal standard) as a co-medication. Therefore, the data from 73 patients were used in this analysis.

Of 73 patients, there were 43 female (58.90%) and 30 male (41.10%) with the mean (SD) age of 47.41 (14.30) years old and the mean (SD) body weight of 62.71 (12.94) kg. The mean (SD) lamotrigine dose and lamotrigine daily dose per body weight were 108.73 (88.65) mg/day and 1.82 (1.55) mg/kg/day, respectively (Table 10). The mean (SD) lamotrigine concentration of the patients in this study was 1.93 (1.83) mg/L, which was considered to be within the therapeutic range of lamotrigine (1-4 mg/L).[2, 11]

Two SNPs including *UGT1A4* 142 T>G (L48V) and *UGT1A4* 70 C>T (P24T) were identified from 80 patients. The results from our study found that nearly half of the patients are wild-type of *UGT1A4* 142T>G (54%). The allele frequency of *UGT1A4* 142 T>G in Thai patients are 27% which is higher than other Asian populations).[23, 26-27] The allele frequency of *UGT1A4* 142 T>G in this study is significantly different from German and Swedish populations (*P<0.001* and *P=0.001,* respectively).[22, 25] However, it is similar to the frequency obtained from Turkish population *(P=0.404)*.[30] Although, the polymorphism of *UGT1A4* 70C>T is commonly found in the Caucasians, it was not detected in this Thai population[22] which is similar to the results obtained from Japanese populations.[23, 26]

Several therapeutic agents are substrates of UGT1A4 such as clozapine, olanzapine, tamoxifen and lamotrigine.[25-26, 30] The glucuronidation activity of UGT1A4 enzyme has been investigated. The impact of *UGT1A4* 142T>G polymorphisms on glucuronidation activity depends upon a substrate. It was shown that an enzyme activity was reduced for β-naphthylamine, benzidine, trans-androsterone, and dihydrotestosterone, while it was increased for the glucuronidation of clozapine, olanzapine, and lamotrigine.[22, 25, 30] Therefore, the polymorphisms of *UGT1A4* 142T>G should be taken into account for dose adjustment of these drugs.

Lamotrigine is mainly metabolized by UGT1A4.[18] Previous studies have shown a reduction of lamotrigine apparent oral clearance in Asian compared to Caucasian.[14-15] The difference of lamotrigine pharmacokinetics among races could probably be related to genetic variation in the metabolism of lamotrigine. There is only one study suggested that patients having *UGT1A4* 142 T>G polymorphism was associated with a lower concentration of lamotrigine compared with patients having wild type when lamotrigine was given as a monotherapy (2.4±1.05 vs 3.5±0.69 mg/L; $p<0.05$).[30]

In this study, the medians of LTG-CDR were compared among *UGT1A4* 142T>G genotypes (T/T, T/G, and G/G). The medians of LTG-CDR were not significantly different among these 3 groups (1.21 vs 1.06 vs 1.06 kg/L; $p=0.707$) (Table 17). When the data were categorized into 2 groups based on *UGT1A4* 142 T>G genotypes (homozygous wide type alleles and patients with at least 1 variants allele), the median of LTG-CDR in patients having at least 1 variants allele of *UGT1A4* 142 T>G (T/G or G/G) tended to be lower than those with homozygous wild type (T/T) (1.06 vs 1.21 kg/L; $p=0.407$). However, it was not statistically significant (Table 18). It is possible that a number of participating patients in this study is small, therefore leads to a lack of statistical power. Furthermore, the influence of *UGT1A4* 142T>G polymorphism on LTG-CDR may be masked by co-medication effect.

As co-medications may interfere the analysis of LTG-CDR among the genotyping groups. The subgroup analysis of the LTG-CDR taking into account the co-medications was performed. The patients were divided into 4 groups based on co-medications (lamotrigine monotherapy, lamotrigine + enzyme inhibitor, lamotrigine + enzyme

inducers, and lamotrigine + enzyme inhibitor + enzyme inducers). The median of LTG-CDR were significantly different among these groups (1.25, 2.62, 0.52, and 1.04 kg/L, respectively; *p<0.001*) (Table 19).

The comparisons of the median of LTG-CDR among different combination therapy groups showed that patients taking lamotrigine + enzyme inhibitor had an approximately two-fold higher of LTG-CDR than patients taking lamotrigine monotherapy (2.62 vs 1.25 kg/L; *p<0.001*). Valproic acid was the only drug identified as an enzyme inhibitor in this study. It is a strong inhibitor of lamotrigine that results in a prolonged half-life and an increase in plasma concentration of lamotrigine. This indicated that when enzyme inhibitor is used in combination with lamotrigine, the dosage of lamotrigine will need to be decreased.[54, 57] In this study, it was found that for the patients taking lamotrigine + enzyme inducers, they had an approximately two-fold lower of LTG-CDR than patients taking lamotrigine monotherapy (0.52 vs 1.25 kg/L; *p<0.001*). In the present study, enzyme inducers including carbamazepine, phenytoin, and oral contraceptive can enhance the metabolism of lamotrigine and reduce lamotrigine concentration. This indicated that the dose of lamotrigine may need to be increased if these drugs are given concomitantly.[54, 57] Moreover, this study found that the median of LTG-CDR were not significantly different among the patients using lamotrigine monotherapy and lamotrigine + enzyme inhibitor + enzyme inducers (1.25 vs 1.04 kg/L; *p=0.052*), which is similar to the previous study by Armijo et al.[75].

Due to the possible confounding of co-medications, further investigation was performed by categorizing patients into 4 groups based on co-medications (lamotrigine monotherapy, lamotrigine + enzyme inhibitor, lamotrigine + enzyme inducers, and lamotrigine + enzyme inhibitor + enzyme inducers). The influence of *UGT1A4* 142T>G polymorphism on LTG-CDR was investigated in each group of patients (Table 21). The median of LTG-CDR in patients having T/T, T/G and G/G genotype was not significantly different among groups. However, the median of LTG-CDR of patients having at least 1 variant allele of *UGT1A4* 142T>G (T/G and G/G) tended to be lower than the patients having homozygous wild type allele (T/T).

Due to the small number of patients in some genotyping groups, the data were divided into 2 groups: patients having at least 1 variant allele (T/G or G/G), and homozygous wild type allele (T/T) (Table 22). The median of LTG-CDR was not significantly different between these two groups. However, the median of LTG-CDR in patients having T/G or G/G genotype tended to be lower than in the patients having T/T genotype.

In our study, the median of LTG-CDR in patients using lamotrigine monotherapy is similar to that using lamotrigine combination with enzyme inhibitor and enzyme inducers. Therefore, the data were divided into 3 groups based on co-medication: (1) lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducers, (2) lamotrigine + enzyme inhibitor, and (3) lamotrigine + enzyme inducers. The influence of *UGT1A4* 142T>G polymorphism on LTG-CDR was investigated in each group of patients (Table 23). The median of LTG-CDR in patients having T/T, T/G and G/G genotype was not significantly different among groups. However, the median of LTG-CDR in patients having at least 1 variant allele of *UGT1A4* 142T>G (T/G and G/G) tends to be lower than those having homozygous wild type allele (T/T).

Due to a small number of the patients in some genotyping groups, the patients were divided into 2 groups: (1) patients having at least 1 variant allele (T/G or G/G), and (2) patients having homozygous wild type allele (T/T) (Table 24). In a group of patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducers, the median of LTG-CDR in patients with at least one variant allele of *UGT1A4* 142 T>G (T/G or G/G) was significantly lower than those with homozygous wide type (T/T) (1.04 vs 1.26 kg/L; *p=0.019*). This findings are similar to the results reported by Gulcebi et al.[30] which suggested that the polymorphism of *UGT1A4* 142 T>G leads to a lower concentration of lamotrigine in patients receiving lamotrigine as monotherapy. These results indicated that the polymorphism *UGT1A4* 142T>G influence lamotrigine concentration.

In the group of patients using lamotrigine + enzyme inducers, the mean of LTG-CDR in patients with wild type (T/T) was 0.56$\pm$0.23 kg/L, while the mean of LTG-CDR in patients with T/G or G/G genotype was lower (0.36$\pm$0.18 kg/L). However,

they were not significantly different (*p=0.132*). In the group of patients taking lamotrigine + enzyme inhibitor, the mean of LTG-CDR in patients with T/G or G/G genotype tend to be higher than patients with wild type (T/T), but they were not significantly different (2.73±1.12 vs 2.34±1.06 kg/L; *p=0.501*).

The multiple linear regression was performed to investigate the influence of genetic and non-genetic factors on the LTG-CDR. The results showed that the use of enzyme inhibitor, enzyme inducers, and age were significantly influence LTG-CDR (Table 25). The final model could explain 20.40% of LTG-CDR variation (Adjusted R-square = 0.204*; p< 0.001*).

