ผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อการเกิดอันตรกิริยาทางเภสัชจลนศาสตร์
ของยาไซโคลสพอรินกับยาดิลไทอะเซ็ม ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต

นางสาวไพลิน วรรณประพันธ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ปีการศึกษา 2554

EFFECT OF *CYP3A5* POLYMORPHISMS ON PHARMACOKINETIC INTERACTION BETWEEN CYCLOSPORINE AND DILTIAZEM IN THAI RENAL ALLOGRAFT RECIPIENTS

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Thesis Title	EFFECT OF *CYP3A5* POLYMORPHISMS ON PHARMACOKINETIC INTERACTION BETWEEN CYCLOSPORINE AND DILTIAZEM IN THAI RENAL ALLOGRAFT RECIPIENTS
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ไพลิน วรรณประพันธ์ : ผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อการเกิดอันตรกิริยาทางเภสัชจลนศาสตร์ของยาไซโคลสพอรินกับยาดิลไทอะเซ็ม ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต (EFFECT OF *CYP3A5* POLYMORPHISMS ON PHARMACOKINETIC INTERACTION BETWEEN CYCLOSPORINE AND DILTIAZEM IN THAI RENAL ALLOGRAFT RECIPIENTS) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ภญ. ดร.ดวงจิตต์ พนมวัน ณ อยุธยา, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : นพ.วิรุฬห์ มาวิจักขณ์ , 93 หน้า.

ในปัจจุบันมีการใช้ไซโคลสพอรินร่วมกับดิลไทอะเซ็มมากขึ้น โดยไซโคลสพอรินและดิลไทอะเซ็มมีการกำจัดยาทางตับเป็นหลักผ่านการทำงานของเอ็นไซม์ Cytochrome P450 CYP3A subfamily ได้แก่ CYP3A4 และ CYP3A5 การใช้ดิลไทอะเซ็มจึงส่งผลในการลดขนาดยาต่อวันของไซโคลสพอรินเนื่องจากดิลไทอะเซ็มมีผลในการยับยั้งการทำงานของเอ็นไซม์ CYP3A5 ส่งผลให้มูลค่าการรักษาผู้ป่วยด้วยไซโคลสพอรินลดลง ดังนั้นในผู้ป่วยปลูกถ่ายไตที่มีจีโนไทป์ของยีน *CYP3A5* ที่แตกต่างกัน น่าจะส่งผลทำให้มีระดับยาไซโคลสพอรินในเลือดที่แตกต่างกัน เนื่องจากในประเทศไทยยังไม่เคยมีการศึกษาเกี่ยวกับผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อระดับยาไซโคลสพอรินในผู้ป่วยปลูกถ่ายไตทั้งในกรณีที่ไม่ใช้ดิลไทอะเซ็ม หรือใช้ไซโคลสพอรินร่วมกับดิลไทอะเซ็ม

วัตถุประสงค์ในการศึกษานี้คือ ศึกษาถึงความแตกต่างของการเกิดปฏิกิริยาระหว่างยาไซโคลสพอรินและดิลไทอะเซ็มในผู้ป่วยไทยที่ปลูกถ่ายไตที่มีจีโนไทป์ของยีน *CYP3A5* ที่แตกต่างกัน โดยวัดผลจากค่าความแตกต่างของอัตราส่วนระหว่างระดับยาไซโคลสพอรินที่ชั่วโมงที่ 2 หลังรับประทานยาต่อขนาดยาต่อวัน (Dose-adjusted C_2) ทั้งก่อนใช้ดิลไทอะเซ็ม และเมื่อใช้ไซโคลสพอรินร่วมกับดิลไทอะเซ็มในขนาด 30 มิลลิกรัมต่อวัน เป็นระยะเวลา 1 เดือน จากผลการศึกษาพบว่าค่าอัตราส่วนระหว่างระดับยาไซโคลสพอรินที่ชั่วโมงที่ 2 หลังรับประทานยาต่อขนาดยาต่อวัน ในผู้ป่วยปลูกถ่ายไตที่มีจีโนไทป์แบบ *CYP3A5*1/*1* ก่อนและหลังได้รับดิลไทอะเซ็มมีแนวโน้มสูงขึ้นแต่ไม่พบนัยสำคัญทางสถิติ (188.10±87.93 และ 217.88±58.67 นก./มล. ต่อ มก./กก./วัน ตามลำดับ, p= 0.107) ในขณะที่ไม่พบความแตกต่างของค่าอัตราส่วนระหว่างระดับยาไซโคลสพอรินที่ชั่วโมงที่ 2 หลังรับประทานยาต่อขนาดยาต่อวัน ก่อนและหลังได้รับดิลไทอะเซ็มในผู้ป่วยปลูกถ่ายไตที่มีจีโนไทป์แบบ *CYP3A5*1/*3* (349.63±158.36 และ304.12±105.89 นก./มล. ต่อ มก./กก./วัน ตามลำดับ, p=0.367) และในผู้ป่วยปลูกถ่ายไตที่มีจีโนไทป์แบบ *CYP3A5*3/*3* (298.91±131.37 และ 316.61±120.73 นก./มล. ต่อ มก./กก./วัน ตามลำดับ , p=0.535)

ดังนั้นดิลไทอะเซ็มส่งผลต่อค่าอัตราส่วนระหว่างระดับยาไซโคลสพอรินที่ชั่วโมงที่ 2 หลังรับประทานยาต่อขนาดยาต่อวัน ในผู้ป่วยปลูกถ่ายไตที่มีจีโนไทป์แบบ *CYP3A5*1/*1* มากกว่าผู้ป่วยปลูกถ่ายไต ที่มีจีโนไทป์แบบ *CYP3A5*1/*3* และ *CYP3A5*3/*3* ดังนั้นเมื่อมีการใช้ไซโคลสพอรินร่วมกับดิลไทอะเซ็มในผู้ป่วยที่มีจีโนไทป์แบบ *CYP3A5*1/*1* มีความจำเป็นในการเฝ้าระวังค่าระดับยาไซโคลสพอรินที่อาจเพิ่มสูงขึ้น และควรมีการปรับขนาดยาไซโคลสพอรินเพื่อป้องกันอาการไม่พึงประสงค์โดยเฉพาะอย่างยิ่งเมื่อใช้ร่วมกับดิลไทอะเซ็มในขนาดยาต่อวันที่สูงขึ้น

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

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

ปีการศึกษา.....2554..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KEYWORDS : CYP3A5/ POLYMORPHISM / CYCLOSPORINE / DILTIAZEM / TRANSPLANTATION

PAILIN WANNAPRAPHAN : EFFECT OF *CYP3A5* POLYMORPHISMS ON PHARMACOKINETIC INTERACTION BETWEEN CYCLOSPORINE AND DILTIAZEM IN THAI RENAL ALLOGRAFT RECIPIENTS.

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

CO-ADVISOR : VIROON MAVICHAK, M.D., 93 pp.

Cyclosporine (CsA) is frequently coadministration with Diltiazem (DTZ) because the latter has possible beneficial effect on the economic impact associated with reduction of the daily dose of CsA. The interaction between CsA and DTZ results in increased CsA blood concentration due to the CYP3A5 inhibitory effect of DTZ. Studies about the effect of *CYP3A5* polymorphism on CsA pharmacokinetics when comedication with DTZ have not been clearly defined. In Thailand there has never been study about the effect of *CYP3A5* polymorphism on CsA blood level at 2 hour post dose (C_2) either in patient using CsA alone or concurrently use with DTZ.

The purpose of this study was to compare the effect of DTZ on CsA level-to-dose ratio (dose-adjusted C_2) in patients with different *CYP3A5* genotype. The outcome was to determine the difference in CsA C_2 before and after coadministration with DTZ 30mg/day for 1 month (without any change in CsA dosage regimen). The results indicated that dose-adjusted C_2 showed the trend increased in *CYP3A5*1/*1* patients (N=5) even though this increment was not reaching the statistically significant level which might due to the small sample size (188.10±87.93 vs 217.88±58.67 ng/ml per mg/kg/day, p= 0.107). In contrast, dose-adjusted C_2 in *CYP3A5*1/*3* and *CYP3A5*3/*3* patients were less affected, dose-adjusted C_2 before and after DTZ used were 349.63±158.36 vs 304.12±105.89 ng/ml per mg/kg/day respectively, p=0.367 in *CYP3A5*1/*3* patients (N=13) while in *CYP3A5*3/*3* patients (N=20) dose-adjusted C_2 before and after DTZ used were 298.91±131.37 vs 316.61±120.73 ng/ml per mg/kg/day, p=0.535.

In conclusion, the effect of DTZ as CsA-sparing agent show more prominent effect in patients carrying *CYP3A5*1/*1* as compared to the others; the dose-adjusted C_2were higher when the drug was concurrently used even with low dose of DTZ (30mg/day). CsA level must be closely monitored and CsA daily dose should be adjusted accordingly to prevent toxicity of CsA overdose, especially when DTZ is coadministered in a higher dosage.

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

ANOVA	=	Analysis of Variance
CsA	=	Cyclosporine
CYP1A2	=	Cytochrome P450, family 1, subfamily A, polypeptide 2
CYP2C8	=	Cytochrome P450, family 2, subfamily C, polypeptide 8
CYP3A4	=	Cytochrome P450, family 3, subfamily A, polypeptide 4
CYP3A5	=	Cytochrome P450, family 3, subfamily A, polypeptide 5
ddH_2O	=	Double distilled water
DNA	=	Deoxyribonucleic acid
DTZ	=	Diltiazem
EDTA	=	Ethylenediaminetetraacetic acid
HWE	=	Hardy-Weinberg Equilibrium
mcl	=	microlite
ml	=	millilite
mRNA	=	messenger Ribonucleic acid
ng	=	nanogram
OD	=	Optical Density
PCR	=	Polymerase Chain Reaction
SNP	=	Single Nucleotide Polymorphism

CHARPTER I
INTRODUCTION

Background and Rationale

Cyclosporine (CsA) is a potent immunosuppressant drug widely used in organ transplantation and some autoimmune diseases. CsA was first introduced for the prevention of graft rejection since 1970's and has had a major impact on the result of solid organ transplantation. While graft survival results are generally better than those achieved with older immunosuppressive drugs, the costs of maintaining grafts with CsA are much greater.[1-5] However, dosage of CsA is complicated by intra- and interindividual variability of its pharmacokinetics and by the narrow therapeutic range to avoid unadequated immunosuppression and toxicity. For this reason, attention to the CsA blood concentration is essential for optimization.

CsA is metabolized by a cytochrome P450 3A (CYP3A) subfamilies CYP3A4 and CYP3A5 subfamilies in both liver and enterocyte[6-8]and many drug interactions occur via this isoenzymes. In particular, ketoconazole and diltiazem (DTZ) are inhibitors of CYP3A4 and CYP3A5[9] and both have been shown to elevate blood CsA concentration. Drugs which affect CsA pharmacokinetics were initially seen as relatively contraindicated, but once the economic potential was realized, deliberate coprescription of drugs to allow a reduction in CsA dosage were soon advocated. The decision to choose these agents is also based upon the potential for additional therapeutic benefit and/or adverse effect.

Calcium channel blockers (CCBs) are effective antihypertensive drugs in renal transplant recipients treated with CsA. CCBs have been reported to have beneficial in term of graft survival, potentially, in part due to their ability to control blood pressure and effectively increasing glomerular filtration rate (GFR) in transplant recipient in the post-transplant period.[10-11] Recently, McDonald et al. and Song et al.[10,12] have demonstrated that renal transplants who were on CsA sparing agent, DTZ, had a better renal allograft outcome than those who were not on DTZ.

Because of the blood concentration of CsA reflect motality, efficacy, adverse reactions and infections.[13-15] Pharmacokinetics studies based on therapeutic drug monitoring (TDM) have been conducted for many year. However, these population pharmacokinetic model was shown

to have only limited predictive value with regard to explaining the variability of CsA dose/drug concentration. In addition, a fundamental limitation of traditional TDM is that it can only be started when an immunosuppressant is administered, and so, can not be used for the prediction of individualized initial dosage. Therefore, an alternative is required for post-transplant management using these immunosuppressants, especially the starting of the optimum dosage regimen.

The clinical application of pharmacogenomic provides an option for improving the large variation in individualized medication including immunosuppressive therapy after organ transplantation. There are many studies have demonstrated that some genetic information is related to the inter- and intra- individual variation in the pharmacokinetics of CsA.

Both CsA and DTZ are mainly metabolized by the liver via CYP3A subfamily; CYP3A4 and CYP3A5 which an amino acid sequence identity of approximately 85% and largely overlapping substrates.[16] There are many pharmacogenetic study attemping to correlate these single nucleotide polymorphism (SNP) of *CYP3A4* and *CYP3A5* genes with the pharmacokinetic parameter of CsA. Attemping to link SNP in the *CYP3A4* gene (especially *CYP3A4*1B)* has been extensively studied and shows mostly negative results on CsA pharmacokinetics.[17-19] While, recently pharmacogenomic studies of the *CYP3A5* polymorphism found the effect on CsA level to dose ratio. Hu et al[21] reported that CsA dose-adjusted C_0 ratio was higher in *CYP3A5* non-expressor (*CYP3A5*3/*3*) than expressors (*CYP3A5*1/*1* and *CYP3A5*1/*3*) in the first week after renal transplantation (9.8-85.8 ng/ml per mg/kg VS 9.0-61.0 ng/ml per mg/kg ; p=0.012, Krusskal-Wallis test) and Min et al[22] reported that CsA clearance in renal transplant patient was larger in patients with *CYP3A5*1* than *CYP3A5*3* (15.6±3.1 ml/hr VS 12.0±2.3 ml/hr)

The concurrent use of DTZ has been reported to allow blood CsA concentrations to be maintained while reducing CsA dosage by approximately 30%[23] but Jones TE[24] reported that DTZ did not provide any CsA-sparing effect in some patients. Studies about the effect of *CYP3A5* polymorphism on CsA pharmacokinetics when co-medication with DTZ have not been clearly defined. In Thailand there has never been study about the effect of *CYP3A5* polymorphism on CsA level to dose ratio either in patients use CsA or concurrent use CsA and DTZ. Knowledge about the effect of *CYP3A5* polymorphism on CsA pharmacokinetics may be useful in therapeutic plans to avoid serum drug concentration-related adverse effect and reduce inappropriate dosage. The purpose of this study was to determine the effect of *CYP3A5*

polymorphism on the interaction between CsA and DTZ. The ultimate goal is to provide a better prediction for optimum dosage regimen for individual patient.

Hypothesis

The null hypothesis is the effect of DTZ on CsA level-to-dose ratio was not different between renal transplant patients with *CYP3A5*1* and *CYP3A5*3* alleles.

Objective

To compare the effect of DTZ on CsA level-to-dose ratio between renal transplant patients with *CYP3A5*1* and *CYP3A5*3* alleles.

Significant of the study

1. Information about the initial CsA dosage regimen in patients with *CYP3A5*1* and *CYP3A5*3*

2. Information about the different between CsA level-to-dose ratio in patients with *CYP3A5*1* and *CYP3A5*3* may be useful for the dosage regimen plans.

3. Information about the different between CsA level-to-dose ratio when concurrent use with DTZ in patients with *CYP3A5*1* and *CYP3A5*3* may be useful for the dosage regimen plans.

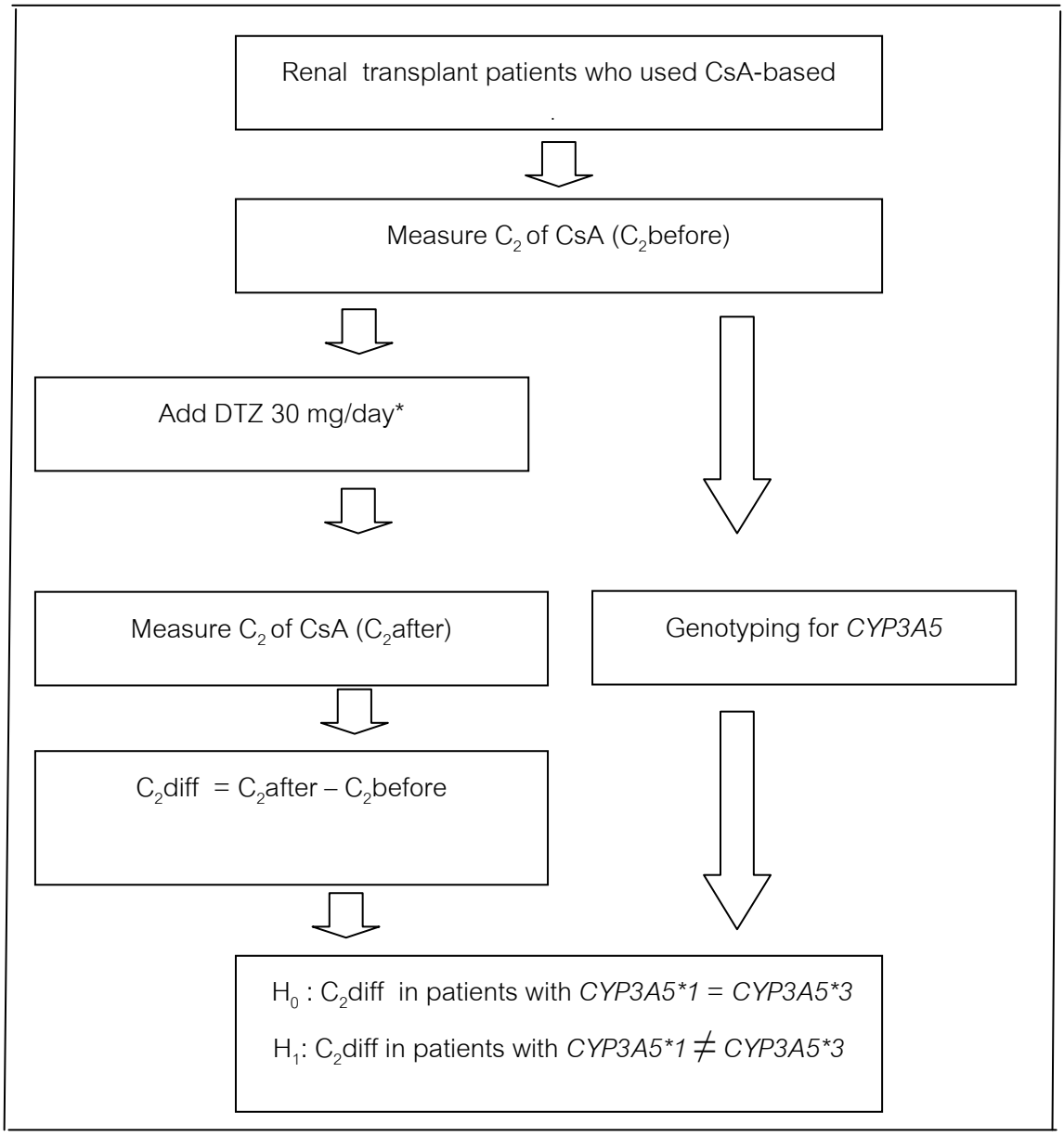
Scope of this study

1. Population of this study are the renal transplant out-patients at Praram 9 Hospital who used CsA-based regimen for immunosuppression.

