PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Possible role of IL-6 and TIE2 gene polymorphisms in predicting the initial high transport status in peritoneal dialysis patients: an observational study
AUTHORS	Ding, Li; Shao, Xinghua; Cao, Liou; Fang, Wei; Yan, Hao; Huang, Jiaying; Gu, Aiping; Yu, Zanzhe; Qi, Chaojun; Chang, Xinbei; Ni, Zhaohui

VERSION 1 - REVIEW

REVIEWER	Yukio Maruyama Division of Nephrology and Hypertension, The Jikei University School of Medicine, Tokyo, Japan.
REVIEW RETURNED	29-Feb-2016

GENERAL COMMENTS	In this work, the authors reported the effects of the SNPs of IL-6 and TIE2 on D/P Cr among patients starting PD as a first RRT. Although the results of this study are meaningful, there are already many reports with similar design and lacks novelty. Additionally, this report has several critical issues. My comments are as follows: Major comments: The authors used the SNPs of IL-6 and TIE2, and concluded that both the genotype AT in IL-6 Rs13306435 (OR=0.227) and the genotype CC in TIE2 Rs639225 (OR=0.501) had protective effect for high transporters. To describe these associations, the authors have to mentioned several things. At first, functional effects or protein levels (at least serum levels) of these SNPs should be presented. Secondly, the authors have to introduce the associations between these SNPs and other phenotypes, such as the prevalence of inflammatory disorders and cardiovascular disease among dialysis or non-CKD patients. Especially, there have been a number of reports presented the effects of the SNPs of IL-6 in many areas. The results of logistic regression analysis (Table 6) were strange. There are no data of OR and 95%CI of TIE2 Rs10967789 and hemoglobin. Are all these 5 parameters explanatory variables of this model? Why did the authors use serum albumin and hemoglobin as explanatory variables? As the authors described in Introduction (P.5), it has been suggested that chronic inflammation mediated by various inflammatory cytokines may have an effect on peritoneal transport, hs-CRP has to be used as an explanatory variable. In the Statistical analysis of Materials and Methods, the authors

	mentioned that continuous variables were presented as mean and standard deviation (SD), and unpaired t-test was used for the comparisons. However, several parameters including hs-CRP, UF, and urine volume seem not to be normally distributed. These types of parameters should be describes as median and range, and non-parametric tests have to be used for the comparisons. If there is parameter with significant difference between the L/LA and H/HA group, the authors cannot describe that “demographic and laboratory data were similar between the two groups” in abstract. Additionally, the authors have to describe the actual p value instead of “NS”. In the Introduction and Discussion, the authors mentioned that because PD patients in developing countries cannot use icodextrin and APD, the high transporters had poor outcome. Is there any study show the difference of the associations between high transporters and patient outcome between developing and developed countries. I do not think only overhydration is a cause of the poor outcome among high transporters. As authors mentioned in Introduction, chronic inflammation is another important cause of poor outcome. The authors have to consider the cause of poor outcome among high transporters in Discussion. In this study, initial PET was performed within 3 months after starting PD. However, the results of PET were changing in this period mainly from the influence of operation. I recommend using the results of PET after 3 months after starting PD. At least, the authors have to described periods between operation and initial PET, and treat this parameter as an explanatory variable in the multivariate analysis. Minor comments There were no description of inclusion criteria in the Patient selection of Materials and Methods. Did the authors include all incident PD patients except for the patients receiving kidney transplantation and HD? There are several descriptions of the 4 hour dialysate-to- plasma ratio of creatinine (D/P cr in P.4, D/P4 or D/P Cr in P.6, and D/P(Cr4h) in Table 3. I recommend using “D/P Cr”.

REVIEWER	Kuo-Hsiung Shu Taichung Veterans General Hospital, Taichung, Taiwan
REVIEW RETURNED	13-Mar-2016

GENERAL COMMENTS	The authors examined the genotypes of IL-6 and TIE2 in a group of uremic patients undergoing peritoneal dialysis (PD) and found that the genotype AT in IL-6 rs13306435 and the genotype CC in TIE2 rs639225 were both negatively associated with a higher initial peritoneal transport characteristics. This paper is interesting and of clinical relevance. However, some points need to be clarified. 1. In table 4, the total patient number of group H/HA is 95 for IL-6 rs13306435 (AT=3, TT=92) rather than 97. The same problem occurs in rs1800795 (GG=96, GC=0). Please explain this discrepancy. 2. Because the p value is borderline (0.047) for rs13306435, I wonder if the 2 missing data finally are proved to be one of the 2 genotype, it would probably change the p value and the conclusion

	would be invalid. 3. Because the peritoneal solute transport data are numeric and belongs to “continuous variables”, while the categorization of transport characteristic into the 4 groups is arbitrary, there is potential bias in misclassifying patients with borderline data (ie, lower end of HA and higher end of LA).To strengthen the conclusion, I suggest look at and compare the numerical data of D/P creatinine and D/D0 glucose at 4 hrs for each pertinent genotype to see if they are really associated with peritoneal solute transport rate. 4. How do you explain the linkage between these gene polymorphisms and peritoneal solute transport rate based on the function of IL-6 and TIE2?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:
Q1: Functional effects or protein levels (at least serum levels) of these SNPs should be presented.
Answer: Your suggestion is very important, we did not measure the serum level of the SNPs in the research. That is a limitation of the study. Most of the patients enrolled in this study starting PD therapy years ago, making a comparison among patients in different treatment stage might be improper. We are collecting specimens of new PD patients now, your advise is very helpful to our future work.

