Reviewer's report

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

**Version:** 1  **Date:** 17 September 2011

**Reviewer:** Tianhua Niu

Reviewer's report:

The authors studied whether the homocysteine-lowering efficacy of two commonly used physiological doses (0.4mg/d and 0.8mg/d) of folic acid (FA) can be modified by methylenetetrahydrofolate reductase (MTHFR) C677T and/or methionine synthase (MTR) A2756G polymorphisms in Chinese hypertensives. A total of 480 subjects with mild or moderate essential hypertension were randomly assigned to 3 treatment groups: (1) enalapril only (10mg, control group); (2) enalapril-FA tablet(10:0.4mg[i.e., 10mg enalapril + 0.4mg FA], low FA group); and (3) enalapril-FA tablet(10:0.8mg, high FA group), once daily for 8 consecutive weeks. After 4 or 8 weeks of treatment, homocysteine concentrations were reduced across all genotypes and FA dosage groups, except in subjects with MTR 2756 AG/GG genotype in the low FA group at week 4. However, compared to subjects with MTHFR 677CC genotype, homocysteine concentrations remained higher in subjects with CT or TT genotype in the low FA group (P<0.05 for both) and TT genotype in the high FA group (P<0.05). Those subjects with TT genotype showed a greater homocysteine lowering response than did subjects with CC genotype in the high FA group (P=0.005), but not in the low FA group (P=0.989).

(I) Major Compulsory Revisions

MTHFR converts 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the primary circulating form of folate. The MTHFR C677T polymorphism (rs1801133, Ala222Val) results in a thermolabile variant and reduces the enzymatic activity of MTHFR. MTR catalyzes the remethylation of homocysteine to methionine, and although the MTR A2756G (rs1805087, Asp919Gly) may alter the enzymatic activity, the association of this polymorphism with the plasma homocysteine level appears to be less well characterized in previous literatures. This study compared plasma homocysteine levels at baseline, week 4, and week 8 treatments of control [i.e., enalapril (10 mg/d) only] group, low-FA [enalapril-FA (10:0.4mg/d)] group, and high-FA [enalapril-FA (10:0.8mg/d)] group among different MTHFR or MTR genotypes. Further, by calculating the median ratio of plasma homocysteine levels at weeks 4 and 8 in comparison to baseline level, the homocysteine-lowering responses to low- or high-dose FA treatments were compared among different MTHFR or MTR genotypes. The findings of the study, especially that hypertensive subjects with the MTHFR TT genotype showed a significantly greater homocysteine-lowering response than those with the CC
genotype in the high-FA group but not in the low-FA group, appear to have made contributions to our understanding of the gene-diet interactions between MTHFR genotype and FA supplementation.

I have the following major concerns.

(1) A study focusing on serum folate level changes in response to FA supplementation among different MTHFR and MTR genotypes has been recently published in the journal, “Pharmacogenetics and Genomics”, by exactly the same investigators based on the same multi-center, randomized, double-blind control trial:


Actually, in the “Materials and Methods” Section, the “Subjects”, “Intervention and data collection”, “Laboratory methods”, and “Statistical Analysis” Subsections are almost identical to those presented in the above published article in “Pharmacogenetics and Genomics”. 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. Actually, as shown in Figure 1 (Flow chart of sampling frame) of this manuscript and the corresponding Figure 1 of Qin et al. (2011) in “Pharmacogenetics and Genomics”, the only difference is that there were “6 excluded Subjects included for analysis N=146” as opposed to “7 excluded Subjects included for analysis N=145”. Although this figure could be kept because of the difference in the number included in the analysis for the High-FA group, because this study is not an independent study, and actually uses the same subjects as those of Qin et al. (2011), all redundant words in the “Materials and Methods” Section of the current manuscript as compared to Qin et al. (2011) should be removed and/or rephrased.

(2) As shown in Figure 1, the total number of subjects included in the analysis is 152 (Control group) + 147 (Low-FA group) + 146 (High-FA group) = 445. On page 10, 2nd paragraph, the authors indicated that “...We obtained similar results when we restricted our analyses to subjects who fully complied with the protocol (consuming at least 80% of all of the prescribed drugs) during the treatment periods (per protocol set, n=381) (data not shown)...” This actually raises a compliance concern here, because only a subset of the 445 subjects, i.e., 381 (85.6%) subjects, had satisfactory compliance with the respective treatment protocol. 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.