2. Variables of this study: Dependent variables are CsA level-to-dose ratio. Independent variables are *CYP3A5* polymorphism and demographic data.

Conceptual framework

Conceptual framework is shown in **figure 1.**



* Take DTZ with morning dose CsA

C_2 = CsA blood concentration at 2-hour after take CsA

DTZ = diltiazem,

CsA = cyclosporine

C_2before = CsA C_2 before DTZ used

C_2after = CsA C_2 after DTZ used

Figure1 ; Conceptual framework

Operational definition

1. *CYP3A5* polymorphism is genotype that control CYP3A5 enzyme producing which has single-nucleotide polymorphism; *CYP3A5*3* allele is substitute amino acid at intron 3 (6986A>G) when the reference allele is *CYP3A5*1.*

2. Level-to-dose ratio or Dose-adjusted C_2 is a ratio of CsA blood level at 2 hour post dose (C_2) and the weight-adjusted dose per day of CsA (ng/ml per mg/kg/day)

4. CsA C_2 blood concentration measurement is a measurement of CsA blood concentration at 2 hour after CsA oral administration. (the time to taking blood sample not more than 10 minutes from the time of exactly C_2 measuring)

5. C_2before is a measurement of CsA blood concentration at 2 hour after CsA oral administration before adding of DTZ and patients are not changed the dosage of CsA within 2 times of follow up of therapy before included to the study.

6. C_2after is a measurement of CsA blood concentration at 2 hour after CsA oral administration after receiving DTZ 30 mg/day for at least 1 month with any change of the dosage regimen of CsA and other comedications.

CHARPTER II
LITERATURE REVIEWS

Cyclosporine

Cyclosporine (CsA) is an immunosuppressant drug widely used in post-allogenic organ transplant to reduce the activity of the patient's immune system, and therefore the risk of organ rejection. CsA is a neutral, lipophilic, cyclic nonribosomal peptide of 11 amino acids and contains a single D-amino acid extracted from a soil fungus *Typocladium inflatum gams*. Borel[22] discovered its immunosuppressive properties in 1972 and he showed that CsA reversibly inhibits T-cell mediated alloimmune and autoimmune responsed. Since its introduction for clinical use in 1983, CsA has become a cornerstone of clinical immunosuppression. In essence, it transformed the entire transplantation field from a state of clinical experimentation to a widespread and successful therapeutic modality.

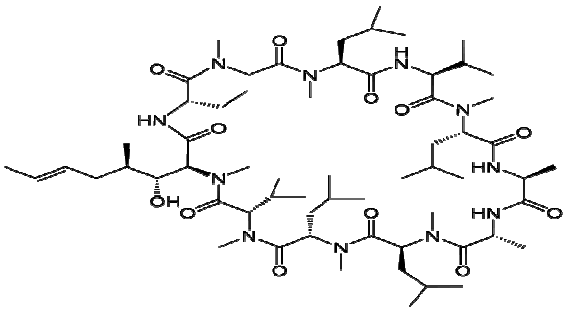


Figure 2 : Chemical structure of CSA

Mechanism of action[26]

CsA block T-cell proliferation by inhibiting the production of IL-2 and other cytokines by T-cells. The drug bind to unique cytoplasmic immunophilins named cyclophilin (CpN). The CsA-cylophilin (CsA-CpN) complex inhibits the action of calcineurin, an enzyme that activates the nuclear factor of activated T-cells, which is, in turn responsible for the transcription of several key cytokines necessary for T-cell activity, including IL-2. IL-2 is a potent growth factor for T cells and ultimately is responsible for activation of clonal expansion. Consequently, as lymphokine synthesis and secretion from T cells is inhibited, T-cell-dependent B cell responses will also be suppressed.

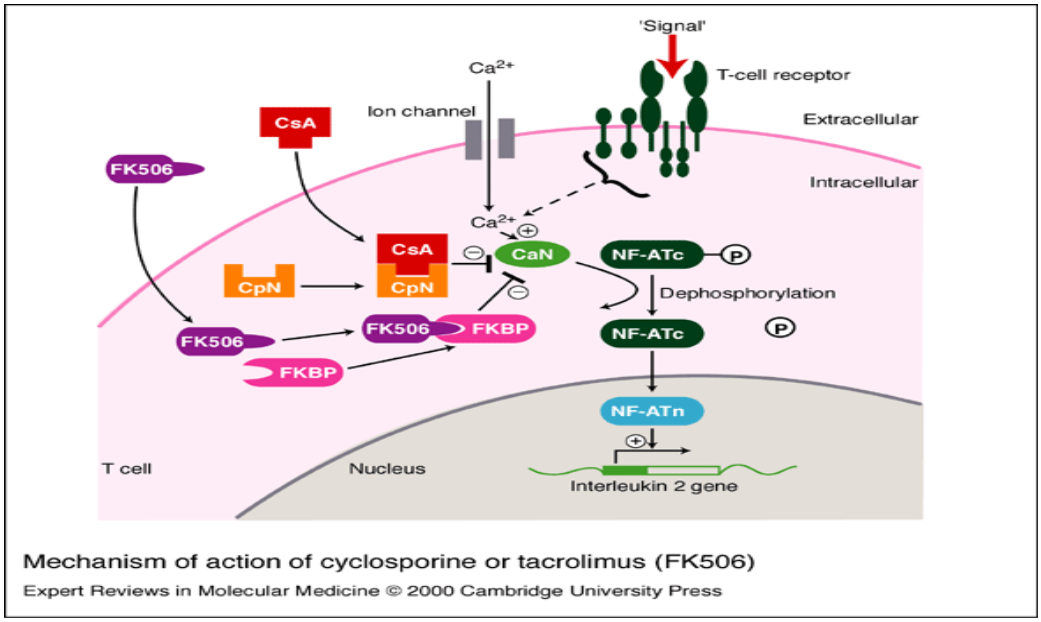


Figure 3 : Mechanism of action of CsA

Pharmacokinetic properties[27]

Absorption

CsA absorption is slow, incomplete, and highly variable after oral administration. The biocavailability has wide variation among the interindividual patients. The bioavailability has an average of 30% (ranges from 5%-90%).[28] The correlation between oral dose and concentration is not found, so the dosage prediction is very difficult to predicted.

There are several reasons for CsA absorption is problematic. First of all, It is a highly lipophilic, requires the formation of micelles with various substances in the gastrointestinal system, including bile salts, cholesterol, and lipids. So the patients with inadequate bile formation reflected to decrease absorption of CsA .[29] Secondly, the absorption of CsA is increased when induced by ursodeoxycholic acid [30] and formulated as a microemulsion [31]

Drug influencing gastric and intestinal motility may change CsA absorption. Metoclopamide, for example, shortened the time to peak concentration and increased the peak concentration, due to enhanced rate of gastric emptying. Other dysfunction and severe disease of the gastrointestinal tract also decrease the CsA absorption. [27]

Table1 ; Factor Affecting CsA Pharmacokinetic: Absorption [27]

Factor	Effect
Increase bile production	Increase
Food	Variable
Grapefruit juice	Increase
Increase time post transplant	Increase
Poor liver function	Decrease
Poor gastrointestinal function (e.g., diarrhea, postoperative ileus, gastroparesis, and short bowel syndrome)	Decrease
Cystic fibrosis	Decrease
Black (vs White) race	Decrease
Diabetes	Decrease
Drug (e.g. metoclopamide)	Increase

Distribution

CsA is very lipophilic and widely distributed throughout the body . In renal transplant recipients, the mean volume of distribution (Vd) was 2.9-4.7 l/kg.CsA concentration in body tissues are relatively high. CsA accumulates in the pancrease, liver, spleen, kidney and fat [32]

In blood, 58% of the circulating CsA is bound to erythrocytes, 9% to leukocytes, and 33% is in plasma.[28] The distribution of CsA between blood cells and plasma is highly temperature-dependent. The plasma separation at 37°C gave 15% higher plasma concentration of CsA than seperation at 36°C. The partitioning of CsA between blood and tissue compartment is affected by changes in hematocrit and lipoprotein concentrations, therefore anemia and lipid disorders may alter CsA pharmacokinetics.[33]

CsA crosses the placenta and is presented in amniotic fluid and fetal circulation, It is also found in breast milk and breast-feeding should be avoided.[27]

Table 2 ; Factor Affecting CsA Pharmacokinetic: Distribution[27]

Factor	Effect
Increased hematocrit	Decrease in free CsA
Increased lipoproteins	Increase in overall CsA level
Decreased lipoproteins	Increase in free CsA
Increased cyclophilin	Increase in bound CsA

Metabolism

CsA is extensively metabolized to more than 30 metabolites[29] by hydroxylation, N-demethylation, cyclization, and oxidation via several cytochrome P450 isoforms (CYP450 3A4 and CYP3A5) in hepatic as well as intestinal metabolism.[34] The most active metabolites is AM1 with 10-20% of the immunosuppressive activity of naive CsA. Other less active metabolites are AM9 and AM4N.

The total body clearance of CsA in renal transplant recipients varies from 0.63-23.9 ml/min/kg.[35] CsA has a low hepatic extraction ratio(<0.3). Its half-life ranges from 4-54 hour in renal transplant patients. The large inter-patient variability in clearance needed the wide range of dosage regimen to achieve an optimal CsA blood concentration.

CsA metabolism is age-dependent, children need the increment of dosage requirements due to an increased of CsA clearance and decreased of CsA elimination half-life.[27]

Table 3 ; Factor Affecting CsA Pharmacokinetic: Metabolism[27]

Factor	Effect
Genetics, specifically structure and quality of inherited P450	Large variation
Age < 18 years	Increase in CsA clearance
Liver disease	Decrease in CsA clearance
Drug interaction	Varied
Circadian variation	Lower clearance during rest(pm) periods as compared to another (am).Clinical significance uncertain

Excretion

Biliary excretion is the major route of elimination. More than 90% of CsA dose is excreted in bile with less than 1% excreted as unchanged CsA and greater than 40% appearing as metabolites. The urinary elimination is of minor importance. The urinary excretion was found to be 6% of an oral dose with less than 1% as parent drug. Thus, dosage adjustment in patients with renal insufficiency is not warranted; however, patients with hepatic failure exhibit decreased CsA clearance.[36]

Adverse Effect

The dose administered, the duration of therapy, comorbidity, co-medication and individual sensitivity is associated with the side effects of CsA .[37-38] The most significant adverse effect of CsA is both acute and chronic forms of nephrotoxicity. Nephrotoxicity is a major drawback of CsA therapy. It has been observed that renal function under CsA therapy declines by 25% in the first 6 months after transplantation followed thereafter by stabilization of serum creatinine.[25]

The nephrotoxicity effect of CsA is not limited to the renal transplants, but has also been observed in native kidneys of patients with heart or liver transplants.[39,40]

CsA causes acute intrarenal vasoconstriction leading to oliguria, decrease sodium filtration, and a rise in urea concentration and serum creatinine. These physiological changes may early occur within 48 hour after the usage of CsA and are detectable throughout the

duration of treatment and can resolve after discontinuation of therapy. They may be accompanied histologically by a characteristic vacuolization of the proximal tubular epithelium. Long term CsA administration may lead to a more serious nephrotoxicity damage with interstitial fibrosis with tubular loss and irreversible nephron damage, particularly in patients receiving high-dose treatment (greater than 6 mg/kg/day) or with coexisiting renal injury. Functional deterioration due to chronic nephrotoxicity appears to stabilize after 12 months however, long-term studies show no increased risk of graft loss when compare to patients receiving non-CsA therapy.[27] Secondary toxicity directly related to or complicated by CsA nephrotoxicity includes hypertension, hyperkalemia, hyperuricemia and hypomagnesemia. Hypertension is a predominate clinical problem commonly encountered in most renal transplant patients, often requiring multidrug therapy. Other side effects are hirsutism, gingival hyperplasia, and a variety of neurologic syndromes such as headaches, tremor, and paresthesias can occur.

Table 4 ; Adverse reactions are ranked under heading of frequency, the most frequent first, using the following convention: very common (>/= 1/10); common (>/=1/100, <1/10);uncommon(>/=1/1,000, < 1/100); rare (>/= 1/10,000, < 1/1,000); very rare (<1/10,000), including isolated reports.

Blood and lymphatic system disorders	
Uncommon	Anemia, Thrombocytopenia.
Rare	Microangiopatic haemolytic anemia, haemolytic uraemic syndrome.
Metabolism and nutritional disorders	
Very common	Hyperlipidaemia.
Common	Anorexia, Hyperuricemia, Hyperkalemia, Hypomagnesemia.
Rare	Hypoglycemia.
Nervous system disorders	
Very common	Tremor, headache.
Common	Paresthesia.
Uncommon	Signs of encephalopathy such as convulsions, confusion, disorientation, decreased responsiveness, agitation, insomnia, visual disturbances, cortical blindness, coma, paresis, cerebellar ataxia.
Rare	Motor polyneuropathy.
Very Rare	Otic disc oedema including papiloedema, with possible visual impairment secondary to benign intracranial hypertension.
Vascular Disorders	
Very Common	Hypertension.
Gastrointestinal Disorders	
Common	Nausea, vomiting, abdominal pain, diarrhea, gingival hyperplasia
Rare	Pancreatitis.
Hepatobilliary disorders	
Common	Hepatic dysfunction.
Skin and subcutaneous tissue disorders	
Common	Hypertrichosis
Uncommon	Allergic rashes.
Musculoskeletal and connective tissue disorders	
Common	Muscle cramps, myalgia
Rare	Muscle weakness, myopathy.
Renal and urinary disorders	
Very Common	Renal dysfunction
Reproductive system and breast disorders	
Rare	Menstrual disturbances, gynecomastia.
General disorders and administration site conditions	
Common	Fatigue.
Uncommon	Oedema, weight increase.

Therapeutic Drug Monitoring

CsA blood concentration is measured routinely in an attempt to optimize therapy. The most common and practical method for monitoring CsA is by measuring trough blood concentration (C_0). Radioimmunoassay (RIA) and fluorescence polarization immunoassay are the most common used methods to measure CsA concentrations. CsA can be measure by high-performance liquid chromatography (HPLC), which is recognized as the reference procedure.[41] It is important to determine which assay methodology the laboratory using because target range vary between nonspecific assays, such as RIA and microparticle enzyme immunoassay, which quantitate parent plus metabolite concentration, and specific assay, such as HPLC using Mass spectrophotometry, which quantitate only the parent compound. Thus the target concentration will be lower for the specific assay (HPLC) compared to nonspecific assays (RIA and microparticle enzyme immunoassay) by approximately 20 to 25%.[42]The specific goal level for CsA is dependent on transplant type, time after transplantation, concomitant immunosuppression and the transplantation center. Blood drug concentration should be measured frequently daily or three times per week following initiation of the drug and during the stabilization period after transplantation. As the time increases after transplantation, blood concentration are measure less frequently, usually monthly.

Matrix for concentration measurement

Whole blood with EDTA as anticoagulant is the most commonly recommended as the medium for CsA concentration measurement. The advantages of using whole blood rather than plasma are that to avoid the problem associated with sample separation and temperature effect on CSA equilibration between blood cells and plasma. However, there is no significant advantage of monitoring CsA in whole blood over the plasma has been reported in clinical perspective.[27, 43]

Analytical Method [40]

There are two general types of assay; those selectively detecting only parent CsA, and those nonselectively measuring composites of CsA plus varying arrays of metabolites. Most assay available for monitoring CsA are selectively for the parent drug, based on the established guidelines. The four most common assays are ranked in order of specificity, precision, accuracy, and cost in **Table 5**

Table 5; Ranking of CsA Assays for Analytical Performance[27]

	Specificity	Precision	Accuracy	Cost
HPLC	1	4	2	1
FPIA	4	1	1	2
RIA	3	3	4	4
EMIT	2	2	3	3

Note: 1 = Highest; HPLC = High performance liquid chromatography; FPIA = Fluorescent polarization immunoassay; RIA = Radioimmunoassay; EMIT = Enzyme multiplied immunoassay technique.

Although HPLC is the method with the highest specific for parent compound CsA and it is the reference method, it has numerous practical disadvantages: methods are rarely standardized, making comparisons of CsA measurements between centers difficult, technical expertise is required, turnaround times are slow and equipment is expensive. Therefore, the use of HPLC has been reserved for using as experimental tool.

Specific RIA involves the use of specific monoclonal antibodies as the detector of CsA. The advantages of specific RIA over HPLC are that they are technically less demanding and have a faster turnaround time.

FPIA method eliminates the problems of using radioactive substance, uses a reproducible, automated format, requires little technician expertise, and has a rapid turnaround time.

