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SHORT COMMUNICATION

Significant association between *CYP3A5* polymorphism and blood concentration of tacrolimus in patients with connective tissue diseases

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Although the association between *CYP3A5* polymorphism and blood concentration of tacrolimus (TAC) in patients with solid organ transplantation was established, whether the association is also true in patients with connective tissue disease (CTD) who usually receive small amount of TAC is uncertain. Here, we performed a quantitative linear regression analysis to address the association between *CYP3A5* and blood TAC concentration in patients with CTD. A total of 72 patients with CTD were recruited in the current study and genotyped for rs776746 in *CYP3A5*, which showed strong association with TAC concentration in patients with solid organ transplantation. The blood trough concentration of TAC after taking 3 mg per day was retrospectively obtained for each patient. As a result, allele A of rs776746 showed a significant association with a decreasing blood concentration of TAC ($P = 0.0038$). Those who are carrying at least one copy of the A allele displayed decreased mean concentration of TAC by 31.0% compared with subjects with GG genotype. Rs776746 is associated with concentrations of TAC in patients with CTD.

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, polymyositis and dermatomyositis to control disease activity.[3,4] TAC is metabolized mainly by cytochrome P450 (CYP) 3A in the liver and intestine.[5] Because TAC concentration is highly variable among patients, to predict TAC concentration to achieve therapeutic effect is a big challenge. Previous studies revealed that the variation of TAC concentration 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 concentration 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 concentration are inconclusive.[6–8] Genetic studies have been performed mainly recruiting patients with organ transplantation to date. The number of previous studies focusing on TAC concentration in non-organ transplantation subjects is limited.[9,10]

When TAC is given to patients with CTD, the dosage is around 3 mg per day,[3,4] which is much lower than that given to patients of organ transplantation. For example, patients with renal transplantation receive $0.3 \, \text{mg} \, \text{kg}^{-1}$ per day at the transplantation and $0.12 \, \text{mg} \, \text{kg}^{-1}$ per day as maintainance.[11] In addition, although recipients of renal transplantation take TAC twice daily,[11] patients with only CTD take a single dose of TAC per day. The effect of *CYP3A5* on TAC concentrations with low TAC exposure in patients with CTD has not been studied so far. Furthermore, chronic, systemic and autoimmune inflammatory process in CTD may influence the metabolism and concentration of TAC. Thus, whether the association between polymorphisms of *CYP3A5* and TAC concentrations can be observed in patients with CTD remained unclear. Here, we performed an association study to address this point.

This study was designed in accordance with the Helsinki Declaration and approved by the ethics committee of Kyoto University Graduate School and Faculty of Medicine. A total of 72 subjects with CTD who were prescribed to take a single dose of 3 mg of TAC every day in the evening at the Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine from December 2005 to December 2012 were enrolled in this study. Written informed consent was obtained from all the participants. Patients

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took PROGRAF (Astellas Pharma Inc., Tokyo, Japan) capsules containing 1 or 0.5 mg TAC. CTD patients fulfilled criteria for each disease, namely, American College of Rheumatology (ACR) criteria for RA in 1987[12] or ACR/EULAR criteria in 2010,[13] for systemic lupus erythematosus,[14] polymyositis and dermatomyositis[15] and for polyarteritis nodosa and microscopic polyangiitis.[16] Information of age, sex, weight, serum creatinine and date of prescription, dosage, and blood concentration of TAC were obtained from clinical charts retrospectively by the system previously described.[17] Information of prescription and dosage of corticosteroid was also obtained. TAC concentration data were obtained at least one week after prescription was initiated or changed. The data of concurrent use of cyclosporine or bosentan, contraindication due to interaction with TAC, was excluded. Estimated glomelular filtration ratio (eGFR) was inferred by serum creatinine, age and sex. The blood trough TAC concentration with 3 mg TAC (around 12 h after taking TAC) was used. TAC concentrations were quantified by two measurements according to measuring time; namely, microparticle enzyme immunoassay (MEIA, IMxTM-TACRO II, Abbott Laboratories, Green Oaks, IL, USA) until May 2009 and chemiluminescent immunoassay (CLIA, ARCHITECT_TACRO, Abbott Laboratories) from May 2009. These measurements can quantify even low TAC concentrations (~ 1.5 and ~ 0.5 ng ml^{-1}, respectively). When TAC concentrations were available for both measurements in a patient, data of CLIA with lower measuring limit of TAC concentrations was used. A total of 63 and 9 patients were quantified by CLIA and MEIA, respectively (hereafter termed as CLIA group and MEIA group, respectively). When multiple TAC concentrations were available, the mean of them was adopted. Calculations were performed on the basis of logarithm of TAC concentrations to obtain normal distribution and to avoid excess influence of extreme data.

