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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Point-by-point Response to the comments

Reviewer: Fernando Magro

Q: The paper “Methionine synthase A2756G polymorphism predicts ulcerative colitis and methylenetetrahydrofolate reductase C677T pancolitis, in Central China” related that methionine synthase and methylenetetrahydrofolate reductase are genes associated with risk and extent of ulcerative colitis. One of the gold marks of this paper is the homogeneity of UC population.

A: We thank F Magro for his comments.

Q: The authors must put in discussion the biologic plausibility of these mutations in UC context.

A: This has been added in the discussion of the revised manuscript. The plausibility of MTR 2756 A>G association with UC risk: “We also evidenced that vitamin B12 was a nutritional determinant of homocysteinemia, that was under the influence of oxidative stress [26]. Consistently, a recent report showed that folate deficiency was not a predictor of homocysteine level in case of increased oxidative stress, underscoring the implication of other nutritional determinants, such as vitamin B12, under pathological conditions [26]. Taken together, these results are in agreement with our findings indicating that methionine synthase A2756G polymorphism, a vitamin B12-dependent enzyme, may predict ulcerative colitis.” The plausibility of MTHFR is discussed in this sentence: “As a consequence, MTHFR C677T polymorphism produces a decreased cellular level of methyl-THF and a cellular accumulation of homocysteine, particularly in
patients with insufficient folate supply [21]. Interestingly, recent experimental findings raise the possibility that homocysteine-induced cellular and vascular stress may contribute to the maintenance of a chronic mucosal inflammatory state in IBD [9, 22].

Q: Why MTHFR 677 TT and MTHFR 1298 AC are related to extension of UC and are not related to UC risk? Is an additive effect?

A: The MTHFR 677 TT genotype is known to be a strong genetic determinant of plasma homocysteine level in patients with inflammatory bowel diseases (Peyrin-Biroulet L et al. Am J Gastroenterol 2007;102:1108-15). In patients carrying MTHFR 677 TT genotype, intestinal mucosal injury and pancolitis (ulcerative colitis that involves the entire colon) may thus be explained, at least in part, by hyperhomocysteinemia-induced oxidative stress. In our study, the MTHFR 1298 AC genotype was independently associated with the risk of extensive UC, but less significant association was found with this genotype than with the MTHFR 677 TT genotype (0.0386 vs 0.0073, respectively). Since the reduction in MTHFR enzyme activity is less important in MTHFR 1298 AC patients than in patients carrying the MTHFR 677 TT genotype, our results are consistent with the hypothesis that hyperhomocysteinemia-induced oxidative stress may cause extensive and severe UC in these patients. By contrast, MTHFR polymorphisms are not associated with the primary risk of UC, confirming previous reports in patients with Crohn’s disease (Peyrin-Biroulet L et al. Am J Gastroenterology 2007;102:1-8). This is now stated in the discussion of the revised manuscript.

Q: Is important to explore these mutations and some UC variables as activity of the disease, refractory to medical treatment, chronic activity and the immunosuppression needed, particularly the azathioprine response.

A: We agree. We have added the following paragraph in discussion:

Vitamin B12 metabolism influenced Crohn’s disease activity by modulating oxidative stress, measured by superoxide dismutase activity [26]. Homocysteine levels were recently correlated with activity, number of flares and duration of the disease [28]. Unfortunately, we could not determine the blood level of these determinants in the present series as no serum or plasma sample was available. Furthermore, we recently found that azathioprine therapy decreased plasma homocysteine level [26], suggesting interactions between azathioprine and homocysteine metabolisms. Genetic variation in the MTHFR gene may result in reduced S-adenosylmethionine concentrations, leading to enhanced TPMT enzyme degradation and possibly modulating azathioprine efficacy [29].

Q: Is important to know the relationship between these mutation and the thrombotic events

A: as indicated in the end of discussion, no thrombotic events occurred in our cohort of Chinese patients. Several studies indicated that homocysteine metabolism does not seem to contribute to the increased risk of thrombosis in patients with inflammatory bowel diseases (Peyrin-Biroulet L et al. Am J
Indeed, as recently discussed by Danese et al. (Am J Gastroenterol 2007;102:174-86), the etiology of thrombosis in inflammatory bowel diseases appears to be multifactorial.

Reviewer: Mickael Fenech

Q: The authors have not provided a plausible reason why altered folate/methionine metabolism could be a cause of UC. A plausible hypothetical mechanism should be explained in the background or discussion.

A: This point has been addressed in a recent review that we have published in Am J Gastroenterology. We have added the following sentences in the introduction section to summarize the potential pathomechanisms:

¿Homocysteine has also a crucial role in cellular stress, epigenetic events, inflammatory processes, thrombosis and host-microbial interactions. Hyperhomocysteinemia might therefore influence the clinical history of IBD, including disease severity [9]. »

Q: The study size is too small to make definite conclusions. The title and the concluding sentence in the discussion should indicate that the study results are only preliminary.