Based on the regression coefficient (B=0.929), the use of enzyme inhibitors increases LTG-CDR by 60% which is consistent with previous studies.[76-77] In this study, valproic acid is only one drug identified as an enzyme inhibitor. Several studies suggested that lamotrigine half-life can be prolonged and lamotrigine concentration can increase when co-administered with valproic acid.[54, 57] A study by May TW et al.[76] found a significant increase of LTG-CDR in patients receiving lamotrigine concomitant with valproic acid. Moreover, in a study by Weintraub D et al.[77], the use of valproic acid decreases lamotrigine clearance by 60% and the dose of lamotrigine needs to be decrease.

Based on the regression coefficient (B=-0.928), the use of enzyme inducers including carbamazepine, phenytoin, and oral contraceptives can increase lamotrigine clearance. Co-administration of these drugs decreases lamotrigine half-life and reduce lamotrigine concentration.[54, 57] Our results showed that the use of enzyme inducers leads to a decrease of LTG-CDR by 60% which is consistent with previous studies.[76-77] A study by May TW et al.[76] found a significant decrease of LTG-CDR in patients receiving lamotrigine with enzyme inducers which is consistent with a study by Weintraub D et al.[77]. In this study, the use of phenytoin or carbamazepine increases clearance of lamotrigine by 125% or 30-50%, respectively. The effect of oral contraceptives on lamotrigine pharmacokinetics was documented in previous studies. Lamotrigine plasma level can be reduced by more than 50% when it is used in combination with oral contraceptives.[64-65] However, in our study, sum of the effects of

all inducers were quantified. The effect of each enzyme inducers were not individually identified, as there was a small number of patients using some inducers (2 patients using oral contraceptives, 4 patients using phenytoin).

Co-medications which are enzyme inducers and enzyme inhibitors can alter drugs' pharmacokinetics. The regression model of LTG-CDR indicated that co-medication treatments with enzyme inhibitors and enzyme inducers are important factors which should to be taken into account for dosage regimens of lamotrigne. However, the current guideline has accounted for the decrease or increase of LTG-CDR when lamotrigine is given concomitantly with enzyme inhibitors or enzyme inducers.[35]

The pharmacokinetics of several drugs were found to be altered in the elderly patients due to physiological changes in this population.[41] However the influence of age on lamotrigine pharmacokinetics is still controversial. Even though, previous studies showed that lamotrigine pharmacokinetics did not depend on age.[14-15, 44-45] Some studies reported the influence of age on lamotrigine pharmacokinetics.[46] Our study found that age is one of the variables significantly influent LTG-CDR. Based on the regression coefficient (B=0.027), the increasing age results in an increase of LTG-CDR which could be due to the decrease of lamotrigine clearance in advanced-age patients.[41] However, our study consisted of a small number of elderly patients (15 patients) aged 60 years or older. Therefore, the influence of age on lamotrigine pharmacokinetics should be investigated in a study consisted of a larger number of elderly patients.

Interestingly, the influence of *UGT1A4* 142 T>G polymorphism on LTG-CDR was found in the group of patients using lamotrigine monotherapy. This results is consistent with a previous study by Gulcibi MI et al.[30] which showed the significant decrease of serum lamotrigine concentrations in patients with monotherapy.

As there is a high allele frequency of *UGT1A4 T>G* polymorphism in Thai population (27%), it is possible that among the patients receiving a recommended dose of lamotrigine, but have a lower lamotrigine concentration than the therapeutic response or fail to control their symptoms, this could be the consequence of *UGT1A4 T>G*

polymorphism. Therefore, the detection of *UGT1A4 T>G* polymorphism may be useful in these groups of patients.

For patients with the variant allele of *UGT1A4* 142 T>G, they may have a lower concentration of lamotrigine compared with those having wild type. Therefore, these patients may require higher dose of lamotrigine. Therefore, identifying *UGT1A4* 142 T>G polymorphism in this group of patients may be clinically useful. Moreover, based on the results from this study, it is recommended that lamotrigine dose adjustment according lamotrigine concentration may be required in elderly patients, and patients using enzyme inducers or enzyme inhibitors.

However, when genetic effect was investigated in the linear regression model, the influence of *UGT1A4* 142 T>G polymorphism was not found. This lack of association could be due to the fact that when age and co-medications were taken into account in the regression model, the influence of *UGT1A4* 142 T>G was adjusted. The developed equation from this study may be used for facilitating an optimal dose adjustment of lamotrigine in Thai patients.

In addition to UGT1A4 enzyme, other UGTs including UGT2B7 may play an important role in the metabolism of lamotrigine. Recently, there is an evidence of the influence of *UGT2B7*_-161C>T polymorphisms on lamotrigine pharmacokinetics.[71] Therefore, a further investigation of the influence of other UGTs on the pharmacokinetics of lamotrigine is required to fully explain the variability of lamotrigine pharmacokinetics.

In conclusion, the influence of *UGT1A4* 142T>G polymorphism on LTG-CDR was observed in patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducers. On the contrary, when its influence was adjusted for age and co-medications (enzyme inducers and enzyme inhibitors), the *UGT1A4* 142T>G polymorphism did not found to be an important factor explaining the variability of lamotrigine concentrations. Therefore, the influence of *UGT1A4* and other UGTs polymorphism on lamotrigine pharmacokinetics requires further investigation.

Limitation

1. In this study, the influence of *UGT1A4* 142T>G polymorphism was found only in a group of patients using lamotrigine monotherapy or lamotrigine + enzyme inhibitor + enzyme inducer, but the effect of this polymorphism was not detected in other subgroups. This could be due to a small number of patients in each subgroup.
2. This study included only patients with normal liver and kidney function. Therefore, the equation obtained from this study should be applied with caution in patients with poor liver and kidney function.
3. As lamotrigine is metabolized by other UGTs such as UGT1A3 and UGT2B7. The polymorphisms of these genes may influence LTG-CDR. However, in this study, only the influence of *UGT1A4* 142T>G polymorphism on LTG-CDR was investigated.

Further study

1. The equation obtained from this study should be further validated to determine the accuracy and precision before it will be used in clinical practice.
2. As there is an evidence of the effect of *UGT1A4* 142T>G polymorphism in this study, the further study with a larger sample size should be performed to confirm this finding.
3. The effects of other genetic factors such as *UGT1A3* and *UGT2B7* polymorphisms on lamotrigine pharmacokinetics in Thai populations should be further investigated.

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APPENDICES

APPENDIX A

Certificate of Approval from the Institutional Review Board of the Prasat Neurological Institute

ที่ สธ ๐๓๑๐/ ๒๓๒๙

สถาบันประสาทวิทยา กรมการแพทย์
เลขที่ ๓๑๒ ถนนราชวิถี แขวงทุ่งพญาไท
เขตราชเทวี กรุงเทพฯ ๑๐๔๐๐

๓๐ ธันวาคม ๒๕๕๓

เรื่อง แจ้งการอนุมัติให้ดำเนินโครงการวิจัยในสถาบันประสาทวิทยา

เรียน น.ส. นภเกตน์ สิงห์คำ

สิ่งที่ส่งมาด้วย เอกสารอนุมัติให้ดำเนินการวิจัยในสถาบันประสาทวิทยา

ตามที่ท่านได้เสนอขออนุมัติดำเนินการวิจัยในสถาบันประสาทวิทยา ต่อคณะกรรมการวิจัยสถาบันประสาทวิทยา ซึ่งเป็นคณะกรรมการวิจัยประจำสถาบัน ที่มีการดำเนินงานตามแนวทางการวิจัยทางคลินิกที่ดี และขณะนี้คณะกรรมการฯ ได้ดำเนินการพิจารณาและอนุมัติให้ดำเนินโครงการวิจัยดังกล่าวเรียบร้อยแล้ว

ในการนี้ สถาบันประสาทวิทยา จึงขอแจ้งการอนุมัติให้ดำเนินโครงการวิจัยดังกล่าวในสถาบันประสาทวิทยา ตามเอกสารของคณะกรรมการฯ ดังแนบ

จึงเรียนมาเพื่อโปรดทราบ

ขอแสดงความนับถือ

(นายมัยธัช สามเสน)
ผู้อำนวยการสถาบันประสาทวิทยา

ที่ สธ ๐๖๓๘.๒ (๑๗๓/๒๕๕๙)/[illegible]

คณะกรรมการการวิจัยสถาบันประสาทวิทยา
สถาบันประสาทวิทยา เลขที่ ๓๑๒ ถนนราชวิถี
แขวงทุ่งพญาไท เขตราชเทวี กรุงเทพฯ ๑๐๔๐๐

๒๗ กันยายน ๒๕๕๙

เรื่อง อนุมัติให้ดำเนินการวิจัยได้

เรียน น.ส. [illegible]

