의학석사 학위논문

A pharmacogenomic study on the pharmacokinetics of tacrolimus in healthy subjects using the DMET™ Plus platform

DMET™ Plus genotyping platform을 이용한 Tacrolimus의 약물유전체 연구

2016년 2월

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협동과정 임상약리학 전공

최 윤 정

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이 논문을 의학석사 학위논문으로 제출함

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최윤정의 의학석사 학위논문을 인준함

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Abstract

A pharmacogenomic study on the pharmacokinetics of tacrolimus in healthy subjects using the DMET^{TM} Plus platform

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Genetic association studies on the pharmacokinetics of tacrolimus have reported conflicting results, except for the role of the *CYP3A5*3* polymorphism. The objective of this study was to identify genetic variants affecting the pharmacokinetics of tacrolimus using the DMET^{TM} Plus microarray in 42 healthy

males. Aside from *CYP3A5*3*, the rs3814055 polymorphism in the *NR1I2* gene was associated with the tacrolimus pharmacokinetics based on false discovery rate-corrected multiple tests and the least absolute shrinkage and selection operator analysis. The area under the concentration-time curve to the last quantifiable time-point (AUC_{last}) was 3.42 times greater in subjects with homozygous mutations in both genes (*CYP3A5*3/*3* and *NR1I2* T/T) than in wild-type subjects. The two variants explained the 54% variability in the tacrolimus AUC_{last}.

Our results agree with previous studies that *CYP3A5*3* (rs776746) has a significant impact on the tacrolimus PK. The association identified for the first time between a SNP (rs3814055) in *NR1I2* gene and the tacrolimus PK is interesting because *NR1I2* gene encodes PXR, a transcriptional regulator of CYP3A enzymes; however, this association warrants further in-vitro and in-vivo studies. Using more than just one data analysis method may improve the interpretation of the results of gene association studies.

Keywords: Tacrolimus; Pharmacogenomics; Pharmacokinetics; FDR; LASSO

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AUC_{last}	AUC from the drug administration to the last measurable concentration
C_{max}	Maximum observed plasma concentration
$DMET^{TM}$	Drug metabolizing enzymes and transporters
SNP	Single nucleotide polymorphism
FDR	False discovery rate
LASSO	Least absolute shrinkage and selection operator
ANOVA	Analysis of variance
PXR	pregnane X receptor

INTRODUCTION

Tacrolimus has been widely used as a first-line immunosuppressive agent for the prophylaxis of organ transplant rejection[1]. The US FDA recommends individual dose titration and routine therapeutic drug monitoring for tacrolimus not only because its therapeutic index is narrow but also because it has a highly variable pharmacokinetic profile amongst patients[2]. Therefore, identifying genetic variants that affect the pharmacokinetics of tacrolimus could be useful for its optimal use in the clinical setting[3].

Although rs776746 C>T (also known as *CYP3A5*3*), which is a nonfunctioning allele of the *CYP3A5* gene, is associated with decreased tacrolimus metabolism[4], the role of other genes, including the *ABCB1*, *CYP2C19*, *POR*, *UGT1A8*, *NOD2*, *PPARA*, and NR1I2 in the pharmacokinetics of tacrolimus was either inconsistent or insignificant[3]. However, many genetic association studies on tacrolimus share the general limitations of the current candidate gene approach, such as a lack of thoroughness and replication[5]. In this sense, high-throughput methods (e.g., genome wide association studies and DNA microarrays) are expected to be more powerful in not only detecting true genetic associations but also identifying novel candidate genes[6]. DMET™ Plus (Affymetrix, Inc., Santa Clara, CA, USA) is a DNA microarray platform that provides a broad coverage of pharmacogenomic markers in one assay, i.e., 1,936 genetic variants across 231 relevant genes that encode drug metabolizing enzymes,

drug transporters, and drug receptors. In fact, DMET™ Plus has been utilized in various population-based pharmacogenomic investigations[7,8], including a recent tacrolimus pharmacogenomic study in kidney transplant patients[9].

Because genetic association studies in patients can be confounded by other factors such as demographic characteristics, disease states, pathophysiological conditions and concomitant drugs[10], minimizing the number of confounding factors (e.g., by performing a study in healthy subjects) can allow for the detection of significant genetic associations[11]. Data analysis is another challenge in high-throughput genetic association studies[12]. For example, a multiplicity correction using either Bonferroni or false discovery rate (FDR), which are commonly used in genetic association studies between multiple variants and quantitative traits[13], is too conservative and thereby may fail to detect some important associations[14]. To overcome the shortfalls of the conventional data analysis methods, the least absolute shrinkage and selection operator (LASSO) analysis, a regularized linear regression method, has been proposed[15,16]. Because LASSO shrinks the coefficient estimates to zero for insignificant variables (i.e., removing them from the model), it is particularly advantageous in variable selection when the number of predictors exceeds the number of study participants, which is very common in genetic association studies[17].

Based on this framework, the objective of the present study was to identify and evaluate genetic variants that could affect the pharmacokinetics of tacrolimus using the DMET™ Plus platform in a less-confounded study

population (i.e., healthy male adults). Additionally, two data analysis methods (i.e., multiplicity correction via FDR and LASSO) were compared.