EMIT has been introduced more recently. It shows the greatest selectivity for the parent drug. The important disadvantage is its limited working range. The highest calibration standard is 500 ug/l. With specimen above that concentration, it must be diluted before analysis

C_2 versus trough (C_0) concentration monitoring

Currently, most centers use trough CsA concentration (C_0) for routine monitoring therapy. C_0 measurement has several advantages: it gives a reliable and reproducible measure of the minimum steady state concentration, it can be performed in outpatients, and it is the most documented monitoring method. However, the concentration-effect relationships are often weak. Patients displaying trough level within a putative CsA therapeutic range are not always spared from either rejection or nephrotoxicity. Besides, trough concentrations are a poor guide to dosage adjustment.[45-46]

Studies have revealed lack of predictive value of trough CsA concentration and rejection.[47] Alternative strategies, including area under the time curve (AUC) determination and peak concentration, have been suggested to better correlation with rejection.[48] AUC 0 to 12 Hours (AUC_{0-12}) determination expresses very accurately the total drug exposure, but this is difficult to determine routinely. Limited sampling techniques using two to five blood samples within the first 4 hours after an oral dose have been used. It was found that AUC 0 to 4 hours (AUC_{0-4}) correlates very well with AUC_{0-12} with a correlation coefficient (R^2) of 0.88 to 0.96 compared to C_0 (R^2 = 0.22). Furthurmore, blood concentration at 2 hour post dose (C_2) determination correlates very well with AUC_{0-4} (R^2=0.8) and therefore one point sample at C_2 rapidly and accurately predict AUC. Thus, the concept of C_2 determination for CsA blood level follow-up emerged.[49,50]

Peak concentration measured 2 hours after an oral dose (C_2) have a better predictive value in terms of rejection compared with trough concentration. [51] The study of Canadian Neoral Renal Transplantation Group[52] reported the correlation between CsA concentration at 2 hour post dose and rejection risk. The CsA C_2 in group with acute rejection was 1063 mg/l compared with those who were rejection free at day 7 after transplantation and no patients conferred acute rejection when mean C_2 > 1,500 mg/l in the following time interval. The conclusion of this study is the using single-point C_2 determination may be the most reliable method to CsA concentration monitoring with maximum therapeutic efficacy. Moreover, Brunet et al,[53] reported the good correlation between CsA C_2 level and CsA pharmacodynamic parameter, but this correlation was not shown at CsA trough level. This data confirms C_2 represent the degree of CsA immunosuppression higher than CsA C_0 and may represent the best option for CsA monitoring to defining immunosuppression. Nowaday, some transplantation centers have adopted CsA C_2

strategy to manage CsA levels because of the convenience of single blood sample. The suggested therapeutic range for CsA C_2 level is 1,500 to 2,000 ng/ml for the first few months after transplant and 700 to 900 ng/ml after 6-12 month.[47]

A new microemulsion formulation of CsA (Sandimmune Neoral®)

A major problem with the original CsA (Sandimmune®), being fat soluble, was its unpredictable absorption from the intestine and its variable bioavailability. These were significantly influenced by bile flow, food and other factors.[54-56]

Sandimmune Neoral® is a new CsA formulation as microemulsion designed to minimize the difficulties of absorption showed with previous formulation of this drug. Sandimmune Neoral® incorporate CsA in a microsuspension preconcentrate containing a surfactant, lipophilic and hydrophilic solvents, and a hydrophilic cosolvent. It was self-emulsifying properties forming a microemulsion on contact with gastrointestinal fluids from which it is consistently absorbed in a much less bile and food dependent manner. Absorption of CsA from Sandimmune Neoral® is more consistent , rapid, complete and dose-linear than from the gelatin capsules and liquid formulation.[57 The mean of peak concentration of Sandimmune Neoral® is 1.4 hour. Moreover, the area under the concentration time (AUC) is increased by approximately 15% and the peak concentration (C_{max}) by 40% when changing from the Sandimmune® to Sandimmune Neoral® at a constant dose.[54,58] Also, very importantly, Sandimmune Neoral® has shown an increased rate and extent of drug absorption with lower inter-and intra-individual pharmacokinetic variability when compared with the conventional formulation.

The safety and tolerability of Sandimmune Neoral®, in subject to the acute rejection episodes and graft survival were compared to the conventional CsA formulation. In a prospective randomized double blind multicenter trial showing that Sandimmune Neoral® has higher bioavailability, increases drug exposure and reduces a deleterious on clinical safety in renal transplantation patients.[59]

Therapeutic range of CsA

An alternative monitoring strategy; AUC_{0-4} or C_2, for CsA was introduced in addition to traditional trough(C_0) monitoring due to its clinical effectiveness. Large scale clinical trials using C_2 monitoring of Neoral® in renal and liver transplant patients demonstrated that optimal target range of CsA C_2 was 1300-1800 ng/ml and 800-1200 ng/ml to the first 3 months for renal and liver transplant patients, respectively[60]

Guidelines for Sandimmun Neoral® target C_2 levels

Transplant	Time post-transplant (months)	Target C_2 concentration (µg/l)
Renal	1	1'700
	2	1'500
	3	1'300
	4–6	1'100
	7–12	900
	> 12	800
Liver	0–3	1'000
	4–6	800
	> 6	600

Figure 4 ; Guidelines for Sandimmun Neoral target C_2 levels[61]

Drug interaction with CsA

Since CYP450 3A4 may be rinvolved for more than 50% of the metabolism of all drugs, the potential for drug interaction is immense. Some of these drug interactions are clinical significance. The most commonly administered drugs that are known to significant alter CsA levels are in **Table 6**. Inhibitors of CYP3A4, such as DTZ or erythromycin, can increase drug concentration significantly, whereas drugs that induce CYP3A4 activity, such as phenytoin or rifampicin, can decrease drug concentrations significantly. Some centers take adventage of these interactions by routinely prescribing CYP3A4 inhibitors to reduce the dosage and costs of CsA therapy while maintaining the same therapeutic concentrations.

In addition to the pharmacokinetic drug interactions, pharmacodynamic interactions may also occur when CsA is administered with certain therapeutic agents. Some drugs can potentiate the nephrotoxicity of CsA such as aminoglycosides, amphotericin B. Other important interactions include potentiation of other toxicities of CsA such as minoxidil causing additive hirsutism, and nifedipine causing increased of gingival hyperplasia.[62-63]

CsA is inhibitors of CYP3A4.[26] The inhibitory effect of CsA on CYP3A4 can be seen with weaker substrates, such as HMG-CoA reductase inhibitors. Concomitant administration of CsA with an HMG-CoA reductase inhibitors results in an increasing the level of the HMG-CoA reductase inhibitors , which increase the risk of HMG-CoA reductase inhibitors adverse effect, especiallly myopathy.[26] Patients should be awared for clinical signs of myopathy when using HMG-CoA reductase inhibitors when comedication with CsA.

Table 6 ; Drug interactions that change the CsA concentration.

CsA levels	
Increase	**Decrease**
Ketoconazole	Rifampicin
Fluconazole	Phenytoin
Itraconazole	Phenobarbitone
Voriconazole	Carbamazepine
Erythromycin	Sulfadimidine
Levofloxacin	Trimethoprim
Diltiazem	
Verapamil	
Danazol	
Nicardipine	
Metoclopamide	
Methylprednisolone	
Sirolimus	
Tacrolimus	
Protease inhibitors	

DTZ pharmacodynamic and pharmacokinetic.

DTZ is a calcium channael blocker widely use in the treatment of angina, supraventricular arrhythmias and hypertension. The mechanism of action are included that DTZ is a potent vasodilator, increasing blood flow and variably decreasing the heart rate via strong depression of A-V node conduction.[64] The absolute bioavailability of DTZ is approximately 40%, with a large inter-individual variability.[65] DTZ undergoes complex and extensive phase I metabolism via the cytochrome P450 (CYP) 3A4 and 3A5, key enzymes in the metabolism that mainly localized in the liver but is also expressed in the small intestine,[66] that include desacetylation, N-demethylation and O- demethylation . Lee et al,[67] reported that the extensive ratios of DTZ in the small intestine and liver after oral administration to rats were about 85% and 63%, respectively. In preclinical studies, the estimated potency of hypotensive effect of desacetyldiltiazem shown to be about one half to equivalent compared to DTZ, whereas the potencies of N-demethyldiltiazem and N-demethyldesacetyldiltiazem were about one third compared to parent DTZ.[68]

CsA and DTZ interaction.

CsA is frequently coadministration with DTZ because the latter has possible beneficial effects on the economic impact associated with reduction of the dosage administration of CsA. DTZ is a relatively safe drug with useful hypotensive effect to control the blood pressure and preservation of kidney function.[69]

Both CsA and DTZ are CYP450 3A4 and 3A5 substrates. The interaction between CsA and DTZ results in increased CsA blood concentration, explained by competitive inhibition of CsA metabolism in the liver.[70] Although prospective controlled studies have shown that concomitant DTZ use reduce CsA dosage requirements by approximately 30%[23] , the magnitude of the interaction declines over times and it dose not occur in all patients.[24]

There are several studies show the beneficial effect of Calcium channel blockers (CCBs) when co-administration as CsA sparing-agent to improve early graft function, reduce acute rejection, decrease the incidence of delayed graft function[23, 71] and may even confer a survival advantage.[10] For this reasons, DTZ was preferably given to renal transplant in doses of 60-180 mg/day.[72] In almost randomized trial in transplant patients to study the effect of DTZ when coadministration with CsA show that co-treatment CsA with oral DTZ has been

associated within clinically and statistically significant smaller of CsA daily doses, without any adverse impact on patient survival, graft rejection or other complications.[72-77] Asberg et al,[78]reported that the mean dose of CsA during the first month of treatment was 30% lower in a DTZ than in a non-DTZ group. Although, the CsA dose was 11% lower in a DTZ than in a non-DTZ group at 1 year after transplantation.[78] Thereafter the efficient combination therapy reduce patients economic burden, at the same time remained kidney function, promoted graft function recovery (6.2±1.5 days vs 3.9±1.4 days, $p<0.01$), decreased hepatic and renal toxicity and decreased the rate of acute rejection (13.2% vs 7.9%, $p<0.01$). Due to the reduced dosage of CsA, the incidence of hepatic and renal toxicity was distinctly reduced, and thus the cost of treating hepatic and renal complication was also decreased, so the total expenditure in kidney transplantation was further reduced.[79-80]

Besides raising CsA blood concentrations, the DTZ combination also protect renal function by defending against direct renal cytotoxicity and the hemodynamic turbulence caused by CsA. The possible mechanism include:

(1) Antagonizing the constrictive effect on afferent glomerular arteriole caused by CsA, depressing the resistance of renal blood vessel, increasing blood flow, and enhancing glomerular filtration.[81]

(2) Restraining effect of mesengial cells and the glomerular filtration caused by CsA. [82]

(3) Abatement of Ca^{2+} inward current and Ca^{2+}channels activation caused by CsA, and blockage of renal toxicity caused by Ca^{2+} dependent reaction.[82]

(4) Increasing the conversion of CsA to M17 and other metabolites. The immunosuppression effect of M17 was same as that of CsA and its renal toxicity was less significant.[83-84]

Pharmacogenomics and Pharmacogenetics

Therapeutic drug monitoring has been used as an essential tool to individualize immunosuppressive drug therapy. This approach offers the opportunity to reduce the pharmacokinetic variability by implementing drug dose adjustment based on plasma/blood concentrations. The main limitation of this method is the fact that it starts only when a given drug is administered to the patients. Thus, inadequate initial doses would be adjusted only after the

necessary time to achieve a steady-state condition, a minimum of 4 half-lives of CsA, causes the risk of acute rejection is increased during the first week after transplantation.[85]

Nongenetic factors such as organ function, drug interactions and the nature of the disease may influence the pharmacokinetic and pharmacodynamic properties of drugs. Recently, genetic factor is founded to associated with the interindividual variations in drug administration.[86] Pharmacogenetics is such a subject to determine the genetic factors describing the inherited nature of individual variations, thereby providing a strong scientific basis for optimizing drug therapy on the basis of each patient's genetic constitution. Genetically determined variability been increasingly, involved in interindividual response to drug therapy. The main contribution of the pharmacogenetics is to predict the initial dose of a given drug, increasing the chances that adequate drug exposure will be achieved early after inception of therapy. Pharmacogenetics may anticipate potentially harmful drug-to-drug interactions, thereby reducing the incidence of adverse drug event, a significant cause of morbidity, mortality, and excessive medical care expenses.[87]

In recent year, extensive studies on pharmacogenetics of immunosuppressive drugs have been focused on the contribution of drug-metabolizing enzyme cytochrome P450 (CYP)3A and the drug transporter P-glycoprotein (P-gp) to the individual administration of CsA in organ transplantation for they are thought to be the main determinant of the pharmacokinetics of currently used immunosuppressive drugs.[88]

Cytochrome P450 3A5 *(CYP3A5)* Polymorphism

Cytochrome P450, family 3, subfamily A, polypeptide 5 named CYP3A5 is a protein that in humans is encoded by the *CYP3A5* gene. The CYP3A enzymes in human consist of CYP3A4, CYP3A5, CYP3A7 and CYP3A43. CYP3A4 and CYP3A5 are regarded as predominant functional form of human CYP3A in the liver and intestine. They are involved in the phase I metabolism of more than 50% of currently prescribed drugs and endogenous compounds.[89-93]

This gene, *CYP3A5*, encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by glucocorticoids and some pharmacological agents. The enzyme metabolizes drugs such as nifedipine and CsA as

well as the steroid hormones testosterone, progesterone and androstenedione. This gene is part of a cluster of cytochrome P450 genes that locus of 231 kb located on chromosome 7q21.1.[94]

CYP3A5 is polymorphically expressed in liver, small intestine and kidney. The allele nomenclature of the *CYP3A5* was shown in **Table 7**. The most frequent and functionally important Single-nucleotide polymorphism (SNP) in the *CYP3A5* gene is a mutation of adenosine (*CYP3A5*1* wild-type allele) to guanosine (*CYP3A5*3* mutated allele) at the position 6986 within intron 3 (**Figure 5**). This mutation creates an alternative splice site in the pre-messenger ribonucleic acid (mRNA) and production of aberrant mRNA (SV1-mRNA) that contains 131 bp of intron 3 sequence (exon 3B) inserted between exon 3 and exon 4 (**Figure 6**). The exon-3B insertion results in a frameshift and encoded a protein that is truncated at amino acid 102 and is inactive. [93, 95-96]

Table 7; *CYP3A5* allele [91]

Allele	Location	Nucleotide changes	Amino Acid substitution	Expression
CYP3A5*1A				
CYP3A5*1B	5'UTR	G-86A		
CYP3A5*1C	5'UTR	C-74T		
CYP3A5*1D	3' UTR	C31611T		
CYP3A5*2	Exon 11	C27289A	T398N	
CYP3A5*3A	Intron 3	A6986G, C31611T	Splicing defect	None
CYP3A5*3B	Intron 3	C3705T, 3709 ins G, A6986G, C31611T	H30Y, splicing defect splicing defect	None
CYP3A5*3C	Intron 3	A6986G		None
CYP3A5*4	Exon 7	A14665G	Q200R	
CYP3A5*5	Intron 5	T12952C	splicing defect	Alternatively spliced mRNA
CYP3A5*6	Exon 7	G14690A	splicing defect	None(skip Exon 7)
CYP3A5*7	Exon 11	27131 ins T	stop codon at 348	None

UTR= untranslated region

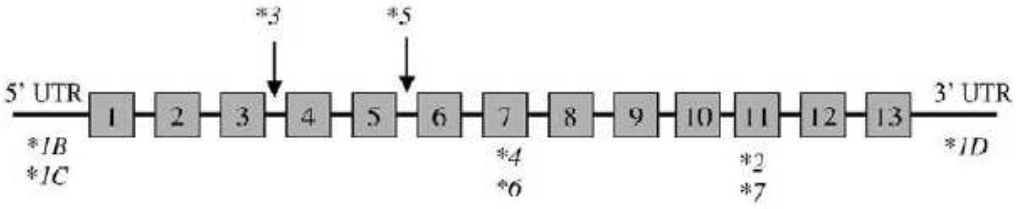


Figure 5; Distribution of mutation in the *CYP3A5* gene [97]

The absence of *CYP3A5* expression was recently correlated to a genetic polymorphism (*CYP3A5*3*). Because CYP3A5 may represent up to 50% of total *CYP3A* protein in individuals polymorphically expressing *CYP3A5*, it may have a major role in variation of CYP3A-mediated drug metabolism.[91] Among *CYP3A5* alleles, *CYP3A5*1* has been found to be the main allele associated with CYP3A5 expression, whereas the mutant allele *CYP3A5*3* prevents expression of the enzyme due to premature termination during translation of the aberrant mRNA and causes alternative splicing and protein truncation resulting in the absence of CYP3A5 enzymes activity.

Genetic polymorphisms of *CYP3A5* have been found to be associated with more significant pharmacokinetic effects on immunosuppressant drugs than those to *CYP3A4*. Among *CYP3A5*3/*3* subjects, CYP3A5 expression comprises only 4.2% of total CYP3A in the liver and 2.7% of total CYP3A in the jejunum. Among heterozygous *CYP3A5*1/*3* subjects, however, CYP3A5 expression is appreciable, with 50% of total CYP3A in the liver and 61% of CYP3A in the jejunum[98]

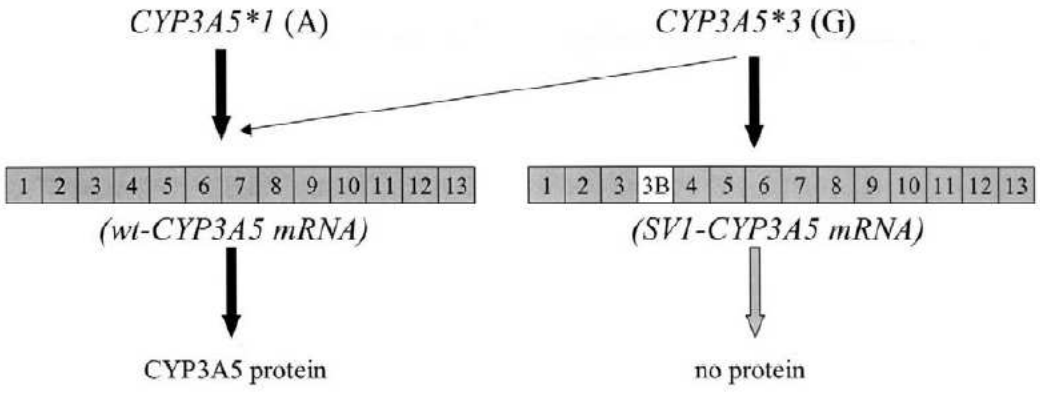


Figure 6; SNP in *CYP3A5* gene within intron 3 (A6986G) [91]

Prevalence of *CYP3A5* polymorphism

Several polymorphic of *CYP3A5* have been recently reported in difference populations. In Thai population the allele frequency of *CYP3A5*3* was 66% and *CYP3A5*1* was 34%, that is similar to other Asian population but significant difference from Caucasian and African American. The frequency of *CYP3A5*3* allele in Thai population was lower and higher than Caucasian and African American respectively. Other *CYP3A5* coding variants have been described, but occur at relatively low allele frequencies.[99-100] The comparison of allele frequency between Thai population and other ethnic populations was shown in **Table 8 and Table 9**

Table 8 ; Allele frequencies of the *CYP3A5* in Thai population and other ethnic populations

Ethnicity	Number of subject	% Allele frequency		p-value
		1	***3***	
Thai [101]	150	34	66	-
Chinese [99]	302	22	78	0.059
Indian [102]	90	41	59	0.307
Malaysian [102]	98	39	61	0.463
Japanese [103]	200	23	77	0.085
Dutch Caucasian [104]	500	8	92	<0.001
African American [100]	20	45	48	0.042

Table 9 ; Genotype frequencies of *CYP3A5* in a Thai population [101]

Genotype	Number of subject	Frequency (%)	95% CI
*1/*1	20	13.33	7.89-18.77
*1/*3	61	40.67	32.80-48.52
*3*3	69	46.00	38.02-53.98
Total	150		

Effect of *CYP3A5* polymorphism on CsA pharmacokinetics.

