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Significant association between *CYP3A5* polymorphism and serum level of tacrolimus in patients with connective tissue diseases.

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Abstract

Objectives. To assess the association between the blood level of tacrolimus (TAC) in patients with connective tissue disease (CTD) and *CYP3A5* polymorphism that affects TAC level in patients of organ transplantation.

Methods. 72 patients with CTD were genotyped for rs776746 in *CYP3A5*. The blood trough level of TAC after taking 3mg/day was retrospectively obtained for each patient.

Results. Allele A of rs776746 showed a significant association with a decreasing serum level of TAC ($p=0.0038$). One copy of the A allele decreased the level of TAC on average by 21.2%.

Conclusions. Rs776746 is associated with levels of TAC level in a dose-dependent manner in patients with CTD.

Introduction

Tacrolimus (FK506, TAC) is a calcineurin inhibitor isolated from *Streptomyces tsukubaensis* (1) and one of the many types of powerful immunosuppressants that are frequently used for solid organ transplantation to prevent organ rejection(2). TAC is also used for patients with connective tissue disease (CTD) including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), polymyositis (PM) and dermatomyositis (DM) to control disease activity(3, 4). TAC is metabolized mainly by cytochrome P450 (CYP) 3A in the liver and intestine(5). Because TAC level is highly variable among patients, to predict TAC level to achieve therapeutic effect is a big challenge. Previous studies revealed that the variation of TAC level is largely attributable to different expressions of *CYP3A* in patients of organ transplantation. Patients carrying *CYP3A5*3* determined by the G allele of rs776746 were shown to have high TAC level than patients with the A allele(5, 6). Although TAC is a substrate for P-glycoprotein encoded by the *ABCB1* gene, effects of polymorphisms in *ABCB1* on TAC level is inconclusive(6, 7). Genetic studies have only been performed recruiting patients with organ transplantation to date.

When TAC is given to patients with CTD, the dosage is around 3mg/day (3, 4) which is much lower than that given to patients of organ transplantation. For example, patients with renal transplantation receive 0.3mg/kg/day around the transplantation and 0.12mg/kg/day as maintainance (8). In addition, though recipients of renal transplantation take TAC twice daily(8), patients with CTD only take a single dose of TAC per day. The effect of *CYP3A5* on TAC levels with low TAC exposure has not been studied so far. Furthermore, chronic, systemic and autoimmune inflammatory process in CTD may influence the metabolism and level of TAC. Thus, whether the associations between polymorphisms of *CYP3A5* and TAC levels can be observed in patients with CTD remained unclear. Evidence of the association between *CYP3A5* and TAC levels in patients with CTD would enable us to develop a predictive model of TAC levels in these patients. Here, we performed an association study to address this point.

Materials and Methods

This study was designed in accordance with the Helsinki Declaration. This study was approved by the ethics committee of Kyoto University Graduate School and Faculty of Medicine. Written informed consent was obtained from all the participants.

Study Subjects

A total of 72 subjects with CTD who were prescribed to take a single dose of TAC every day in the evening at the Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine from December in 2005 to December in 2012 were enrolled in this study. Patients took PROGRAF® (Astellas) capsules containing 1mg or 0.5mg tacrolimus. CTD patients

fulfilled criteria for each disease, namely, American College of Rheumatology (ACR) criteria for RA in 1987(9) or ACR/EULAR criteria in 2010(10), criteria for SLE(11), PM and DM(12) and for Polyarteritis Nodosa and microscopic polyangiitis(13).

Clinical Information

Information of age, sex, weight, serum creatinine and date of prescription, dosage, and serum level of TAC were obtained from clinical charts retrospectively by the system previously described(14). Information of prescription and dosage of corticosteroid was also obtained. TAC level data was obtained at least one week after prescription was initiated or changed. The data of concurrent use of cyclosporine, bosentan or antibiotics was excluded. eGFR was inferred by serum creatinine, age, and sex.

TAC level

The serum trough TAC level with 3mg TAC (around 12 hours after taking TAC) was used. TAC levels were quantified by two measurements according to measuring time; namely, microparticle enzyme immunoassay (MEIA, IMxTM-TACRO II, Abott) until May 2009 and chemiluminescent immunoassay (CLIA, ARCHITECT_TACRO, Abott) from May 2009. These measurements can quantify even low TAC levels (1.5~ and 0.5~ng/ml, respectively). When TAC levels were available for both measurements in a patient, data of CLIA with lower measuring limit of TAC levels was used. 63 and 9 patients were quantified by CLIA and MEIA, respectively (hereafter termed as CLIA group and MEIA group, respectively). When multiple TAC levels with 3mg TAC were available, the mean of the levels was adopted. Calculations were performed based on logarithm of TAC levels to avoid negative level by linear regression model and excess influence of extreme data.

Selection of SNPs and genotyping

Rs776746, whose G allele determined *CYP3A5*3*, was selected based on previous studies. Genotyping was performed by the Taqman assay (Applied Biosystems).