SUBJECT AND METHODS

Clinical study

A double-blind, randomized, two-way, two-period, crossover bioequivalence study of tacrolimus was previously performed using a reference drug (Prograf^{TM}, Astellas Pharma Korea Inc., Seoul, Korea) and a generic formulation. Study subjects were non-smoking healthy Korean males 19 to 55 years of age with a body mass index between 18 and 30 kg/m2. Subjects were excluded if they showed evidence of or had a history of clinically significant cardiovascular, respiratory, hepatic, renal, or neurological abnormalities. From two weeks prior to the study drug administration to the completion of the study, subjects were restricted from smoking, drinking alcohol, and taking other medications or nutritional supplements. Subjects were admitted to the clinical trials center at Seoul National University Hospital (SNUH), Seoul, South Korea the day before the administration of the study drug in each period. After fasting overnight, all subjects took a single oral dose of 1 mg tacrolimus with 240 ml tap water. Then, 5 ml of venous blood was collected at 0 (i.e., pre-dose), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96 hours after the drug administration. Two standard meals were provided at 4 and 9 hours after the drug administration. All subjects were discharged after the blood sampling at 24 hours post dose and they visited the clinical trial center for the remainder of the sampling time points. Subjects were asked

if they had experienced any adverse events. Furthermore, vital signs and standard blood biochemistry were checked regularly during the entire study period. The study procedures were repeated during a second period after a washout.

The Institutional Review Board at SNUH approved the study protocol. All of the subjects signed informed consent before any study-related procedure was performed, and the study was conducted in full compliance with the principles stipulated in the Declaration of Helsinki and the Good Clinical Practice guidelines.

Determination of plasma concentrations of tacrolimus

Plasma concentrations of tacrolimus were determined using a previously published LC/MS/MS method[18] with some modifications. The plasma sample preparation involved a liquid/liquid extraction with methyl tert-butyl ether. Ascomycin was used as the internal standard (IS). The mobile phase consisted of 10 mM ammonium acetate and methanol (5:95, v/v, with 0.1% formic acid). Tacrolimus and the IS were separated with a YMC Pro C18 RS column (5 μm, 2.1 mm × 150 mm; YMC Co., Ltd., Kyoto, Japan) and quantified using multiple reaction monitoring (MRM) in the positive electrospray ionization (ESI) mode. The MRM transition mass-to-charge ratios (m/z) were 821.5 → 768.6 for tacrolimus and 809.5 → 756.6 for ascomycin (IS). The lower limit of quantitation was 25 pg/ml. The intra-day accuracy and precision of the quality control (QC) samples were within the ranges of 96.4 - 101.6% and 0.7 - 2.9%, respectively. The inter-day accuracy and precision of the QC samples were within the ranges of 97.5 - 103.5% and 1.0 - 3.0%, respectively.

Pharmacokinetic analysis

Tacrolimus concentrations from the reference formulation were used for the pharmacokinetic analysis in the present study. The maximum plasma concentration (C_{max}) of tacrolimus was determined directly from the observed plasma concentration data. The area under the concentration curve from time zero to the last quantifiable time point (AUC_{last}) was determined using the

non-compartmental model in Phoenix WinNonlin (version 6.4, Certara, Princeton, USA) based on the linear trapezoidal method.

Genotyping analysis

A peripheral blood sample was collected from all of the subjects for genotyping during the screening, and genomic DNA was isolated using QuickGene-mini80 (Fujifilm, Tokyo, Japan), a nucleic acid isolation device. Prior to adding the markers in the DMET™ Plus (Affymetrix Inc., Santa Clara, CA, USA) assay flow system, multiplex polymerase chain reaction was applied as a pre-amplification step. Genomic sequences that included the polymorphic markers were amplified by using highly selective Molecular Inversion Probe (MIP) amplification. A QC gel was run to verify the amplified PCR products. The resulting target DNA was labeled by the program DMET™ Plus Label and hybridized to the DMET™ Plus Array to identify the genotypes. The data generated in the Affymetrix Targeted Genotyping System represented the nucleic acid bases (G, A, T, or C) detected with a particular probe, which was then translated into genotypes using the Affymetrix DMET™ Console software (Affymetrix, Santa Clara, CA, USA). The genotypes of each variant were coded as -1, 0 or 1, representing minor homozygotes, heterozygotes, and major homozygotes, respectively. Genotyping using the DMET™ Plus was performed at DNA Link, Inc. (Seoul, Korea).

Statistical analysis

Two statistical analysis methods were used to identify genetic variants strongly associated with the pharmacokinetics of tacrolimus. First, an individual *P* value obtained from a conventional ANOVA analysis was multiplied by the ratio equal to the total number of the variants divided by its rank with the smallest *P* value ranked as one, resulting in an FDR-adjusted *P* value (multiple testing via FDR correction analysis)[19]. FDR-adjusted *P* values less than 0.05 were considered to be statistically significant. Second, statistical comparison of the PKs parameters including C_{max} and AUC_{last} among minor homozygotes, heterozygotes, and major homozygotes was done by LASSO. A LASSO regression model was independently fit, where the tuning parameter was selected to minimize the 10-fold cross-validation errors. According to LASSO's principles, it tends to produce some coefficient estimates of insignificant variables to be exactly zero. The larger the tuning parameter applied, the more coefficients estimates were shrunk towards zero.