ตามที่ท่านซึ่งเป็น หัวหน้าโครงการวิจัยตามรายละเอียดข้างท้าย ได้เสนอ โครงการวิจัยเรื่องต่าง ๆ ต่อคณะกรรมการการวิจัยสถาบันประสาทวิทยา

เลขที่โครงการ [illegible]
ชื่อโครงการ [illegible]

ในการนี้ คณะกรรมการการวิจัยสถาบันประสาทวิทยา ซึ่งเป็นคณะกรรมการการวิจัยสถาบัน (Institutional Review Board : IRB) ที่มีการดำเนินงานตามแนวทางการวิจัยทางคลินิกที่ดี (ICH GCP) ได้พิจารณา และอนุมัติให้ดำเนินการโครงการวิจัยดังกล่าวในสถาบันประสาทวิทยาได้ โดยผู้วิจัยจะต้องรักษาข้อมูลความลับของผู้ป่วยภายหลังได้รับการอนุมัติ คือ ต้องปฏิบัติตามพระราชบัญญัติสุขภาพแห่งชาติ พ.ศ. ๒๕๕๐ มาตรา ๗ "ข้อมูลด้านสุขภาพของบุคคล เป็นความลับส่วนบุคคล ผู้ใดจะนำไปเปิดเผยในประการที่น่าจะทำให้บุคคลนั้นเสียหายไม่ได้ เว้นแต่การเปิดเผยนั้นเป็นไปตามความประสงค์ของบุคคลนั้นโดยตรง" โดยเคร่งครัด และจะต้องรายงานความก้าวหน้าของโครงการวิจัยเมื่อมีการร้องขอและ/หรือเมื่อเกิดเหตุการณ์ดังต่อไปนี้ ทุกครั้ง ได้แก่

๑. เมื่อโครงการวิจัยยุติลง ซึ่งอาจจะเป็นการดำเนินการวิจัยเสร็จสิ้นสมบูรณ์ หรืออาจจะไม่สามารถดำเนินการวิจัยต่อไปได้ พร้อมทั้งแจ้งสาเหตุของการยุติโครงการวิจัยให้ทราบด้วย
๒. เมื่อมีการเปลี่ยนแปลงในโครงการวิจัยที่ขออนุมัติ ต้องแจ้งว่า มีการเปลี่ยนแปลงอะไร อย่างไร พร้อมเหตุผลที่ต้องเปลี่ยนแปลง
๓. เมื่อมีการเปลี่ยนแปลงหัวหน้าโครงการวิจัยหรือเพิ่มเติมคณะผู้วิจัย ต้องส่งประวัติของคนที่เปลี่ยนแปลงพร้อมเหตุผลให้คณะกรรมการฯ ทราบด้วย
๔. เมื่อมีอาการไม่พึงประสงค์เกิดขึ้นในโครงการวิจัย ขอให้ผู้วิจัยวิเคราะห์ความสัมพันธ์ของการเกิดอาการไม่พึงประสงค์ที่ unlikely, possible/likely, probably related, fatal กับโครงการวิจัยที่ท่านรับผิดชอบอย่างไร รวมทั้งแผนการแก้ไขการดูแลป้องกันการเกิดในครั้งต่อไปด้วย
๕. จัดส่งรายงานการศึกษาวิจัย จำนวน ๑ ชุด ให้แก่สำนักงานคณะกรรมการการวิจัยสถาบันประสาทวิทยา เมื่อสิ้นสุดการดำเนินงาน

จึงเรียนมาเพื่อโปรดทราบ

ขอแสดงความนับถือ

(นายสุชาติ หาญไชยพิบูลย์กุล)
ประธานคณะกรรมการการวิจัยสถาบันประสาทวิทยา

คณะกรรมการวิจัยสถาบันประสาทวิทยา
สถาบันประสาทวิทยา กรมการแพทย์ กระทรวงสาธารณสุข

โครงการวิจัย	ปัจจัยที่มีผลต่อพัฒนาส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาโมดาฟินิลในผู้ป่วยชาวไทย (เลขที่โครงการ 54021)
ผู้วิจัยหลัก	น.ส. นภาพงษ์ สิงห์คำ
สถานที่ดำเนินการวิจัย	สถาบันประสาทวิทยา
เอกสารที่พิจารณา	1. แบบเสนอโครงการวิจัย ฉบับวันที่ 24 ธันวาคม 2553 2. แบบบันทึกการเก็บข้อมูล ฉบับวันที่ 24 ธันวาคม 2553 3. เอกสารชี้แจงข้อมูลสำหรับอาสาสมัครผู้เข้าร่วมการวิจัย ฉบับวันที่ 24 ธันวาคม 2553 4. หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย ฉบับวันที่ 24 ธันวาคม 2553
วันที่พิจารณาอนุมัติ	27 ธันวาคม 2553

คณะกรรมการวิจัยสถาบันประสาทวิทยา ได้พิจารณาโครงการฉบับภาษาไทยและ/หรือฉบับภาษาอังกฤษแล้ว คณะกรรมการฯ พิจารณาอนุมัติในแง่จริยธรรมและให้ดำเนินการวิจัยข้างต้นภายในสถาบันประสาทวิทยาได้ ทั้งนี้โดยยึดตามเอกสารฉบับภาษาไทยเป็นหลัก

ประธานคณะกรรมการ
(นายสุชาติ หาญไชยพิบูลย์กุล)

กรรมการและเลขานุการ
(นางสาวพิมพ์ชนก พุ่มชาว)

APPENDIX B
เอกสารชี้แจงข้อมูล/คำแนะนำแก่ผู้เข้าร่วมการวิจัย

โครงการวิจัยเรื่อง ปัจจัยที่มีผลต่ออัตราส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาลาโมทริจีนในผู้ป่วยชาวไทย

ผู้วิจัย ภญ.นภเกตน์ สิงห์คำ นิสิตระดับปริญญาโท สาขาเภสัชกรรมคลินิก
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

อาจารย์ที่ปรึกษาโครงการ อ. ดร. บราลี ปัญญาวุธโธ

อาจารย์ที่ปรึกษาโครงการร่วม นพ. สมชาย โตวณะบุตร

สถานที่วิจัย สถาบันประสาทวิทยา

บุคคลและวิธีการติดต่อเมื่อมีเหตุฉุกเฉินหรือความผิดปกติที่เกี่ยวข้องกับการวิจัย

1. ภญ.นภเกตน์ สิงห์คำ
   ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ สาขาเภสัชกรรมคลินิก
   คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
   โทรศัพท์ติดตามตัว 08-4138-7475
2. นพ.สมชาย โตวณะบุตร
   ที่อยู่ สถาบันประสาทวิทยา
   โทรศัพท์ที่ทำงาน 02-3547075 ต่อ 1138

ท่านได้รับเชิญให้เข้าร่วมการศึกษาวิจัยนี้เนื่องจากได้รับการรักษาด้วยยาลาโมทริจีน โดยท่านจะได้อ่านข้อมูลข้างล่างก่อน (หรือทีมผู้ศึกษาวิจัยอ่านให้ท่านรับทราบ) ถ้าท่านมีข้อข้องใจสงสัยใดๆ เกี่ยวกับการศึกษาวิจัยนี้ สามารถซักถามผู้ทำการศึกษาวิจัยหรือแพทย์ที่ทำการศึกษาวิจัยได้ หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัย ท่านจะได้รับสำเนาใบยินยอมที่ท่านเซ็นชื่อกำกับเก็บไว้ 1 ฉบับ

ความเป็นมาของโครงการ

ลาโมทริจีนเป็นยาที่ได้รับการรับรองให้ใช้รักษาโรคลมชักชนิดที่มีอาการชักเฉพาะที่หรืออาการชักเกร็งกระตุกทั้งตัว โดยสามารถใช้เป็นยากันชักตัวเดียวหรือใช้เป็นยาเสริมร่วมกับยากันชักมาตรฐานได้ นอกจากนี้ยาลาโมทริจีนยังมีประสิทธิภาพในการรักษาในโรคอารมณ์สองขั้ว

เนื่องจากเภสัชจลนศาสตร์ของยาลาโมทริจีนมีความผันแปรที่เกิดจากคุณลักษณะของผู้ป่วย เช่น อายุ การตั้งครรภ์ สภาวะโรค อันตรกิริยาของยา เชื้อชาติ จึงมีคำแนะนำให้ทำการตรวจติดตามระดับยาในเลือดเพื่อทำการประเมินผู้ป่วยกรณีเกิดความล้มเหลวจากการรักษา ซึ่งอาจมีสาเหตุจากความไม่ร่วมมือในการใช้ยา หรือเกิดอันตรกิริยาของยา หรือสงสัยว่าเกิดพิษจากยา นอกจากนี้เพื่อใช้เป็นค่าอ้างอิงในการติดตามผลการรักษาและปรับขนาดยาให้ถูกต้องเหมาะสมกับผู้ป่วยแต่ละราย

