Author's response to reviews

**Title:** MTHFR C677T and MTR A2756G Polymorphisms and the Homocysteine Lowering Efficacy of Different Doses of Folic Acid in Hypertensives Chinese Adults

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**Version:** 2  **Date:** 29 November 2011

**Author's response to reviews:** see over
November 29, 2011

Dear Editor:

    We greatly appreciate the careful review and comments from you and the reviewers. We believe that with the changes suggested by you, we have a stronger manuscript for the Journal. We look forward to your positive reply to the revised work submitted.

    We have made point-to-point responses to each of these comments in the attached document and have revised our manuscript accordingly.

    This manuscript has not been published before, nor is it being considered for publication elsewhere in any language. There are not any conflicts of interest regarding this work. All authors have read the manuscript and approved its submission to the European Journal of Clinical Nutrition.

    Please do not hesitate to contact us if we can be of any further assistance.

Sincerely,

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Reviewer Comments:

Reviewer #1 (Comments to the Author): Tianhua Niu

Major concerns

(1) Therefore, the authors should give only succinct and concise descriptions by shortening all the subsections of the “Materials and Methods” Section by referring to the above Article for the exquisite details of the “Subjects”, “Intervention and data collection”, “Laboratory methods”, and “Statistical Analysis” Subsections.

Response: We have revised this section as suggested.

(2) Then, the authors should explicitly point out that the non-compliance problem as a potential limitation of the study in the “Discussion” Section (although the authors stated that similar results were obtained), because this could lead to either an under- or an over-estimation of the genotypic effects of homocysteine-lowering responses depending on whether there is differential or non-differential non-compliance in these three treatment groups.

Response:

We have supplemented this limitation in our Discussion section as stated below:

“Furthermore, though similar results were obtained when we restricted our analyses to subjects who fully complied with the protocol during the treatment periods, we still could not fully exclude the effect of non-compliance problem on our results.”

(3) There was no mentioning of what statistical model (additive or multiplicative, with or without adjustments for potential non-genetic confounding factors) was used to test the gene-gene interaction between the MTHFR and the MTR polymorphisms in the “Statistical Analysis” Subsection of the “Materials and Methods” Section on page 7. The authors should explicitly present the statistical model selected for testing gene-gene interaction in the “Statistical Analysis” Subsection.

Response:

The additive models were used to test the gene-gene interaction between the MTHFR C677T and the MTR A2756G polymorphisms with and without adjustments for potential non-genetic confounding
Response of plasma Homocysteine to different doses and duration of folic acid supplementation by MTHFR C677T and MTR A2756G genotypes

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<tr>
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<td>ref</td>
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<td>0.90(0.16)</td>
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<td>ref</td>
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¹Regression model was adjusted for age, sex, BMI, baseline SBP, DBP, creatinine, TG, HDL, TC, FPG and study centers; 2Means (SD); ³Without adjustments for the potential non-genetic confounding factors. *Significantly different from participants with CC and AA genotypes.

(4) Page 7, 2nd paragraph. The authors stated that the MTHFR C677T and MTR A2756G polymorphisms were genotyped using the PCR-Restriction Fragment Length Polymorphism Method. However, the authors did not provide details regarding what quality control procedures (e.g., number and percentage of duplication samples genotyped to calculate the consistency rate as internal control) was used for genotyping in order to minimize potential genotyping errors.

Response:
For genotyping quality control, we used 20% randomly selected samples to regenotype and the
consistency rate was a 100%.

(5) Then, this raises the question when the natural logarithms of homocysteine or folate levels have been applied? Please clarify. Further, have the authors applied the logarithmic transformation in calculating median ratios for weeks 4 and 8 in comparison to the baseline (Tables 2 and 3 and Figure 2)? This should be explained in greater detail.

Response:
We have presented the homocysteine and folate levels as geometric mean values in Table 1-3.

We applied the natural logarithms of homocysteine or folate levels in comparing the difference among MTHFR C677T genotypes or between MTR A2756G genotypes. The non-parametric methods also were used to confirm these results. However, the median ratios - but not the natural logarithmic transformed ratios – were used in the regression models found in Table 2 and 3. Furthermore, the relative change of homocysteine, which is expressed as the change of homocysteine after FA treatment relative to the baseline concentration \[(\text{homocysteine after treatment} - \text{homocysteine at baseline})/(\text{homocysteine at baseline})\], was applied as the primary outcome in the revised manuscript.

(6) The “Discussion” Section is insufficient and there are several places need corrections.

- (i) Page 10, 1st paragraph of “Discussion”, the authors stated that “...Two previous dose-finding trials [12, 13]...” Actually, reference 12 is a “dose-finding” trial, but reference 13 is a “dose-response” trial. Please clarity.

Response:
We have corrected this sentence to read as follows: “A previous dose-finding trial and a dose-response trial have discussed the relationship between homocysteine lowering efficacy and optimal dose of folic acid supplementation”.

- (ii) The authors did not discuss the discrepancies of the relationship between homocysteine-lowering response to FA supplementation and MTHFR C677T polymorphism in previous literatures.
Response:
We have supplemented our Discussion section with this information.

- (iii) Among the 480 study subjects, at the baseline, were there any subjects taking multivitamin supplementation? This could potentially confound the genotypic effects of MTHFR and MTR polymorphisms on folate and homocysteine levels shown in Table 1.

Response:
Only one subject in the low FA group used vitamins at baseline. However, all of the subjects agreed not to take any vitamin B supplements in the week before the study and during the study period.

- (iv) MTHFR gene has another widely studied missense polymorphism A1298C (rs1801131, Glu429Ala) in previous literatures, but was not examined in this study. The authors should acknowledge this as a potential limitation.

