ABSTRACT
Tacrolimus requires close therapeutic drug monitoring because of its narrow therapeutic index and marked interindividual 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 posttransplantation stage. A total of 258 renal transplant recipients receiving tacrolimus with prednisone were genotyped for HSD11B1 rs846908, rs846910, rs4844880, and CYP3A5*3 polymorphisms. Tacrolimus trough concentrations were determined on days 6–9 after transplantation, measured by a chemiluminescent microparticle immunoassay. Among the CYP3A5 expressers, the dose-adjusted trough concentration (C₀/D) of tacrolimus in HSD11B1 rs846908 AA homozygous individuals was considerably lower than found in GG+GA carriers (56.2 (23.9–86.6) versus 76.7 (12.6–220.0) (ng/ml)/(mg/kg), P = 0.0204; HSD11B1 rs846910 AA homozygotes had a lower tacrolimus C₀/D compared with GG+GA carriers (51.2 (23.9–86.6) versus 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) versus 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) versus 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, a calcineurin inhibitor, is the cornerstone of the pharmacologic treatment in solid-organ transplantation to prevent allograft rejection. Its narrow therapeutic index and highly variable pharmacokinetics make therapeutic drug monitoring essential and indispensable for fine-tuning the dosage (Kahan et al., 2002). It is critical to reach the target tacrolimus therapeutic range as early as possible, as the highest rejection rate occurs during the early posttransplantation stage (Wang et al., 2010). However, therapeutic drug monitoring is only possible after drug has been administered and a steady state is achieved, thus complementary strategies are needed (Cattaneo et al., 2004).

As optimizing the 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 the liver by cytochrome P450 enzymes 3A4 and 3A5 (CYP3A4 and CYP3A5), and is transported by P-glycoprotein (P-gp/MDR1 [multidrug resistance 1], encoded by ABCC1) (Staat 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 CYP3A4, CYP3A5, and ABCB1 on tacrolimus pharmacokinetics (Kurzawski and Drozdzik 2013; Zuo et al., 2013; Kurzawski et al., 2014), but the existing data remain conflicting. The only consistent conclusion to date appears to be the association of the CYP3A5*3 polymorphism with tacrolimus pharmacokinetics. However, the results of our preliminary studies and those of others have indicated that the CYP3A5*3 genotype does not completely explain the 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 combined therapy with tacrolimus. Prednisone is a CYP3A4 and P-gp inducer (Pichard et al., 1992; Joy et al., 2005); clinical drug-drug interactions between tacrolimus and prednisone have been observed. The tacrolimus dose requirement is higher when used in combination with prednisone, and its trough concentrations are elevated after prednisone withdrawal (Park et al., 2010). In this study, we investigated the associations of polymorphisms in the gene encoding 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) with prednisone concentrations in Chinese renal transplant recipients receiving tacrolimus with prednisone combined therapy.

Short Communication
Associations of HSD11B1 Polymorphisms with Tacrolimus Concentrations in Chinese Renal Transplant Recipients with Prednisone Combined Therapy

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ABBREVIATIONS: 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; C₀/D, trough concentration/dose corrected by weight; PCR, polymerase chain reaction; P-gp, P-glycoprotein; SNP, single-nucleotide polymorphism.
PCR products were digested with HpyCH4IV. Rs846910 and rs4844880 were associated through alteration of enzyme activity with various metabolic syndromes (Feldman et al., 2012; Gambineri et al., 2014), one can speculate whether these variants could be involved. Based on this background, we hypothesized that the genetic variants of HSD11B1 might exert a polymorphic regulatory effect on 11β-HSD1 expression and may alter the prednisolone-to-prednisone ratio, which would affect the interaction between tacrolimus and prednisone. In our present study, the association of HSD11B1 genotypes with the tacrolimus concentration was investigated in adult renal transplant recipients with a fixed prednisone dose treatment during the early posttransplantation stage.

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 and 80 females) who underwent surgery in the Kidney Transplant Department of the First Affiliated Hospital of Sun Yat-Sen University (KTD-SYSU) from September 2008 to September 2014 provided their written informed consent to be included in the study. Patients undergoing single primary renal transplantation were eligible. Patients who received medications known to affect tacrolimus blood concentrations (other than prednisone), those with abnormal hepatic function, or those who had combined organ transplantation were excluded. The immunosuppression regimen after surgery was based on tacrolimus.