ความผันแปรทางพันธุกรรมของเอนไซม์ในการเปลี่ยนแปลงยาอาจเป็นปัจจัยหนึ่งที่มีผลต่อเภสัชจลนศาสตร์ของยาลาโมทริจีนถูกกำจัดที่ตับเป็นหลัก โดยอาศัยเอนไซม์ UGT 1A4 และ *UGT2B7* ในการเปลี่ยนแปลงยา ปัจจุบันมีการศึกษาพบภาวะพหุสัณฐานของยีนที่ควบคุมการทำงานเอนไซม์ดังกล่าวซึ่งทำให้ประสิทธิภาพการทำงานของเอนไซม์มีการเปลี่ยนแปลงนำไปสู่ความผันแปรทางเภสัชจลนศาสตร์ของยาลาโมทริจีนได้ ซึ่งความผันแปรทางพันธุกรรมของยีน *UGT1A4* และ *UGT2B7* นั้นยังมีความแตกต่างระหว่างเชื้อชาติ อย่างไรก็ตามยังไม่พบการศึกษาดังกล่าวในประชากรไทย

ผู้วิจัยจึงสนใจศึกษาผลของปัจจัยทางพันธุกรรมและปัจจัยที่ไม่เกี่ยวข้องกับพันธุกรรมต่ออัตราส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาลาโมทริจีนในผู้ป่วยชาวไทย เพื่อนำข้อมูลที่ได้จากการศึกษานี้ไปใช้ประโยชน์ในการออกแบบแผนการใช้ยาลาโมทริจีน รวมทั้งสามารถช่วยทำนายเภสัชจลนศาสตร์ของยา หรือผลของการรักษาและอาการไม่พึงประสงค์จากยาเพื่อช่วยวางแผนการรักษาที่เหมาะสมกับผู้ป่วยแต่ละรายและให้เกิดประโยชน์สูงสุดในการใช้ยา

วัตถุประสงค์

ศึกษาผลของปัจจัยทางพันธุกรรมและปัจจัยที่ไม่เกี่ยวข้องกับพันธุกรรมต่ออัตราส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาลาโมทริจีนในผู้ป่วยชาวไทย

รายละเอียดที่จะปฏิบัติต่อผู้เข้าร่วมการวิจัย

หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัยครั้งนี้กรุณาเซ็นชื่อลงในใบยินยอม

ท่านจะได้รับการสอบถามข้อมูลพื้นฐานทั่วไปโดยใช้แบบสอบถาม เมื่อท่านมาพบแพทย์ตามนัดท่านจะได้รับการชั่งน้ำหนัก วัดส่วนสูง และได้รับการเจาะเลือด

การนัดหมายผู้ป่วยมาเจาะเลือดมีทั้งหมด 2 ครั้ง ดังนี้

- นัดหมายครั้งที่ 1 เจาะเลือดผู้ป่วยปริมาณ 10 มิลลิลิตร โดยแบ่งเลือดออกเป็น 2 ส่วน คือ
  1) เลือดปริมาณ 5 มิลลิลิตร เพื่อนำไปตรวจภาวะพหุสัณฐานของยีน *UGT1A4* และ *UGT2B7*
  2) เลือดปริมาณ 5 มิลลิลิตร เพื่อนำไปตรวจวัดระดับยาลาโมทริจีนในเลือด ก่อนวันนัดหมายผู้วิจัยจะโทรศัพท์ไปเตือนให้ผู้ป่วยงดรับประทานยาลาโมทริจีนในมื้อเช้าก่อนเจาะเลือด ภายหลังเจาะเลือดให้ผู้ป่วยรับประทานยาลาโมทริจีนได้ตามปกติ โดยให้นำยาลาโมทริจีนที่จะรับประทานมาเอง
- นัดหมายครั้งที่ 2 เจาะเลือดผู้ป่วยปริมาณ 5 มิลลิลิตร เพื่อนำไปตรวจวัดระดับยาลาโมทริจีนในเลือด ก่อนวันนัดหมายผู้วิจัยจะโทรศัพท์ไปเตือนให้ผู้ป่วยรับประทานยาลาโมทริจีนในมื้อเช้ามาตามปกติในวันนัดหมายเพื่อทำการเจาะเลือดหลังการรับประทานยา

หมายเหตุ

การนัดเจาะเลือดจะทำในวันที่ท่านต้องมาพบแพทย์อยู่แล้วและท่านไม่ต้องเสียค่าใช้จ่ายใด ๆ ที่นอกเหนือไปจากค่ารักษาพยาบาลของท่านตามปกติ ระยะเวลาที่ท่านต้องเกี่ยวข้องในการศึกษาวิจัยนี้คือ 1-3 เดือนตามระยะเวลาในการนัดหมายพบแพทย์ตามปกติ

ประโยชน์ที่จะเกิดแก่ผู้เข้าร่วมการวิจัยและประโยชน์ในทางวิชาการต่อส่วนรวม

1) ได้ข้อมูลลักษณะยีน *UGT1A4* และ*UGT2B7* ของตัวท่านเอง ซึ่งเกี่ยวข้องกับการกำจัดยาลาโมทริจีน
2) ได้ข้อมูลระดับยาลาโมทริจีนในเลือดของท่านเมื่อได้รับขนาดยาในปัจจุบันและสามารถใช้เป็นค่าอ้างอิงในการติดตามผลการรักษาและปรับขนาดยาให้ถูกต้องเหมาะสมกับผู้ป่วยแต่ละราย
3) ได้แบบจำลองทางเภสัชจลนศาสตร์ประชากร สามารถนำไปใช้ในการวางแผนการใช้ยาลาโมทริจีนทั้งกรณีที่ให้เป็นยาเดี่ยวหรือใช้ร่วมกับยาตัวอื่นเพื่อให้มีความเหมาะสมกับประชากรไทย

ค่าชดเชยแก่ผู้เข้าร่วมการวิจัย

การศึกษาวิจัยครั้งนี้จะให้ค่าชดเชยหรือค่าเดินทางแก่ผู้ป่วยแต่ละรายในการนัดหมายแต่ละครั้งเป็นจำนวนเงิน 250 บาทต่อครั้ง

ความเสี่ยงจากการเข้าร่วมการวิจัย

ความเสี่ยงในการเจาะเลือดคือ อาจมีอาการปวด หรือมีจ้ำเลือดบริเวณที่เจาะ แต่มีความเสี่ยงน้อยมากที่จะเกิดการติดเชื้อจากการเจาะเลือด ถ้าหากเกิดขึ้น ท่านจะได้รับการรักษาพยาบาลโดยแพทย์ผู้ทำหัตถการหรือแพทย์และบุคลากรทางการแพทย์คนอื่นที่ได้รับมอบหมาย

การเก็บตรวจดีเอ็นเอ อาจทำให้ท่านเกิดความกังวลว่าความลับในส่วนนี้จะถูกเปิดเผย งานวิจัยนี้จะตรวจเฉพาะยีนหรือสารทางพันธุกรรมที่เกี่ยวข้องกับการกำจัดยาที่ทำการศึกษาวิจัยคือ ยีน *UGT1A4* และ *UGT2B7* ข้อมูลของท่านจะถูกเก็บไว้เป็นความลับและจะใช้สำหรับงานวิจัยนี้เท่านั้น เลือดหรือสารสกัดดีเอ็นเอที่เหลือจากการวิจัยจะไม่มีการเก็บไว้

หากท่านไม่ต้องการเข้าร่วมการศึกษาวิจัย หรือเปลี่ยนใจระหว่างร่วมศึกษาวิจัย

ท่านไม่จำเป็นต้องเข้าร่วมการศึกษาวิจัยครั้งนี้หากท่านไม่สมัครใจ หลังจากตัดสินใจเข้าร่วมการศึกษาแล้วท่านสามารถถอนตัวได้ตลอดเวลา การตัดสินใจของท่านจะไม่มีผลต่อการรักษาในอนาคตหรือการดูแลอื่นใด หากท่านไม่ต้องการเข้าร่วมการศึกษาหรือต้องการหยุดการศึกษา ณ เวลาใดก็ตาม

การเก็บข้อมูลเป็นความลับ

ข้อมูลของท่านที่ถูกบันทึกไว้ระหว่างการศึกษาจะถูกเก็บไว้เป็นความลับตลอดเวลา เช่นเดียวกับข้อมูลที่เกี่ยวข้องจากแฟ้มเวชระเบียนของโรงพยาบาล คณะกรรมการจริยธรรมการวิจัยและพนักงานหรือผู้วิจัยสามารถขอตรวจสอบข้อมูลเหล่านี้ได้ โดยข้อมูลเหล่านี้จะยังเก็บรักษาไว้เป็นเรื่องลับเฉพาะ