The human CYP3A subfamily plays a most important role in the metabolic elimination of CsA. These enzymes (CYPs) catalyze a variety of reactions including N-dealkylation, O-dealkylation, S-oxidation, epoxidation, and hydroxylation, rendering drugs more ionic, water soluble, and ready to be excreted.[91, 105] For renal transplant patients, achieving target blood concentrations of CsA as soon as possible after transplantation is key in the prevention of rejection.[106-107]

Genetic polymorphism of *CYP3A5* have been found to be associated with more significant pharmacokinetic effect on immunosuppressive drug than those *CYP3A4*. The *CYP3A5*3* (6986 A>G within intron 3) seems to be the most important functional polymorphism in the *CYP3A5* gene. *CYP3A5*3* (G at position 6986) causes an aberrantly spliced site in the pre-mRNA with a stop codon and leads to a truncated CYP3A5 protein.[98] It has been reported that *CYP3A5*1* (A at position 6986) allele actually related to the increases expression of *CYP3A5* enzyme and only people with at least one *CYP3A5*1* allele actually express CYP3A5 protein.[20]

There are few studies have shown the role of *CYP3A5* polymorphism on CsA pharmacokinetic characteristics and the effect of these SNPs on CsA disposition has been interestingly inconsistent .Hesselink et al[108] has not found the association between *CYP3A5* genetic polymorphism and CsA dose-adjusted C_0. In contrast, in healthy volunteer it was shown that *CYP3A5*1* carriers had a lower CsA AUC and higher CsA clearance.[22] A study of 10 patients found that CsA metabolism was increased by 52% in *CYP3A5*1* carriers.[97] A study of 50 renal transplant recipients using CsA for immunosuppression found that patients carrying the *CYP3A5*1* allele had lower dose-adjusted C_0 when considering the second CsA administration of the day (p=0.037).[109] Hu et al[21] studied in Chinese renal transplant patients, reported patients carrying the *CYP3A5*3* variant genotype require a lower dose of CsA to reach target levels than those carrying with the *CYP3A5*1* allele. Moreover, Qiu et al[110] also reported that the dose-adjusted CsA concentration in patients with the *CYP3A5*3/*3* genotype was 25.5% and 30.7% higher than those with the *CYP3A5*1/*1* genotype during days 8-15 and days 16-30 post-transplantation, respectively. From these report, its may be assumed that *CYP3A5* polymorphism may be associated with the large inter-individual variability in CsA pharmacokinetic in renal transplant patients.

Table 10; Influence of Genetic Polymorphism of Metabolizing Enzymes on CsA Pharmacokinetics.[87]

Population	Investigated Polymorphisms	Findings	Reference
14 healthy subjects (11 African American, 4 Caucasian)	*CYP3A4*	AUC/D: *1/*1>*1/*1B>*1B/*1B	111
110 renal transplants (87 Caucasian, 11 African American, 12 Asian)	*CYP3A4* *CYP3A5*	- -	19
106 renal transplants (Chinese)	*CYP3A5*	C_0/D: *1/*1<*1/*3<*3/*3	21
16 healthy subjects (11 African American, 5 Caucasian)	*CYP3A5*	AUC: *1/*1<*1/*3<*3/*3	12
135 renal and heart transplant (107 Caucasian, 17 African American, 11 Asian)	*CYP3A4* *CYP3A5*	↑ oral clearance for *1/*1B or *1B/*1B -	112
106 renal transplants (Caucasian)	CYP3A5	-	113
197 renal transplants (15 African American, 133 Caucasian, 49 South Asia)	*CYP3A5*	-	114
50 renal transplants	*CYP3A5*	C/D ratio was 1.6 fold higher in *3/*3 than *1/*3	109

CYP3A5 genotyping

Published methods for genotyping *CYP3A5* have relied on gene sequencing or the use of mismatched primers to generate restriction sites to enable restriction fragment length polymorphism (RFLP) analysis. Sequencing is expensive and requires specialized equipment. RFLP may be an option, but can be time-consuming. In the case of *CYP3A5* analysis, the amplification, digestion and visualization methods are technically more involved than standard RFLP protocols. This is due to the absence of naturally occurring splice site for known restriction endonucleases. Allelic discrimination assay is an alternative method which is rapid and reliable for genotyping *CYP3A5* polymorphism. In allele specific polymerase chain reaction amplification, oligonucleotides specific for hybridizing with the common or variant alleles are used for parallel amplification reaction and then identify for the presence or absence of the appropriate amplified DNA products by real-time fluorescence-based analysis, melt curve analysis or gel electrophoresis. [115-118]

CHARPTER III
PATIENTS AND METHOD

This study was conducted from February to April 2011 at Praram 9 Hospital, Bangkok, Thailand.

1. Study design

A before-after experimental method was used. Demographic data and measured drugs serum concentrations from patients were collected, *CYP3A5* genes were genotyped, and the data were then analyzed.

2. Patients

2.1 Population and samples.

2.1.1 Population is renal allograft patients received CsA-based regimen for immunosuppression.

2.1.2 Samples are renal allograft patients who were out patients at Praram 9 Hospital during February to April 2011 and met the inclusion criteria.

2.2 Inclusion criteria.

2.2.1 Renal transplant patients who were on microemulsion CsA-based regimen.

2.2.2 Age not less than 18 years old.

2.2.3 Patients who have had stable renal allograft function for at least 3 months (the difference of 3 points of serum creatinine within 60 days were not more than 0.3 mg/dl)

2.2.4 Patients who were not had contraindication for DTZ treatment (SBP < 100 mmHg or DBP < 60 mmHg, heart rate < 60 beats/min, severe congestive heart failure, acute myocardial infarction and pulmonary congestion)

2.2.5 Patients who were not allergic to DTZ.

2.2.6 Patients who were not on other agents that had the effect on pharmacokinetic of CsA at least 2 weeks before inclusion such as carbamazepine, phenytoin, ketoconazole, fluconazole, voriconazole, itraconazole, phenobarbital, erythromycin, clarithromycin.

2.2.7 All patients consented to enroll in this study.

2.3 Exclusion criteria

2.3.1 Patients with drug non-compliance deleted from interviewing by the investigator.

2.3.2 Patients with abnormal liver function test (ALT or AST elevated more than 3 times from baseline)

2.3.3 Patients with elevated serum creatinine more than 25% from baseline.

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

2.4 Sample size determination

The purpose of this study was to determine whether patients with difference allele of *CYP3A5* genotype, *CYP3A5*1* and *CYP3A5*3* would show the different interaction between CsA and DTZ by determine level-to-dose ratio of CsA before and after DTZ used in different group of patients.

Faradori et al[119] studied of CsA pharmacokinetics in chronic stable adult renal transplant patients treated with CsA as immunosuppressive (N=4) has the C_{max} (mean$\pm$SD) of CsA were 441.7$\pm$155.9 ng/ml per mg/kg/12hr while in the group of patients (N=9) treated with CsA and used 180mg/day of DTZ has the C_{max} (mean$\pm$SD) of CsA were 606.6$\pm$164.4 ng/ml per mg/kg/12hr. In this study we assumed the different (D) of CsA concentration at 2 hour post dose (C_2) after used DTZ should be much more than C_2 before used DTZ might be at least 20% to find the different between these groups.

From the formula to calculate the Population :

$$N = \frac{(Z_{\alpha} + Z_{\beta})^2 S^2 p}{D^2}$$

When; S^2p = Pool variance = $\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}$

D = the difference of CsA concentration at 2 hour

When; $n_1 = 4$, $S_1 = 155.9$, $n_2 = 9$, $S_2 = 164.4$, D = (0.2×441.7) = 88.34, $Z_{\text{œ;œ=0.05(one-sided)}} = 1.64$, Z_{β}; $\beta_{\text{=0.10(one-sided)}} = 1.28$

$N = 28.7 \approx 29$

Calculated for 20% drop out, so the sample size for this research was at least 35.

3. Study protocol

3.1 Study protocol was approved by the ethical committee of Praram 9 Hospital.

3.2 Patients were selected following inclusion and exclusion criterias

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

3.4 Demographic data were collected from medical records.

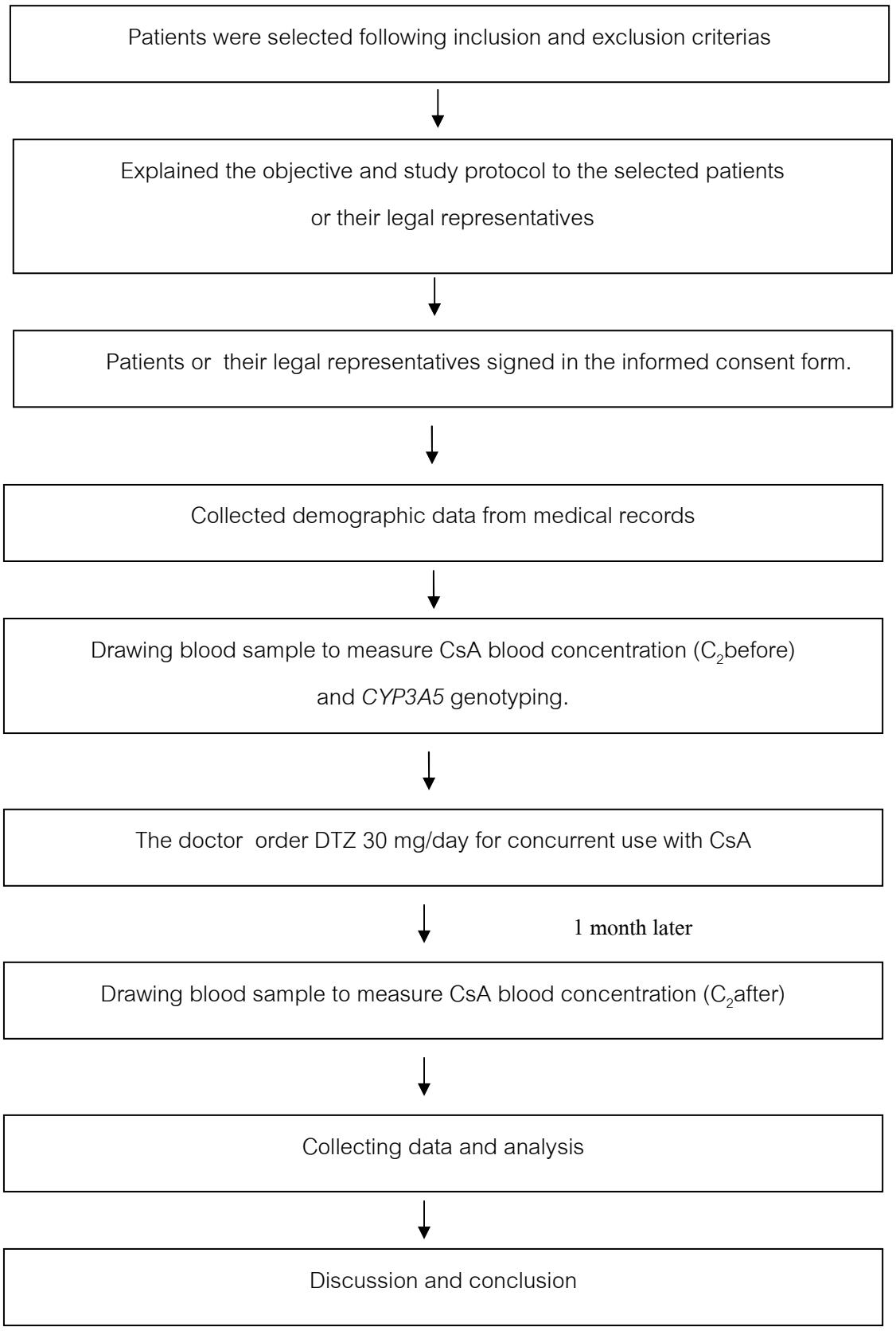
3.5 Coordinated the medical technologists for 10 ml blood sample drawing (2 tubes of 5 ml blood volume) at 2 hour post CsA administration (C_2) to measure CsA blood concentration(C_2before) and *CYP3A5* genotyping.

3.6 Coordinated the doctor to order DTZ 30 mg/day and made an appointment for the next visit.

3.7 Coordinated the medical technologists for 5 ML blood sample drawing at 2 hour post CsA administration (C_2) to measure CsA blood concentration again (after concurrent use with DTZ for at least 1 month to ensured the full interaction; C_2after)

3.8 Collected all the required data and analyzed.

Figure 7 ; Study protocol



4. Sampling

Fourty two patients who met the inclusion criteria were participated in this study. Blood sampling for CsA concentration were obtained at steady state. Whole blood was drawn from patients after 2 hour the morning dose of CsA. Volume of blood sample was 10 ml collected in 2 tubes of 5 ml of Vacutainer tube (purple-stopper) containing EDTA for measured CsA level and *CYP3A5* genotyping.

Whole blood in EDTA tube for *CYP3A5* genotyping was prepared as buffy coat by centrifuge at 2,500 x g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Pipette 200 mcl of buffy coat into microcentrifuge tube size 1.5 ml and stored in a freezer at -20 °C until extracted for DNA.

5. Bioanalysis

5.1 DNA extraction

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

5.1.1 Materials

Chemical and reagents

1.	Absolute etanol	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 tube (1.5 ml) Treff AG. Switzerland
2. Pipette tip (Blue and Yellow) Scientific Plastics USA
3. Micropipette 1,000 mcl Eppendorf Germany
4. Micropipette 200 mcl Eppendorf Germany
5. Micropipette 20 mcl Eppendorf Germany
6. QIAamp Mini spin Column Qiagen Germany
7. Collection tube 2 ml Qiagen Germany
8. Disposable gloves

5.1.2 DNA Extraction method

1. Equilibrate samples and reagents to room temperature.
2. Heat a heating block to 56°C.
3. Pipette 20 mcl QIAGEN Protease into a 1.5 ml microcentrifuge tube containing buffy coat 200 mcl.
4. Mix by vortex mixer for 15 seconds.
5. Add 200 mcl buffer AL to the sample. Mix by vortex mixer for 15 seconds.
6. Incubate at 56°C for 10 minutes.
7. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
8. Add absolute ethanol (96–100%) 200 mcl to the sample, and mix again by vortex mixer for 15 seconds. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
9. Carefully apply the mixture to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.

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

5.1.3 Optical Density measurement

After DNA isolation should bring a sample to measure the amount and quality of DNA by OD measurement. These steps should be done with spectrophotometer as following.

1. Dilute a sample of DNA isolation in 1:5 concentrations, by using DNA 20 mcl add ddH_2O 80 mcLl
2. Prepare dH_2O 100 mcL for control.
3. Set spectrophotometer measure OD at 260 and 280 nm.
4. Calculate OD 260/280 ratio to observe purity and estimate concentration of DNA following this formula.

DNA concentration in mcg/mL or ng/mcL = OD260 x 50 x dilution factor

5.2 *CYP3A5* genotyping

CYP3A5 genotyping was determined by Allelic discrimination assay using real-time polymerase chain reaction (real-time PCR) technique with specific probe and primer (TaqMan® MGB probes, FAM™ and VIC® dye-labeled). See methods at **Appendix D.**

5.3 Determination of CsA whole blood concentration

CsA whole blood concentration were quantified using a chemiluminescent microparticle immunoassay (CMIA) according to the manufacturer's instruction The Architect I system (Abbott Laboratories, Chicago, IL, USA). The measurement range of The Architect CsA assay is 30.0 ng/ml to 1500.0 ng/ml

6. Statistical analysis

Statistical analyses were determined using the Statistical Package for Social Sciences (SPSS Co., Ltd., Bangkok Thailand) software version 17.0. Both descriptive and inferential statistics were determined. The level of significance was set at an $\alpha = 0.05$.

Continuous variables was determined for normality of the distribution using Kolmogorov–Smirnov test and determined for homogeneity of variance using Levene's test.

Demographic data were determined and presented as mean ± SD, median, percentage or frequency where appropriate for qualitative or quantitative variables.

Statistical comparisons of CsA clearance and level-to-dose-ratio between patients with *CYP3A5*1* and *CYP3A5*3* were performed using independent t-test or Mann-Whitney U test. Statistical comparisons of CsA level-to-dose-ratio in the same patients before and after use DTZ were performed using Paired t-test or Wilcoxon-Signed Rank test.

CHARPTER IV
RESULTS

Demographic data

Of the 42 patients recruited, 4 patients reported side effect which might relate to DTZ usage; 2 patients had severe headache while the other 2 patients had edema. These 4 patients were excluded from the study.

Data used for analysis included from the total of 38 patients. Twenty-two patients received cadaver and 16 received living-related renal transplant. The mean time after transplantation (range) was 7.53±4.87 years (range from 1 year 7 months to 17 years 5 months). Their characteristics are shown in **Table 11** . All patients were treated with triple drug regimen (CsA, Mychophenolate mofetil or Mychophenolate sodium and prednisolone) for immunosuppression. The CsA dose was range from 50 to 200 mg/day with a mean value of 136.84±34.74 mg/day. Thirty-one patients had hypertension as a concomitant disease and other concomitant diseases are shown in **Table 11**.

