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Influence of *CYP3A5* genetic differences in tacrolimus on quantitative interstitial fibrosis and long-term graft function in kidney transplant recipients

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ABSTRACT

The impact of *CYP3A5* polymorphisms on clinical outcomes is controversial. The present study investigated the impact of *CYP3A5* genetic differences on the development of interstitial fibrosis (IF) from 0 h to 1 year post-transplantation in biopsy sections from 96 living kidney recipients under the same target trough regimen of tacrolimus. The relationships between *CYP3A5* polymorphisms and long-term graft function and death-censored graft survival were also examined. A quantitative analysis of IF was performed using computer-assisted imaging on virtual slides. Percent IF (%IF) in the cortical region at 0 h was defined as the baseline, and increases in the ratio of %IF 1 year post-transplantation were calculated. The relationships between *CYP3A5* genetic differences and the development of IF, the incidence of clinical events, and the long-term function and death-censored survival of grafts were assessed. The mean increase in the ratio of %IF from 0 h to 1 year was 1.38 ± 0.74-fold. Despite therapeutic drug monitoring (TDM), trough levels of tacrolimus were lower in carriers with the *CYP3A5*1* allele (expressers) than in those with the *CTP3A5*3/*3* genotype (non-expressers) throughout the 1-year post-transplantation period. However, *CYP3A5* genetic differences were not associated with the development of IF, any clinical events, or the long-term function and survival of grafts. The clinical impact of *CYP3A5* genetic differences may be small under the current immunosuppressive regimen consisting of mycophenolate mofetil, steroids, basiliximab, and lower target trough levels of tacrolimus with suitable TDM in a low immunological risk population.

1. Introduction

Tacrolimus, a commonly used calcineurin inhibitor (CNI), is a substrate of cytochrome P450 (CYP) 3A, and most of the interindividual variability observed in its pharmacokinetics is attributed to the presence of a single-nucleotide polymorphism in intron 3 of *CYP3A5 6986A > G*, resulting in the absence of a functional CYP3A5 protein in homozygous carriers (*CYP3A5*3/*3*) [1]. Previous studies reported that dose-adjusted trough levels and the area under the blood concentration-time curve were lower in carriers of the *CYP3A5*1* allele (expressers) than in those with the *CYP3A5*3/*3* genotype (non-expressers) [1–3].

CNIs have a significant adverse impact on renal function and chronic nephrotoxicity, and induce a fibrogenic response that may lead to scarring of the renal allograft [4]. Renal interstitial fibrosis (IF) is the main histological feature of progressive renal diseases and chronic allograft injury [5,6]. In allografts, IF is associated with tubular atrophy (TA) as IF/TA, and the degree of IF/TA showed the strongest correlation with clinical outcomes [7,8]. IF/TA is graded using the Banff classification. In the assessment of Banff classification scores for IF/TA, visual evaluations of trichrome-stained slides are often the standard practice; however, previous studies demonstrated that this approach may have poor reproducibility [9,10]. Therefore, an automated computerized digital analysis of IF in biopsy sections has been conducted

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[6,8–12], and this quantitative analysis may be a more reliable and reproducible method for evaluating renal IF [6,8]. However, the influence of *CYP3A5* pharmacogenetic differences in tacrolimus on the development of quantitative IF is poorly documented.

Regarding the impact of *CYP3A5* polymorphisms on tacrolimus trough levels and transplant outcomes, MacPhee et al. [13] showed that acute rejection (AR) episodes occurred earlier in CYP3A5 expressers than in non-expressers. Despite the use of therapeutic drug monitoring (TDM), CYP3A5 expressers in their study had significantly lower mean tacrolimus trough levels during the first 2 weeks after transplantation and exhibited a delay in achieving target levels [13]. However, the impact of *CYP3A5* polymorphisms on clinical outcomes under the current immunosuppressive regimen with lower target trough levels of tacrolimus is controversial [14–17]. Furthermore, the long-term influence of *CYP3A5* genetic differences on graft function and survival has yet to be clarified.

The present study retrospectively assessed whether *CYP3A5* polymorphisms influenced tacrolimus trough levels adjusted by TDM in the 1 year following transplantation in living renal transplant recipients with the same lower target trough regimen of tacrolimus. We then focused on the impact of *CYP3A5* genetic differences on the development of IF from 0 h to 1 year post-transplantation using an automated digital analysis of virtual biopsy slides. The relationships between *CYP3A5* genetic differences and clinical events, and the long-term function and death-censored survival of grafts were also investigated.