(3) Bottom line of page 9, and first two lines of page 10. The authors indicated “...There was no significant interactive effect between MTHFR C677T and MTR A2756G polymorphism on the homocysteine-lowering efficacy of different doses of folic acid supplementation....” However, 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.

(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.

(5) Page 7, 3rd paragraph, lines 1-2. The authors stated that “…Homocysteine and folate concentrations in the natural logarithms (due to their positively skewed distributions) were analyzed as continuous variables...” However, the homocysteine levels in Tables 1-3 and the folate levels in Table 1 were presented as raw values. 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.

(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.

(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. Actually, Malinow et al. (1997) 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 compared with CC). These results appear to be in agreement with the results of this study for the high-FA (i.e., FA supplementation 0.8mg/d) group of the current study. However, as indicated by Duell and Malinow (1999), the study of Woodside et al. (1998) showed that TT subjects are less responsive to the effects of FA and B-vitamin supplementation than CC subjects, and they hypothesized that doses of FA >1 mg/d may be required to reduce plasma total homocysteine concentrations in TT subjects. The authors should indicate that the results of the current study appear to be consistent with Malinow et al. (1997), which demonstrated that TT subjects showed a much greater decrease in plasma homocysteine concentrations in response to a FA supplementation of 0.8 mg/d at week 8 compared to the CC subjects.

(iii) Page 13, 1st paragraph, lines 3-6. The authors had only a bare statement about potential confounding effects of other vitamins: “...Additional studies are needed to examine the possible interaction effects between enalapril and folic acid on the change in homocysteine, and whether other vitamins may impact the modifying effect of the MTHFR C677T polymorphism on the homocysteine-lowering efficacy of folic acid[31]...” 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.

(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.

References:


(II) Minor Essential Revisions

The study Funding Source has been indicated as “None”, which is also indicated in Qin et al. (2011) published in the Journal “Pharmacogenetics and Genomics”.
However, in an earlier published article, Mao et al. (2008) [Reference: Mao G, Hong X, Xing H, Liu P, Liu H, Yu Y, Zhang S, Jiang S, Wang X, Xu X. Efficacy of folic acid and enalapril combined therapy on reduction of blood pressure and plasma glucose: a multicenter, randomized, double-blind, parallel-controlled, clinical trial. Nutrition. 2008 Nov-Dec;24(11-12):1088-96. Epub 2008 Jul 24. PubMed PMID: 18656328], which was based on the same multi-center, randomized, double-blind controlled trial, it was stated that “The study was supported by Beijing Huaanfo Biomedical Research Center Inc. Beijing, China.” (shown on the first page of the article) In the “Acknowledgments” Section on page 1095 of the above article [i.e., Mao et al. (2008)], it was stated that “This study was supported 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.” However, the current manuscript does not even contain an “Acknowledgments” Section. This needs to be clarified and an “Acknowledgments” Section needs to be added regarding funding source.

Page 6, 2nd paragraph. The authors indicated that “...This study was approved by the Ethics Committee of Peking University First Hospital, Beijing, China. The purpose and procedures of the study were carefully explained to all participants, and written informed consent was obtained from each subject...” It is important, as the authors stated that, “the purpose and procedures of the study were carefully explained to all participants, and written informed consent was obtained from each subject”. 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.

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

Page 3 (Abstract), 1st paragraph, line 2. 
"...physiological doses (0.4mg/d and 0.8mg/d) of folic acid can be modified..." should be changed to 
"...physiological doses (0.4mg/d and 0.8mg/d) of folic acid (FA) can be modified..."

Page 3 (Abstract), 2nd paragraph, lines 2-4. 
"...1) enalapril only(10mg, control group); 2) enalapril-folic acid tablet[10:0.4mg(10mg enalapril combined with 0.4mg of folic acid), low FA group]; and 3) enalapril-folic acid tablet(10:0.8mg, high FA group), once daily for 8 weeks..." should be changed to 
"...1) enalapril only(10mg, control group); 2) enalapril-FA tablet[10:0.4mg(10mg enalapril combined with 0.4mg of FA), low FA group]; and 3) enalapril-FA tablet(10:0.8mg, high FA group), once daily for 8 weeks..."