Table 11; Demographical characteristics of the patients (N=38)

Demographical data	Frequency, (mean ± SD)	Percentage
Gender		
Male	21	55.3
Female	17	44.7
Age	55.13±11.18	
Weight	66.63±13.67	
Cause of chronic renal failure		
Diabetic nephropathy	8	21.0
Chronic glomerulonephritis	24	63.2
IgA nephropathy	3	7.9
Others	3	7.9
Follow- up time(Years)	7.53±4.87	
Graphic illustration		
CDKT*	22	57.9
LRKT*	16	42.1
Concomitant disease**		
Hypertension	31	
Diabetes	11	
Cardiovascular disease	7	
Hypercholesterol	22	
Other	5	

* CDKT = Kidney taken from cadavers, LRKT = Kidney taken from living donors

** Some patients had more than one concomitant disease

Concentration at 2-hour post CsA dose (C_2) was measured twice; the first time was measured before the patient received diltiazem (DTZ) as CsA-sparing agent, and the second time was performed 1 month after concomitantly used of DTZ to ensure that steady state was reached. The mean±S.D of the dose-adjusted C_2 before and after received DTZ were 301.68±142.78 and 299.35±112.07 ng/ml per mg/kg/day, respectively. There was a wide standard deviation of the pharmacokinetic parameter among these subjects, indicating high inter-individual variation in CsA pharmacokinetic profile.

Paired t -test was performed to determine any significant difference between the dose-adjusted C_2 before and after concomitantly use of DTZ. Although, the concomitantly use of DTZ and CsA may increase the CsA blood level but this effect is not shown in this report. The blood creatinine level was measured to detected the nephrotoxicity and graft rejection. Paired t -test was performed to determine any significant difference between serum creatinine (Scr) level before and after DTZ used. The mean±S.D of Scr level before receiving DTZ was 1.58±1.03 mg/dl while the mean after used DTZ for 1 month was 1.63±1.15 mg/dl. The mean Scr level after concomitantly use of DTZ was increasing but was not significantly different at œ = 0.05 and no patient was diagnosed to confer nephrotoxicity or graft rejection.

Table 12; CsA doses, CsA C_2 blood level, Dose-adjusted C_2 and Serum creatinine (Scr) level before and after DTZ therapy. (N = 38)

Parameter	CsA	CsA+DTZ	p-value[a]
CsA daily dose (mg, mean±SD)	136.84±34.74 AM 69.08±17.85 PM 67.76±18.30	136.84±34.74 AM 69.08±17.85 PM 67.76±18.30	-
CsA C_2 level (ng/ml, mean±SD)	602.37±261.39	594.71±198.38	0.856
Dose-adjusted C_2 (ng/ml per mg/kg/day, mean±SD)	301.68±142.78	299.35±112.07	0.918
Scr level (mg/dl, mean±SD)	1.58±1.03	1.63±1.15	0.098

[a] Paired t-test

Population allelic frequencies

Genotyping of *CYP3A5* was obtained from 38 patients. When characterized the patients into 3 groups by *CYP3A5* genotyping, there were 5 patients (13%) with homozygous **1/*1*, 13 patients (34%) with heterozygous **1/*3* and 20 patients (53%) with homozygous **3/*3*. The allele frequency of *CYP3A5*1* was 30% and *CYP3A5*3* was 70%. Patient's gender, age, body weight, were not significantly different among these 3 groups. The details about demographic data of patients when categorized by *CYP3A5* genotypes are shown in **Table 13**.

Table 13; Demographic characteristics of patients when categorized patients into 3 groups based on *CYP3A5* genotypes

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	5	13	20	
Gender (male/female) [a]	2/3	7/6	12/8	0.724
Age (yr) [b] (mean±SD)	45.00±13.56	55.85±9.87	57.20±10.54	0.086
Body weight (kg) [b] (mean±SD)	63.40±15.81	67.99±13.79	66.55±13.66	0.823

[a] Chi-square test, [b] One-way ANOVA.

The frequency expected for each genotype was evaluated on the basis of Hardy-Windberg equilibrium proportions. None of the observed frequencies was significantly different from the expected frequencies. The details were shown in **Table 14**.

Table 14 ; Prevalence of *CYP3A5* genotype

(38 patients x 2 alleles)			Genotypes	Observed N=38	%	Predicted (HWE)
Alleles	N=76	%				
***1**	23	30	***1/*1**	5	13	3
			***1/*3**	13	34	16
***3**	53	70	***3/*3**	20	53	19
Chi-square= 1.948, p= 0.377						

Allelic frequencies of *CYP3A5* genotypes were in Hardy-Weinberg Equilibrium (HWE), p =0.377 .The calculation if allelic frequencies were in HWE:

The number of the *1 allele = (5 x 2) + (13 x 1) = 23 alleles

The number of the *3 allele = (20 x 2) + (13 x 1) = 53 alleles

The frequency of the *1 allele = p = 23 / (23 + 53) = 0.30

The frequency of the *3 allele = q = 53 / (23 + 53) = 0.70

The proportion of expected *1/*1, *1/*3 and *3/*3 genotypes could be predicted from HWE: p+q = 1 and $(p + q)^2 = 1$ or $p^2 + 2pq + q^2 = 1$

$p^2 = 0.30 \times 0.30 = 0.09$

$2pq = 2 \times 0.30 \times 0.70 = 0.42$

$q^2 = 0.70 \times 0.70 = 0.49$

The total number of patients included to this study was 38

Expected number of *1/*1 = 0.09 x 38 = 3.42 ≈ 3

Expected number of *1/*3 = 0.42 x 38 = 15.96 ≈ 16

Expected number of *3/*3 = 0.49 x 38 = 18.62 ≈ 19

The observed number of *1/*1 = 5

The observed number of *1/*3 = 13

The observed number of *3/*3 = 20

Chi-square = 1.948, p= 0.377

Therefore, could not reject the null hypothesis that the population is in HWE.

Effect of *CYP3A5* genotypes on CsA blood concentration at trough (C_0)

Data from 34 patients of the 38 patients from previous part were recruited into this part of the study. The 4 patients were excluded since the trough CsA concentration was not available. Patient's gender and body weight, were not significantly different while the significantly difference of patients'age was observed. The demographic data of 34 patients was shown in **Table 15**.

Table 15; Demographic characteristics of patients when categorized patients into 3 groups based on *CYP3A5* genotypes (N=34)

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	p-value
No. of patients	5	13	16	
Gender (male/female) [a]	2/3	7/6	10/6	0.493
Age (yr) [b] (Mean±SD)	45.00±13.56	55.85±9.87	60.56±8.09	0.013*
Body weight (kg) [b] (Mean±SD)	63.40±15.82	67.99±13.79	67.98±14.5	0.807

[a] Chi-square test, [b] One-way ANOVA.

The weight-adjusted dose was significantly higher in the *CYP3A5*1/*1* group when compare to *CYP3A5*3/*3* (post hoc; p = 0.021) while the dose-adjusted C_0 and CsA C_0 were not significantly different. However, the mean dose-adjusted C_0 showed an increasing trend in the patients with non-expressor alleles (*3). This result showed the higher dose requirement in patients with *CYP3A5*1/*1* genotype. The details about CsA dose, CsA blood C_0 and dose-adjusted CsA C_0 are shown in **Table 15A**.

Table 15A; Comparisons of CsA dose, CsA C_0 and dose-adjusted CsA C_0 among the renal transplant patients with different *CYP3A5* genotypes.

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	P-value[a]
Number of patients	5	13	16	
CsA daily dose	165.00±33.54	142.31±21.37	134.38±38.6	0.197
(mg, mean±SD)	AM80.00±20.92	AM73.08±12.34	AM 67.19±19.83	0.336
	PM85.00±13.69	PM69.23±10.96	PM 67.19±19.83	0.108
Weight-adjusted dose	2.66±0.49[b]	2.16±0.53	2.00±0.53[b]	0.067
(mg/kg/day, mean±SD)				
CsA C_0	98.00±32.91	101.69±21.69	99.50±28.78	0.959
(ng/ml, mean±SD)				
Dose-adjusted C_0	36.87±11.98	48.96±14.47	52.26±17.03	0.169
(ng/ml per mg/kg/day, mean±SD)				

[a] One-way ANOVA.

[b] Post-hoc ; p=0.021

When we categorized patients into 2 groups based on *CYP3A5* genotypes by included *CYP3A5*1/*3* into the same group as *CYP3A5*3/*3*; the weight-adjusted dose in *CYP3A5*1/*1* group was significantly higher while the C_0 was nearly equal as compared to the other group and inturn the dose-adjusted C_0 of the *CYP3A5*1/*1* group was lower than the other group but did not reach the statistically significant level. The demographic data of these 2 groups are shown in **Table 15B** while the details about CsA dose, CsA C_0 and dose-adjusted CsA C_0 are shown in **Table 15C**.

Table 15B; Demographic characteristics of patients when categorized patients into 2 groups as *CYP3A5*1/*1* versus *CYP3A5*1/*3 + CYP3A5*3/*3* genotypes (N=34)

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3+CYP3A5*3/*3*	p-value
No. of patients	5	29	
Gender (male/female) [a]	2/3	17/12	0.850
Age (yr) [b] (Mean±SD)	45.00±13.56	58.45±9.08	0.008
Body weight (kg) [b] (Mean±SD)	63.40±15.81	67.98±13.93	0.509

[a] Chi-square test, [b] t-test

Table 15C; Comparisons of CsA dose, CsA C_0 and dose-adjusted CsA C_0 when categorized patients into 2 groups as *CYP3A5*1/*1* versus *CYP3A5*1/*3 + CYP3A5*3/*3* genotypes (N=34)

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3+CYP3A5*3/*3*	P-value[a]
Number of patients	5	29	
CsA daily dose (mg, mean±SD)	165.00±33.54	137.93±31.78	0.090
	AM 80.00±20.91	AM 69.83±16.88	0.237
	PM 85.00±13.69	PM 68.10±16.22	0.036*
Weight-adjusted dose (mg/kg/day)	2.66±0.49	2.07±0.53	0.028*
CsA C_0 (ng/ml, mean±SD)	98.00±32.92	100.48±25.43	0.848
Dose-adjusted C_0 (ng/ml per mg/kg/day, mean±SD)	36.87±11.98	50.78±15.75	0.070

[a] t-test

Effect of *CYP3A5* genotypes on CsA blood at hour 2 (C_2) *(Before receiving DTZ)*

When we categorized the 38 patients into 3 groups based on *CYP3A5* genotypes. The demographic data was shown in **Table 13**. There were no statistically difference in patients' gender, age and body weight among these 3 genotype groups.

The impact of genetic polymorphism of *CYP3A5*3/*3* on CsA dose-adjusted concentration at hour 2 (C_2) in 38 renal transplant patients are summarized in **Table 16**. There was significantly higher CsA weight-adjusted dose in subjects with the *CYP3A5*1/*1* genotype compared to subjects with the *CYP3A5*3/*3* genotype (post hoc; p=0.025) , whereas, CsA C_2 and dose-adjusted CsA C_2 in subjects with the *CYP3A5*1/*1* genotype were lower than those in CYP3A5*3/*3 genotype patients.

Table 16; Comparisons of CsA dose, CsA C_2 and dose-adjusted CsA C_2 before receiving DTZ among renal transplant patients with different genotypes of *CYP3A5* (Before DTZ used)

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	P-value[a]
Number of patients	5	13	20	
CsA daily dose	165.00±33.54	142.31±21.37	126.25±38.45	0.061
(mg, mean±SD)	AM 80.00±20.92	AM73.08±12.34	AM 63.75±18.98	0.115
	PM 85.00±13.69	PM 69.23±10.96	PM 62.50±20.68	0.041*
Weight-adjusted dose	2.66±0.49[b]	2.16±0.53	1.91±0.52[b]	0.022*
(mg/kg/day,				
mean±SD)				
CsA C_2	498.20±230.93	731.54±310.57	544.45±207.62	0.081
(ng/ml, mean±SD)				
Dose-adjusted C_2	188.10±87.93	349.63±158.36	298.91±131.37	0.096
(ng/ml per mg/kg/day,				
mean±SD)				
[c](ng/ml per	376.20±175.86	699.25±316.72	597.82±262.73	0.096
mg/kg/12hr,mean±SD)				
[d](ng/ml per	399.39±196.73	681.84±311.90	583.79±226.12	0.123
mg/kg/12hr,mean±SD)				

[a] ANOVA

[b] Post-hoc; p = 0.025

[c] calculated form CsA C_2/average dose (AM+PM/2)

[d] calculated from CsA C_2 / AM dose

When we categorized patients into 2 groups based on *CYP3A5* genotypes by included only *CYP3A5*1/*1* into the first group, and the second group was *CYP3A5*1/*3 and CYP3A5*3/*3*, the CsA weight-adjusted dose in *CYP3A5*1/*1* group was significantly higher while the C_2 were lower but not reach the statistically significant level and inturn the dose-adjusted C_2 were nearly significantly lower when compare to another group . The demographic data of these 2 groups was shown in **Table 16A** while the details about CsA dose, CsA C_2 and dose-adjusted CsA C_2 are shown in **Table 16B**.

Table 16A; Demographic characteristics of patients when categorized patients into 2 groups as *CYP3A5*1/*1* versus *CYP3A5*1/*3* + *CYP3A5*3/*3* genotypes (N=38)

Demographic data	*CYP3A5*1/*1*	*CYP3A5*1/*3+CYP3A5*3/*3*	p-value
No. of patients	5	33	
Gender (male/female) [a]	2/3	19/14	0.850
Age (yr) [b] (Mean±SD)	45.00±13.56	56.67±10.15	0.128
Body weight (kg) [b] (Mean±SD)	63.40±15.82	67.12±13.52	0.578

[a] Chi-square test, [b] t-test

Table 16B; Comparisons of CsA dose, CsA C_2 and dose-adjusted CsA C_2 when categorized patients into 2 groups as *CYP3A5*1/*1* versus *CYP3A5*1/*3 + CYP3A5*3/*3* genotypes (Before DTZ used)

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3+CYP3A5*3/*3*	P=value[a]
Number of patients	5	33	
CsA daily dose (mg, mean±SD) Weight-adjusted dose (mg/kg/day)	165.00±33.54 AM 80.00±20.92 PM 85.00±13.69 2.66±0.49	132.58±33.36 AM 67.42±17.10 PM 65.15±17.61 2.01±0.53	0.050* 0.144 0.022* 0.015*
CsA C_2 (ng/ml, mean±SD)	498.20±230.93	618.15±265.30	0.346
Dose-adjusted C_2 (ng/ml per mg/kg/day, mean±SD) [b](ng/ml per mg/kg/12hr,mean±SD) [c](ng/ml per mg/kg/12hr,mean±SD)	188.10±87.93 376.20±175.86 399.39±196.73	318.89±142.42 637.78±284.84 622.42±263.07	0.055 0.055 0.078

[a] t-test

[b] calculated form CsA C_2/average dose (AM+PM/2)

[c] calculated from CsA C_2 / AM dose

To compare the different of the dose-adjusted C_0 and the dose-adjusted C_2 between the *CYP3A5*1/*1* patients and the groups of *CYP3A5*1/*3 + CYP3A5*3/*3* patients, the data are shown in **Table 16C** . Although, these data show the same result that the dose-adjusted C_0 and dose-adjusted C_2 were lower in the *CYP3A5*1/*1* patients when compare to the another group, the p-value of the difference of dose-adjusted C_2 between these 2 groups of patients was more significant at œ=0.05 than the p-value of the difference of dose-adjusted C_0, these data might be show the more sensitivity when use the dose-adjusted C_2 as the parameter to detect the

different of CsA pharmacokinetic parameter between the patients with different of *CYP3A5* genotype.

Table 16C; Comparisons of Dose-adjusted C_0 and Dose-adjusted C_2 when categorized patients into 2 groups as *CYP3A5*1/*1* versus *CYP3A5*1/*3 + CYP3A5*3/*3* genotypes

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3+CYP3A5*3/*3*	P=value[a]
Dose-adjusted C_0 (ng/ml per mg/kg/day, mean±SD)	36.87±11.98 (N=5)	50.78±15.75 (N=29)	0.070
Dose-adjusted C_2 (ng/ml per mg/kg/day, mean±SD)	188.10±87.93 (N=5)	318.89±142.42 (N=33)	0.055

Effect of *CYP3A5* genotypes on CsA blood concentration at hour 2 (C_2) after DTZ used

When we categorized 38 patients into 3 groups based on *CYP3A5* genotypes. There were no statistically difference in the gender, age and body weight among these 3 genotype groups as shown in **Table 13**.

Table 17 shows the comparisons of patient's pharmacokinetic parameters of CsA after concurrent use with DTZ for 1 month when categorized patients into 3 groups based on their *CYP3A5* genotypes. The CsA daily dose was the same as before receiving DTZ, so the weight-adjusted dose was still significantly higher in patients with *CYP3A5*1/*1* genotype. The mean dose-adjusted C_2 showed slightly increasing trend with the number of variant allele but no statistically difference among these 3 groups of patients.

Table 17; Comparisons of CsA dose, CsA C_2 and adjusted-CsA C_2 among renal transplant patients with different *CYP3A5* genotypes. (After DTZ used)

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3*	*CYP3A5*3/*3*	P-value [a]
Number of patients	5	13	20	
CsA daily dose (mg, mean±SD)	165.00±33.54	142.31±21.37	126.25±38.45	0.061
	AM 80.00±20.92	AM73.08±12.34	AM 63.75±18.98	0.115
	PM 85.00±13.69	PM 69.23±10.96	PM 62.50±20.68	0.041*
Weight-adjusted dose (mg/kg/day)	2.66±0.49	2.16±0.54	1.91±0.52	0.022*
CsA C_2 (ng/ml, mean±SD)	579.20±178.7	633.08±196.01	573.65±209.87	0.701
Dose-adjusted C_2 (ng/ml per mg/kg/day, mean±SD)	217.88±58.67	304.12±105.89	316.61±120.73	0.212
[b](ng/ml per mg/kg/12hr,mean±SD)	435.76±117.35	608.25±211.79	633.23±241.46	0.212
[c](ng/ml per mg/kg/12hr,mean±SD)	462.04±152.02	595.55±213.87	624.57±238.60	0.353

[a] One-way Anova

[b] calculated form CsA C_2/average dose (AM+PM/2)

[c] calculated from CsA C_2 / AM dose

When we categorized patients into 2 groups based on *CYP3A5* genotypes by included only *CYP3A5*1/*1* into the first group, and the second group was *CYP3A5*1/*3 and CYP3A5*3/*3*, the CsA weight-adjusted dose in *CYP3A5*1/*1* group was significantly higher while the C_2 was nearly equal as compare to the other group and inturn the dose-adjusted C_2 was nearly significantly lower than the other group. The demographic data of these 2 groups are shown in **Table 16A** while the details about CsA dose, CsA C_2 and dose-adjusted CsA C_2 are shown in **Table 17A**.

