Associations of \textit{HSD11B1} Polymorphisms with Tacrolimus Concentrations in Chinese Renal Transplant Recipients with Prednisone Combined Therapy

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ABBREVIATIONS

\begin{itemize}
\item 11\beta-HSD1, 11\beta-hydroxysteroid dehydrogenase type 1;
\item C/D, trough concentration/dose corrected by weight;
\item CMIA, chemiluminescent microparticle immunoassay;
\item CYP, cytochrome P450;
\item LD, linkage disequilibrium;
\item PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism;
\item P-gp/MDR1, P-glycoprotein/Multi-drug resistance gene 1;
\item POR, cytochrome P450 oxidoreductase;
\item PXR, pregnane X receptor;
\item SNP, single nucleotide polymorphism;
\item TDM, therapeutic drug monitoring.
\end{itemize}
ABSTRACT

Tacrolimus requires close therapeutic drug monitoring because of its narrow therapeutic index and marked inter-individual pharmacokinetic variation. In this study, we investigated the associations of polymorphisms in the gene encoding 11β-hydroxysteroid dehydrogenase type 1 (*HSD11B1*) with tacrolimus concentrations in Chinese renal transplant recipients during the early stage post-transplantation. A total of 258 renal transplant recipients receiving tacrolimus with prednisone (30 mg) combined therapy were genotyped for *HSD11B1* rs846908, rs846910, rs4844880 and *CYP3A5*/*3* polymorphisms. Tacrolimus trough concentrations were determined on days 6-9 following transplantation measured by a chemiluminescent microparticle immunoassay. Among CYP3A5 expressers, the dose-adjusted trough concentration (C0/D) of tacrolimus in *HSD11B1* rs846908 AA homozygous individuals was considerably lower than that in GG+GA carriers [56.2 (23.9-86.6) vs 76.7 (12.6-220.0) (ng/mL)/(mg/kg), \( P = 0.0204 \)]; *HSD11B1* rs846910 AA homozygotes had a lower tacrolimus C0/D comparing with GG+GA carriers [51.2 (23.9-86.6) vs 76.3 (12.6-220.0) (ng/mL)/(mg/kg), \( P = 0.0367 \)]; carriers with the *HSD11B1* rs4844880 AA genotype had a significantly lower tacrolimus C0/D with respect to carriers of TT+TA genotypes [61.3 (23.9-97.5) vs 77.2 (12.6-220.0) (ng/mL)/(mg/kg), \( P = 0.0002 \)]; the *HSD11B1* AA-AA-AA haplotype carriers had a lower tacrolimus C0/D than noncarriers [51.2 (23.9-86.6) vs 76.3 (12.6-220.0) (ng/mL)/(mg/kg), \( P = 0.0367 \)]. These findings illustrate that the *HSD11B1* genotypes are closely correlated with tacrolimus trough concentrations, suggesting that these polymorphisms may be useful for safer dosing of tacrolimus.
Introduction

Tacrolimus, as a calcineurin inhibitor, is the cornerstone of the pharmacological treatment in solid organ transplantation to prevent allograft rejection. Its narrow therapeutic index and highly variable pharmacokinetics make therapeutic drug monitoring (TDM) essential and indispensable to fine-tune the dosage (Kahan et al., 2002), and it is critical to reach target tacrolimus therapeutic range as early as possible, as the highest rejection rate occurs during the early stage post-transplantation (Wang et al., 2010). However, TDM is only possible after drug is administered and steady state is achieved, thus complementary strategies are needed (Cattaneo et al., 2004).

As optimizing balance between therapeutic efficacy and adverse events is the main goal of individualized medicine, pharmacogenetic/pharmacogenomic studies hold great promise as complementary tools in drug monitoring to better guide individualized therapy. Tacrolimus is mainly metabolized in the intestine and liver by cytochrome P450 3A4 and 3A5 (CYP3A4 and CYP3A5) enzymes, and transported by P-glycoprotein (P-gp/MDR1, encoded by \textit{ABCB1}) (Staatz and Tett 2004), making it susceptible to many clinically significant drug-drug interactions (Christians et al., 2002). Therefore factors that can modulate the expression or function of CYP3A4, CYP3A5 or P-gp may affect tacrolimus pharmacokinetics. Extensive studies have been conducted to explore the influence of genetic variants in \textit{CYP3A4}, \textit{CYP3A5} and \textit{ABCB1} on tacrolimus pharmacokinetics (Kurzawski and Drozdzik 2013; Zuo et al., 2013; Kurzawski et al., 2014), and recent studies also focused on pregnane X receptor (\textit{PXR}), cytochrome P450 oxidoreductase (\textit{POR}) and interleukins polymorphisms (de Jonge et al., 2011; Barraclough et al., 2012; Li et al., 2014), but the existing data remain conflicting. The only consistent conclusion to date appears to be the association of \textit{CYP3A5*3} polymorphism with tacrolimus pharmacokinetics. However, the results of our preliminary studies and others indicated that \textit{CYP3A5*3} genotype did not completely explain individual differences in tacrolimus metabolism (Thervet et al., 2010; Li et al., 2011).
Therefore, it is possible that additional genetic factors may explain the remaining variability in tacrolimus pharmacokinetics.