Additionally, a multiple linear regression model was developed to evaluate the relative contribution by the candidate SNPs (chosen in the multiple testing FDR correction and LASSO analyses) to the total variation in the pharmacokinetics of tacrolimus. The coefficient of determination (r square) was used to describe the proportion of total variability that could be explained by all of the candidate SNPs, whereas standardized coefficients were used to indicate the relative contribution by each candidate SNP individually. Non-

parametric Kruskal Wallis test and Mann-Whitnet U test were used to evaluate whether the differences in the pharmacokinetic parameters of tacrolimus were statistically significant between various genotypes of the candidate SNPs (because the sample size was small and the variance was not considered similar between these groups), which were also summarized using the geometric mean ratio (GMR) and their 95% confidence intervals (CI). The SAS software (version 9.3, SAS Institute, Inc., Cary, NC, USA) was used for multiple tests via FDR correction and regression analyses. The LASSO regression analysis was performed using the R package *glmnet* implemented in the R software (version 3.0.1).

Results

Subjects

The clinical study enrolled 50 healthy males, 42 of whom completed the entire study. The mean ± standard deviation of the age, body weight and height and BMI of those 42 subjects were 27.1 ± 7.3 years, 66.7 ± 6.8 kg, 173.3 ± 5.6 cm and 22.2 ± 1.9 kg/m^2, respectively. Eight subjects dropped out of the studies; seven withdrew their consent and one took other drugs during the study. No subjects dropped out of the study due to adverse events, and there were no serious adverse events reported.

Genetic associations with tacrolimus pharmacokinetics

A total of 1,888 (97.5%) out of 1,936 genetic markers in the DMET^{TM} Plus microarray were successfully assayed (> 95% genotyping calls), 1,223 markers of which did not show any polymorphisms. Thus, the remaining 665 markers were included in the final genetic data analysis.

Three SNPs in the *CYP3A5, CYP3A7* and *CYP3A4* genes (rs776746, rs2257401 and rs2242480, respectively) were significantly associated with the AUC_{last} of tacrolimus (Table 1). Furthermore, one SNP in the *NR1I2* gene (rs3814055) showed a potential genetic association with tacrolimus AUC_{last} because its FDR-adjusted P value was ranked 4th among all the markers although it failed to reach statistical significance (FDR-adjusted P = 0.12, Table 1). However, no SNP was significantly associated with tacrolimus C_{max}; the lowest FDR-adjusted P value was 0.60.

The coefficients in the final LASSO model for the AUC_{last} and C_{max} of tacrolimus were greater than zero for twelve and two SNPs, respectively (Table 2). Among these, only two SNPs in the *NR1I2* and *CYP3A5* genes (rs3814055 and rs776746, respectively) resulted in a LASSO model coefficient greater than zero for both the AUC_{last} and C_{max} of tacrolimus (Table 2). Additionally, these two SNPs in the *NR1I2* and *CYP3A5* genes showed the second and third largest coefficients, respectively, in the LASSO model of tacrolimus AUC_{last} (Table 2). Although rs4986949 for the *GSTP1* gene had the highest coefficient in the LASSO model for tacrolimus AUC_{last}, it was absent

in the final LASSO model of tacrolimus C_{max} (Table 2). In addition, its minor allelic frequencies were very low (1.2%, n = 1) in our study population.

Both the *CYP3A5* (rs776746) and *NR1I2* (rs3814055) genes were significantly associated with the variability in the AUC_{last} of tacrolimus and explained 54% of the total variability (Table 3), whereas the *CYP3A5* genotype showed a slightly greater effect size compared to the *NR1I2* genotype (standardized coefficients of 0.54 and 0.39, respectively). In contrast, only the *NR1I2* (rs3814055) but not the *CYP3A5* (rs776746) gene significantly contributed to the variability of the tacrolimus C_{max}, which was approximately half that observed with tacrolimus AUC_{last} (24%, Table 3). No interaction term between these SNPs was significantly associated with either tacrolimus AUC_{last} or C_{max}.

Table 1. Generic variants associated with tacrolimus AUC_{last} based on the FDR-adjusted multiple testing analysis. The four most significant associations are shown here.

SNP	Gene	Reference allele	Mutant allele	*P* value	FDR-adjusted *P* value
rs776746	*CYP3A5*	T	C	0.00001	0.00466
rs2257401	*CYP3A7*	G	C	0.00001	0.00466
rs2242480	*CYP3A4*	T	C	0.00029	0.06444
rs3814055	*NR1I2*	C	T	0.00073	0.12125

AUC_{last}: area under the concentration curve from time zero to the last quantifiable time point; SNP: single nucleotide polymorphism; FDR: false discovery rate.

