Title: Functional Genetic Variants Affecting Tacrolimus Trough Levels and Side Effects in Chinese Renal Transplantation.

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Study Protocol and Statistical Analysis Plan

Study Protocol:

Aim: Renal transplant is the treatment of choice for patients with end-stage renal disease. Immunological rejection is the leading cause of renal allograft dysfunction after transplant, and immunosuppressant play the main role in preventing against it. Tacrolimus (TAC) has become the main immunosuppressant as it can effectively improve allograft survival and prevent acute rejection. The therapeutic range for TAC is narrow, underexposure to TAC may lead to acute rejection in transplant recipients, whereas overexposure to TAC puts patients at risk of TAC-related side effects including nephrotoxicity, neurotoxicity. Clinical factors and genetic factors play the main role in the variation in TAC trough blood concentrations. There is a necessity for searching genetic marker affecting TAC metabolism and side effects to guide individualized administration of TAC.

Methods: Two cohorts will be enrolled in our study. The Chinese renal transplant recipients recruited at Nanfang Hospital will be set as the Discovery cohort. The Chinese renal recipients recruited at Guilin No. 924 Hospital will be set as Replication cohort. In order to identify the genetic variants accounted for the unexplained variability of TAC and the variants associated with the side effects in Chinese kidney transplant recipients, we will conduct a systematic candidate-SNP approach to investigate the effect of genetic polymorphisms on TAC trough blood concentration and side effects in two Chinese kidney transplant cohorts. The SNPs from two GWAS cohorts, eQTLs from GTEx database, SNPs locating at the promoters of *CYP3A4*, *CYP3A5* and *CYP3A7* gene, and genetic variants previously reported to be associated with variation in TAC trough blood concentration will be analyzed in this study. Additionally, the molecular mechanisms of the novel functional genetic variants will be evaluated, and the impact of clinical factors on TAC trough blood concentrations will be also analyzed.

Statistical Analysis Plan:

Statistical analyses will be performed using software R3.4.1 (R foundation for statistical computing, http://www.r-project.org) and GraphPad Prism5 (https://www.graphpad.com/). The dose-adjusted tacrolimus C_0/D without normal distribution will be log-transformed to attain normality. Multivariate linear regression model will be used to evaluate the association between TAC $\log(C_0/D)$ and clinical factors. In Discovery cohort, association analysis between candidate SNPs and TAC $\log(C_0/D)$ as well as the risk of acute rejection will be performed in five genetic models using software R 3.4.1 with SNPassoc package. An acute rejection free survival rates

among different genotypes will be calculated using the Kaplan-Meier method. In Replication cohort, SNPassoc package was used to evaluate the association between functional SNPs and TAC $\log(C_0/D)$, as well as the association between functional SNPs and the risk of acute rejection. A two-sided p value<0.05 was considered statistically significant.