ข้อมูลส่วนตัวที่ท่านไม่ต้องการเปิดเผยจะถูกเก็บรวบรวมไว้ในฐานข้อมูล และนำมาใช้เพื่อวัตถุประสงค์ทางการวิจัยทางการแพทย์เฉพาะในส่วนที่เกี่ยวข้องกับการศึกษา โดยจะมีการกำหนดสิทธิการเข้าถึงการใช้งานเฉพาะแพทย์ผู้ศึกษาวิจัยและบุคคลที่แพทย์ผู้ศึกษาวิจัยอนุญาตเท่านั้นที่จะมีรหัสผ่านในการเข้าถึงข้อมูล ทั้งนี้เพื่อวัตถุประสงค์ทางการศึกษาวิจัยทางการแพทย์

โดยไม่มีการอ้างถึงชื่อและเลขประจำตัวผู้ป่วยของท่าน ทางสถาบันประสาทวิทยาจะทำทุกวิถีทางเพื่อให้เกิดความมั่นใจว่าข้อมูลส่วนตัวของท่านจะถูกปกป้องไว้

หากท่านได้รับการปฏิบัติที่ไม่ตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงนี้ ท่านสามารถแจ้งให้ประธานคณะกรรมการจริยธรรมฯ ทราบได้ที่ สำนักงานคณะกรรมการจริยธรรมการวิจัยสถาบันประสาทวิทยา ตึกกุมารประสาทวิทยา ชั้น 4 โทร 02-3547076 ต่อ 2402

APPENDIX C

หนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัย

ข้าพเจ้า (นาย/นาง/นางสาว) _____ อายุ ____ ปี ที่อยู่บ้านเลขที่ _____ ถนน _____ ตำบล _____ อำเภอ _____ จังหวัด _____ โทรศัพท์_____

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย รวมทั้งประโยชน์ที่เกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้โดยสมัครใจและมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยเมื่อใดก็ได้ โดยการบอกเลิกการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการรักษาโรคและการรับบริการต่างๆที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้วและมีความเข้าใจดีทุกประการ 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. .....

ข้าพเจ้า.....อายุ.....ปี อนุญาตให้นายแพทย์/แพทย์หญิง.....เก็บตัวอย่างตรวจคือ.....จากข้าพเจ้า เพื่อประโยชน์ในการศึกษาวิจัยเรื่อง “ปัจจัยที่มีผลต่ออัตราส่วนความเข้มข้นของยาในเลือดต่อขนาดยาของยาลาโมทริจีนในผู้ป่วยชาวไทย” ที่ข้าพเจ้าเข้าร่วมในการวิจัย

ข้าพเจ้าได้รับทราบข้อมูลเกี่ยวกับการวิจัยดังกล่าวดังนี้

1. วัตถุประสงค์ในการวิจัย
2. ประโยชน์ที่คาดว่าจะได้รับ
3. การตรวจดังกล่าวจะกระทำโดยไม่เปิดเผยข้อมูลส่วนตัวของข้าพเจ้าแก่บุคคลอื่น ที่ไม่เกี่ยวข้องกับการวิจัย
4. การเก็บตัวอย่างตรวจนี้กระทำโดยการเจาะเลือดดำ ซึ่งมีผลข้างเคียงคือ ความเจ็บปวด เลือดซึม หรือการติดเชื้อ ซึ่งเกิดได้น้อยมาก และถ้าหากเกิดขึ้น ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยแพทย์ผู้ทำหัตถการหรือแพทย์และบุคลากรทางการแพทย์คนอื่นที่ได้รับมอบหมาย
5. การตรวจดีเอ็นเอจะตรวจเฉพาะยีน *UGT1A4* และ *UGT2B7* โดยเลือดหรือสารสกัดดีเอ็นเอที่เหลือจากการทำวิจัยจะไม่มีการเก็บไว้

ข้าพเจ้าได้รับทราบข้อมูลในเอกสารให้ความยินยอมนี้ และได้มีโอกาสซักถามแพทย์จนเข้าใจดี ข้าพเจ้าจึงลงนามไว้ข้างท้ายนี้เพื่อเป็นหลักฐาน

ลงชื่อ.....ผู้เข้าร่วมโครงการวิจัย/ผู้แทนโดยชอบธรรม

(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....แพทย์ผู้ทำการตรวจรักษา

(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน

(..... ชื่อ-นามสกุล ตัวบรรจง)

ลงชื่อ.....พยาน

(..... ชื่อ-นามสกุล ตัวบรรจง)

APPENDIX D

แบบบันทึกการเก็บข้อมูลการติดตามระดับยาลาโมทริจีนในเลือดของผู้ป่วย

ข้อมูลทั่วไปของผู้ป่วย

รหัสผู้ป่วย_____ เพศ ☐ หญิง ☐ ชาย เชื้อชาติ_____

วัน/เดือน/ปีเกิด_____ อายุ_____ปี น้ำหนัก_____ ส่วนสูง_____

CC : _____

HPI : _____

Underlying : _____

ALL: _____

V/S : T _____°C BP_____mmHg P_____/min RR_____/min

Diagnosis :_____

ความร่วมมือในการใช้ยา (จากแบบประเมิน MMAS)

☐ มีความร่วมมือดี (8 คะแนน)

☐ มีความร่วมมือปานกลาง (6-7 คะแนน)

☐ ความร่วมมือต่ำ (< 6 คะแนน)

สูบบุหรี่ ☐ ไม่เคยสูบ ☐ เคยสูบ แต่เลิกแล้วมา___ปี/เดือน ☐ สูบ วันละ ___ มวน

ดื่มแอลกอฮอล์ ☐ ไม่เคยดื่ม ☐ เคยดื่ม แต่เลิกแล้วมา___ปี/เดือน ☐ ดื่ม สัปดาห์ละ ___ แก้ว

สมุนไพร ☐ ไม่เคยใช้ ☐ เคยใช้ แต่เลิกแล้วมา___ปี/เดือน ☐ กำลังใช้ (_____)

อาหารเสริม ☐ ไม่เคยใช้ ☐ เคยใช้ แต่เลิกแล้วมา___ปี/เดือน ☐ กำลังใช้ (_____)

ผลตรวจทางห้องปฏิบัติการ

DATE				
Liver function	Aspartate aminotransferase; AST (0-42 U/L)			
	Alanine aminotransferase; ALT (0-48 U/L)			
Renal function	BUN (7-25 mg/dL)			
	Serum creatinine (0.7-1.4 mg/dL)			
	Creatinie clearance; CrCl (ml/min)			
Other				

ประวัติการใช้ยา Lamotrigine					
วันที่ได้รับยา	รูปแบบ	ขนาดยา (mg)	วิธีใช้ยา	เวลาที่ได้ยา	หมายเหตุ

ข้อมูลการได้รับยา Lamotrigine ก่อนเจาะเลือด			
Dose ที่	วันที่ได้รับยา	เวลาที่ได้รับยา	ขนาดยาที่ได้รับ (mg)
Dose 1 Dose 2 Dose 3			

การติดตามตรวจวัดระดับยา Lamotrigine ในเลือด			
วันที่เจาะเลือด	เวลาที่เจาะเลือด	ระดับยาในเลือด (mg/L)	หมายเหตุ

ยาอื่นที่ได้รับร่วมด้วย			
ชื่อยา	รูปแบบ/ขนาดยา (mg)/ วิธีใช้	วันที่เริ่มใช้	เวลาที่ได้ยา
☐ _____			
☐ _____			
☐ _____			
☐ _____			
☐ _____			
☐ _____			

การตรวจภาวะพหุสัณฐานของ *UGT1A4* และ *UGT2B7*	
***UGT1A4* 142T>G (L48V)**	***UGT2B7* -161C>T**
☐ Wide type/ Wide type (T/T) _____	☐ Wide type/ Wide type (C/C) _____
☐ Wide type/ Mutation (T/G) _____	☐ Wide type/ Mutation (C/T) _____
☐ Mutation/ Mutation (G/G) _____	☐ Mutation/ Mutation (T/T) _____
***UGT1A4* 70C>T (P24T)**	***UGT2B7* 372 A>G**
☐ Wide type/ Wide type (C/C) _____	☐ Wide type/ Wide type (A/A) _____
☐ Wide type/ Mutation (C/T) _____	☐ Wide type/ Mutation (A/G)_____
☐ Mutation/ Mutation (T/T) _____	☐ Mutation/ Mutation (G/G) _____

APPENDIX E

แบบวัดความร่วมมือในการใช้ยาของมอริสกี ชนิด 8 คำถาม
(MMAS ชนิด 8 คำถาม)

คำชี้แจงกรุณากาเครื่องหมาย √ ลงในกล่อง □ ในคำถามต่อไปนี้ให้ตรงกับความเป็นจริง

1. บางครั้งคุณลืมกินยาใช่ไหม

   □ ใช่ □ ไม่ใช่

2. บางครั้งคนไม่ได้กินยาเพราะมีเหตุผลอื่นที่ไม่ใช่การลืม ลองคิดย้อนหลังในช่วง 2 อาทิตย์ที่ผ่านมา มีวันใดบ้างไหมที่คุณไม่ได้กินยา