Table 2. Genetic variants having a coefficient greater than zero in the LASSO models for tacrolimus AUC_{last} and C_{max}

	SNP	Gene	Reference allele	Mutant allele	Coefficient
AUC_{last}	rs4986949	*GSTP1*	T	G	0.28485
	rs776746	*CYP3A5*	C	T	0.18146
	rs3814055	*NR1I2*	C	T	0.10679
	rs2257401	*CYP3A7*	G	C	0.05650
	rs16947	*CYP2D6*	T	C	0.05085
	rs7496	*GSTA4*	C	T	0.03105
	rs1736565	*FMO6P*	C	T	0.02985
	rs2020861	*FMO2*	A	G	0.02571
	rs6068816	*CYP24A1*	G	A	0.01274
	rs3803390	*SLC28A1*	C	T	0.00302
	rs1783811	*SLC22A11*	A	G	0.00050
	rs1080983	*CYP2D6*	T	C	0.00008
C_{max}	rs776746	*CYP3A5*	T	C	0.10999
	rs3814055	*NR1I2*	T	C	0.00039

AUC_{last}, area under the concentration curve from time zero to the last quantifiable time point; C_{max}, maximum plasma concentration; SNP: single nucleotide polymorphism.

Table 3. P values from a general linear model of the pharmacokinetic parameters for tacrolimus, where the CYP3A5 (rs776746) and NR1I2 (rs3814055) genotypes and their interaction term were the independent variables.

	P value			Adjusted r^2 [a]
(rs776746)	*CYP3A5*	*NR1I2* (rs3814055)	Interaction	
AUC_{last}	<0.01	<0.05	0.16	0.54
C_{max}	0.20	<0.05	0.34	0.24

AUC_{last}, area under the concentration curve from time zero to the last quantifiable timepoint; C_{max}, maximum plasma concentration

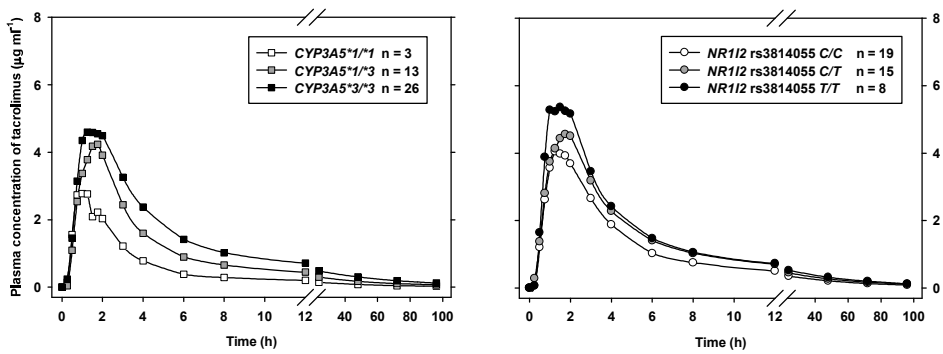
[a] Proportion of variability that can be explained by the model consisting of the CYP3A5 and NR1I2 genotypes.

Genetic effects of *CYP3A5* and *NR1I2* on tacrolimus pharmacokinetics

The greater the number of nonfunctioning **3* alleles in the *CYP3A5* gene, the greater the mean exposure to tacrolimus (Figure 1a). Consequently, the geometric mean AUC_{last} and C_{max} of tacrolimus was 2.78 (95% CI: 1.66 - 4.66) and 1.64 (95% CI: 1.04 - 2.60) times greater, respectively, in the *CYP2A5*3/*3* homozygote than in the **1/*1* wild-type ($P < 0.05$; Figure 2a and 2b). Likewise, the greater the number of T alleles at rs3814055 in the *NR1I2* gene, the greater the mean exposure to tacrolimus, although the differences between the *NR1I2* genotypes was not as obvious as those observed between the *CYP3A5* genotypes (Figure 1a). The geometric mean AUC_{last} and C_{max} of tacrolimus was 1.72 (95% CI: 1.25 - 2.38) and 1.54 (95% CI: 1.18 - 2.00) times greater, respectively, in the T/T homozygote compared to the C/C wild-type ($P < 0.01$; Figure 2c and 2d). Taken together, the highest and lowest systemic exposures to tacrolimus were observed in the combined genotype of *CYP3A5*3/*3* and *NR1I2* T/T and the combined genotype of *CYP3A5*1/*1* and *NR1I2* C/C, respectively (Figure 1b). The geometric mean ratio (95% CI) of the AUC_{last} and C_{max} for the two combined genotypes were 3.42 (2.43 - 4.82) and 1.93 (1.20 - 3.11), respectively.