Page 3 (Abstract), 3rd paragraph, line 2.
"...and folic acid dosage groups, except in subjects with 2756AG/GG genotype..."
should be changed to
"...and FA dosage groups, except in subjects with MTR 2756 AG/GG genotype..."

Page 3 (Abstract), 3rd paragraph, line 3.
"...compared to subjects with 677CC genotype..."
should be changed to
"...compared to subjects with MTHFR 677 CC genotype..."

Page 3 (Abstract), 4th paragraph, lines 2-3.
"...at baseline and post-folic acid treatment, but also can modify therapeutic responses to various dosages of folic acid supplementation..."
should be changed to
"...at baseline and post-FA treatment, but also can modify therapeutic responses to various dosages of FA supplementation..."

Page 4, 1st paragraph, line 4.
"...hyperhomocysteine on the risk of CVD..."
should be changed to
"...hyperhomocysteinemia on the risk of CVD..."

Page 4, 1st paragraph, line 5.
"...suggested that folic acid supplementation..."
should be changed to
"...suggested that folic acid (FA) supplementation..."

Page 4, 1st paragraph, lines 7-8.
"...with no or partial folic acid fortification (RR:0.75, 95% CI: 0.62-0.91; P=0.003)..."
should be changed to
"...with no or partial FA fortification [Relative Risk (RR): 0.75, 95% Confidence Interval (CI): 0.62-0.91; P=0.003]..."

Page 4, 1st paragraph, line 9.
"...Furthermore, folic acid supplementation..."
should be changed to
"...Furthermore, FA supplementation..."

Page 4, 1st paragraph, lines 10-13.
"...in patients with end stage renal disease (ESRD) or advanced chronic kidney disease (ACKD, creatinine clearance <30mL/min) by 15% (RR:0.85; 95CI: 0.76-0.96, P=0.009), particularly in trials with a decrease in homocysteine level
>20% (RR: 0.83; 95% CI: 0.73-0.95; p=0.007); and no or partial folic acid fortification (RR: 0.80; 95% CI: 0.65-0.99; p=0.04)[7]..."

should be changed to

"... in patients with end stage renal disease or advanced chronic kidney disease (creatinine clearance <30mL/min) by 15% (RR:0.85; 95% CI: 0.76-0.96, P=0.009), particularly in trials with a decrease in homocysteine level >20% (RR: 0.83; 95% CI: 0.73-0.95; P=0.007); and no or partial FA fortification (RR: 0.80; 95% CI: 0.65-0.99; P=0.04)[7]...."

(Please note: ESRD and ACKD acronyms are not necessary because each of the two is referenced only once throughout the manuscript text.)

Page 4, 1st paragraph, bottom line.
"...but without folic acid fortification..."

should be changed to
"...but without FA fortification...

Page 4, 2nd paragraph, lines 6-7.
"... demethylation of 5-methyl-tetrahydrofolate to tetrahydrofolate and the remethylation (using the methyl group donated by 5-methyl-tetrahydrofolate)...

should be changed to
"... demethylation of 5-methyl-THF to THF and the remethylation (using the methyl group donated by 5-methyl-THF)...

Page 5, 1st paragraph, lines 2-4.
"...(0.4mg/d and 0.8mg/d) of folic acid can be modified by individual MTHFR C677T and MTG A2756G polymorphisms in hypertensive adults without folic acid fortification...

should be changed to
"...(0.4mg/d and 0.8mg/d) of FA can be modified by individual MTHFR C677T and MTR A2756G polymorphisms in hypertensive adults without FA fortification...

Page 5, 1st paragraph, lines 6-10.
"...2) enalapril-folic acid tablet (10 mg enalapril combined with 0.4 mg of folic acid, low FA group); and 3) enalapril-folic acid tablet (10 mg enalapril combined with 0.8 mg of folic acid, high FA group), once daily for 8 weeks. We sought to assess if MTHFR or MTG genotypes can influence a change in plasma homocysteine concentration in response to each of the two different dosages of folic acid supplementation...

should be changed to
"...2) enalapril-FA tablet (10 mg enalapril combined with 0.4 mg of FA, low FA group); and 3) enalapril-FA tablet (10 mg enalapril combined with 0.8 mg of FA, high FA group), once daily for 8 weeks. We sought to assess if MTHFR or MTR
genotypes can result in a change in plasma homocysteine concentration in response to each of the two different dosages of FA supplementation..." 