Response:
We agree that it would be helpful to include the MTHFR A1298C variant in the report. We have acknowledged this limitation in the Discussion.

Minor Essential Revisions
(1) This needs to be clarified and an “Acknowledgments” Section needs to be added regarding funding source.

Response:
We have included this supplemental information as presented below:

Acknowledgments: The study was supported by Beijing Huaanfo Biomedical Research Center Inc. Beijing, China and in part by a grant from Anhui Provincial Ministry of Education (No. 2002kj174ZC), Anhui Provincial Ministry of Science and Technology, Anhui Medical University Biomedical Institute. The sponsors did not participate in the design or conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, and approval of the manuscript. We gratefully acknowledge the assistance and cooperation of the faculty and staff of the Anhui Medical University and
thank all of the participants in our study. This study was conducted in accordance with the current regulations of the People’s Republic of China.

(2) As the authors indicated, this is a multi-center, randomized, double-blind control trial. The authors should clarify about protocol approvals from all local ethics committees.

Response:
This study was approved by the Ethics Committee of Peking University First Hospital, Beijing, China and all local ethics committees. The purpose and procedures of the study were carefully explained to all participants, and written informed consent was obtained from each subject.

(3) There are a large number of typographical and grammatical errors that should be corrected.

Response:
We have corrected all of these errors as suggested.

Reviewer #2 (Comments to the Author): Ke Hao
1. Table 1, the footnote stated, "* Significantly different from the CT or CC genotype", however, there is no "*" presented in the table. Does it mean no significant difference? However, the folate and homocysteine level were very different among C677T genotypes.

Response:
We have included this supplemental information in Table 1.

2. Table 1 and Table 2, for sample traits, e.g. homocysteine, the mean and median values were quite different, indicating the trait value follows non-symmetric distributions, which may bias the ANOVA analysis. The authors should try (1) transformation to normal distribution or (2) non-parametric method to confirm the statistical analysis results.

Response:
Homocysteine and folate concentrations in the natural logarithms (due to their positively skewed distributions) were analyzed as continuous variables. We have presented the homocysteine and folate
levels as geometric mean and median values in Table 1-3.

We applied the natural logarithms of homocysteine or folate levels in comparing the difference among MTHFR C677T genotypes or between MTR A2756G genotypes using one-way analysis of variance (ANOVA) or t test, respectively. The non-parametric methods also were used to confirm these results.

Reviewer #3 (Comments to the Author): Madhu Khullar

My major comments are:
(1) The sample size is rather small, when sub-grouped into various groups (Control, low FA and High FA), for genetic association studies.

Response:
We have acknowledged this limitation in the discussion as stated below:

“Furthermore, the treatment period of FA was only 8 weeks and the sample size was rather small when sub-grouped into various groups (Control, low FA and High FA) for genetic association studies. Future studies with longer treatment duration and a larger sample size are needed to confirm our results.”

Minor comments
(2) Table 2 and Fig. 2 are presenting same data, so either of these should be given.

Response:
We have deleted Figure 2.

(3) It will be better to give Response of Plasma Homocysteine lowering to Different Doses of Folic Acid Supplementation by MTHFR C677T /MTR Genotypes as Delta reduction/percent reduction. The current Tables are very confusing.

Response:
The relative change of homocysteine is expressed as the change of homocysteine after FA treatment relative to the baseline concentration [(homocysteine after treatment - homocysteine at
baseline)/(homocysteine at baseline)], which was applied as the primary outcome in the revised manuscript.

(4) Fig. 1 is also not needed.

**Response:**

We have deleted Figure 1.

(5) Authors should cite some Asian studies as there is ethnic difference between Asians and Caucasians as far MTHFR genotypes and Folate levels are concerned.

**Response:**

We have supplemented this information in Discussion section as stated below:

“In fact, Malinow et al [1] first reported that TT subjects experienced much greater decreases in plasma total homocysteine concentrations after receiving FA at a dose of 1 or 2 mg/d for 3 wk than did CC subjects. Among subjects who were not previously taking multivitamins, the mean reductions in plasma total homocysteine concentrations were -20.9%, -13.1%, and -7.1% in persons with the TT, CT, and CC genotypes, respectively (P=0.019 for TT versus CC). A similar result also has been reported in a study in Taiwan, in which 5 mg of FA daily were supplemented for 8 wks [2]. Our results appear to be consistent with these studies. However, the study of Woodside et al. [3] showed that TT subjects are less responsive to the effects of FA and B-vitamin supplementation (daily 1 mg FA, 7.2 mg Vitamin B6, and 0.02 mg Vitamin B12 for 8 wks) than CC subjects. And Ho GY et al. [4] also reported that MTHFR C677T did not impact the total homocysteine-lowering effect of vitamins (daily 2.5 mg FA, 25 mg Vitamin B6, and 0.5 mg Vitamin B12 for 1 year) in a study performed in Singapore. The reasons for the disparities among the results of these studies are unknown. Additional large dose-finding studies are required to further elucidate the possible contributory factors, such as folate status, ethnicity of the participants, and in particular the possible effects of concomitant vitamin B6 and/or B12.”

**References**


(6) Enalapril has been reported to influence HC levels, authors should discuss this vis a vis their results.

Response:

We have added the following text to our revised manuscript:

“The association between angiotensin-converting enzyme inhibitor treatment and the change of homocysteine was controversial [1, 2]. We did not observe a significant relationship between enalapril treatment and homocysteine change in our study. Additional large sample studies also are needed to further examine the relationship of enalapril treatment with homocysteine change and the possible interactive effects between enalapril and folic acid on the change in homocysteine.”

References