Figure 1. Mean concentration-time profiles of tacrolimus a) by different CYP3A5 and NR1I2 genotypes (n = 42) and b) by two different combined CYP3A5 and NR1I2 genotypes (n = 9) where the genotypes represented the highest (CYP3A5 *3/*3 and NR1I2 TT) and the lowest (CYP3A5 *1/*1 and NR1I2 CC) exposure to tacrolimus. The error bars represent the standard deviations.

a)



b)

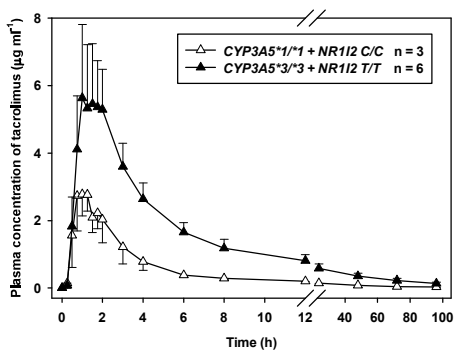
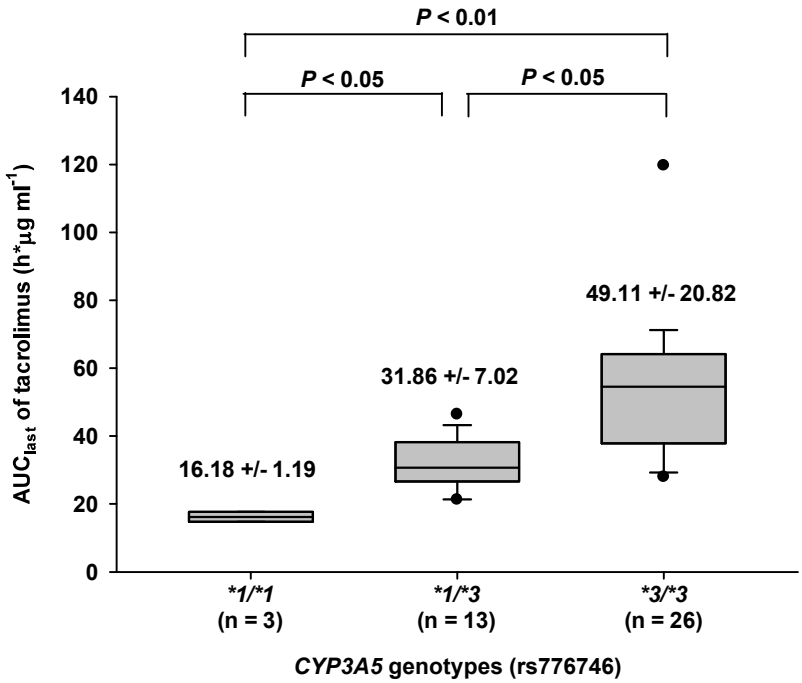
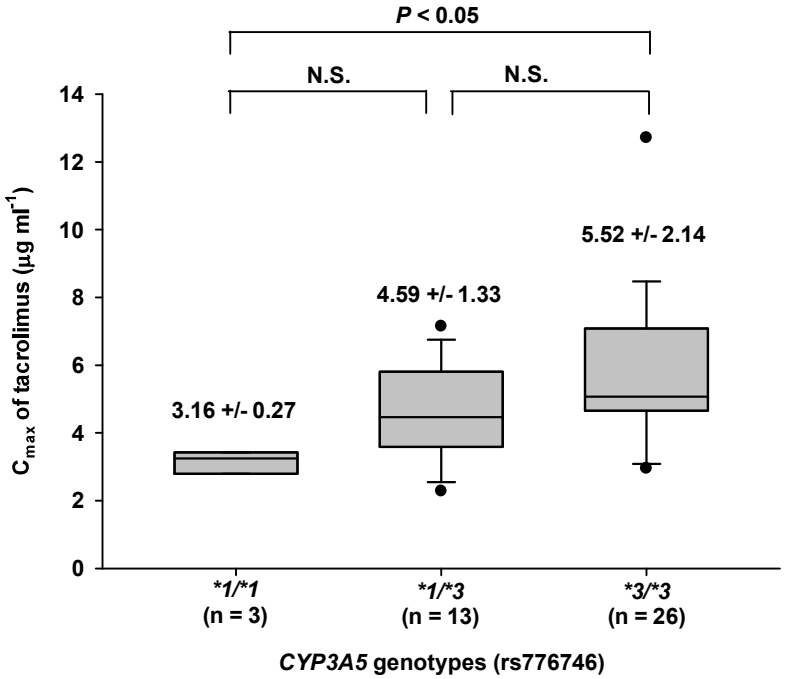


Figure 2. Comparison of tacrolimus AUC_{last} and C_{max} between different genotypes of a) *CYP3A5* (rs776746) and b) *NR1I2* (rs3814055). (n = 42, *P* value were obtained from ANOVA with Post-hoc test, N.S., not significant). The line across each box, the bottom edge, and the top edge represent the median, the first quartile, and the third quartile, respectively. The horizontal lines connected to the whiskers extending from the box denote the 1.5 times interquartile range. The solid circles (•) indicate outliers from the 1.5 times interquartile range.