2. Materials and methods

2.1. Patients

One hundred and twenty-six adult patients underwent living renal transplantation under the same target trough regimen of tacrolimus at our institute between July 2004 and December 2010. Four out of 126 patients lost graft function or died with a functional graft within 1 year of transplantation. Three patients with infections, 2 with malignant tumors, 1 with cerebrovascular disease, and 1 who changed residences were not subjected to biopsy 1 year post-transplantation. Nineteen patients missed protocol biopsy at a precise time 1 year post-transplantation. The remaining 96 patients, who underwent protocol biopsies 0 h and 1 year post-transplantation, were eligible for this study. All recipients and donors gave informed consent, and the Ethics Committee of Akita University School of Medicine approved this study.

2.2. Immunosuppressive regimen

Patients initially received combination immunosuppressive therapy consisting of tacrolimus and mycophenolate mofetil (MMF) starting 2 days prior to surgery. An initial oral dose (0.15 mg/kg) of tacrolimus was given twice a day every 12 h at 9:00 A.M. and 9:00 P.M. A 24-hour continuous intravenous infusion of 0.05 mg/kg per day of tacrolimus was administered for the first 3 days from the day of transplantation. Intravenous administration was discontinued at 9:00 A.M. on the 4th post-operative day, and the same dose as the initial oral administration was started. The daily dose was adjusted according to our targeting strategy, with the whole blood trough target level being 15–20 ng/ml within the first week after transplantation, 12 ng/ml in the second week, 10 ng/ml in the third week, and less than 8 ng/ml thereafter. An initial oral dose of 1,500 mg/day of MMF was given in equally divided doses every 12 h. Methylprednisolone was given concomitantly: a dose of 500 mg on the day of surgery, tapered to 40 mg/day during the first week, 20 mg/day of prednisolone in the second week, 15 mg/day of prednisolone in the third week, and 10 mg/day thereafter. All patients received basiliximab (20 mg) intravenously on the day of surgery and postoperative day 4.

In ABO-incompatible or second transplantation, patients initially received tacrolimus starting 7 days prior to surgery with splenectomy at the time of transplantation or rituximab (200 mg/body) intravenously.

No patients received drugs or food that obviously affect the function of CYP3A in the first month after transplantation. All patients received rabeprazole and the sulfamethoxazole-trimethoprin drug combination within 6 months post-transplantation.

2.3. Sample collection and analysis of tacrolimus trough levels

Tacrolimus trough levels were measured 1, 3, 7, 14, and 21 days post-transplantation and when recipients visited the outpatient unit each month. Additional tacrolimus trough concentrations were measured to adjust target trough levels on −1, 0, 2, 4, 5, 6, 8, 10, 16, 18, or 24 days post-transplantation if needed. The blood level of tacrolimus was measured using a microparticle enzyme immunoassay (IMx Abbott Laboratories, Abbott Park, IL, USA), and pharmacokinetics were estimated as previously reported [3].

2.4. Genotyping of genetic polymorphisms

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to genotype *CYP3A5* polymorphisms [3].

2.5. Biopsies

Histological specimens were obtained by allograft core biopsy without clinical events at the cold preservation time immediately pretransplantation (0 h biopsy) and 1 year post-transplantation, and were then processed for routine light microscopy. Briefly, tissue was embedded in paraffin, cut into 2-μm-thick sections, and then stained with hematoxylin and eosin, Periodic acid-Schiff, and Masson's trichrome (MT). The pathological diagnosis was performed by one nephropathologist (AK) according to the Banff criteria, and a histomorphometric analysis was conducted by one observer (NK). These two observers (AK and NK) did not participate in the treatment or follow-up of donors and recipients.

2.6. Quantitative image analysis

Whole glass slides stained with MT were scanned using a Nano Zoomer-RS C10730 (Hamamatsu Photonics Co., Shizuoka, Japan) in order to prepare virtual slides [18]. The image acquisition of virtual slides was performed using Dell PC (Dell Inc., Round Rock, TX) and analyses were conducted with the software HCImage (Hamamatsu Photonics Co., Shizuoka, Japan). Briefly, the renal cortex was defined as the part inside the renal capsule and outside the medulla. In each biopsy, the entire cortical region was analyzed and MT-stained green areas were recognized as regions of IF using the RGB (red, green, and blue) color scale of the software (Fig. 1). Several regions of IF from each cortical section were selected and used to define a threshold for the exclusion of all material differing in color and intensity. A separate threshold was set for each biopsy and adjusted to ensure the exclusion of the pale blue staining of tubular epithelial cells. The total area of the cortical region was then calculated, after which the area of IF was quantified and expressed as a percentage of the cortex. According to this procedure, %IF in an allograft cortex was calculated 0 h and 1 year post-transplantation for each patient. %IF at 0 h was defined as the baseline, and increases at 1 year were calculated as follows:

$$\text{Increases in the ratio of\%IF} = \%\text{IF at 1 year}/\%\text{IF at 0 h}$$

2.7. Criteria for clinical events

Delayed graft function (DGF) was defined as the need for hemodialysis in the first week after transplantation. Intravenous methylprednisolone pulse therapy was performed on patients with clinical AR,