Author's response to reviews

Title: MDR1 polymorphisms are associated with inflammatory bowel disease in a cohort of Croatian IBD patients

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Author's response to reviews: see over
To: BMC Gastroenterology

Zagreb, 14th of December, 2012

Dear Madam/Sir,

We would like to resubmit our manuscript, entitled: "**MDR1 polymorphisms are associated with inflammatory bowel disease in a cohort of Croatian IBD patients**", for publication in BMC Gastroenterology.

We are grateful for the opportunity to resubmit our manuscript to BMC Gastroenterology. We have received the comments made by the reviewers and have amended our manuscript accordingly.

We also provide a point-by-point reply to each of the questions raised by the reviewers below.

We feel that the remarks have greatly improved the quality of our manuscript and hope that it will be acceptable in its current form for publication in the BMC Gastroenterology.

We are looking forward to hearing from you soon.

Most respectfully,

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RESPONSE TO REVIEWERS

Reviewer No 1:
Reviewer: Manuela Neuman
The reviewer found no serious deficiencies or major compulsory revisions.

Reviewer No 2:
Reviewer: Devendra K Amre
1. As the frequency of the A allele of 2677 was low any related analysis should be excluded from Table 3.
   - Manuscript was amended accordingly.

2. Given the limited sample size, the genotype-phenotype analysis should be excluded, notwithstanding the somewhat negative associations between perianal disease in CD. These analysis only enhance the issue of multiple comparisons and encourage misleading interpretation.
   - Genotype-phenotype analysis is excluded from the manuscript.

3. The authors indicate that the T allele frequency of 2677 in their controls was among the lowest (35.8% in the text and 35.2% in Table 3..discrepancy probably relates to the fact that 35.8% is after exclusion of individual with the A allele..need to confirm).
   - Confirmed. The discrepancy in T allele frequency is amended in the manuscript.

It would be important to know the source of their controls
(no details currently provided) and how these frequencies relate to ethnically similar populations. For example, are the Croats ethnically close to the Hungarians or Slovenians? If so, I believe there have been studies carried out in these populations for MDR1 and IBD (The Potocnik et al, study for example) and a comparable allele freqs within these populations, would provide greater assurance on the representativeness of the current study's control population.

- Unrelated healthy volunteers formed the control group. All volunteers prior to inclusion were specifically questioned regarding IBD symptoms and regarding symptoms of other immune mediated diseases (rheumathologic conditions, psoriasis). Methods part of the manuscript was updated to better reflect this.

- Croats are not ethnically close to Hungarians. In fact, two nations have a completely different background and the Hungarian language is by no means similar to Croatian. Slovenians, on the other hand, are a Slavic nation, and as such ethnically close to Croatians. Of note, the allele frequency of 2677T allele in Slovenian control group was 40% which, although higher than in our control group, is one of the lowest reported frequencies. The statement regarding this was added in the discussion.

Minor comments

1. The significant p-values can be consistently highlighted in bold in the tables.

   - Manuscript was amended accordingly.

2. The authors can consider summarizing the findings of previous studies relating to the 2 MDR1 SNPs in a Table to allow comparability.

   - The table was added in the manuscript.

**Reviewer No 3:**

Reviewer: Alfreda Krupoves
1. The process of controls selection and sample collection should be described, as well as the possibility of genotyping errors and what attempts were made aiming to prevent it. The period of time during which patients were followed or recruited should be provided.
   - *The methods part of the manuscript was ammended accordingly.*

2. This paper would benefit from discussion of study internal validity (to add in the “discussion” section). The possibility of bias should be discussed. Given small sample size and generally low magnitude (OR < 1.5) of associations in gene-disease studies, a power analysis should be made and presented in order to give an idea about the magnitude of association study was adequately powered to detect.
   - *The discussion part of the manuscript was ammended accordingly.*

3. The detected association with C3435T is a silent substitution, so its role in pathogenesis should be discussed, what is the biological plausibility supporting this association.
   - *The role of C3435T in pathogenesis is discussed in the discussion part of the manuscript.*

4. Authors state that some (not anticipated) associations were found between phenotype of extra intestinal manifestations, but these EM are not described in details. What was the definition of EM should be clarified.
   - *The genotype-phenotype associations were removed from the manuscript on the request of the reviewer No2. However, extraintestinal manifestations were defined as affection of joints (type I and type II arthropathy), eye (uveitis, episcleritis), skin (pyoderma gangrenosum, erythema nodosum) and liver (primary sclerosing cholangitis).*

Minor Essential Revisions:
1. A table for the presentation of results of associations between the disease characteristics and studied snps would be useful as well.
   - *Table was added in the manuscript.*