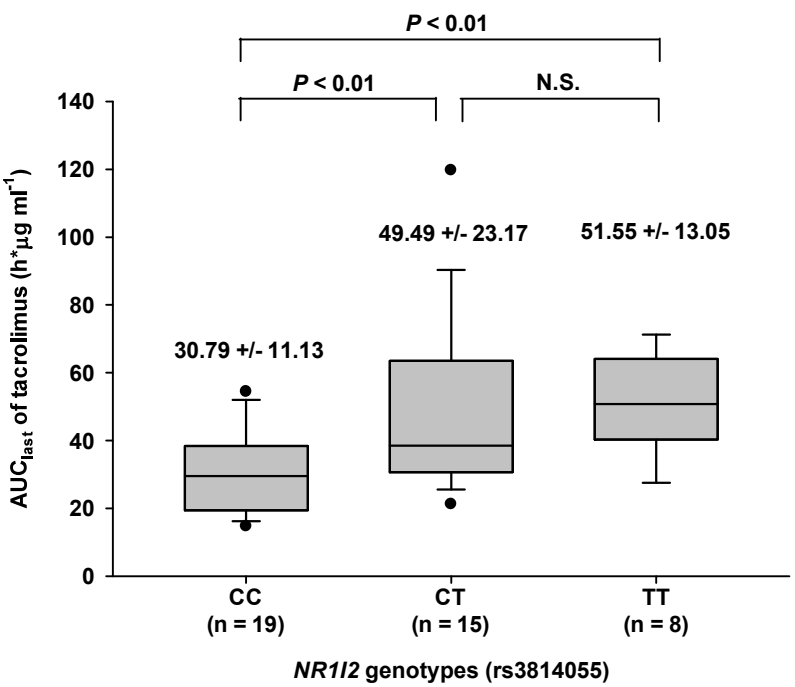
a)



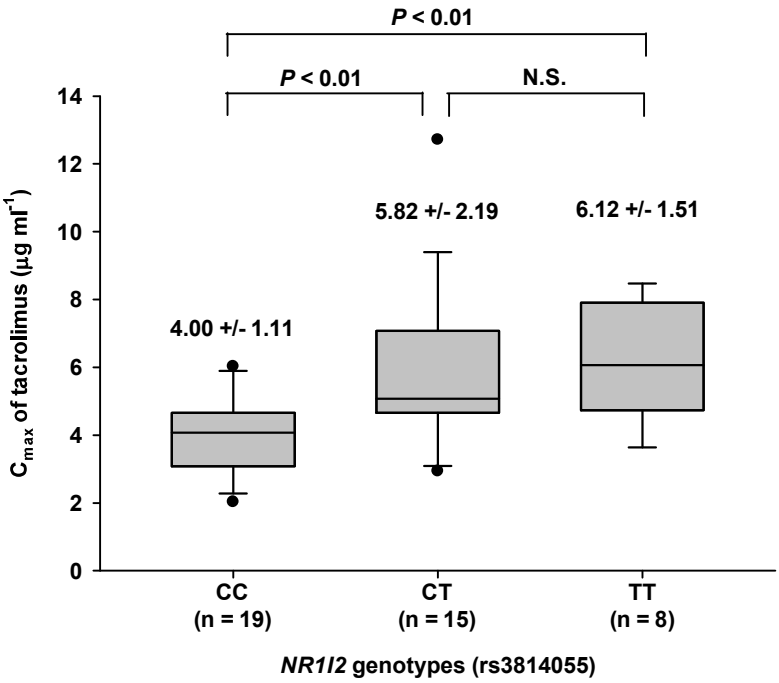
b)


P < 0.05
N.S.
N.S.
14
12
10
8
6
4
2
0
C max of tacrolimus (µg ml-1)
5.52 +/- 2.14
4.59 +/- 1.33
3.16 +/- 0.27
*1/*1
(n = 3)
*1/*3
(n = 13)
*3/*3
(n = 26)
CYP3A5 genotypes (rs776746)

c)


P < 0.01
P < 0.01
N.S.
140
120
100
80
60
40
20
0
AUClast of tacrolimus (h*µg ml-1)
49.49 +/- 23.17
51.55 +/- 13.05
30.79 +/- 11.13
CC
(n = 19)
CT
(n = 15)
TT
(n = 8)
NR1I2 genotypes (rs3814055)

d)


P < 0.01
P < 0.01
N.S.
14
12
10
8
6
4
2
0
C_max of tacrolimus (µg ml^-1)
4.00 +/- 1.11
5.82 +/- 2.19
6.12 +/- 1.51
CC
(n = 19)
CT
(n = 15)
TT
(n = 8)
NR1I2 genotypes (rs3814055)

Discussion

By using the DMET^{TM} Plus platform in a homogeneous heathy subject population, we replicated the well-characterized influence of the *CYP3A5*3* (rs776746) polymorphism on the pharmacokinetics of tacrolimus in the present study. Unexpectedly, we also discovered the association between a genetic variant (rs3814055) in the *NR1I2* gene and a significantly increased exposure to tacrolimus, although the effect of this variant was slightly lower than that of the *CYP3A5*3* variant. We demonstrated that employing more than one data analysis method could enhance the detection of genetic variants that may affect the pharmacokinetics of a drug, as exemplified by multiple testing via FDR correction and LASSO analyses in the present study.

In this study, the systemic exposure to tacrolimus was significantly greater in the subjects with the *CYP3A5*3/*3* genotype compared to those carrying at least *CYP3A5*1* allele, with a GMR for C_{max} at 1.64 (Figure 2a and 2b). A similar difference in the dose-adjusted trough concentration of tacrolimus has been noted in both adult and pediatric patients of various ethnic backgrounds and who underwent organ transplantations[20]. To support this notion, the current Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline recommends a starting dose 1.5 - 2 times higher for *CYP3A5*1* carriers[3].