Patients were observed for the first 9 days after transplantation for tacrolimus concentrations and clinical data. All relevant data were retrieved without interfering with the patients’ treatment, and the data were handled according to the standard regulations 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) at 0.5–1.0 g/day, and prednisone (Guangdong Huanan Pharmacy Ltd., Dongguan, People’s Republic of China) at 30 mg/day. According to the routine at KTD-SYSU, the initial dose (0.05–0.075 mg/kg twice daily) of tacrolimus was started on the second day after transplantation, and it was subsequently adjusted to achieve a target trough concentration/dose corrected by weight (C0/D) [(ng/ml)/(mg/kg)].

Tacrolimus blood concentrations were measured in whole-blood samples collected immediately before the tacrolimus morning dose administration using the chemiluminescent microparticle immunoassay on the Architect analyzer (Abbott Diagnostics Laboratories, Abbott Park, IL). Blood samples were obtained on days 6 to 9 after a steady-state concentration of tacrolimus had been achieved (i.e., the dosage had been unchanged for more than 3 days). The dose-normalized blood concentration of tacrolimus was expressed as the ratio of the three major haplotypes of HSD11B1 were started on the second day after transplantation, and it was subsequently adjusted to achieve a target trough concentration between 5 and 10 ng/ml.

Trough tacrolimus concentrations were measured in whole-blood samples collected immediately before the tacrolimus morning dose administration using the chemiluminescent microparticle immunoassay on the Architect analyzer (Abbott Diagnostics Laboratories, Abbott Park, IL). Blood samples were obtained on days 6 to 9 after a steady-state concentration of tacrolimus had been achieved (i.e., the dosage had been unchanged for more than 3 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 (rs676746) and HSD11B1 rs846908 were genotyped by published methods (Li et al., 2011) and by the newly developed polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, respectively. For HSD11B1 rs846908 genotyping, forward primer 5′-GTAGATAGGGCTAGAAATCAATATAAGC-3′ and reverse primer 5′-AAACCTCGGAGGAAGACTGTAAT-3′ were used, and the PCR products were digested with HpyCH4IV. Rs846910 and rs4844880 were genotyped by PCR direct sequencing.

Statistical Analysis. Statistical analyses and calculations were performed using SPSS software (version 21; SPSS/IBM, Armonk, NY) and Prism 6 (GraphPad Software, La Jolla, CA). The 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 identified were tested for deviations from Hardy-Weinberg equilibrium with the use of chi-square test. The pairwise linkage disequilibrium for single-nucleotide polymorphism was evaluated using Haplovie 4.2 software. The HSD11B1 rs846908-rs4844880-rs846910 haplotype analysis was performed with PHASE 2.1 (Stephens and Donnelly 2003). P < 0.05 (two-tailed) was considered statistically significant.

Results and Discussion

The alleles and genotype frequencies, shown in Table 1, were in agreement with previous reports in Han Chinese populations. No deviations from the Hardy-Weinberg equilibrium were observed.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0/D</td>
<td>0.81–0.97</td>
</tr>
</tbody>
</table>

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 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 statistically significant association was observed between HSD11B1 genotypes and the tacrolimus C0/D. Tacrolimus is predominantly metabolized by CYP3A5 (Dai et al., 2006), and the only polymorphism that has reached extensive consensus presently is CYP3A5*3 (Ware and MacPhee, 2010). Thus, a stratification analysis was performed to eliminate the confounding effect of CYP3A5*3.

The CYP3A5*3 genotype causes a splicing defect that results in the absence of functional CYP3A5 protein in CYP3A5*3/*3 carriers (Kuehl et al., 2001). The presence of the CYP3A5*3 allele was associated with higher dose-adjusted tacrolimus blood concentrations and lower tacrolimus requirements (MacPhee et al., 2005). Carriers of the CYP3A5*1/*3 and *1/*3 genotypes were combined in a group as CYP3A5 expressers. The carriers of the CYP3A5*3/*3 genotype were defined as CYP3A5 nonexpressers.

All statistically significant associations were found in the CYP3A5 expressers group. A considerably lower tacrolimus C0/D was observed in HSD11B1 rs846908 AA homozygous individuals when compared to the CYP3A5*3 carriers.

