Author's response to reviews

Title: Methionine synthase A2756G polymorphism may predict ulcerative colitis and methylenetetrahydrofolate reductase C677T pancolitis, in Central China

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Author's response to reviews: see over
Comments of Michael Fenech:

**Minor essential Revisions:**

- Page 3 line 10: Change "was found ot be associated with" to "is".
- Page 4 line 5: Change "MTFHR" to "MTHFR"
- Page 4 lines 10-12: Indicate direction in which allele frequencies differed between Central Chinese and Caucasian populations.
- Page 6 line 10: Insert "analysis" after "The stepwise".
- Page 7 line 7: Delete "we were no and"

Answer: we have made all the changes and we appreciate the help for improving our manuscript.

Comments of Elias Zintzaras

The authors did not reply to my comments. The methodology and analysis followed is not appropriate and the results might be peculiar.

We made a detailed answer to the first revision of the reviewer and we are surprised by this comment. Rather than providing an evaluation of our answer, the second revision adds new comments and suggestions. Some of them are questionable.

The authors are advised to consult an expert in genetic analysis.

The reviewer has produced many interesting meta-analyses. The corresponding and senior author of this article (JL Guéant) has an established expertise in association studies (N Engl J Med, Ann Intern Med, J Med Genetics, Am J Clin Nutrition, Pharmacogenetics, J Pharmacogenomics, Am J Med Genet, etc…) and considers unfair this kind of comment.

Sample size calculation is wrong! In genetic association studies sample size is not determined as in case-control studies in clinical research. See papers by the following authors: Gordon, de Bakker, Jackson, or Skol.

We disagree. Sample size was evaluated for investigating an association between a limited number of categorical variables (specific gene variants) and UC. The significant association between MTR and UC was in agreement with our estimation. We would appreciate a specific reference of the cited authors to be able to understand the comment. Gordon have published theoretical studies on the influence of phenotype errors in the calculation of sample size (BMC genetics, 2005, 6, 1-18). The rate of phenotype errors is neglectable in our work. The diagnosis was based on colonoscopy and histological examination procedures that we have previously reported in specialized journals (Laurent Peyrin et al, Gastroenterology 2007, Am J Gastroenterology 2007, Gut 2006). De Bakker et al. and Skol et al. explored analytical strategies to improve power of whole-genome association studies (Nat Genet. 2006 Jun;38(6):663-7 and Genet Epidemiol. 2007;31 :776-88). Our paper is not a genome-wide association study.

For each polymorphism should calculate the unadjusted OR (95% CI) for susceptibility to UC, and extensive UC, for the allele contrast, the dominant and recessive models of the minor allele. The OR adjusted by age, sex and clinical
variables (which is mentioned in the Statistical analysis section) should be calculated for the same genetic models.

We have added the suggested unadjusted OR in the tables 2 and 3. The unadjusted OR for MTHFR/extensive UC association is given in the revised table 4 and the significant adjusted ORs are given in the text of the result section. Sex and age adjustment is not needed in the estimate of UC risk predictors as the study design was based on sex- and age-matched case control groups.

Correct the analysis for multiple comparisons. This was done by Bonferroni correction, as previously reported in our MTHFR paper published in Am J Clin Nutrition (ref 18).

Omit the stepwise procedure. Otherwise follow the methodology introduced by papers of PC Sham (London).

A new suggestion. In our opinion, the stepwise was adapted to our restricted model. However, to take into account the remark, we have deleted the table 4 and modified the revised table 4 by giving the unadjusted odds ratio and considering a multivariate model that included only the variables with P-value lower than 0.10 in the univariate analysis. The corresponding paragraph of method section has been revised accordingly.

Present combined genotypes and test associations using unadjusted ORs.

We have already answered to this comment in our first revision. The sample size of our study does not permit to analyse gene gene associations.

Test for LD and provide Ds and p-values.

These comments are a new suggestion. These calculations are not relevant for the genes evaluated in our study. No LD exists between MTHFR, MTR, MTRR and TCN2. For example, you can obtain this information in the paper by Frederiksen et al, Large-Scale Population-Based Metabolic Phenotyping of Thirteen Genetic Polymorphisms Related to One-Carbon Metabolism, Hum Mutation, Hum Mutat. 2007;28:856-65. There is a complete disequilibrium between MTHFR 677T and 1298C alleles. Presently this complete disequilibrium was confirmed. A short sentence has been added in the result section « MTHFR 677T and 1298C alleles were in complete disequilibrium, as previously reported in other populations »

Estimate haplotype frequencies for cases and controls using an EM algorithm and estimate the respective SE or p-values for each haplotype association.

Again a new suggestion. MTHFR, MTR, MTRR and TCN are in distant regions of the human genome (1p36.3, 1q43, 5p15.3, 22q12.2) and have no LD (see Human mutation paper). There is no rational for evaluating haplotypes. In addition, the sample size does not permit this kind of analysis.

Test for HWE using an exact test.
Difficult to understand this comment. We have calculated HWE as described in Guéant et al, J Med Genet, 2007. Our calculation is correct. There is no disequilibrium. The following table provides the expected distribution used in the calculation.

<table>
<thead>
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<th>UC patients</th>
<th>Expected</th>
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<tr>
<td>Number</td>
<td>(percentage)</td>
</tr>
</tbody>
</table>

### MTHFR 677
- **CC**: 64 (38.1) | 62
- **CT**: 76 (45.2) | 80
- **TT**: 28 (16.7) | 26
- **Total number**: 168

### MTHFR 1298
- **AA**: 111 (69.4) | 111
- **AC**: 45 (28.1) | 44
- **CC**: 4 (2.5) | 5
- **Total number**: 160

### MTR 2756
- **AA**: 115 (73.7) | 113
- **AG**: 36 (23.1) | 39
- **GG**: 5 (3.2) | 4
- **Total number**: 156

### MTRR 66
- **AA**: 88 (53.7) | 87
- **AG**: 63 (38.4) | 65
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<td>27 (18.5)</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>66 (45.2)</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>GG</td>
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<td>51</td>
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<tr>
<td><strong>Total</strong></td>
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<table>
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