The summary of the subjects in the current study is shown in Table 1. Log-transformation of TAC concentration supported applying linear regression analysis. When we analyzed correlations between TAC concentration and age, sex, weight, eGFR, dosage or usage of corticosteroid or the presence of RA or systemic lupus erythematosus by single linear regression analysis, none of them displayed overall significant associations ($P \geqslant 0.083$). However, because presence of RA showed a suggestive association in MEIA group ($P = 0.0091$), we used presence of RA as a covariate. Rs776746, whose G allele determined CYP3A5*3, was selected on the basis of previous studies and genotyped by the Taqman assay (Applied Biosystems Inc, Foster city, CA, USA). As a result, no deviation from Hardy–Weinberg equilibrium was observed ($P = 0.13$). Although previous reports comparing different measurement methods of blood concentration of TAC showed good correlations ($r \geqslant 0.84$) and did not detect discrepancy even in ranges of low concentrations,[18,19] MEIA was suggested to underestimate TAC concentrations in low levels.[19] Thus we analyzed the associations in CLIA and MEIA groups separately, and the overall

associations were estimated by meta-analysis using inverse-variance method. We found a significant decreasing effect of the A allele of rs776746 on TAC concentration ($P = 0.0038$, Figure 1). Both MEIA and CLIA groups showed the comparable effect sizes, supporting the accuracy of the result (Table 2). Patients who carried A allele had 31.0% lower mean concentration than those who were homozygote for G allele. Although the current and the previous studies[11] showed a good fit of the dose-dependent model of rs776746, there are also conflicting reports.[6,20] Considering the limited number of subjects with AA genotype, the dose-dependent effect of rs776746 should be regarded as inconclusive. Meta-analysis of the recessive model resulted in a comparable result ($P = 0.0035$, AA + GA vs GG).

The current study provided evidence that TAC concentration was strongly influenced by CYP3A5 in patients with CTD even taking 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 concentrations was not clear. As this study contained relatively small number of subjects and low TAC concentrations around the measurement limits might be associated with diminished accuracy, these results should be replicated by a larger number of patients with CTD, also including other populations. Because the predictive model of TAC concentration is proposed in
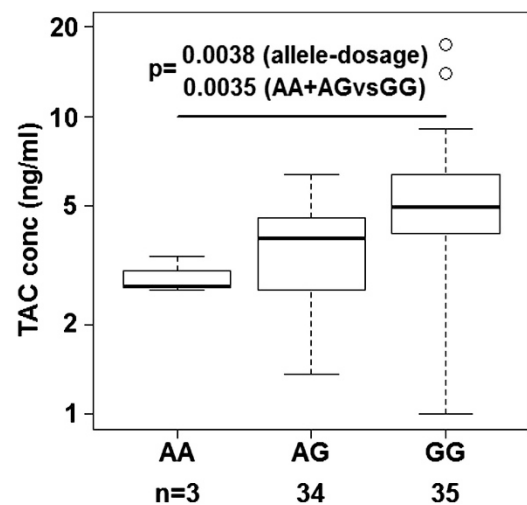

Figure 1 Association between TAC concentration and the polymorphism in CYP3A5 in patients with CTD. The obtained or inferred TAC concentrations adjusted for 3mg TAC are shown according to rs776746 genotypes. Y axis is shown in log scale. The mean concentrations are 2.88, 3.57 and 5.10 ng ml^{-1} for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

Table 1 Summary of subjects in the current study

	Study subjects
Age[a]	48.94 ± 17.24
Sex	Male 13, female 59
Disease	RA: 22, SLE: 43, DM: 3, PM: 2, PAN: 1, mPA: 1

Abbreviations: DM, dermatomyositis; mPA, microscopic polyangiitis; PAN, polyarteritis nodosa; PM, polymyositis; RA, rheumatoid arthritis; SLE, systemic lupus.erythematosus.
[a]mean ± s.d.

Table 2 Association between rs776746 and TAC concentration in multiple regression analysis

	Number	Beta	s.e.	P-value
CLIA	63	0.106	0.041	0.012
MEIA	9	0.161	0.12	0.22
Overall	72	0.112	0.039	0.0038

Abbreviations: CLIA, chemiluminescent immunoassay; MEIA, microparticle enzyme immunoassay; TAC, tacrolimus.
Statistics adjusted by rheumatoid arthritis presence.

patients of organ transplantation, it will be interesting to construct a predictive model of TAC concentration in patients with CTD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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