Our results also indicated that the effect of rs3814055 variant in the *NR1I2* gene was similar to that of *CYP3A5*3* by increasing the exposure to

tacrolimus (Figure 2). Furthermore, the effects of rs3814055 and *CYP3A5*3* on the pharmacokinetics of tacrolimus appeared to be independent from each other, leading to a significantly higher exposure to tacrolimus (3.42 and 1.93 times higher for AUC_{last} and C_{max}, respectively) in subjects with homozygous mutations in both genes compared to those with wild-type alleles for both genes (Figure 1b). rs3814055 is located in the promoter region of the *NR1I2* gene, which encodes PXR, a xenosensor that plays a key role in the regulation of several genes involved in drug metabolism such as *CYP3A4* and *3A5*[21]. Although the role of rs3814055 has been investigated in diseases such as inflammatory bowel disease (IBD)[22] and ulcerative colitis (UC)[23], as well as in drug responses to cyclosporine and bupropion[24, 25], the genetic association between rs3814055 and the pharmacokinetics of tacrolimus has not been clearly determined. Recently, a clinical trial in 32 kidney transplant patients showed that subjects with the rs3814055 C/T and T/T genotypes had 1.2 and 1.5, respectively, times greater clearance of tacrolimus than that of the rs3814055 T carriers[26], which supports the findings in our study. The result suggests that this polymorphism might stimulate CYP3A-mediated metabolism and consequently decrease exposure to drugs metabolized mainly via CYP3A. Additionally, although a few previous investigations suggested a possible association between another variant (rs2276707) in the *NR1I2* gene and the pharmacokinetics of tacrolimus in kidney transplant patients[27], this association has not been replicated in the studies that followed[28, 29].

Based on the in vitro study results obtained from liver samples from male subjects[30], it has been observed that the rs3814055 C>T mutation may be associated with decreased *CYP3A5* metabolic activity by reducing PXR expression. However, conflicting results have also been reported, in which the rs3814055 C>T mutation resulted in 4-fold higher PXR promoter activity in vitro[31]. In either case, these findings above suggest that the *NR1I2* gene (specifically the rs3814055 C>T variant) may play a role in tacrolimus metabolism, and specifically the rs3814055 C>T variant may cause the variability of tacrolimus pharmacokinetics. Therefore, further studies are warranted to confirm the effect of this SNP on the pharmacokinetics of tacrolimus.

In the present study, the association of rs3814055 with the pharmacokinetics of tacrolimus was the third highest for both tacrolimus AUC_{last} and C_{max} in the LASSO analysis (Table 2), whereas the multiple testing via FDR correction analysis failed to detect this association (Table 1). This discrepancy is possibly because multiple testing via the FDR correction method is rather conservative, particularly in the situation that the number of genetic markers far exceeds the sample size, as exemplified in the present study (e.g., 665 markers vs. 42 subjects). Based on the results from the multiple linear regression analysis in the present study, however, it is more reasonable to conclude that rs3814055 is very likely associated with the pharmacokinetics of tacrolimus. This illustrates that using more than one data

analysis method can be useful for detecting significant genetic associations that are unlikely to be identified otherwise.

In addition to the two SNPs in the *CYP3A5* and *NR1I2* genes (rs776746 and rs3814055, respectively), a few other variants are also worth noting in the present study. First, rs4986949 in the *GSTP1* gene showed the highest coefficient in the LASSO analysis for tacrolimus AUC_{last}, suggesting a much larger effect size than all the other genetic variants. However, the frequency of this mutation was very low in our study population, with only one subject carrying a heterozygous mutation. Although this subject had the highest tacrolimus exposure to tacrolimus, it is difficult to determine if this variant was truly associated with the pharmacokinetics of tacrolimus.

rs2242480 (*CYP3A4*1G*) also deserves mention because it was observed to be significantly associated with tacrolimus pharmacokinetics by the multiple testing via FDR correction but not by the LASSO analysis in the present study. The Pharmacogenomics Knowledge Base (PharmGKB) has curated rs2242480 as "a SNP that is likely to be related to tacrolimus therapeutics". It is known that this SNP, together with rs2257401 in the *CYP3A7* (which was also identified as significant by both statistical analyses in the present study), was strongly but not completely linked with the *CYP3A5*3*[32]. Therefore, the discrepancy in the two statistical analyses in the present study suggests that multiple testing via FDR correction is more advantageous in discovering variants in linkage disequilibrium although the discovered associations are often not clinically meaningful, which may be

missed by the LASSO analysis[33]. Furthemore, although the LASSO analysis identified eight other genetic variants that had the coefficients greater than zero for the AUC_{last} of tacrolimus, we did not analyze them further because not much is known about the role of these variants, similar to rs4986949 in the *GSTP1* gene, except rs16947 in the *CYP2D6* gene. However, rs16947, also known as the *CYP2D6*2*, does not change *CYP2D6* function. This may reflect one disadvantage of the LASSO analysis, which usually identifies general associations rather than the true genetic associations[33]. Therefore, it is important to evaluate the results of statistical analyses based on the biological meanings of the genetic variants.