Prednisone as a corticosteroid is also an important component of the induction and maintenance immunosuppressive therapy in solid organ transplantation, which is usually used in the combined therapy with tacrolimus. Prednisone is a CYP3A and P-gp inducer (Pichard et al., 1992; Joy et al., 2005), and clinically drug-drug interaction between tacrolimus and prednisone have been observed. Tacrolimus dose requirement was higher when used in combination with prednisone, and its trough concentrations were elevated after prednisone withdrawal (Park et al., 2009; Stratta et al., 2012). Prednisone is largely converted by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1, encoded by gene HSD11B1) in the liver to its active form prednisolone (Czock et al., 2005; Hardy et al., 2013), which is a weak competitive inhibitor of CYP3A4 (Lam et al., 2008) and the principal form found in plasma (Gambertoglio et al., 1982). Moreover, greater than threefold inter-individual variability in dose-adjusted exposure to prednisolone was evident in transplant recipients (Bergmann et al., 2012). Since genetic variants of HSD11B1 have been shown to be associated through alteration of enzyme activity with various metabolic syndromes (Feldman et al., 2012; Gambineri et al., 2014), one can speculate if these variants could be involved. Based on these backgrounds, we hypothesized that the genetic variants of HSD11B1 might exert a polymorphic regulatory effect on 11β-HSD1 expression and alter the prednisolone to prednisone ratio, which would affect the interaction between tacrolimus and prednisone.

In the present study, the associations of HSD11B1 genotypes with tacrolimus concentrations were investigated in adult renal transplant recipients with a fixed prednisone dose treatment during the early stage post-transplantation.
Materials and Methods

Study Design and Patients. A total of 258 de novo adult renal transplant recipients (aged 39.6±10.8 years; 178 males, 80 females) who underwent surgery in Kidney Transplant Department, the First Affiliated Hospital of Sun Yat-sen University (KTD-SYSU) from September 2008 to September 2014 and provided their written informed consent were included. Patients undergoing single primary renal transplantation were eligible. Patients who received medications known to affect tacrolimus blood–concentrations other than prednisone, with abnormal hepatic function or had combined organ transplantation were excluded. Immunosuppression regimen after surgery was based on tacrolimus.

Patients were followed up for the first 9 days after transplantation for the tacrolimus concentrations and clinical data. All relevant data were retrieved without interfering with patient treatment and were handled according to the standard regulation for preservation of patient anonymity and privacy.

The study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University (No.[2008]23) and was conducted in accordance with the Declaration of Helsinki.

Immunosuppression Regimen and Measurement of Tacrolimus Concentrations. All patients were maintained on a triple immunosuppressive regimen consisting of tacrolimus (Prograf, Astellas, Killorglin, Ireland), mycophenolate mofetil (Cellcept, Roche, Basel, Switzerland) 0.5-1.0 g/day and prednisone (Guangdong Huanan Pharmacy Ltd., Dongguan, China) 30 mg/day. According to the routine in KTD-SYSU, the initial dose (0.05-0.075 mg/kg twice daily) of tacrolimus was started on the second day after transplantation and subsequently adjusted to achieve target trough concentration between 5-10 ng/ml.

Tacrolimus blood concentrations were measured in whole-blood samples collected immediately before tacrolimus morning dose administration using the chemiluminescent microparticle immunoassay (CMIA) on the Architect® analyzer (Abbott diagnostics Laboratories, IL, USA). Blood samples were obtained on days 6-9.
when steady-state concentration of tacrolimus was achieved (dosage had been unchanged for more than three days).

The dose-normalized blood concentration of tacrolimus was expressed as the ratio of trough concentration/dose corrected by weight (C0/D) [(ng/ml)/(mg/kg)].