   □ มี □ ไม่มี

3. คุณเคยลดยาหรือหยุดกินยาโดยไม่ได้บอกหมอเพราะคุณรู้สึกแย่ลงเมื่อกินยาบ้างไหม

   □ เคย □ ไม่เคย

4. เมื่อคุณเดินทางหรือออกจากบ้าน บางครั้งคุณลืมเอายาไปด้วยใช่ไหม

   □ ใช่ □ ไม่ใช่

5. เมื่อวานนี้คุณกินยาหรือไม่

   □ กิน □ ไม่กิน

6. เมื่อคุณรู้สึกว่าอาการดีขึ้นหรือควบคุมอาการได้แล้ว บางครั้งคุณหยุดกินยาใช่ไหม

   □ ใช่ □ ไม่ใช่

7. การกินยาทุกวันเป็นความไม่สะดวกอย่างยิ่งสำหรับบางคน คุณเคยรู้สึกรำคาญที่ต้องเคร่งครัดกับการกินยารักษาโรคของคุณหรือไม่

   □ เคย □ ไม่เคย

8. คุณมีความลำบากในการจำว่าต้องกินยาทุกชนิดบ่อยแค่ไหน

   □ ไม่เคยเลย □ แทบไม่เคย □ บางครั้ง □ บ่อยครั้ง □ เป็นประจำ

การแปลผลคะแนนของ MMAS ชนิด 8 คำถาม

ข้อที่	คำถาม	การให้คะแนน
1.	บางครั้งคุณลืมกินยาใช่ไหม	ใช่ = 0, ไม่ใช่ = 1
2.	บางครั้งคนไม่ได้กินยาเพราะมีเหตุผลอื่นที่ไม่ใช่การลืม ลองคิดย้อนหลังในช่วง 2 อาทิตย์ที่ผ่านมา มีวันใดบ้างไหมที่คุณไม่ได้กินยา	มี = 0, ไม่มี = 1 เ
3.	คุณเคยลดยาหรือหยุดกินยาโดยไม่ได้บอกหมอเพราะคุณรู้สึกแย่ลงเมื่อกินยาบ้างไหม	คย = 0, ไม่เคย = 1
4.	เมื่อคุณเดินทางหรือออกจากบ้าน บางครั้งคุณลืมเอายาไปด้วยใช่ไหม	ใช่ = 0, ไม่ใช่ = 1
5.	เมื่อวานนี้คุณกินยาหรือไม่	กิน=1, ไม่กิน=0
6.	เมื่อคุณรู้สึกว่าอาการดีขึ้นหรือควบคุมอาการได้แล้ว บางครั้งคุณหยุดกินยาใช่ไหม	ใช่ = 0, ไม่ใช่ =1
7.	การกินยาทุกวันเป็นความไม่สะดวกอย่างยิ่งสำหรับบางคน คุณเคยรู้สึกรำคาญที่ต้องเคร่งครัดกับการกินยารักษาโรคของคุณหรือไม่	เคย = 0, ไม่เคย = 1
8.	คุณมีความลำบากในการจำว่าต้องกินยาทุกชนิดบ่อยแค่ไหน	ไม่เคยเลย =1 แทบไม่เคย = 0.75 บางครั้ง = 0.5 บ่อยครั้ง = 0.25 เป็นประจำ = 0
	รวมคะแนนความร่วมมือในการใช้ยา	
	คะแนนต่ำกว่า 6 บ่งชี้ว่า ผู้ป่วยมีความร่วมมือในการใช้ยาต่ำ	
	คะแนน 6-7 บ่งชี้ว่า ผู้ป่วยมีความร่วมมือในการใช้ยาปานกลาง	
	คะแนน 8 บ่งชี้ว่า ผู้ป่วยมีความร่วมมือในการใช้ยาดี	

APPENDIX F

Determination of lamotrigine concentration and method validation

Determination of lamotrigine plasma concentration

The determination of lamotrigine plasma concentration was performed using HPLC with UV detection method.

1. **Materials**

Chemical and reagents

1) Lamotrigine standard
2) Phenobarbital (as the internal standard)
3) Acetonitrile (ACN); HPLC grade
4) Methanol (MeOH) ; HPLC grade
5) Dichloromethane; HPLC grade
6) Diethyl ether; Analytical Grade
7) Potassium dihydrogen phosphate (KH_2PO_4); Analytical Grade

Instruments

1)	High Performance Liquid Chromatographic System, Surveyor® Thermo Electron Corporation		USA
2)	Freezer -20°C, FR-148E	Sharp Corporation	Indonesia
3)	Freezer -20°C, MF-U14B Mitsubishi	Electric Kanyong Watana	Thailand
4)	Deep Freezer -80°C, ULT-2586-9-V40	Revco	USA
5)	Analytical Balance, XP 105 DR	Mettler Toledo	Switzerland
6)	Analytical Balance, ED 224S	Sartorius	Germany
7)	Vortex, Zx^3 VELP®	Scientifica	Italy
8)	Speed Evaporator (Centrivap®), LCC-1 7812011	Labconco Corporation	USA
9)	Refrigerated Centrifuge, Z383K	Hermle Labortechnik	Germany

10) Sonicator, DSC-106 D.S.C. Group Co., Ltd., Thailand

11) Water Purification System	Millipore S.A.S.	France
12) Autopipette	Mettler Toledo	USA

Apparatus

1) Volumetric flask (5, 10, 1000 mL)
2) Cylinder (50, 100 mL)
3) Glass bottle, screw cap (100, 250, 500, 1000 mL)
4) Beaker (10, 25, 250, 600 mL)
5) Microcentrifuge tube (1.5 mL)
6) Glass test tube, screw cap (16x100, 12x75 mm)
7) Plastic centrifuge tube (50 mL)
8) Disposable plastic pipette tip (250, 1000, 5000 mcL)
9) 0.22-µm Nylon membrane filter (47-mm)
10) Screw-thread vial (1.5 mL)
11) Insert vial (100 mcL)

2. Analytical method

Total plasma lamotrigine concentration determination was developed in the therapeutic drug monitoring laboratory of Medica Innova Co., Ltd., Bangkok Thailand. (Good Laboratory Practice certified by the Departement of Medical Sciences)

1) Adding 50 mcL of internal standard (phenobarbital 10000 ng/mL), to 300 mcL of plasma sample and vortex mixing at 40 hertz, 10 seconds.
2) Add 4000 µL of diethyl ether:dichloromethane (70:30, v/v) and vortex at 40 hertz, 30 seconds.
3) Centrifuge the resulting solution at 5000 rpm, 4°C, 5 min and kept in freezer at below -70°C for 15 min.
4) Transfer organic layer to 12 x 75-mm glass test tube and then evaporate at 50°C for 50 min.
5) Reconstitute with 200 mcL of 80% MeOH and vortex at 40 hertz, 10 seconds.

6) Transfer solution into 100 mcL insert vial

7) A volume of 20 mcL of solution was injected into HPLC

3. Method Validation

Table A Summary result of method validation

Item	Result
Analysis	Lamotrigine
Internal standard	Phenobarbital
Method description	Rosuvastatin was extracted from human plasma by liquid-liquid extracting technique using diethyl ether: dichloromethane (70:30, v/v). An aliquot of 20 µL was analyzed by reverse phase C18, HPLC.
QC sample, concentration (ng/mL)	QCL = 150 ng/mL QCL = 1500 ng/mL QCL = 3000 ng/mL
Selectivity	No interfering peak was observed in each source of plasma
Carry over	The method showed no carry over
Intra-batch: accuracy range (%)	94.96 – 101.50%
Intra-batch: precision range (%)	0.94 – 3.31%
Inter-batch: accuracy range (%)	97.94 – 99.68%
Inter-batch: precision range (%)	1.45 – 2.83%
Recovery for rosuvastatin (%)	92.77%, CV (%) = 2.35%
Recovery for internal standard (%)	99.11%, CV (%) = 1.02%
Range of calibration curve (ng/mL)	50 – 4000 ng/mL, $r^2 \geq 0.9993$
Regression analysis	Linear regression, weight 1/x
Lower limit of quantification (ng/mL)	50 ng/mL
Freeze-thaw stability (cycles)	3 cycles
Short-term stability (hours)	8 hours at room temperature
Long-term stability (months)	1 month (Plan of long-term stability study is 4 months)
Stock solution stability (hours)	6 hours at room temperature, 1 month at -20ºC
Working solution stability (hours)	6 hours at room temperature, 1 month at -20ºC
Post-preparative stability (hours)	24 hours at 4°C (in autosampler)