Table 17A; Comparisons of CsA dose, CsA C_2 and dose-adjusted CsA C_2 DTZ between renal transplant patient with different genotypes as *CYP3A5*1/*1* versus *CYP3A5*1/*3* + *CYP3A5*3/*3* genotypes (After DTZ used)

Parameter	*CYP3A5*1/*1*	*CYP3A5*1/*3+CYP3A5*3/*3*	P-value[a]
Number of patients	5	33	
CsA daily dose	165.00±33.54	132.58±33.36	0.050*
(mg, mean±SD)	AM 80.00±20.92	AM 67.42±17.1	0.144
	PM 85.00±13.69	PM 65.15±17.61	0.022*
Weight-adjusted dose	2.66±0.49	2.01±0.53	0.015*
(mg/kg/day)			
CsA C_2	579.20±178.70	597.06±203.64	0.854
(ng/ml, mean±SD)			
Dose-adjusted C_2	217.88±58.67	311.69±113.57	0.081
(ng/ml per mg/kg/day, mean±SD)			
[b](ng/ml per	435.76±117.35	632.38±227.14	0.081
mg/kg/12hr,mean±SD)			
[c](ng/ml per	462.04±152.02	613.14±226.19	0.160
mg/kg/12hr,mean±SD)			

[a]T-test

[b] calculated form CsA C_2/average dose (AM+PM/2)

[c] calculated from CsA C_2 / AM dose

Effect of DTZ on the pharmacokinetics of CsA in renal transplant patients with different *CYP3A5* genotypes

When we categorized 38 patients into 3 groups based on *CYP3A5* genotypes. The demographic data was shown in **Table 13**. There were no statistically difference in patient's gender, age and body weight among these 3 genotype groups.

Table 18 show comparisons of weight-adjusted dose, CsA C_2 and dose-adjusted CsA C_2 before and after concurrent used with DTZ in renal transplant patients with different *CYP3A5* genotypes. There was significantly higher CsA weight-adjusted dose in subject with *CYP3A5*1/*1* as previously mentioned. However, the CsA daily dose was fixed before and after DTZ used, the mean dose-adjusted C_2 after concurrently used with DTZ showed the trend to be increasing in the patient with *CYP3A5*1/*1* genotype, while the mean dose-adjusted C_2 after DTZ used seem to be nearly the same as before DTZ used in the patients with *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes.

Table 18; Comparisons of CsA dose, CsA C_2 and dose-adjusted CsA C_2 before and after concurrent use with DTZ when categorized patients into 3 groups based on their *CYP3A5* genotypes

Parameter	*CYP3A5*1/*1* (n=5)			*CYP3A5*1/*3* (n= 13)			*CYP3A5*3/*3* (n=20)		
	before	after	p-value[a]	before	after	p-value[a]	before	after	p-value[a]
CsA daily dose (mg, mean±SD)	165.00±33.54 AM 80.00±20.92 PM 85.00±13.69	165.00±33.54 AM 80.00±20.92 PM 85.00±13.69	-	142.31±21.37 AM 73.08±12.34 PM 69.23 ±10.96	142.31±21.37 AM 73.08±12.34 PM 69.23±10.96	-	126.25±38.45 AM 63.75±18.98 PM 62.50 ± 20.68	126.25±38.45 AM 63.75±18.98 PM 62.50 ± 20.68	—
CsA C_2 (ng/ml, mean±SD)	498.20±230.93	579.20±178.7	0.094	731.54±310.57	633.08±196.01	0.342	544.45±207.62	573.65±209.87	0.509
Dose-adjusted C_2 (ng/ml per mg/kg/day, mean±SD)	188.10±87.93	217.88±58.67	0.107	349.63±158.36	304.12±105.89	0.367	298.91±131.37	316.61±120.73	0.535

[a] Paired t-test

When we categorized patients into 2 groups based on *CYP3A5* genotypes by included only *CYP3A5*1/*1* into the first group while the second group was *CYP3A5*1/*3 and CYP3A5*3/*3*. The mean dose-adjusted C_2 after concurrently used with DTZ showed the trend to be increasing in the patients with *CYP3A5*1/*1* but did not reach the statistically significant difference, while the mean dose-adjusted C_2 before and after concurrently used with DTZ were nearly equal in patients with *CYP3A5*1/*3 and CYP3A5*3/*3* genotypes. These details are shown in **Table18A.**

Table18A; Comparisons of CsA dose, CsA C_2 and dose-adjusted CsA C_2 before and after concurrent use with DTZ when categorized patients into 2 groups (*CYP3A5*1/*1* versus *CYP3A5*1/*3* + *CYP3A5*3/*3* genotype)

Parameter	*CYP3A5*1/*1* (n=5)			*CYP3A5*1/*3*+ *CYP3A5*3/*3* (n= 33)		
	before	after	p-value[a]	before	after	p-value[a]
CsA daily dose (mg, mean±SD)	165.00±33.54 AM 80.00±20.92 PM 85±13.69	165.00±33.54 AM 80.00±20.92 PM 85±13.69	-	132.58±33.36 AM 67.42±17.10 PM 65.15±17.61	132.58±33.36 AM 67.42±17.10 PM 65.15±17.61	-
CsA C_2 (ng/ml, mean±SD)	498.20±230.93	579.20±178.70	0.094	618.15±265.30	597.06±203.64	0.660
Dose-adjusted C_2 (ng/ml per mg/kg/day, mean±SD)	188.10±87.93	217.88±58.67	0.107	318.89±142.42	311.69±113.57	0.781

[a] Paired t-test

Moreover, this study found that DTZ dose not always show CsA-sparing effect in all patients. The CsA C_2 after (C_2after) concurrently used of DTZ and CsA for 1 month were not in all cases increasing from CsA C_2 before (C_2before) DTZ was given to the patients. There were 22 patients of the total 38 patients (57.9%) where DTZ showed CsA-sparing effect to could be reduced the CsA dosage requirement (C_2after > C_2before). The wide standard deviation indicating high inter-individual variations in CsA- sparing effect of DTZ.

Despite the group data showing no significant difference in CsA C_2 between before and after DTZ used, the individual data showed that the concurrent use with DTZ trend to increasing CsA C_2 in *CYP3A5*1/*1* genotype more than the other genotypes. There were 4 patients out of the total 5 patients (80%) with homozygous **1/*1*, 5 patients out of the total 13 patients (39%) with heterozygous **1/*3* and 13 patients out of the total 20 patients (*65%*) with homozygous **3/*3* showing the CsA-sparing effect (C_2after > C_2before) when DTZ used. When we categorized patients into 2 groups based on *CYP3A5* genotypes by included only *CYP3A5*1/*1* into the first group, and the second group was *CYP3A5*1/*3* and *CYP3A5*3/*3*, the difference of CsA C_2 between before and after DTZ used (C_2diff; C_2after – C_2before) was trend to more different in the *CYP3A5*1/*1* patients when compare to the other genotypes eventhough the significant difference was not met. The difference of CsA-sparing effect of DTZ when characterized the patients based on their *CYP3A5* genotypes are shown in **Table 19**, while the individual data are shown in **Appendix E**.

Table 19; The difference of CsA-sparing effect of DTZ when characterized the patients into 3 groups based on their *CYP3A5* genotypes

Parameter	*CYP3A5* genotypes			p-value
	*1/*1 (N=5)	*1/*3 (N=13)	*3/*3 (N=20)	
No. of patients C_2after > C_2before	4	5	13	
C_2diff (ng/ml, Mean±SD)	81.00±82.64	-98.46±358.81	29.20±194.11	0.278
C_2diff (ng/ml, Mean±SD)	81.00±82.64	-21.09±273.25		0.105

C_2diff = C_2after – C_2before

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DISCUSSION AND CONCLUSION

CsA is a potent immunosuppressant drug widely used in organ transplantation. While graft survival results are generally better than those achieved with older immunosuppressive drugs; the cost of maintaining grafts with CsA are much greater.[1-5] CsA is metabolized by a cytochrome P450 3A (CYP3A) subfamilies, CYP3A4 and CYP3A5, in both liver and enterocyte.[6-8] Nowaday, CsA is frequently coadministration with DTZ because the latter has possible beneficial effect on the economic impact associated with reduction of the CsA dosage. The interaction between CsA and DTZ results in increase CsA blood concentration due to the CYP3A5 inhibitory effect of the DTZ.[22-23] Moreover, DTZ is a relatively safe drug with useful antihypertensive effect on the control of blood pressure and protection of kidney function.[69, 81-84] McDonald et al,[10] demonstrated that renal transplanted patients who were on CsA-sparing agent, DTZ, had a better renal allograft outcome than those who were not on DTZ.

In recent year, extensive studies on pharmacogenetics of immunosuppressive drug have been focused. The main contribution of the pharmacogenetics is to predict the initial dose of a given drug, increasing the chances that adequate drug exposure will be achieved early after inception of therapy. Pharmacogenetics may anticipate potentially harmful drug-to-drug interactions, thereby reducing the incidence of adverse drug event, a significantly cause of morbidity, mortality and excessive medical care expenses.[87] Recent pharmacogenomic studies found that *CYP3A5* polymorphism effect on CsA level-to-dose ratio. The most frequent and functionally important Single-nucleotide polymorphisms (SNPs) in the *CYP3A5* gene is a mutation of adenosine (*CYP3A5*1*, wild type allele) to guanosine (*CYP3A5*3*, mutated allele) at the position 6986 within intron 3. *CYP3A5*1* has found to be the main allele associated with CYP3A5 expression, whereas the mutant allele *CYP3A5*3* prevents expression of this enzyme due to premature termination during translation of the aberrant mRNA and cause alternative splicing and protein truncation resulting in the absence of CYP3A5 enzyme activity.[91, 98] However, few studies have shown the role of these SNPs on CsA pharmacokinetics characteristics.[21-22] Moreover, the effect of *CYP3A5* polymorphism on CsA disposition has been interestingly inconsistent.[19, 113-114]

Several polymorphic of *CYP3A5* have been recently reported in difference populations. In Thai population the allele frequency of *CYP3A5*3* was 66% and *CYP3A5*1* was 34%,[101] which is similar to other Asian population but significant difference from Caucasian and African American. The frequency of *CYP3A5*3* allele in Thai population was lower and higher than Caucasian and African American respectively. Other *CYP3A5* coding variants have been described, but occur at relatively low allele frequencies.[99-100]In this study, we determined the frequencies of *CYP3A5**3 alleles in Thai renal allograft recipients. When characterized the 38 patients into 3 groups by *CYP3A5* genotyping, there were 5 patients (13%) with homozygous **1/*1*, 13 patients (34%) with heterozygous **1/*3* and 20 patients (53%) with homozygous **3/*3*. The allele frequency of *CYP3A5*1* was 30% and *CYP3A5*3* was 70%. Our finding indicated that these frequencies are similar to previous study in Thai population and in all Asians, including Chinese, Indian, Malaysians and Japanese populations,[99, 101-103] but are different from those report to other populations, including Caucasian and African-American populations.[100, 104] The expected allelic frequencies of *CYP3A5* genotype estimated at Hardy-Weinberg equilibrium were quite similar to the observed distribution in the population (chi-square = 1.948, p=0.377)

Moreover, we explored the effect of *CYP3A5* genotypes on the pharmacokinetic parameters of CsA blood concentration at trough (C_0) and 2 hour post dose (C_2). The finding show that the CsA pharmacokinetic parameter in patients with *CYP3A5*1/*3* genotype is more similar to the *CYP3A5*3/*3* genotype than the *CYP3A5*1/*1* genotype, so we combined patients with *CYP3A5*1/*3* genotype and *CYP3A5*3/*3* genotype into the same group. When categorized patients into 2 groups of different genotypes as *CYP3A5*1/*1* group vs *CYP3A5*1/*3* and *CYP3A5*3/*3* group, there were statistically significantly higher in weight-adjusted daily dose of the *CYP3A5*1/*1* group ($p = 0.028$ (N=34, C_0 part) and $p = 0.015$ (N=38, C_2 part)), while the dose- adjusted C_0 and the dose- adjusted C_2 of *CYP3A5*1/*1* were nearly statistically significantly lower than those obtained in *CYP3A5*1/*3* and *CYP3A5*3/*3* group ($p = 0.070$ and $p = 0.055$, respectively). These results confirm the fact that patients with *CYP3A5*1/*1* genotype show the higher CsA dosage requirement than patients with *CYP3A5*1/*3* and *CYP3A5*3/*3* genotypes due to the fact that the expression of the larger amount of CYP3A5 enzyme. Hence expression of CYP3A5 may result in increased metabolism of its substrate drug; carrier of the enzyme would display lower drug concentrations per administered dose; and inturn, lower dose-adjusted concentration ratios (C/D ratios).

Conversely, non-expressors *CYP3A5*3* carriers may show higher C/D ratios, due to reduced metabolism of the substrate drug. Yate et al[97] studied in 10 patients and found that CsA metabolism was increase by 52% in *CYP3A5*1* carriers. Our results were consisted to few studies which showed that patients carrying *CYP3A5*1* allele had lower dose-adjusted trough blood concentration than patients carrying *CYP3A5*3* allele.[109-110] Moreover, we found that the difference in dose-adjusted C_2 between *CYP3A5*1/*1* group vs *CYP3A5*1/*3* and *CYP3A5*3/*3* group is more obvious than the difference in dose-adjusted C_0 ($p = 0.055$ vs $p = 0.070$, respectively). This may due to the higher value of C_2 as compared to C_0 and inturn more sensitive to detect the difference. Pharmacokinetics studies have suggested that CsA C_2 is the best single point to predict AUC of CsA in kidney transplant patients. CsA C_2 had also been reported to be able to predict acute rejection episode and nephrotoxicity better than CsA C_0.[52, 120] The monitoring to achieve the optimal levels of CsA C_2 might be more appropriated and may help reducing the incidence of graft rejection better than CsA C_0. Whereas, correlations between the *CYP3A5* genotype and dose-adjusted CsA concentrations was found by some studies, these effect were not observed by some other studies.[108, 113] Trials evaluating the pharmacogenetics of CsA have inconsistent methods, which may be a contributing factor to the largely inconsistent results. Besides, these conflicting finding may due to the vary in the examined pharmacokinetic parameters, differences in the frequencies of *CYP3A5*1* and *CYP3A5*3* variants in different population and the lower power of the test due to small number of patients participated in the study especially those patients in *CYP3A5*1/*1* group.

The cotreatment of oral CsA with different drugs oriented to a reduction of dosage regimen is well reported in the literature.[62] Faradori et al,[119] demonstrated that DTZ enhances the absorption phase of CsA with increases in C_0 and C_{max} and a tentative reduction in T_{max}. To our knowledge, this is the first study concentrated on CsA-sparing effect of DTZ among different *CYP3A5* genotypes. We study the differences of CsA daily dose, CsA C_2 and dose-adjusted C_2 before and after DTZ used. In our study, all patients received 30 mg/day of DTZ for 1 month. Although, CsA daily dose were kept constant, the CsA C_2 seemed to be higher in *CYP3A5*1/*1* patients but did not reach statistically significant level (498.20±230.93 and 579.20±178.70 ng/ml, respectively, $p = 0.094$), contrastly, the CsA C_2 in *CYP3A5*1/*3* and *CYP3A5*3/*3* patients were nearly indifferent. The CsA C_2 were 731.54±310.57 and 633.08±196.01 ng/ml, $p=0.342$ in *CYP3A5*1/*3* patients while the CsA C_2 were 544.45±207.62 and 573.65±209.87 ng/ml, $p=0.509$

in *CYP3A5*3/*3* patients before and after DTZ used, respectively. The statistically different of CsA C_2 was not reach in *CYP3A5*1/*1* patients should due in part to the small number of patients participated in the study and the low dose of DTZ used in this study make the pharmacokinetic interaction between CsA-DTZ, if any, less strong since the interaction has a dose-response relationship.[121] Moreover, we found that the DTZ coadministration has the effect to the difference of dose-adjusted CsA C_2 among *CYP3A5* genotype patients. Before DTZ was administered, the dose-adjusted C_2 between *CYP3A5*1/*1* group vs *CYP3A5*1/*3* and *CYP3A5*3/*3* group seem to be nearly statistically different (p =0.055) while the dose-adjusted C_2 between *CYP3A5*1/*1* group vs *CYP3A5*1/*3* and *CYP3A5*3/*3* group after DTZ was administered was clearly indifferent (p = 0.081). This result might be due to the effect of DTZ prominently increase the C_2 in the patients with *CYP3A5*1/*1* patients, the dose-adjusted C_2 in this group was increased and the different between these 2 groups of patients was decreased.

Although there were no different in dose-adjusted C_2 among the different *CYP3A5* genotypes after DTZ was administered, the individual patients sub-analysis was found some interesting data. The results from this study indicated that DTZ does not always show CsA-sparing effect in all patients as described before.[24] The number of patients who had C_2after > C_2before is 22 out of the total 38 patients (58%). However, when characterized the patients into 3 groups by *CYP3A5* genotyping, the percentage of patients whose C_2after > C_2before was higher in *CYP3A5* homozygous **1/*1* genotype (80%, 4 patients out of the total 5 patients) than those in the heterozygous **1/*3* (38%, 5 patients out of the total 13 patients) and homozygous **3/*3* groups (65%, 13 patients out of the total 20 patients). These results suggested that coadministration with DTZ was differently showed the CsA-sparing effect differently among different *CYP3A5* genotype, with a more prominent inhibitory effect of DTZ on enzyme activity in *CYP3A5**1/*1 genotype.

In conclusion, the present study has demonstrated that genetic polymorphisms of *CYP3A5* may effect the pharmacokinetic of CsA; first, higher dosage of CsA were required in patients with *CYP3A5*1/*1* genotypes. Pharmacogenetic detection of *CYP3A5* before transplantation may be useful in clinical practice to optimize the initial dose of CsA administered to individual renal transplant patients. However, the clinical applicability of this approach and changed in the dosage of CsA based on the outcome of genotype screening remain to be proven. Moreover, if the DTZ is coadministered, the effect of DTZ as CsA-sparing agent show

more prominent effect in patients carrying *CYP3A5*1/*1* genotype as compared to the others; the dose-adjusted C_2 showed trend to be higher when the drug was concurrently used even with low dose of DTZ (30mg/day). Closely monitored for CsA level and dosage adjusted accordingly to prevent toxicity of CsA overdose may be required, especially when DTZ is coadministered in a higher dosage (60-180 mg/day as normally used) in patient with *CYP3A5**1/*1 genotype.