**Genotyping.** Genomic DNA was collected from EDTA-anticoagulated whole blood samples from transplant recipients. Total genomic DNA was extracted from peripheral leukocytes. *CYP3A5*^*3* (rs766746) and *HSD11B1* rs846908 were genotyped by published (Li et al., 2011) and newly developed polymerase chain reaction restriction-fragment length polymorphism (PCR-RFLP) method, respectively. For *HSD11B1* rs846908 genotyping, forward primer 5'-GTAGATAGGCTAGAATCAATAGAAGC-3' and reverse primer 5'-AAACTCCGAGGAGACTGTAAT-3' were used, and the PCR products were digested with HpyCH4IV.

Rs846910 and rs4844880 were genotyped by PCR-direct sequencing.

**Statistical Analysis.** Statistical analysis and calculations were performed using SPSS software (version 21; SPSS, IBM, Armonk, NY) and Prism 6 (GraphPad Software, Inc., La Jolla, CA). Data are expressed as the median and range or mean±S.D., depending on data type. Groups were compared using nonparametric tests. For the analysis of continuous pharmacologic variables, patient genotypes were used as categorical independent variables. The Mann-Whitney U test was used for comparisons between two groups, and the Kruskal Wallis H test was used for comparisons among several groups. All single nucleotide polymorphisms (SNPs) identified were tested for deviations from Hardy-Weinberg equilibrium with the use of $\chi^2$ test. The pair-wise linkage disequilibrium (LD) for SNPs was evaluated using Haploview 4.2 software. *HSD11B1* rs846908-rs4844880-rs846910 haplotype analysis was performed by PHASE 2.1 (Stephens and Donnelly 2003). The significance level for all statistical tests was set at $P<0.05$ (two-tailed).
Results and Discussion

The alleles and genotypes frequencies shown in table 1 were in agreement with previous reports in Han Chinese populations. No deviations from the Hardy-Weinberg equilibrium were observed. \textit{HSD11B1} rs846908, rs4844880 and rs846910 genotypes were in strong linkage disequilibrium with each other, with D’ value ranging from 0.81 to 0.97. According to the haplotype analysis, the frequencies of the three major haplotypes of \textit{HSD11B1} (rs846908-rs4844880-rs846910) were 58.3% for GGT, 22.4% for AAA and 13.7% for GGA. The remaining haplotypes constituted 5.6% of the patients’ haplotypes.

In the overall study population, no significant association was observed between \textit{HSD11B1} genotypes and haplotypes with tacrolimus C\textsubscript{0}/D.Tacrolimus is predominantly metabolized by CYP3A5 (Dai et al., 2006), and the only polymorphism that reached extensive consensus nowadays is \textit{CYP3A5*3} (Ware and MacPhee 2010), thus stratification analysis was performed to eliminate the confounding effect of \textit{CYP3A5*3}. \textit{CYP3A5*3} genotype causes splicing defect that results in the absence of functional CYP3A5 protein in \textit{CYP3A5*3/*3} carriers (Kuehl et al., 2001), and the presence of \textit{CYP3A5*3} allele was associated with higher dose-adjusted tacrolimus blood concentrations and lower tacrolimus requirements (MacPhee et al., 2005). Carriers of the \textit{CYP3A5*1/*1} and \textit{*1/*3} genotypes were combined as CYP3A5 expressers, and carriers of the \textit{CYP3A5*3/*3} genotype were defined as CYP3A5 non-expressers. All significant associations were found in CYP3A5 expressers group: a considerably lower tacrolimus C\textsubscript{0}/D was observed in \textit{HSD11B1} rs846908 AA homozygous individuals when compared with GG+GA carriers [56.2 (23.9-86.6) \textit{vs} 76.7 (12.6-220.0) (ng/mL)/(mg/kg), \textit{P}=0.0204] (Figure 1A); tacrolimus C\textsubscript{0}/D was lower in \textit{HSD11B1} rs846910 AA homozygotes with respect to that in carriers of GG+GA genotypes [51.2 (23.9-86.6) \textit{vs} 76.3 (12.6-220.0) (ng/mL)/(mg/kg), \textit{P}=0.0367] (Figure 1B); \textit{HSD11B1} rs4844880 AA homozygous patients had a significantly lower tacrolimus C\textsubscript{0}/D than those with the TT+TA genotypes [61.3 (23.9-97.5) \textit{vs} 77.2 (12.6-220.0) (ng/mL)/(mg/kg), \textit{P}=0.0002] (Figure 1C); when
combining the effects of \textit{HSD11B1} rs846908, rs4844880 and rs846910, carriers of the AA-AA-AA haplotype associated with a lower tacrolimus C\textsubscript{0}/D comparing with noncarriers [51.2 (23.9-86.6) vs 76.3 (12.6-220.0) (ng/mL)/(mg/kg), \(P=0.0367\)] (Figure 1D).