Q2: The authors have to introduce the associations between these SNPs and other phenotypes, such as the prevalence of inflammatory disorders and cardiovascular disease among dialysis or non-CKD patients.
Answer:We accept the reviewer’s suggestion and re-written the first part of discussion(page 8).

Q3:The results of logistic regression analysis (Table 6) were strange.
Answer: There are differences between two groups in level of albumin and hemoglobin as we showed in Table1. They may be associated with the initial high transport status we think. We accept the reviewer’s suggestion and do statistic analysis again. The new results are showed in Table 6.

Q4: Several parameters including hs-CRP, UF, and urine volume seem not to be normally distributed.
Answer: We are very sorry for our negligence.These errors have been corrected in TABLE3.

Q5:The authors cannot describe that “demographic and laboratory data were similar between the two groups” in abstract
Answer: We accept the suggestion to delete it.

Q6: The authors have to describe the actual p value instead of “NS”.
Answer: We accept the suggestion. All the “NS” had been replaced by actual p value.

Q7: I recommend using the results of PET after 3 months after starting PD. At least, the authors have to described periods between operation and initial PET, and treat this parameter as an explanatory variable in the multivariate analysis.
Answer: We agree that influence of operation is very important and we accept the suggestion to described periods between operation and initial PET, and treat this parameter as an explanatory variable. The results were showed in TABLE1 and TABLE6.

Q8: There were no description of inclusion criteria in the Patient selection of Materials and Methods.
Answer: We include all incident PD patients except for the patients receiving kidney transplantation and HD. We have re-written this part to declare it (page 5).

Q9: I recommend using “D/P Cr” to replace (D/P cr in P.4, D/P4 or D/P Cr in P.6, and D/P(Cr4h) in Table 3.
Answer:We accept the suggestion.

Special thanks to you for your good comments.

Reviewer #2:

Q1:In table 4, the total patient number of group H/HA is 95 for IL-6 rs13306435 (AT=3, TT=92) rather than 97. The same problem occurs in rs1800795 (GG=96, GC=0).
Answer: We are very sorry for our negligence of our calculation, especially in this important part. We’ve check the original data and corrected these errors. We did statistic analysis again, including the logistic regression model.Please see TABLE4 and TABLE 6.

Q2:Because the p value is borderline (0.047) for rs13306435, I wonder if the 2 missing data finally are proved to be one of the 2 genotype, it would probably change the p value and the conclusion would be invalid.
Answer: We did statistic analysis again, including the logistic regression model. Fortunately, the result did not change. We feel very sorry again.

Q3: To strengthen the conclusion, I suggest look at and compare the numerical data of D/P creatinine and D/D0 glucose at 4 hrs for each pertinent genotype to see if they are really associated with peritoneal solute transport rate.
Answer: Considering the Reviewer’s suggestion, we have compare the numerical data of D/P and D/D0. The result showed a tendency, however, it is not statistical significant. We think it is caused by the limited number of cases. It still need to be improved by expanding the sample size.

Q4: How do you explain the linkage between these gene polymorphisms and peritoneal solute transport rate based on the function of IL-6 and TIE2?
Answer: Chronic inflammation mediated by various inflammatory cytokines can influence the peritoneal transport. Gene polymorphisms are associated with the concentration of IL6 in the whole body including peritoneal dialysate. That might explain the linkage between these gene polymorphisms and peritoneal solute transport rate.
TIE2 is the receptor of angiogenin (Ang) 1 and 2. Ang/Tie2 has been confirmed to play an important role on the angiogenesis in peritoneum.The increasing of effective solute exchange area caused by peritoneal vascular proliferation is also an important factor for high peritoneal transport status. So it is possible that the genetic polymorphisms of TIE2 might be involved in the mechanism of high peritoneal transport status.

In all, I found the reviewer’s comments are quite helpful, and I revised my paper point-by-point. Thank you again for your help!

VERSION 2 – REVIEW

REVIEWER	Kuo-Hsiung Shu Taichung Veterans General Hospital, Taiwan
REVIEW RETURNED	24-Apr-2016

GENERAL COMMENTS	In my previous review, I suggest look at the numeric data of D/P creatinine and D/D0 glucose and compare them between different genotypes. Although the authors have already done this job and

	found no statistical significance, this was not mentioned in the revised manuscript. I suggest make another Table to show these results and address adequately in the discussion as a limitation of this study.

VERSION 2 – AUTHOR RESPONSE

We acepted the advise of Dr. Shu to make another Table to show the numeric data of D/P creatinine and D/D0 glucose between different genotypes and discussed it as a limitation of this study (TABLE 6).

VERSION 3 – REVIEW

REVIEWER	Kuo-Hsiung Shu Taichung Veterans General Hospital, Taiwan
REVIEW RETURNED	25-Jun-2016

GENERAL COMMENTS	I have no further comments for the authors.