Table B Summary result of accuracy and precision

QC sample	QCL			QCM			QCH		
Batch number	1	2	3	1	2	3	1	2	3
Concentration (ng/mL)	151.3485			1513.4850			3026.9700		
Calculated concentration (ng/mL)	150.6338	139.6487	151.2586	1485.2179	1505.5270	1441.7758	3035.8186	3025.6540	3025.1268
	154.7477	148.0495	148.3266	1506.2387	1513.9455	1437.6059	3064.5133	3111.2190	2958.2893
	144.6695	142.8380	154.5414	1555.6199	1483.1467	1500.3083	3088.7471	3005.7452	2864.7924
	154.1093	151.0124	146.7926	1528.2300	1469.9265	1430.4132	3111.6658	2997.6330	2951.8515
	145.8089	149.7699	149.2017	1524.7362	1477.1189	1375.5478	3061.7524	3014.5549	2941.8283
Intra-batch Analysis									
Mean of calculated concentration (ng/mL)	149.9939	146.2637	150.0242	1520.0086	1489.9329	1437.1302	3072.4994	3030.9612	2948.3777
Precision: CV (%)	3.09	3.31	2.00	1.73	1.27	3.08	0.94	1.52	1.94
Mean of accuracy (%)	99.10	96.64	99.12	100.43	98.44	94.96	101.50	100.13	97.40
Inter-batch Analysis									
Mean of calculated concentration (ng/mL)	148.7606			1482.3572			3017.2794		
Precision: CV (%)	1.45			2.83			2.09		
Mean of accuracy (%)	98.29			97.94			99.68		

Table C Summary the limit of quantification *(*LOQ*)*

Intra-batch Analysis			
Batch number	1	2	3
Concentration (ng/mL)	50.4495	50.4495	50.4495
Accuracy: mean of accuracy (%)	94.22	93.05	102.72
Precision: CV (%)	2.22	6.42	3.02
Inter-batch analysis			
Mean of accuracy (%)	96.66		
Precision: CV (%)	5.46		

APPENDIX G

DNA extraction

The DNA were extracted using QIAamp® DNA Blood Mini kit (Qiagen, Hilden, Germany) by the following procedure as recommended by the manufacturer.

1. Materials

Chemical and reagents

1.	Absolute etanol (100%)	Carlo erba	Italy
2.	Buffer AL	Qiagen	Germany
3.	Buffer AW1	Qiagen	Germany
4.	Buffer AW2	Qiagen	Germany
5.	Buffer AE	Qiagen	Germany
6.	QIAGEN®protease	Qiagen	Germany
7.	Protease solvent	Qiagen	Germany

Apparatus

1.	Centrifuge (Universal 320)	Hettick	Germany
2.	Vortex mixer (S0100-220)	Labnet	USA
3.	Heating block (Dri-block DB-2D)	Techne	UK
4.	Microcentrifuge (5415R)	Eppendorf	Germany
5.	Spectrophotometer(Smart spec 3000 Bio-rad ™)		USA
6.	Freezer	Sanyo	Japan
7.	Real-Time PCR system (Applied Biosystems 7500)		USA

Supplies

1.	Microcentrifuge tubes 1.5 mL	Treff AG.	Switzerland
2.	Pipette tips (Blue and Yellow)	ScientificPlastics	USA
3.	Micropipette 1,000 mcL	Eppendorf	Germany

4.	Micropipette 200 mcL	Eppendorf	Germany
5.	Micropipette 20 mcL	Eppendorf	Germany
6.	QIAamp Mini spin Columns	Qiagen	Germany
7.	Collection tubes 2 mL	Qiagen	Germany
8.	Disposable gloves		

2. DNA Extraction method

2.1 Prepare samples and equilibrate reagents at room temperature.

2.2 Pipette 20 mcL QIAGEN Protease into a 1.5 mL microcentrifuge tube

2.3 Add 200 mcL buffy coat to the microcentrifuge tube.

2.4 Add 200 mcL Buffer AL to the sample and mix by vortex mixer for 15 seconds.

2.5 Incubate by heating block at 56°C for 10 minutes and briefly centrifuge to remove drops from the inside of the lid.

2.6 Add 200 mcL 100% ethanol to the sample then mix by vortex mixer for 15 seconds, and briefly centrifuge to remove drops from the inside of the lid.

2.7 Pipette the mixture to the QIAamp Mini spin column (in a 2 mL collection tube) and centrifuge at 6000 x g (8000 rpm) for 1 minute.

2.8 Place the QIAamp Mini spin column in a new 2 mL collection tube and dispose of the old tube containing the filtrate.

2.9 Add 500 mcL Buffer AW1 to the QIAamp Mini spin column and centrifuge at 6000 x g (8000 rpm) for 1 minute.

2.10 Place the QIAamp Mini spin column in a new 2 mL collection tube and dispose of the old tube containing the filtrate.

2.11 Add 500 mcL Buffer AW2 to the QIAamp Mini spin column and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes.

2.12 Place the QIAamp Mini spin column in a new 2 mL collection tube and dispose of the old tube containing the filtrate. Centrifuge at full speed for 1 minute.

2.13 Place the QIAamp Mini spin column in a new 1.5 mL microcentrifuge tube and dispose of the old tube containing the filtrate.

2.14 Add 200 mcL Buffer AE (the elution buffer for genomic DNA) to the QIAamp Mini spin column.

2.15 Incubate at room temperature for 1 minute then centrifuge at 6000 x g (8000 rpm) for 1 minute.

2.16 Storing DNA (in Buffer AE) at –20°C until genotyping.

APPENDIX H

UGT1A4 Genotyping analysis

The single nucleotide polymorphisms (SNPs) detection was carried out by Taqman allelic discrimination assays with fluorogenic probes (Applied Biosystems, Foster City, CA). The probe primers for all 4 SNPs were designed by Applied Biosystems.

Two polymorphisms of *UGT1A4* were investigated as following

1. *UGT1A4* 142T>G (L48V) SNP Assay: rs2011425
2. *UGT1A4* 70C>T (P24) SNP Assay: rs6755571

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 assays are designed for the allelic discrimination of specific 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.

Chemical and reagents

1. Custom TaqMan® SNP Genotyping Assays, 375 rxn
   Applied Biosystems USA
2. TagMan Drug Metabolism Genotyping Assays, 187 rxn
   Applied Biosystems USA
3. TaqMan® Universal PCR Master Mix (1 x 5 mL), 500 rxn at 20 mcL
   Applied Biosystems USA

Apparatus

1. MicroAmp Fast Optical 96-well Reaction plates
2. MicroAmp Optical Adhesive Film
3. Vortex mixer
4. Real-Time PCR system (Applied Biosystems 7500) USA

Supplies

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

Table A. Allelic Discrimination PCR Reaction

Reaction Components	Volume/Well (20 mcL volume reaction)	Final concentration
TaqMan® Universal PCR Master Mix (2X)	10 mcL	1 X
20 X TaqMan® Drug metabolism Genotyping Assay Mix	0.5 mcL	1 X
Genomic DNA (10 ng/mcL) **	2 mcL	20 ng
dH_2O	7.5 mcL	-
Total	20 mcL	-

* 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.

Procedure

To prepare the reaction components for one reaction refer to the table A. The ABI PRISM® 7900HT Sequence Detection System uses 5 mcL in a 384 well plate. The Applied Biosystems 7300 and 7500 Real-Time PCR System and ABI PRISM® 7000 Sequence Detection System use 25 mcL reactions in a 96 well plate.

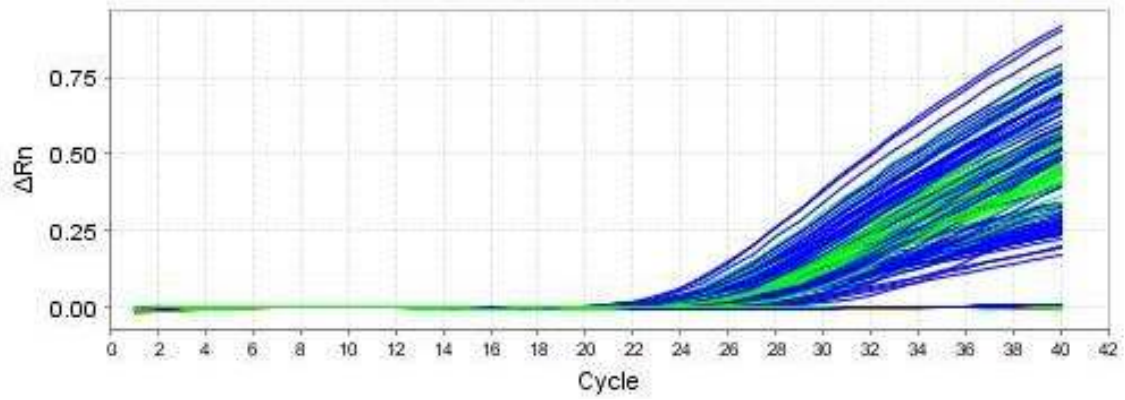
Table B. Thermal Cycler Conditions

Times and Temperatures		
Initial Steps	Denature	Anneal/Extend
HOLD	50 CYCLES	
10 min 95 ^{0}C	15 sec 92 ^{0}C	90 sec 60 ^{0}C