Limitation

1. The number of patients in the *CYP3A5*1/*1* group was too few, higher number of patients are needed to increase the power of statistical analysis before any strong conclusion could be made.

2. This is the cross-sectional study that the primary analysis focused on drug dosing and CsA blood level but did not concentrate on the clinical outcomes of the patients such as, the rate of patients survival, graft rejection, hepatic or renal toxicity and other long-term complications.

3. The dose of DTZ was lower than those normally used for CsA-sparing agent (60-180 mg/day). [72] The study kept CsA daily dose constant between before and after receiving 30 mg/day of DTZ. The low dosage of DTZ might not contribute enough effect on the inhibition of CsA metabolism. Co-administration with DTZ in CsA-based immunosuppression therapy may need further study for the appropriated individualizing of DTZ/CsA doses, so that, DTZ could function as an effective CsA-sparing dose.

4. The subjects included into this study were outpatients, so that co-medications which are not inhibitor or inducer of CYP3A5 enzyme only were controlled. Other factors that may affect the CsA pharmacokinetics, such as, the exact time of CsA and DTZ administrations, the patients compliance and the food or behavior that may have impacted on the absorption or metabolism of CsA had not been controlled. Moreover, some blood chemistry might not be appropriately controlled such as hemoglobin, hematocrit and lipoprotein value that may be affected to the CsA pharmacokinetic properties.

5. C_2 had been chosen for CsA blood level monitoring , since it has been suggested that the CsA C_2 is the best single point that correlates with AUC, while C_0 correlates poorly, whether patients were treated with or without DTZ.[122] However, Faradori et al[119] who reported that there was a tentative reduction of T_{max} when CsA-DTZ were co-administration; the T_{max} was 2±0.4 hours and 1.62±0.64 hours when CsA was used alone and when CsA-DTZ were co-administration, respectively. When T_{max} after DTZ used was decreased, the single point determination of C_2 for monitoring the difference of CsA blood level before and after DTZ used were not the same point, therefore, this might confound the effect.

Further study

Another issue that needs to be addressed is the combined effect of the genetic polymorphism in *CYP3A5* and *P-glycoprotein*(*P-gp*) SNPs on CsA-DTZ interaction. The DTZ can decrease intestinal absorption of CsA because both DTZ and CsA are good substrates for P-gp, which is an important molecule for CsA absorption. While the *P-gp* polymorphism effects the different of DTZ/CsA absorption part, the *CYP3A5* polymorphism effects the metabolism part. This dual pathway partially obscures the effect of genetic polymorphism to CsA-DTZ interaction. In conclusion, the present study has demonstrated that genetic polymorphism of *CYP3A5*3* may be responsible; patients with *CYP3A5*1/*1* genotype may need to be given higher dose of CsA to reach target concentration and need to be adjusted dose when DTZ was coadministered compare with patients that were *CYP3A5*1/*3* and *CYP3A5*3/*3*. Pharmacogenetic detection of *CYP3A5* before transplantation is likely to be useful in clinical practice to optimize the initial dose of CsA, especially when co-administration with DTZ to individual renal transplant patient. However, the clinical application of this approach and change in the initial dose of CsA based on the outcome of genotype screening remain to be proven.

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APPENDICES

APPENDIX A

แบบเก็บข้อมูลผู้ป่วยเพื่อศึกษาผลของภาวะพหุสัณฐานของยีน *CYP3A5*
ต่อการเกิดอันตรกิริยาของยาไซโคลสพอรินและยาดิลไทอะเซ็ม

ส่วนที่1: ข้อมูลพื้นฐานทั่วไป

อายุ…….. ปี **เพศ** ☐ ชาย ☐ หญิง **เชื้อชาติ/สัญชาติ**……..

ประวัติแพ้ยา……. **น้ำหนัก**……**กิโลกรัม**

สาเหตุของโรคที่ทำให้ได้รับการปลูกถ่ายไต

- ☐ Chronic glomerulonephritis
- ☐ ESRD from chronic disease
- ☐ Nephrotic syndrome
- ☐ Congenital abnormalities
- ☐ Other

ประวัติความเจ็บป่วยร่วม

- ☐ ความดันโลหิตสูง
- ☐ เบาหวาน
- ☐ ระบบหลอดเลือดและหัวใจ
- ☐ ระดับไขมันผิดปกติในเลือด
- ☐ อื่นๆระบุ……

ส่วนที่ 2: ข้อมูลการปลูกถ่ายไตและการใช้ยากดภูมิคุ้มกัน

ชนิดของไตปลูกถ่าย ☐ LRKT ☐ CDKT

วันที่ปลูกถ่ายไต……..

สูตรยากดภูมิคุ้มกันที่ใช้ในปัจจุบัน

- ☐ CSA…….mg/day เวลาที่รับประทานยา…….
- ☐ MMF…….mg/day เวลาที่รับประทานยา…….
- ☐ AZA…….mg/day เวลาที่รับประทานยา……
- ☐ Pred…….mg/day เวลาที่รับประทานยา…….

ยาที่ใช้ร่วม

รายการยา	Dosage regimen	วัน/เดือน/ปี								

<u>ส่วนที่ 3</u>: ข้อมูลการใช้ยา diltiazem

1. เวลาที่รับประทานยา……..

2. วันที่เริ่มใช้ยา……วันที่หยุดใช้ยา…….

3. อาการไม่พึงประสงค์จากการใช้ยา……....

……..

……

<u>ส่วนที่ 4</u>: ข้อมูลระดับยาในเลือด

	ก่อนใช้ DTZ วันที่เจาะเลือด…….. เวลา……		หลังใช้ DTZ วันที่เจาะเลือด…….. เวลา……	
	ขนาดยา (mg/day)	ระดับยา (ng/ml)	ขนาดยา (mg/day)	ระดับยา (ng/ml)
CSA (C_2)				

<u>ส่วนที่ 5</u> : ข้อมูล *CYP3A5* genotyping

ผลการตรวจ *CYP3A5* genotyping

☐ *CYP3A5*1/*1* ☐ *CYP3A5*1/*3* ☐ *CYP3A5*3/*3*

APPENDIX B

เอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในโครงการวิจัย

ชื่อโครงการวิจัย ผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อการเกิดอันตรกิริยาทางเภสัชจลนศาสตร์ของยาไซโคลสพอรินกับยาดิลไทอะเซ็ม ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต

ชื่อผู้วิจัย เภสัชกรหญิงไพลิน วรรณประพันธ์ นิสิตระดับปริญญาโท
สาขาเภสัชกรรมคลินิก คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สถานที่วิจัย โรงพยาบาลพระรามเก้า

<u>บุคคลและวิธีการติดต่อเมื่อมีเหตุฉุกเฉินหรือความผิดปกติที่เกี่ยวข้องกับการวิจัย</u>

ชื่อ นางสาวไพลิน วรรณประพันธ์
สถานที่ติดต่อ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
เบอร์โทรศัพท์ 08-6904-9574
(สามารถติดต่อได้ 24 ชั่วโมง)
ชื่อ นายแพทย์วิรุฬห์ มาวิจักขณ์
สถานที่ติดต่อ แผนกอายุรกรรม โรงพยาบาลพระรามเก้า
เบอร์โทรศัพท์ 0-2202-9999
(สามารถติดต่อได้ 24 ชั่วโมง)

เรียน ผู้เข้าร่วมโครงการวิจัยทุกท่าน

ท่านได้รับเชิญให้เข้าร่วมในโครงการวิจัยนี้เนื่องจากท่านเป็นผู้ที่มีคุณสมบัติครบตามเกณฑ์การคัดเลือกตามที่กำหนด ดังต่อไปนี้ ได้แก่

<u>เกณฑ์การคัดตัวอย่างเข้าร่วมการศึกษา</u>

1. ผู้ป่วยที่ได้รับการปลูกถ่ายไตและมีการใช้ยาไซโคลสพอรินเป็นยาหลักในสูตรยากดภูมิคุ้มกัน โดยอาจใช้ร่วมกับยากดภูมิคุ้มกันอื่น ได้แก่ azathioprine , mychophenolate mofetil หรือ prednisolone
2. อายุมากกว่าหรือเท่ากับ 18 ปี
3. ได้รับการปลูกถ่ายไตเป็นระยะเวลาไม่น้อยกว่า 3 เดือน
4. ไม่มีข้อห้ามใช้ในการใช้ยาดิลไทอะเซ็ม ได้แก่ ภาวะความดันโลหิตต่ำ (SBP < 100 mmHg

หรือ DBP < 60 mmHg), อัตราการเต้นของหัวใจ (Heart rate) < 60 ครั้ง/นาที, ภาวะหัวใจล้มเหลวรุนแรง (severe congestive heart failure), ภาวะหัวใจขาดเลือด (acute myocardial infarction) และ ภาวะการคั่งของน้ำในปอด (pulmonary congestion)

5. ไม่แพ้ยาและส่วนประกอบของยาที่ใช้ในการวิจัย

6. ไม่ใช้ยาตัวอื่นที่มีฤทธิ์ยับยั้งหรือกระตุ้นการทำงานของเอ็นไซม์ CYP3A5 ได้แก่ Carbamazepine, Phenytoin, Ketoconazole, Fluconazole, Voriconazole, Itraconazole, Phenobarbital, Erythromycin, Clarithromycin, Rifampicin เป็นระยะเวลาอย่างน้อย 2 สัปดาห์ก่อนเข้าร่วมงานวิจัย

7. ผู้ป่วยหรือผู้แทนโดยชอบธรรมยินยอมเข้าร่วมการวิจัย

ก่อนที่ท่านจะตัดสินใจเข้าร่วมในการศึกษาวิจัยดังกล่าว ขอให้ท่านอ่านเอกสารฉบับนี้อย่างถี่ถ้วน เพื่อให้ท่านได้ทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้ หากท่านมีข้อสงสัยใดๆ เพิ่มเติม กรุณาซักถามผู้วิจัยซึ่งจะเป็นผู้สามารถตอบคำถามและให้ความกระจ่างแก่ท่านได้

ท่านสามารถขอคำแนะนำในการเข้าร่วมโครงการวิจัยนี้จากครอบครัว เพื่อน หรือแพทย์ประจำตัวของท่านได้ ท่านมีเวลาอย่างเพียงพอในการตัดสินใจโดยอิสระ ถ้าท่านตัดสินใจแล้วว่าจะเข้าร่วมในโครงการวิจัยนี้ ขอให้ท่านลงนามในเอกสารแสดงความยินยอมของโครงการวิจัยนี้

<u>เหตุผลความเป็นมา</u>

ยาไซโคลสพอริน เป็นยากดภูมิคุ้มกันเพื่อป้องกันการปฏิเสธอวัยวะที่มีประสิทธิภาพดี และมีการนำมาใช้อย่างแพร่หลายในผู้ป่วยปลูกถ่ายไต แต่อย่างไรก็ตามมีการศึกษาหลายการศึกษาที่พบว่าภาวะพหุสัณฐานของยีน *CYP3A5* มีผลต่อความแตกต่างในค่าอัตราการกำจัดยาในผู้ป่วยแต่ละราย ส่งผลให้ผู้ป่วยที่มีภาวะพหุสัณฐานของยีน *CYP3A5* ที่แตกต่างกันจะมีความต้องการขนาดยาต่อวันที่แตกต่างกัน

จากการศึกษาความชุกของภาวะพหุสัณฐานของยีน *CYP3A5* ในคนไทยพบความถี่ของยีนชนิด *CYP3A5*3* ถึงร้อยละ 66 ซึ่งยีนชนิด *CYP3A5*3* จะมีความบกพร่องในการทำงานของเอนไซม์ CYP3A5 ซึ่งส่งผลต่ออัตราการกำจัดยาไซโคลสพอริน ดังนั้นผู้ป่วยปลูกถ่ายไตที่มีภาวะพหุสัณฐานของยีนแบบ *CYP3A5*3* จะมีความต้องการขนาดยาต่อวันที่ต่ำกว่าผู้ป่วยที่มียีนแบบ *CYP3A5*1*

ยาดิลไทอะเซ็มเป็นยาที่มีการนำมาใช้ในผู้ป่วยปลูกถ่ายไตเนื่องจากมีคุณสมบัติในการรักษาโรคความดันโลหิตสูง นอกจากนี้ยังมีการศึกษาที่พบว่าการใช้ดิลไทอะเซ็มช่วยป้องกันภาวะการเกิดพิษต่อไตจากการใช้ยาไซโคลสพอริน และสามารถลดขนาดยาต่อวันของยาไซโคลสพอริน อันเนื่องมาจากการเกิดปฏิกิริยาระหว่างยาซึ่งส่งผลต่อการเพิ่มระดับยาไซโคลสพอรินในเลือด

การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบค่าสัดส่วนระดับยาต่อขนาดยา (level-to-dose ratio) ของยาไซโคลสพอรินเมื่อใช้ร่วมกับยาที่มีฤทธิ์เป็น CYP3A5 inhibitor โดยใช้ดิลไทอะเซ็มเป็นตัวยาต้นแบบในการศึกษา โดยทำการศึกษาในผู้ป่วยปลูกถ่ายไตที่ใช้ยาไซโคลสพอริน เป็นตัวยากดภูมิคุ้มกันหลัก เพื่อดูผลของยาดิลไทอะเซ็มซึ่งส่งผลต่อค่าอัตราการกำจัดยา และค่าสัดส่วนระดับยาต่อขนาดยาของยาไซโคลสพอริน ในผู้ป่วยปลูกถ่ายไตที่มีภาวะพหุสัณฐานของยีน *CYP3A5* ที่แตกต่างกัน ซึ่งจะช่วยให้แพทย์สามารถใช้ยาไซโคลสพอรินในขนาดยาที่เหมาะสมกับผู้ป่วยแต่ละคน และทำให้ระดับยาในเลือดอยู่ในช่วงที่เหมาะสมได้รวดเร็วขึ้น

<u>วัตถุประสงค์ของการศึกษา</u>

วัตถุประสงค์หลักจากการศึกษาในครั้งนี้คือเพื่อเปรียบเทียบค่าสัดส่วนระดับยาต่อขนาดยา (level-to-dose ratio) ของยาไซโคลสพอรินเมื่อใช้ร่วมกับดิลไทอะเซ็ม ในผู้ป่วยปลูกถ่ายไตที่มีภาวะพหุสัณฐานของยีน *CYP3A5* ที่ต่างกันคือ *CYP3A5*1* และ *CYP3A5*3*

จำนวนผู้เข้าร่วมในโครงการวิจัย คือ 40 คน

<u>วิธีการที่เกี่ยวข้องกับการวิจัย</u>

หากท่านตัดสินใจเข้าร่วมการศึกษาวิจัยนี้กรุณาเซ็นชื่อลงในใบยินยอม ท่านจะได้รับยาดิลไทอะเซ็มในขนาด 30 มิลลิกรัม/วัน และแพทย์จะนัดติดตามผลเลือดหลังจากได้รับยาดิลไทอะเซ็มเป็นระยะเวลา 1 เดือน โดยในการติดตามท่านจะได้รับการตรวจดังต่อไปนี้

ท่านจะได้รับการชั่งน้ำหนัก ตรวจวัดความดันโลหิต และได้รับการเจาะเลือดปริมาณ 10 มิลลิลิตร (2 ช้อนชา) เพื่อตรวจหา

- ระดับยาไซโคลสพอรินในเลือด
- ลักษณะของยีน *CYP3A5*

<u>หมายเหตุ</u> ท่านไม่ต้องเสียค่าใช้จ่ายใดๆที่นอกเหนือไปจากค่ารักษาพยาบาลของท่านตามปกติ

<u>ความเสี่ยงจากการเข้าร่วมวิจัย</u>

ความเสี่ยงในการเจาะเลือดคือ อาจมีอาการปวด หรือมีจ้ำเลือดบริเวณที่เจาะ แต่มีความเสี่ยงน้อยมากที่จะเกิดการติดเชื้อจากการเจาะเลือด

ความเสี่ยงจากการได้รับผลข้างเคียงจากการใช้ยาดิลไทอะเซ็ม คือ อาการปวดศีรษะ เวียนศีรษะ คลื่นไส้ หน้าแดง อาเจียน เบื่ออาหาร ผื่นแดง ความดันโลหิตต่ำ นอนไม่หลับ ส่วนผลข้างเคียงต่อหัวใจ เช่น หัวใจเต้นช้า หัวใจเต้นผิดจังหวะ เกิดได้น้อยมาก (น้อยกว่าร้อยละ 1)

ความเสี่ยงจากค่าระดับยาไซโคลสพอรินในเลือดที่อาจสูงขึ้นเมื่อใช้ร่วมกับดิลไทอะเซ็ม เนื่องจากดิลไทอะเซ็มมีผลในการเพิ่มระดับยาในเลือดของยาไซโคลสพอริน ซึ่งอาจทำให้ผู้ป่วยเกิดอาการ

ข้างเคียงจากยาไซโคลสพอริน อาการข้างเคียงดังกล่าวได้แก่ อาการคลื่นไส้ อาเจียน เวียนศีรษะ ระดับซีรัมครีอะตินินเพิ่มขึ้น แต่อย่างไรก็ตามแพทย์จะนัดติดตามระดับยา ไซโคลสพอรินในเลือด และติดตามค่าการทำงานของไตหลังจากท่านได้รับยาดิลไทอะเซ็มเป็นเวลา 1 เดือน ทำให้ความเสี่ยงในการเกิดอาการข้างเคียงดังกล่าวน้อยมาก และท่านสามารถติดต่อผู้วิจัยได้ทุกเวลาหากท่านมีอาการข้างเคียงดังกล่าวเกิดขึ้น

<u>ประโยชน์ที่อาจได้รับ</u>

ประโยชน์ที่จะเกิดแก่ผู้เข้าร่วมการวิจัย

ทราบลักษณะของยีน *CYP3A5* ของตัวท่าน ซึ่งจะมีประโยชน์ต่อการใช้ยากดภูมิคุ้มกันและยาอื่นๆ ที่มีการกำจัดยาผ่านทางเอ็นไซม์ *CYP3A5* ซึ่งส่งผลให้แพทย์สามารถเลือกใช้ขนาดยาที่เหมาะสมกับตัวท่านมากยิ่งขึ้น