Statistical Analysis

The association was analyzed between serum level of TAC in the patients and genotypes of rs776746, age, sex, weight, eGFR, whether the patients were affected with RA or with SLE by single or multiple linear regression analysis in each measurement separately. The overall associations were estimated by meta-analysis using inverse-variance method. P-value less than 0.05 was regarded as significant. These analyses were performed by R statistical software.

Results

Tacrolimus and CYP3A5 polymorphism

The summary of the subjects in the current study is shown in Table 1. When we analyzed correlations between TAC level with 3mg TAC and age, sex, weight, eGFR, dosage or usage of corticosteroid or presence of RA or SLE by single linear regression analysis, none of them displayed overall significant associations ($p \geq 0.083$). However, because presence of RA showed a suggestive association in MEIA group ($p=0.0091$), we used presence of RA as a covariate. All the 72 patients were genotyped for rs776746 by Taqman assay. No deviation from Hardy-Weinberg equilibrium was observed ($p=0.13$). When the association between TAC level and genotypes of rs776746 was analyzed, we found a significant decreasing effect of the A allele of rs776746 on TAC level in patients with CTD ($p=0.0038$, Figure 1). Both MEIA and CLIA groups showed the comparable effect sizes (Table 2). Patients who were homozygote for the A allele had 16.9% and 35.0% lower level than those who were heterozygote and homozygote for G allele, respectively.

Discussion

The current study provided evidence that TAC level was strongly influenced by *CYP3A5* in patients with CTD even taking only a small amount of TAC. Our results showed the same direction of A allele of rs776746 and comparable effect sizes in the previous studies using patients of solid organ transplantation(5, 6). Disease-specific influence on TAC levels was not clear. Although a previous study has shown that a polymorphism in the *ABCB1* gene was associated with the pharmacodynamics effect of TAC(15), there are also contradicting reports(6, 7). Thus, to our knowledge, the polymorphism of *CYP3A5* is the only established polymorphism to influence TAC level. The dose-dependent association also supported the accuracy of the current study. These results should be replicated by a larger number of patients with CTD, also including other populations. Because the predictive model of TAC level is proposed in patients of organ transplantation, it will be interesting to construct a predictive model of TAC level in patients with CTD.

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Reference

1. Kino T, Hatanaka H, Miyata S, Inamura N, Nishiyama M, Yajima T, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. II. Immunosuppressive effect of FK-506 in vitro. The Journal of antibiotics. 1987 Sep;40(9):1256-65.

2. Scott LJ, McKeage K, Keam SJ, Plosker GL. Tacrolimus: a further update of its use in the management of organ transplantation. Drugs. 2003;63(12):1247-97.

3. Takeuchi T, Kawai S, Yamamoto K, Harigai M, Ishida K, Miyasaka N. Post-marketing surveillance of the safety and effectiveness of tacrolimus in 3,267 Japanese

patients with rheumatoid arthritis. Mod Rheumatol. 2013 May 14.
4. Takahashi S, Hiromura K, Sakurai N, Matsumoto T, Ikeuchi H, Maeshima A, et al. Efficacy and safety of tacrolimus for induction therapy in patients with active lupus nephritis. Mod Rheumatol. 2011 Jun;21(3):282-9.
5. Hesselink DA, van Gelder T, van Schaik RH. The pharmacogenetics of calcineurin inhibitors: one step closer toward individualized immunosuppression? Pharmacogenomics. 2005 Jun;6(4):323-37.
6. Tsuchiya N, Satoh S, Tada H, Li Z, Ohyama C, Sato K, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation. 2004 Oct 27;78(8):1182-7.
7. Tada H, Tsuchiya N, Satoh S, Kagaya H, Li Z, Sato K, et al. Impact of CYP3A5 and MDR1(ABCB1) C3435T polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation proceedings. 2005 May;37(4):1730-2.
8. Niioka T, Kagaya H, Miura M, Numakura K, Saito M, Inoue T, et al. Pharmaceutical and genetic determinants for interindividual differences of tacrolimus bioavailability in renal transplant recipients. European journal of clinical pharmacology. 2013 Jun 4.
9. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988 Mar;31(3):315-24.
10. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis and Rheumatism. 2010 Sep;62(9):2569-81.
11. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997 Sep;40(9):1725.
12. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med. 1975 Feb 13;292(7):344-7.
13. Bloch DA, Michel BA, Hunder GG, McShane DJ, Arend WP, Calabrese LH, et al. The American College of Rheumatology 1990 criteria for the classification of vasculitis. Patients and methods. Arthritis Rheum. 1990 Aug;33(8):1068-73.
14. Terao C, Hashimoto M, Yamamoto K, Murakami K, Ohmura K, Nakashima R, et al. Three groups in the 28 joints for rheumatoid arthritis synovitis–Analysis using more than 17,000 assessments in the KURAMA database–. PLoS One. 2013;in press.
15. Vafadari R, Bouamar R, Hesselink DA, Kraaijeveld R, van Schaik RH, Weimar W, et al. Genetic Polymorphisms in ABCB1 Influence the Pharmacodynamics of Tacrolimus.

Therapeutic drug monitoring. 2013 Jun 5.