It was reported that rs4844880 and rs846908 might function as transcriptional silencers, with the A alleles resulted in decreased \textit{HSD11B1} activity (Ku et al., 2009; Feldman et al. 2012). Hence the AA genotypes are speculated to be associated with a higher prednisone proportion in blood, which increases CYP3A and P-gp expression, ultimately leading to lower tacrolimus concentrations. Consistent with these studies, our results showed that a significant lower tacrolimus C\textsubscript{0}/D were found in AA homozygous individuals. As for rs846910, a haplotype containing the A allele has been previously shown to be associated with higher \textit{HSD11B1} mRNA levels and activity in the adipose tissue of Southern European whites (Gambineri et al., 2011). However, another research demonstrated that rs846910 did not influence \textit{HSD11B1} promoter activity, and it was unlikely to affect the potential binding site of a transcription factor as no sites for mammalian transcription factors were predicted in this region (Malavasi et al., 2010). In the present study, patients carrying the AA homozygous presented a lower tacrolimus C\textsubscript{0}/D, which was probably due to its strong linkage disequilibrium with rs4844880. However, further mechanistic study would be required.

The results showed that the associations between \textit{HSD11B1} polymorphisms and tacrolimus concentrations were only observed in CYP3A5 expressers, indicating that CYP3A5 expressers were more susceptible to the influence of \textit{HSD11B1} polymorphisms. However, further investigations are needed to explore whether this effect only exists with the expression of CYP3A5. Furthermore, it is reported that CYP3A5 non-expressers achieved the target tacrolimus concentration easily, whereas there was a significant delay for CYP3A5 expressers (MacPhee et al., 2004), thus carriers with the rs846908 AA, rs846910 AA or rs4844880 AA genotype in CYP3A5 expressers may be at greater risks of low tacrolimus concentrations when coadministered with
prednisone, which could lead to allograft rejection.

To our knowledge, the present study is the first one to report the associations of *HSD11B1* polymorphisms with tacrolimus concentrations, which is probably due to their impacts on the interaction between tacrolimus and prednisone. Similar to this study, pharmacogenomics association through drug-drug interactions have been previously reported, as *CYP2C19* genotypes had an indirectly impact on tacrolimus concentrations through altering CYP3A activities when it was administered concomitantly with proton pump inhibitors (PPIs), which are CYP2C19 substrates (Hosohata et al., 2009; Boso et al., 2013).

The present study should be interpreted within the context of its potential limitations. It is lack of *in vitro* data to support the clinical observations. Besides, the recipients in this study were those at the early stage after transplantation, who routinely received a high and fixed dose of prednisone, but the dose would be tapered progressively, thus it is not clear whether the impact of *HSD11B1* polymorphism on tacrolimus concentrations is prednisone dose-dependent. Therefore, further clinical and mechanistic studies are needed.

In summary, this study reports the potential effect of *HSD11B1* polymorphisms on tacrolimus concentrations in renal transplant recipients during the early stage post-transplantation with prednisone combined therapy. CYP3A5 expressers who carry the rs846908 AA, rs846910 AA or rs4844880 AA genotype may have lower tacrolimus concentrations. Due to the prevalence of prednisone combined therapy and potential serious consequences of this interaction, further studies are warranted to validate the clinical relevance of these findings.
Authorship Contributions

*Participated in research design:* Ji. Li, Huang, Xi. Liu, Ch. Wang.

*Conducted experiments:* Xi. Liu, Fu, Sh. Liu, Zhang, Ho. Wang, Ju. Li.

*Performed data analysis:* Xi. Liu, Xu. Wang, Zhu.

*Wrote or contributed to the writing of the manuscript:* Xi. Liu, Ji. Li.
References


Footnotes

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Xiaoman Liu and Jiali Li contributed equally to this study.
Legends for Figures:

Figure 1. Correlations of the *HSD11B1* (A) rs846908 genotype (GG+GA, n=106; AA, n=6), (B) rs846910 genotype (GG+GA, n=107; AA, n=5), (C) rs4844880 genotype (TT+TA, n=91; AA, n=21) and (D) haplotype (non-AA-AA-AA haplotype, n=107; AA-AA-AA haplotype, n=5) with tacrolimus C₀/D on days 6–9 post-transplantation in CYP3A5 expressers in renal transplant recipients. *P*<0.05, ***P*<0.001.
Table 1. Allele frequencies of polymorphisms in *HSD11B1* and *CYP3A5* genes of recipients (n=258)

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