The present study had several limitations. First, the small sample size allowed us to only detect the SNPs that exist at relatively high frequencies and exert a large effect. Second, because only males were studied, we were unable to identify genetic associations that may be unique to females. Likewise, body weight and BMI, which could affect the pharmacokinetics of tacrolimus, were not controlled in the present study although their variability was relatively modest at ~ 10% coefficient of variation. Third, because the present study was conducted in a homogeneous population, other factors such as age, sex, and concomitant drugs that can impact tacrolimus pharmacokinetics as well, were not investigated; therefore, it should be cautious to extrapolate our findings to heterogeneous clinical settings. Fourth, the genetic effect of rs3814055 in the NR1I2 may differ in a multiple-dose study as shown previously on the *CYP3A5*3*[34], although a similar effect of the rs3814055 in the *NR1I2* on the

pharmacokinetics of tacrolimus was found in a retrospective multiple-dose study in kidney transplant[26]. Collectively, future prospective studies in various populations are warranted to adequately characterize the effect of not only genetic but also non-genetic factors, as well as dose regimen effect on the pharmacokinetics of tacrolimus.

In conclusion, rs776746 (*CYP3A5*3*) and rs3814055 (*NR1I2*) were identified as significant genetic variants that could affect the pharmacokinetics of tacrolimus using *in vivo* investigations based on multiple testing correction via FDR and LASSO analysis methods.

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Abstract in Korean

DMET™ Plus genotyping platform을 이용한 Tacrolimus의 약물유전체 연구

서론: Tacrolimus는 신장, 간, 심장이식을 받은 환자에서 거부반응을 예방하기 위해 사용되는 면역억제제이며, 치료역이 좁고 개인차가 심한 약물이다. Tacrolimus는 생체 내에서 흡수, 대사되는 과정에서 cytochrome P450 3A (CYP3A)와 P-glycoprotein (P-gp)의 기질이 되며, 개인 간 *CYP3A* 효소와 P-gp의 발현 정도는 Tacrolimus의 흡수와 대사 차이에 영향을 준다고 알려져 있다. *CYP3A5*의 유전적 다형성이 Tacrolimus의 약동학에 미치는 영향은 잘 알려져 있으나, 이외 CYP 효소와 같은 약물대사효소나 P-gp과 같은 약물수송체의 유전자 다형성과 Tacrolimus의 약동학 결과에 미치는 영향에 대해서는 상반된 연구결과들이 보고되고 있다. 따라서 본 연구에서는 DMET™ Plus를 이용하여 시험대상자들의 유전자 정보를 탐색하고, Tacrolimus의 약동학과 유전자 형간의 상관관계를 분석하고자 하였다.

방법: 본 연구는 서울대학교병원에서 Tacrolimus를 투여 받은 건강한 자원자를 대상으로 하였으며, 대상자들의 혈액 채취 후 DMET™ Plus를 이용하여 유전형을 분석하였다. DMET 분석 결과로부터 Tacrolimus의 약동학과 유전자형간의 상관관계를 탐색하였다. 유전적 다형성(Genetic Polymorphism)과 tacrolimus의 약동학간의 유의한 영향을 탐색하기 위하여 FDR(false discovery rate)를 이용한 다중검정(Multiple testing) 및 LASSO(Least Absolute Shrinkage and Selection Operator)를 이용하여 분석하였으며, 독립된 2가지의 통계분석을 통해 결과의 신뢰성을 높이고자 하였다.

결과: *CYP3A5*의 유전적 다형성 이외에, *NR1I2* −25385C>T (rs3814055) 유전자의 유전적 다형성 또한 tacrolimus 약동학에 유의한 연관성이 있는 것으로 나타났다. 본 연구에서 *CYP3A5*1* 대립유전자를 가진 대상자군은 **1* 대립유전자를 갖지 않은 대상자군에 비해 체내 약물노출 정도가 유의하게 낮게 나타났고, *NR1I2* 유전자 변이가 있을 경우 tacrolimus 약물혈중농도가 유의하게 높은 것으로 나타났다. 또한, *CYP3A5*와 *NR1I2* 두 가지 유전자 모두에서 homozygous mutations을 가지는 대상자군(*CYP3A5*3/*3, NR1I2* TT)의 혈중농도 곡선하면적(AUC_{last})은 wild−type을 가지는 대상자군에 비해 3.42배 높은 것으로 나타났다.

결론: 이와 같은 결과로부터, *CYP3A5*와 *NR1I2*(PXR)의 유전적 다형성은 tacrolimus의 약동학을 결정하는데 있어 중요한 요소라는 것을 알 수 있었다. 본 연구는 *CYP3A5*와 *NR1I2*(PXR) 유전자의 유적전 변이에 따른 tacrolimus 용량 조절의 필요성을 제시하였으며, 개인별 맞춤약물요법 치료의 기초 정보를 생성하였다는 점에서 의의가 있다.

중심단어: 타크로리무스; 약물유전체학; 약동학; FDR; LASSO

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