ประโยชน์ในทางวิชาการส่วนรวม

1. ได้แนวทางในการกำหนดขนาดยาเริ่มต้นที่เหมาะสมของยาไซโคลสพอรินในผู้ป่วยปลูกถ่ายไตที่มีภาวะพหุสัณฐานของยีน *CYP3A5* ที่แตกต่างกัน

2. ได้ข้อมูลความแตกต่างของสัดส่วนของระดับยาต่อขนาดยาของยาไซโคลสพอรินในผู้ป่วยปลูกถ่ายไตที่มีภาวะพหุสัณฐานของยีน *CYP3A5* ที่แตกต่างกัน

3. ได้ข้อมูลความแตกต่างของสัดส่วนของระดับยาต่อขนาดยาของยาไซโคลสพอรินเมื่อใช้ร่วมกับดิลไทอะเซ็มในผู้ป่วยปลูกถ่ายไตที่มีภาวะพหุสัณฐานของยีน *CYP3A5* ที่แตกต่างกัน

<u>อันตรายที่อาจเกิดขึ้นจากการเข้าร่วมในโครงการวิจัยและความรับผิดชอบของผู้ทำวิจัย</u>

หากพบอันตรายที่เกิดขึ้นจากการวิจัย ท่านจะได้รับการรักษาอย่างเหมาะสมทันที ผู้ทำวิจัยยินดีรับผิดชอบค่าใช้จ่ายในการรักษาพยาบาลของท่าน และการลงนามในเอกสารให้ความยินยอมไม่ได้หมายความว่าท่านได้สละสิทธิ์ทางกฎหมายตามปกติที่ท่านพึงมี

ในกรณีที่ท่านได้รับอันตรายใด ๆ หรือต้องการข้อมูลเพิ่มเติมที่เกี่ยวข้องกับโครงการวิจัย ท่านสามารถติดต่อกับผู้ทำวิจัยคือ นางสาวไพลิน วรรณประพันธ์ เบอร์โทรศัพท์ 08-6904-9574 ได้ตลอด 24 ชั่วโมง

ทั้งนี้ทางผู้วิจัยจะขอเก็บตัวอย่างที่เหลือจากการวิจัยเป็นระยะเวลา 1 ปีเพื่อการตรวจเพิ่มเติมในอนาคต หรือเพื่อการศึกษาใหม่ในอนาคต แต่การใช้ตัวอย่างนั้นทางผู้วิจัยต้องยื่นเรื่องให้คณะกรรมการจริยธรรมพิจารณาก่อนการนำตัวอย่างมาใช้ เพื่อพิทักษ์สิทธิของผู้เข้าร่วมวิจัย

หากท่านไม่ได้รับการชดเชยอันควรต่อการบาดเจ็บหรือเจ็บป่วยที่เกิดขึ้นโดยตรงจากการวิจัย หรือท่านไม่ได้รับการปฏิบัติตามที่ปรากฏในเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในการวิจัย ท่านสามารถร้องเรียนได้ที่ คณะกรรมการจริยธรรมการวิจัย โรงพยาบาลพระรามเก้า โทร 0-2202-9999 ในเวลาราชการ

ขอขอบคุณในการร่วมมือของท่านมา ณ ที่นี้

APPENDIX C

เอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัย

การวิจัยเรื่อง ผลของภาวะพหุสัณฐานของยีน *CYP3A5* ต่อการเกิดอันตรกิริยาทางเภสัชจลนศาสตร์ของยาไซโคลสพอรินกับยาดิลไทอะเซ็ม ในผู้ป่วยไทยที่ได้รับการปลูกถ่ายไต

วันให้คำยินยอม วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า นาย/นาง/นางสาว.....ได้อ่านรายละเอียดจากเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัยวิจัยที่แนบมาฉบับวันที่..... และข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนาม และ วันที่ พร้อมด้วยเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย อันตรายหรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ารับทราบจากผู้วิจัยว่าหากเกิดอันตรายใดๆจากการวิจัยดังกล่าว ผู้เข้าร่วมวิจัยจะได้รับการรักษาพยาบาลโดยไม่เสียค่าใช้จ่าย โดยผู้วิจัยจะเป็นผู้ให้ความช่วยเหลือในการติดต่อประสานงานเพื่อให้ผู้เข้าร่วมการวิจัยได้เข้ารับการตรวจรักษาจากแพทย์เจ้าของไข้ สำหรับค่าใช้จ่ายในการดูแลรักษาอาการผิดปกติ ที่เกิดขึ้นจากการวิจัย ผู้วิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายที่เกิดขึ้นเองทั้งหมด

ข้าพเจ้ามีอิสระที่จะปฏิเสธ หรือถอนตัวจากโครงการวิจัยเมื่อใดก็ได้ โดยไม่มีผลใดๆ ต่อการรักษาพยาบาลที่ควรจะได้รับตามมาตรฐาน หรือสูญเสียผลประโยชน์ใด ๆ

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น บุคคลอื่นในนามของคณะกรรมการพิจารณาจริยธรรมการวิจัยในคน สำนักงานคณะกรรมการอาหารและยาอาจได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลของผู้เข้าร่วมวิจัย ทั้งนี้จะต้องกระทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของข้อมูลเท่านั้น โดยการตกลงที่จะเข้าร่วมการศึกษานี้ข้าพเจ้าได้ให้คำยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของผู้เข้าร่วมวิจัยได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ ของผู้เข้าร่วมวิจัย เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการวิจัยและต้องการให้ทำลายเอกสารและ/หรือ ตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถสืบค้นถึงตัวข้าพเจ้าได้

ข้าพเจ้าเข้าใจว่า ข้าพเจ้ามีสิทธิ์ที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของข้าพเจ้าและสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของข้าพเจ้าได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของข้าพเจ้าที่ไม่มีการเปิดเผยชื่อจะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคตหรือการวิจัยทางด้านเภสัชภัณฑ์ เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

ลงชื่อ……ผู้เข้าร่วมโครงการวิจัย/
ผู้แทนโดยชอบด้วยกฎหมาย
(…….…… ชื่อ-นามสกุล ตัวบรรจง)

ในกรณีที่ผู้เข้าร่วมโครงการวิจัยไม่สามารถลงลายมือชื่อด้วยตนเองได้ ให้ผู้แทนโดยชอบตามกฎหมายซึ่งมีส่วนเกี่ยวข้องเป็น…….ของผู้เข้าร่วมโครงการวิจัยเป็นผู้ลงนามแทน

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการไม่พึงประสงค์หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียดให้ผู้เข้าร่วมในโครงการวิจัยตามนามข้างต้นได้ทราบและมีความเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

.....ลงนามผู้ทำวิจัย
(.....) ชื่อผู้ทำวิจัย ตัวบรรจง
วันที่ .....เดือน.....พ.ศ.....

.....ลงนามพยาน
(.....) ชื่อพยาน ตัวบรรจง
วันที่ .....เดือน.....พ.ศ.....

APPENDIX D

TaqMan® Drug Metabolism Genotyping Assays

(TaqMan® MGB probes, FAM™ and VIC® dye-labeled)

Assay ID: C_26201809_30

rs: 776746

Chemical and reagents

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

Apparatus

1. MicroAmp Optical 96-well reaction plate
2. MicroAmp Optical Adhesive Film kit
3. Vortex mixer
4. 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

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 Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (indels). Each assay enables scoring of both alleles of a biallelic polymorphism in a single well. All assays are optimized to work with TaqMan® Universal PCR Master Mix No AmpErase® UNG (P/N 4324018)† and with genomic DNA. These products utilize the modified thermal cycling parameters described below in Table B.

Procedure

To prepare the reaction components for one reaction refer to the table below. The 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 A. Allelic Discrimination PCR Reaction

Reaction Components	Volume/Well (10 mcL volume reaction) *	Final concentration
TaqMan® Universal PCR Master Mix (2 X)	5 mcL	1 X
20 X TaqMan® Drugmetabolism Genotyping Assay Mix	0.5 mcL	1 X
Genomic DNA (20 ng/mcL) **	1 mcL	-
dH_2O	3.5 mcL	-
Total	10 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.

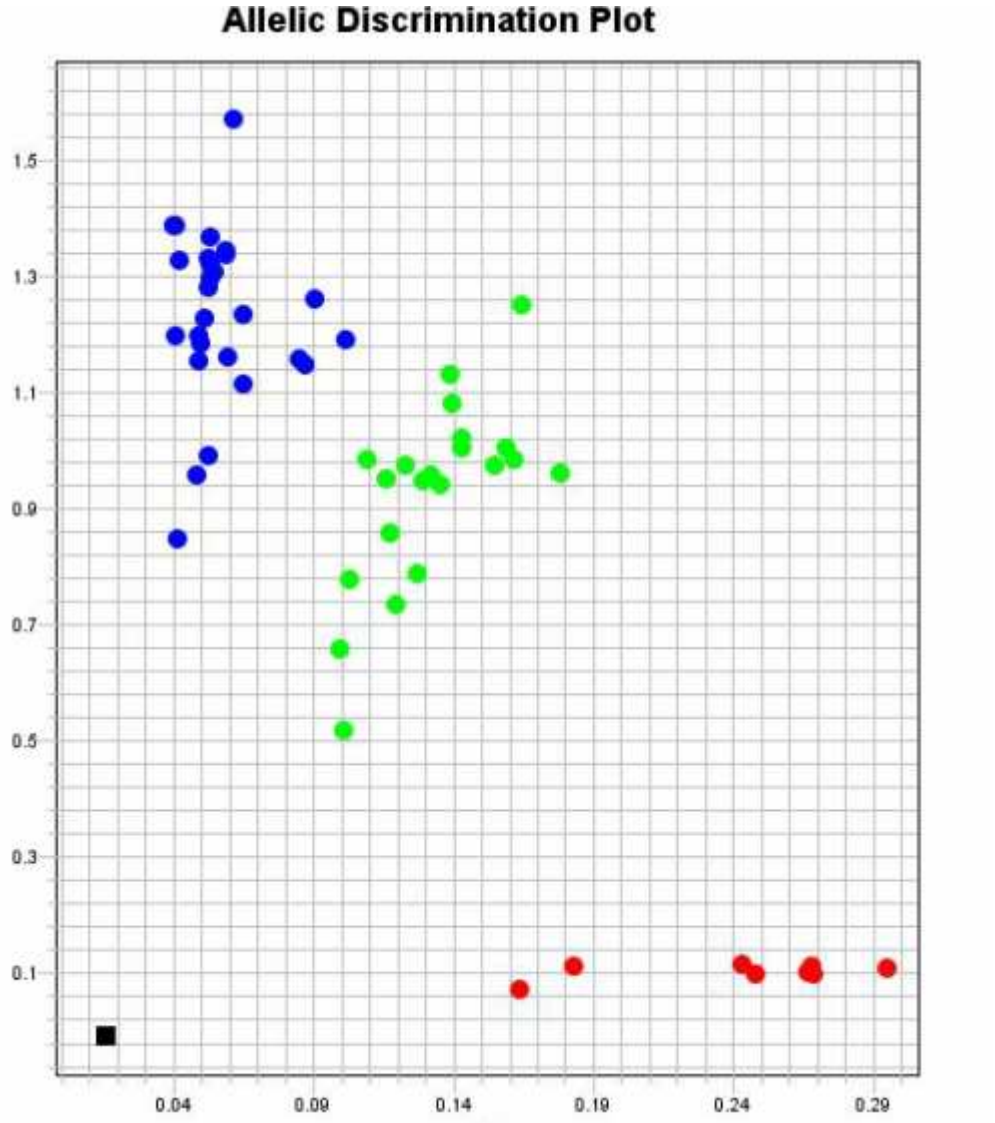
Table B. Thermal Cycler Conditions

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.

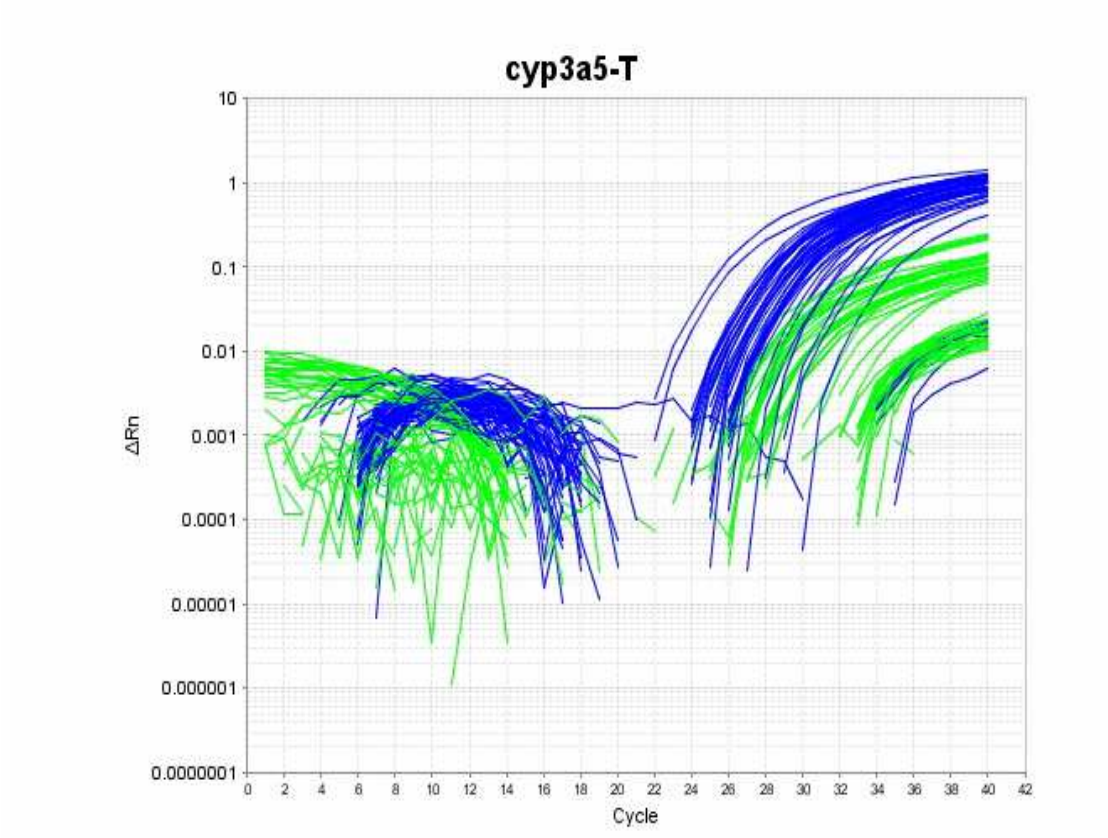


No template control

*CYP3A5*1/*1*

*CYP3A5*1/*3*

*CYP3A5*3/*3*



*CYP3A5*1* *CYP3A5*3*

APPENDIX E

The demographic data, CsA pharmacokinetic parameter and *CYP3A5* genotype of DTZ in individual patients

PT NO.	sex	age (yr)	weight (kg)	AM dose (mg)	PM dose (mg)	C_0 (ng/ml)	C_2before (ng/ml)	C_2after (ng/ml)	*CYP3A5* genotype
1	M	47	66	75	75	75	447	862	*1/*3
2	M	70	71	50	50	63	174	712	*3/*3
3	M	68	83	100	100	116	557	446	*3/*3
4	F	70	44	50	50	44	339	180	*3/*3
5	F	58	54.8	75	75	123	654	724	*3/*3
6	M	66	75.6	75	75	91	344	115	*3/*3
7	F	53	53.1	50	50	-	695	869	*3/*3
8	M	54	73.4	75	75	103	827	931	*3/*3
9	M	41	73.5	50	50	-	440	529	*3/*3
10	M	48	58.4	50	25	-	865	528	*3/*3
11	F	61	48	75	75	107	289	559	*3/*3
12	M	41	85.2	75	75	152	622	700	*1/*3
13	F	58	68.7	50	75	110	651	611	*3/*3
14	M	62	75	75	75	125	727	291	*1/*3
15	F	49	43.7	75	75	109	750	958	*1/*3
16	F	48	68.8	75	50	104	669	738	*3/*3
17	M	56	64.2	100	100	127	754	780	*1/*1
18	M	22	69.7	75	75	44	580	557	*1/*1
19	F	65	62	75	50	88	973	722	*1/*3
20	M	69	64.3	50	50	86	712	509	*3/*3
21	M	52	90.7	75	75	74	858	639	*1/*3
22	F	65	57.7	50	50	99	184	528	*1/*3
23	F	50	64.3	75	75	117	774	648	*3/*3

PT NO.	sex	age (yr)	weight (kg)	AM dose (mg)	PM dose (mg)	C_0 (ng/ml)	C_2before (ng/ml)	C_2after (ng/ml)	*CYP3A5* genotype
24	F	33	58.4	50	50	-	395	427	*3/*3
25	F	66	49.5	75	75	111	712	450	*1/*3
26	M	52	83	75	75	100	814	443	*1/*3
27	F	52	46.5	75	75	92	451	615	*1/*1
28	M	45	54.8	25	25	82	241	348	*3/*3
29	M	63	96.8	75	75	104	483	528	*3/*3
30	M	59	70.4	75	75	174	799	829	*3/*3
31	F	60	68.8	75	75	108	1526	694	*1/*3
32	M	50	66.6	75	75	91	748	673	*1/*3
33	F	73	60.2	50	50	79	542	846	*1/*3
34	M	69	58.3	50	50	78	422	544	*3/*3
35	F	44	86	100	100	108	133	295	*1/*1
36	F	51	50.6	50	75	119	573	649	*1/*1
37	M	61	91.5	100	100	90	559	698	*3/*3
38	M	44	75.5	100	75	111	607	424	*1/*3

VITA

Miss Pailin Wannapraphan was born on 12th of November 1981 at Bangkok.

She got Bachelor of Sciences (Pharmacy) (2nd Class Honours) from The Faculty of Pharmaceutical Sciences, Chulalongkorn University in 2003. She started her work as hospital pharmacist at Kasetsomboon Hospital, Chaiyapoom Province from May 2003 – May 2004 and then work at Soongnoen Hospital, Nakorn Ratchasima Province from May 2004 – Oct 2005 after that she had worked at Samitivej Srinakarin Hospital, Bangkok for two years. She has enrolled in a study for the degree of Master of Science in Clinical Pharmacy at the Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University since June 2008.