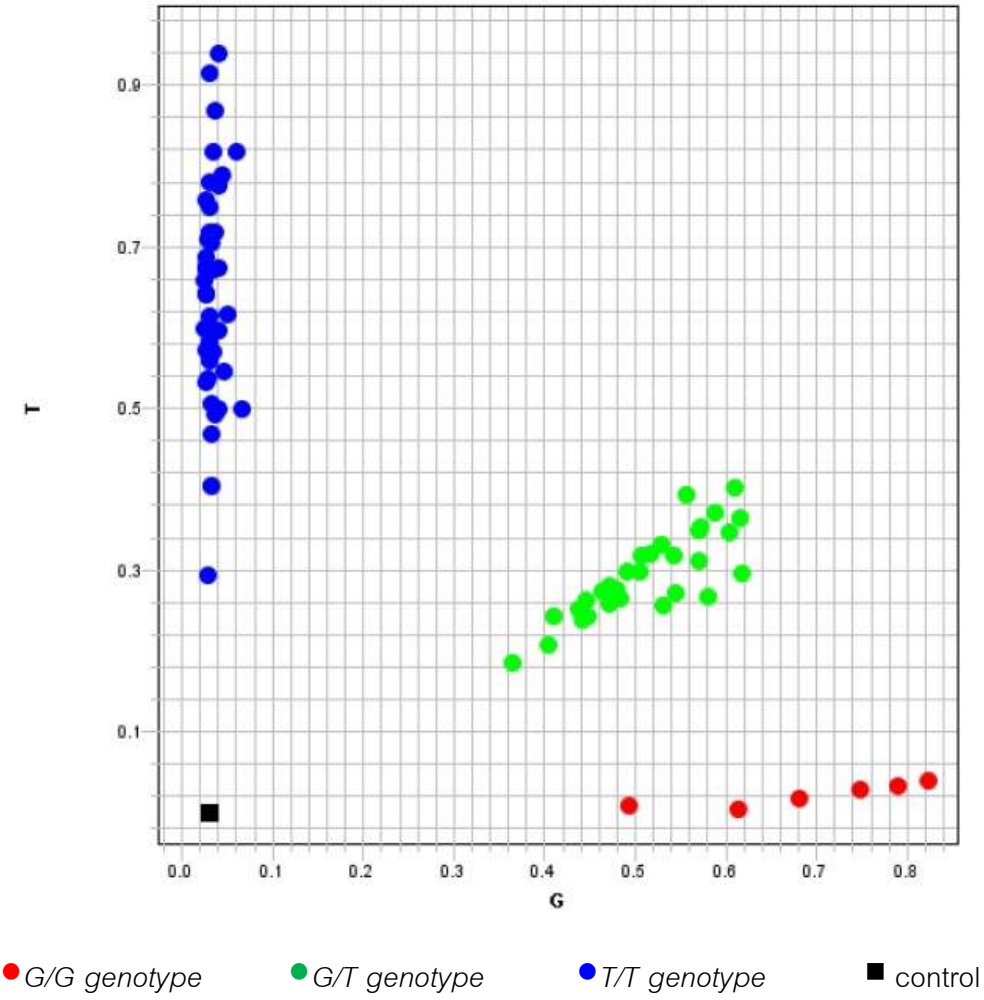
† Note: If using TaqMan® Universal Master Mix (P/N 4304437), add a 2 min @ 50°C HOLD step prior to the initial 10 min @ 95°C HOLD step.

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

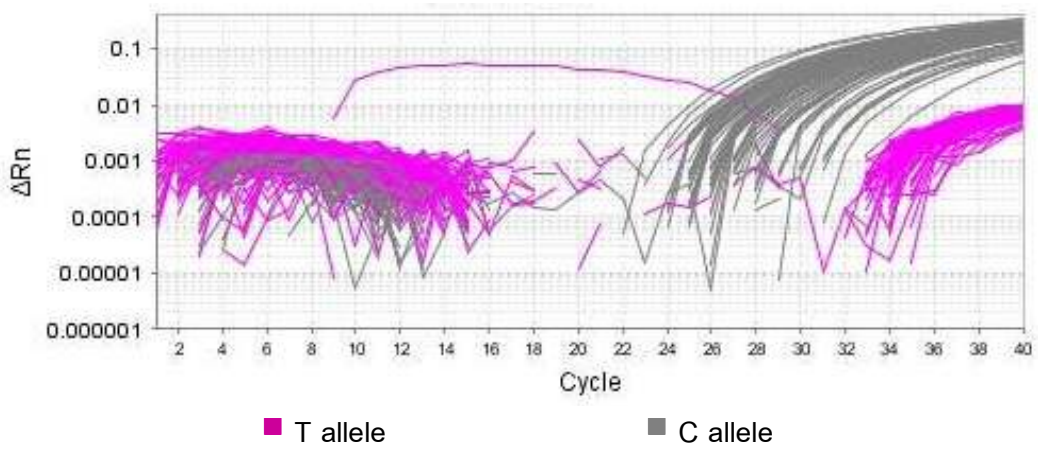
Amplification plot of *UGT1A4* 142T>G



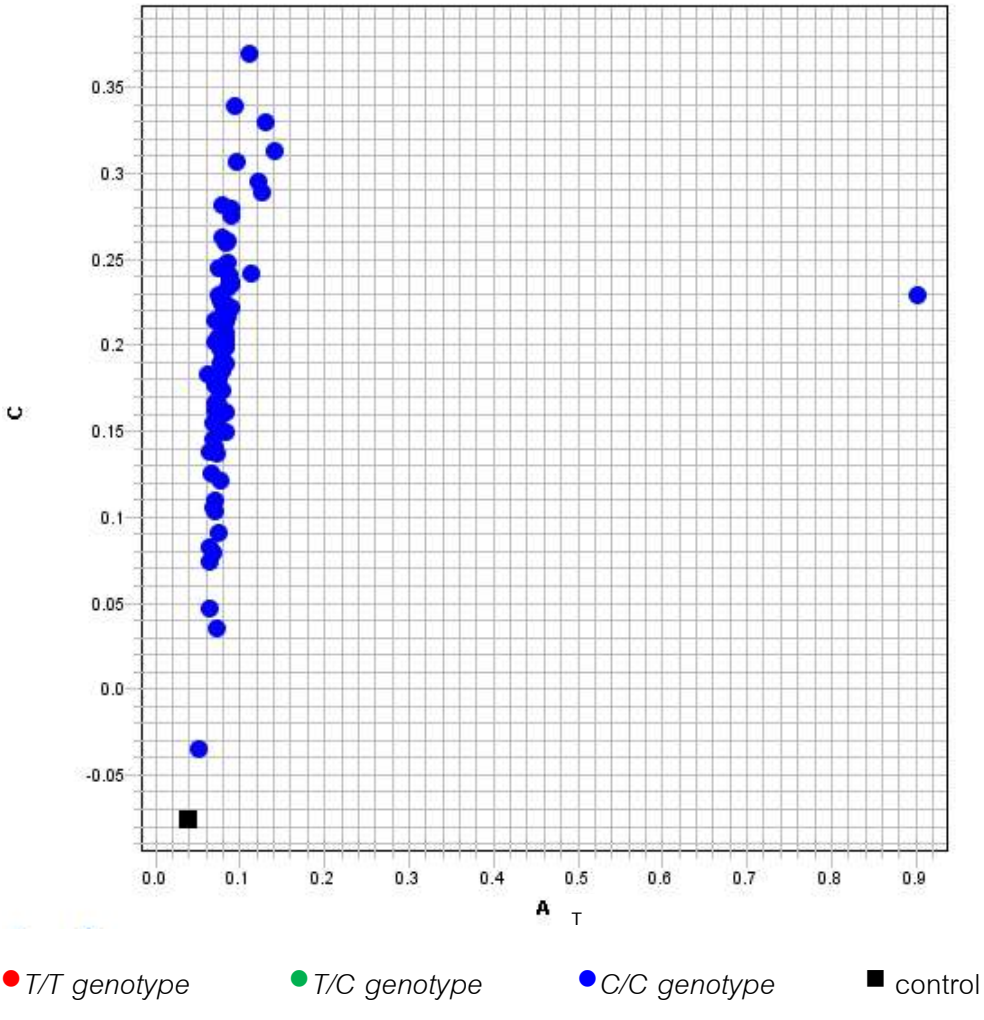
Allelic discrimination plot of *UGT1A4* 142T>G



Amplification plot of *UGT1A4* 70C>T



Allelic discrimination plot of *UGT1A4* 70C>T



VITAE

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