Reviewer's report

Title: Effect of CYP3 A4, CYP3 A5 and ABCB1 gene polymorphisms on the clinical efficacy of tacrolimus in the treatment of nephrotic syndrome

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Reviewer: Jiraganya Bhongsatiern

Reviewer's report:
This article investigated effect of CYP3A5, CYP3A5, and ABCB1 genetic polymorphisms on the clinical efficacy of tacrolimus in 100 Chinese patients with nephrotic syndrome. The patients were followed for three months and were evaluated clinical efficacy according to (1) the 24-hour urine protein, (2) albumin, (3) renal function, and (4) the degree of edema. Gene sequencing was analyzed and the sequencing map of the four genetic variants were illustrated in the Supplementary Figures. Genotype distribution with Hardy-Weinberg equilibrium was assessed. The CYP3A4/5 and ABCB1 genetic polymorphisms, body mass index (BMI), and calcium channel blockers (CCB) antihypertensive drugs were evaluated as potential factors in multivariate logistic regression analyses. The CCB drugs were included owing to their property as CYP450 enzyme inhibitors. The authors concluded that ABCB1 C1236T, ABCB1 G2677T/A genotypes, and BMI are significantly influenced the clinical efficacy of tacrolimus in this patient cohort.

Strengths of this article include (1) the sequencing analysis and genetic equilibrium test of the four genetic polymorphisms of interests in Chinese population, which are well-corresponded to the report published in the NCBI dbSNP database, (2) age range of 6-63 years old providing an opportunity to study in both children and adults, and (3) the findings suggesting ABCB1 not CYP3A5 and CYP3A4 polymorphisms significantly affected the clinical efficacy of tacrolimus.

However, there are weaknesses that are critical and need to be improved before this manuscript can be accepted for publication. The followings are three of my main concerns.
(1) Tacrolimus clinical use:
   a. Tacrolimus in clinical use is complicated by its high between-patient variability in pharmacokinetics as well as its narrow therapeutic index. The tacrolimus doses in this study were suggested by the 'formula' in the National Health and Family Planning Commission of People's Republic of China (Reference 18). However, I was not able to find the reference on PubMed and it could not be checked by the Journal system. The dosing management of tacrolimus which usually includes therapeutic drug monitoring system is very critical. I think it is necessary to include more information on this issue.
   b. The authors informed the same tacrolimus doses were given by weight for both children and adults and BMI was one of the potential covariates in the regression analyses. The authors should be careful. Children typically require higher tacrolimus dosages (mg/kg) than adult patients, most likely reflecting the higher mean total body clearance and volume of distribution in children. I think the authors may want to at least report percentage of children (and any dose changes?), or reanalyze the children group separately from the adult group, or compare and address pharmacokinetic parameters such as trough concentrations of tacrolimus in both children and adults whether they are similar.

(2) Results of logistic regression analysis and clinical efficacy evaluation:
   a. Table 4, can the authors please include the results for all covariates (CYP3A4 and CYP3A5 genotypes, and CCB)? The significant results were already reported but I think it is important to show statistical parameters of all covariates. This study found no significant effect of the CYP3A5 genotype on tacrolimus clinical efficacy. The authors mentioned the study limitation of small sample size and the guideline (reference 18) in renal transplantation not nephrotic syndrome, which are somewhat reasonable. Nonetheless, the full table report of the regression analysis table is recommended, particularly when this CYP3A5 genotype was reported in previously published studies with regard to its significant effect of the genotype on tacrolimus concentration.
   b. The authors may consider providing a report of the genotype effects in multiple time points in the duration of 3 months. The clinical efficacy of tacrolimus can change owing to tacrolimus concentration that is associated with one or more gene polymorphisms in this
study. Including an analysis at multiple time point or at least address the time points that the clinical efficacy data were collected is strongly suggested.

c. Clinical efficacy was classified into 4 categories (complete remission, partial remission, no remission, and recrudescence). There was no information mentioned anywhere in the study of how many patients were in each category. I think this information would be very helpful when interpreting the results of regression analysis.

(3) Writing and conclusion:

a. Repetitive summary of genetic polymorphisms were written in the Background and Discussion sections. For example, Discussion section 'Relationship between ABCB1 gene polymorphism and clinical efficacy of tacrolimus', line 39, 'It can result in 839 amino acid…protein expression [17]' is almost the same sentence as that of Background section page 2 line 56.

b. Abstract line 22, the word 'Results' should be moved to line 24.

c. Wrong spelling of 'Females' in Table 1.

d. Discussion section page 4 line 56-59, '…the effect of TAC (tacrolimus) in patients with 2677G>T/A mutation genotype is better (reference 13 and 18). In reference 13, Kurzawski et al 2014 reported that CYP3A5/A4 not ABCB1 polymorphisms affect tacrolimus trough concentrations. This result is opposite from what the authors found.

e. Two repetitive sentences are detected in Conclusion section "Detecting the related genes…improve the curative effect." While I agree that individual drug guided by 'gene monitoring' can add more values in clinical work, I cannot agree with the authors based on results of this study that it is safer, more effective, more economical, and more reasonable.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

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