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype</th>
<th>Frequency</th>
<th>Allele</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>HSD11B1</td>
<td>846908</td>
<td>GG</td>
<td>143</td>
<td>55.4</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA</td>
<td>96</td>
<td>37.2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>19</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>846910</td>
<td>GG</td>
<td>147</td>
<td>57.0</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GA</td>
<td>96</td>
<td>37.2</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>15</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4844880</td>
<td>TT</td>
<td>104</td>
<td>40.3</td>
<td>T</td>
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</tr>
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<td></td>
<td></td>
<td>AA</td>
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</tr>
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<td>*1/*1</td>
<td>13</td>
<td>5.0</td>
<td>*1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1/*3</td>
<td>99</td>
<td>38.4</td>
<td>*3</td>
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<td></td>
<td></td>
<td>*3/*3</td>
<td>146</td>
<td>56.6</td>
<td></td>
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</table>

SNP, single-nucleotide polymorphism.
with GG+GA carriers [56.2 (23.9–86.6) versus 76.7 (12.6–220.0) (ng/ml)/(mg/kg), \( P = 0.0204 \)] (Fig. 1A). The tacrolimus C0/D was lower in HSD11B1 rs846910 AA homozygotes compared with carriers of GG+GA genotypes [51.2 (23.9–86.6) versus 76.3 (12.6–220.0) (ng/ml)/(mg/kg), \( P = 0.0367 \)] (Fig. 1B). The HSD11B1 rs4844880 AA homozygous patients had a statistically significantly lower tacrolimus C0/D compared with the TT+TA genotypes [61.3 (23.9–97.5) versus 77.2 (12.6–220.0) (ng/ml)/(mg/kg), \( P = 0.0002 \)] (Fig. 1C). When we combined the effects of HSD11B1 rs846908, rs4844880, and rs846910, the carriers of the AA-AA-AA haplotype were associated with a lower tacrolimus C0/D when compared with noncarriers [51.2 (23.9–86.6) versus 76.3 (12.6–220.0) (ng/ml)/(mg/kg), \( P = 0.0367 \)] (Fig. 1D).

It has been reported that rs4844880 and rs846910 may function as transcriptional silencers, with the A alleles resulting in decreased HSD11B1 activity (Ku et al., 2009; Feldman et al., 2012). The AA genotypes thus are speculated to be associated with a higher prednisone proportion in blood, which increases CYP3A and P-gp expression and ultimately leads to lower tacrolimus concentrations. Consistent with these studies, our results showed that a statistically significantly lower tacrolimus C0/D was found in AA homozygous individuals.

As for rs846910, a haplotype containing the A allele has been previously shown to be associated with higher HSD11B1 mRNA levels and activity in the adipose tissue of southern European whites (Gambineri et al., 2011). However, another study demonstrated that rs846910 did not influence HSD11B1 promoter activity and that it was unlikely to affect the potential binding site of a transcription factor because no sites for mammalian transcription factors were predicted in this region (Malavasi et al., 2010). In our study, the patients carrying the AA homozygous presented a lower tacrolimus C0/D, which was probably due to its strong linkage disequilibrium with rs4844880. However, further mechanistic study is required.

Our results have shown that the association between HSD11B1 polymorphisms and tacrolimus concentrations was only observed in CYP3A5 expressers, indicating that CYP3A5 expressers were more susceptible to the influence of HSD11B1 polymorphisms. However, further investigation is needed to explore whether this effect only exists with the expression of CYP3A5. Furthermore, CYP3A5 nonexpressers 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 risk of low tacrolimus concentrations when coadministered with prednisone, which could lead to allograft rejection.

To our knowledge, our study is the first to report the association of HSD11B1 polymorphisms with tacrolimus concentrations, which probably impacts the interactions of tacrolimus and prednisone. Similar to our study, a pharmacogenomic association through drug-drug interaction was previously reported, in which CYP2C19 genotypes had an indirect impact on tacrolimus concentrations via altering the activity of CYP3A when tacrolimus was administered concomitantly with proton pump inhibitors, which are CYP2C19 substrates (Hosohata et al., 2009; Bosso et al., 2013).

Our study should be interpreted within the context of its potential limitations. We lack in vitro data to support our clinical observations. Also, the recipients in this study were in the early posttransplantation stage, so they routinely received a high, fixed dose of prednisone, which was tapered progressively; thus, it is not clear whether the

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**Fig. 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 after transplantation in CYP3A5-expressers in renal transplant recipients. *\( P < 0.05 \); **\( P < 0.001 \).
impact of HS1DB1 polymorphism on tacrolimus concentrations is nondose dependent and consistent. Consequently, further clinical and mechanistic studies are needed.

In summary, this study reports on the potential effect of HS1DB1 polymorphisms on tacrolimus concentrations in renal transplant recipients during the early posttransplantation stage who are receiving prednisone combined therapy. The CYP3A5 expressers who carry the rs844908 AA, rs846910 AA, or rs844880 AA genotype may have lower tacrolimus concentrations. Due to the prevalence of prednisone combined therapy and the potential serious consequences of this interaction, further studies are warranted to validate the clinical relevance of our findings.

References


Bhattacharya SK, Das SK, Chakraborty A, Majumder S, Hazra D, Kar SA, De S, Banerjee P, Chakrabor