A: we have modified the title as followed: ¿Methionine synthase A2756G polymorphism may predict ulcerative colitis and methylenetetrahydrofolate reductase C677T pancolitis, in Central China¿. In addition, the last sentence of the Discussion has been modified as followed:¿These findings might open new insights into the pathogenesis and assessment of UC, particularly for the potential interest in treating the patients presenting with the 677TT MTHFR genetic trait and a deficit in folate. However, our results await confirmation in a large cohort of patients with UC¿.

Q: The explanation of results in the abstract is confusing because it is not clear whether the data relate to controls or cases.

A: We agree. The sentence has been modified as followed

¿Methylenetetrahydrofolate reductase 677TT genotype was 2.7-fold more prevalent in individuals with pancolitis than in those with left colitis or proctitis, with respective percentages of 27.3 (95% C.I.16.4-42.0) and 10.5 (95% C.I. 6.3-17.1) (P= 0.0123). The carriage of 677TT or 677CT/1298AC genotypes of methylenetetrahydrofolate reductase was 2.2-fold higher in cases with pancolitis than in subjects with left colitis or proctitis (P= 0.0048), with an Odds ratio at 3.3 (95% C.I. 1.4-7.9), P=0.0084) in multivariate logistic regression.¿.

Q: Background. Explain the function of the transcobalamin gene product.

A: We have mentioned that transcobalamin is a specific plasma transporter of cobalamin (vitamin B12) and facilitates the cellular uptake of the vitamin by receptor-mediated endocytosis in the second paragraph of the Introduction of the revised manuscript.
Q: Results. What was the reproducibility of the genotyping assays.
A: As previously shown, genotyping assays were highly reproducible. In fact, five % of the samples were re-genotyped to check for genotype calling consistency. The genotyping success rate was more than 98%. This is indicated in Method section of the revised manuscript.

Q: Page 6 line 7 from bottom. ¿with a¿¿¿¿¿episode¿ does not make sense. Please correct this phrase.
A: We apologize for this typo error, which has been corrected as followed: ¿None of the cases presented with a reported thromboembolic episode.¿

Q: Explain ¿pancolitis¿.
A: It is now stated that the term ¿Pancolitis¿ means ¿ulcerative colitis that involves the entire colon¿ in the method section (first paragraph) of the revised manuscript.

Q: Last sentence on page 7 is unclear and needs to be re-written.
A: This sentence has been modified as it : ¿The frequency of subjects who presented with either 677TT or the double heterozygous 677CT/1298AC genotype was also significantly different between subjects with pancolitis and those with left colitis or proctitis, and to a much greater extent than with the TT genotype of MTHFR C677T polymorphism, with respective percentages of 43.2 (95% C.I. 29.6-43.2) and 20.2 (95% C.I. 14.1-28.1) (P=0.0048).¿

Q: Was the impact of MTHFR C677T mutation on extensive lesions greater in those with the MTR A2756G mutation?
A: No, it was not.

Q: Last sentence of discussion should be changed to indicate that these are only preliminary data and that a larger study is needed to verify the results.
A: The last sentence of the Discussion has been modified as followed: ¿These findings might open new insights into the pathogenesis and assessment of UC, particularly for the potential interest in treating the patients presenting with the 677TT MTHFR genetic trait and a deficit in folate. However, our results await confirmation in a large cohort of patients with UC¿.

Reviewer Elias Zintzaras
Q: ¿The authors should analyze the data in the following way: For each polymorphism, to calculate unadjusted OR with 95% C.I. for susceptibility to UC (or extensive UC) for the allele contrast, the dominant and recessive model of the minor allele. » Unadjusted ORs were not calculated as no genotype was associated with the susceptibility to UC, as evidenced in table 2. We have added in result section (page 7) the unadjusted OR for the association between MTR
2756 AG and GG and UC risk, since a significant association was evidenced with the MTR allele G in the univariate analysis presented in table 3: « The unadjusted OR of MTR 2756AG and GG were estimated respectively to 1.72 (95% C.I. 1.02-2.92, P=0.0422) and 2.64 (95% C.I. 0.62-11.25, P=0.1899).»

Q: Then, for the same models to calculate adjusted OR after correcting for clinical and demographic characteristics. A: As indicated by another reviewer, our study was performed on an homogeneous population from China, Hubei province (comment: « One of the gold marks of this paper is the homogeneity of UC population. »). There is no need for adjusting for demographic data.

Q: « Finally, an association based on haplotypes and combined genotypes should be tested. »

A: The single haplotype that was significantly associated with UC (pancolitis) was MTHFR 677CT/1298AC. In fact the sample size does not permit to evaluate gene-gene associations.

Q: The sample size should be calculated taking into account the prevalence of the minor allele of the most commonly investigated polymorphism. This is indicated in the method section of the revised version: « The minimal size of our sample was estimated at 150 patients, with a study power 1- \( \beta = 0.8 \) and \( \beta = 0.05 \), assuming a 1.5-fold difference in the less frequent allele between controls and patients. »

Q: « Was HWE tested in the controls or cases? How the test was performed? What are are p-values? It was tested in patients, comparing the expected genotypes with the observed values, using a two-tailed chi-square (one degree of freedom for two alleles). The P-values are given in result section, page 7.

Q: Quality of English. It was considered acceptable by the two other reviewers and has been revised by a colleague from US.