Conflict of interest statement
None

Figure 1. Association between TAC level and the polymorphism in *CYP3A5* in patients with CTD.
The obtained or inferred TAC levels adjusted for 3mg TAC are shown according to rs776746 genotypes. Y axis is shown in log scale. The mean levels are 2.88, 3.57, and 5.10 ng/ml for AA, AG, GG genotypes, respectively. TAC levels were adjusted for MEIA group.

Table 1. Summary of subjects in the current study.

	Study Subjects
Age*	48.94±17.24
Sex	Male 13 Female 59
Disease**	RA:22, SLE,43, DM3, PM2, PAN1, mPA1

*mean±standard deviation

** RA:Rheumatoid Arthritis, SLE: Systemic Lupus Erythematosus, DM: Dermatomyositis, PM: Polymyositis, PAN: Polyarteritis Nodosa, mPA: Microscopic Polyangiitis

Table 2. Association between rs776746 and TAC level in multiple regression analysis.

	Number	Beta	SE*	P
CLIA	63	0.106	0.041	0.012
MEIA	9	0.161	0.12	0.22
Overall	72	0.112	0.039	0.0038

Statistics adjusted by RA presence. *standard error

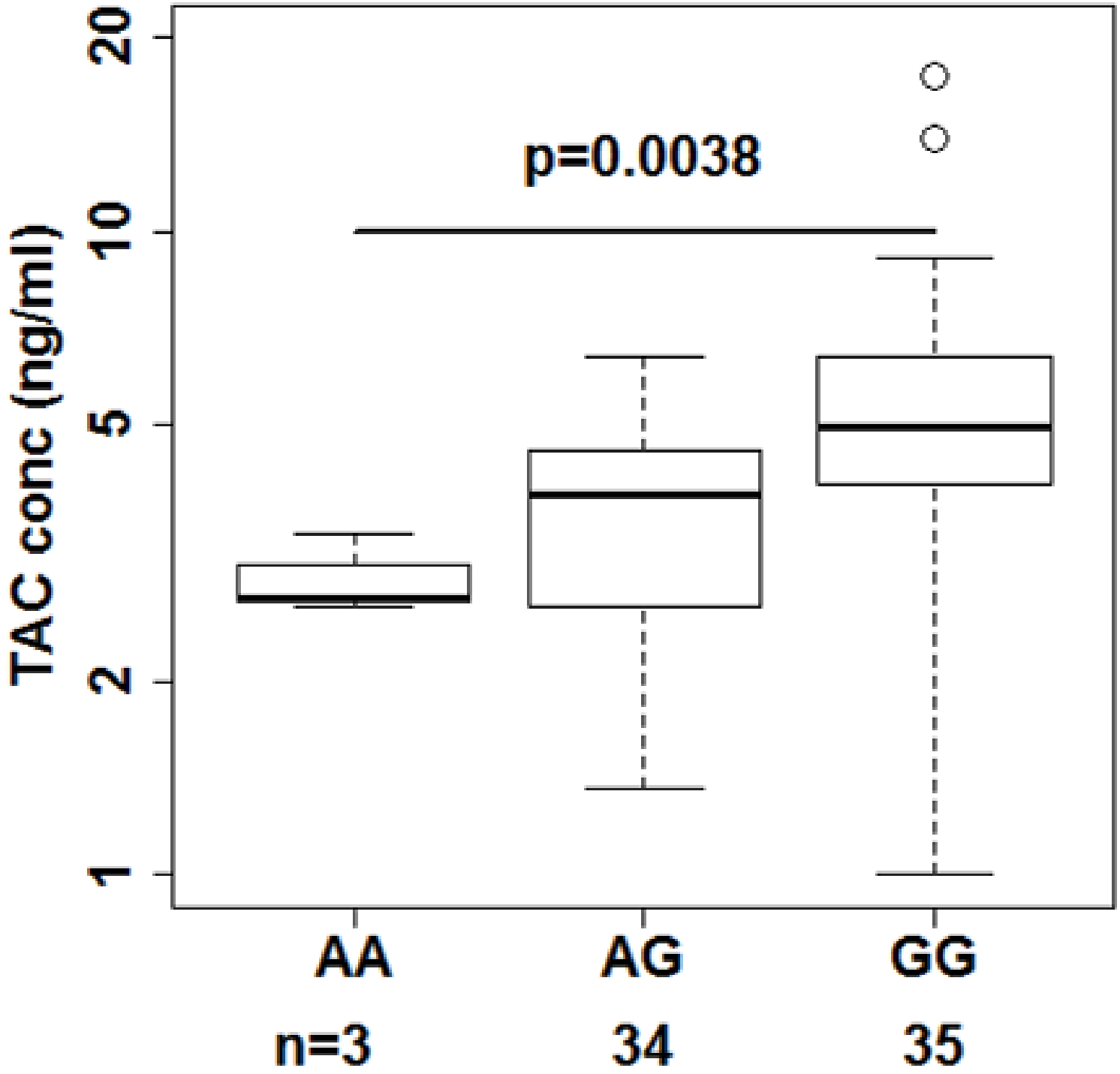

Figure 1