Page 6, 3rd paragraph, lines 2-4.
"...2) enalapril-folic acid tablet (10 mg enalapril combined with 0.4 mg of folic acid, low FA group); or 3) enalapril-folic acid tablet (10 mg enalapril combined with 0.8 mg of folic acid, high FA group), once daily for 8 weeks..." should be changed to
"...2) enalapril-FA tablet (10 mg enalapril combined with 0.4 mg of FA, low FA group); or 3) enalapril-FA tablet (10 mg enalapril combined with 0.8 mg of FA, high FA group), once daily for 8 weeks..."

Page 6, bottom line, and Page 7, top line.
"...for the measurement of glucose, lipids (including total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and triglycerides (TG))..." should be changed to
"...for the measurement of glucose, lipids [including total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG)]..."

Page 7, 2nd paragraph, line 3.
"...PCR-RFLP method..." should be changed to
"...PCR-Restriction Fragment Length Polymorphism method..."

Page 7, line 12.
The Subsection title "Statistical Analysis" should be changed to "Statistical analysis", to be consistent with the formats of all other Subsection titles in the "Materials and Methods" Section, or, all other Subsection titles need to be modified to have the same format as this Subsection title.

Page 8, 1st paragraph, line 5.
"...4 in high FA group..." should be changed to
"...6 in high FA group..."
(Please note: Based on numbers shown in the boxes at the bottom of Figure 1, this correction appears to be needed.)

Page 8, 2nd paragraph, line 3.
"...genotypes were 0.820, 0.171, and 0.090, respectively..." should be changed to
"...genotypes were 0.820, 0.171, and 0.009, respectively..."
"...Subjects with TT genotype had significantly higher..."
should be changed to
"...Subjects with MTHFR 677 TT genotype had significantly higher..."
Page 8, 3rd paragraph, line 6.
"...MTHFR C677T and MTR A2756G polymorphism on..."
should be changed to
"...MTHFR C677T and MTR A2756G polymorphisms on..."
Page 8, bottom paragraph, lines 1-2.
"...after 4 or 8 weeks of folic acid treatment decreases in plasma homocysteine concentrations were seen across all MTHFR C677T genotypes and folic acid..." should be changed to
"...after 4 or 8 weeks of FA treatment decreases in plasma homocysteine concentrations were seen across all MTHFR C677T genotypes and FA..."
Page 8, bottom paragraph, lines 4-5.
"...with TT and CT genotypes remained significantly higher at week 4 or week 8 (P<0.05 for both other genotypes)..." should be changed to
"...with TT or CT genotypes remained significantly higher at week 4 or week 8 (P<0.05 for having either of these genotypes)..."  
Page 9, 2nd paragraph, line 3.
"...with or without adjustment for the relevant demographic and clinical characteristics..." should be changed to
"...with or without adjustments for the relevant demographic and clinical characteristics..."  
Page 9, 2nd paragraph, line 10.
"...with adjustment for..." should be changed to
"...with adjustments for..."  
Page 9, 3rd paragraph, line 2.
"...and folic acid dosage groups..." should be changed to
"...and FA dosage groups..."  
Page 9, bottom paragraph, lines 1-5.
"...after 4 or 8 weeks of folic acid treatment decreases in plasma homocysteine
concentrations were seen in all MTR A2756G genotypes and folic acid dosage groups, except in subjects with AG/GG genotype in the low FA group at week 4. However, neither homocysteine concentration post-folic acid treatment nor the homocysteine lowering response of folic acid supplementation...

should be changed to

"...after 4 or 8 weeks of FA treatment decreases in plasma homocysteine concentrations were seen in all MTR A2756G genotypes and FA dosage groups, except in subjects with AG/GG genotype in the low FA group at week 4. However, neither homocysteine concentration post-FA treatment nor the homocysteine lowering response of FA supplementation...

Page 10, 1st paragraph, line 1.
"...polymorphism on the homocysteine-lowering efficacy of different doses of folic acid...

should be changed to

"...polymorphisms on the homocysteine-lowering efficacy of different doses of FA...

Page 10, Discussion Section, 1st paragraph.
All occurrences of "...folic acid...

should be changed to

"...FA...

Page 10, Discussion Section, 1st paragraph, line 8.
"...the study population was young women...

should be changed to

"...the study population consisted of young women...

Page 10, Discussion Section, 1st paragraph, lines 11-12.
"...were lower than those observed in a U.S. population[15], which means that this Chinese population was not a representative or an appropriate population...

should be changed to

"...were lower than those observed in a U.S. population[15] and a Chinese population[16], which means that the Chinese population of [14] was not a representative population...

(Please note: the authors could not draw the conclusion that the the study population of reference 14 is not appropriate.)

Page 10, Discussion Section, 1st paragraph, line 16.
"...MTHFR C677T and MTG A2657G polymorphisms...

should be changed to

"...MTHFR C677T and MTR A2657G polymorphisms..."
Page 11, 1st paragraph, line 2.
"...in all genotypes and folic acid dosage..."
should be changed to
"...in all genotypes and FA dosage..."

Page 11, 1st paragraph, line 4.
"...subjects with TT genotype..."
should be changed to
"...subjects with MTHFR 677 TT genotype..."

Page 11, 1st paragraph, lines 12-13.
"...0.4mg folic acid supplementation may be insufficient, and that a daily dose of 0.8mg folic acid may be required..."
should be changed to
"...0.4mg FA supplementation may be insufficient, and that a daily dose of 0.8mg FA may be required..."

Page 11, 2nd paragraph, line 4.
"...0.8 mg folic acid also may be necessary..."
should be changed to
"...0.8 mg FA also may be necessary..."

Page 11, 2nd paragraph, bottom line.
"...hyperhomocysteine in TT subjects..."
should be changed to
"...hyperhomocysteinemia in TT subjects..."

Page 11, 3rd paragraph, line 2.
"...folic acid supplementation..."
should be changed to
"...FA supplementation..."

Page 12, 1st paragraph, line 2.
"...4 weeks of folic acid supplementation..."
should be changed to
"...4 weeks of FA supplementation..."

Page 12, 2nd paragraph.
All occurrences of "...folic acid..."
should be changed to
"…FA…"

Page 12, 2nd paragraph, line 2.
"…with CT or TT genotype…"
s should be changed to
"…with MTHFR 677 CT or TT genotype…"

Page 12, bottom paragraph, and Page 13.
All occurrences of "…folic acid…"
s should be changed to
"…FA…"

Page 14, Authors’ contributions, line 7.
"…primary responsibility…"
s should be changed to
"…primary responsibilities…"

Page 20, Figure Legends.
The following should be added to the Legend to Figure 2: Upper Panel: week 4, Lower Panel, week 8.

Page 21, Table 1 Caption, line 7.
"…MTR A2567G…"
s should be changed to
"…MTR A2756G…"

Page 21, Table 1 footnotes, bottom line.
"…Means (SD)…"
s should be changed to
"…Mean (SD)…"

Also, the “*” superscript does not appear in the table content. The authors need to check whether the “*” superscript should be added for any numbers in the table; if none, “*Significantly different from the CT or CC genotype” should be removed from the footnotes.

Page 22, Table 2 Caption.
"…plasma Homocysteine…"
s should be changed to
"…plasma homocysteine…"

Page 22, Table 2 footnotes, top line.
"…Means (SD)…"
should be changed to
"...Mean (SD)...

Page 23, Table 3 Caption.
"...plasma Homocysteine..."
should be changed to
"...plasma homocysteine...

Page 23, Table 3.
A row for “Total” should be added for each of the three treatment groups for “baseline”, “4 weeks”, and “8 weeks” for Mean(SD)[Median] values of plasma homocysteine levels, i.e., “Control”, “Low FA”, “high FA”, as in Table 2.

Page 23, Table 3 footnotes, top line.
"...Means#SD..."
should be changed to
"...Mean (SD)...

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.