

September 16, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5317-review.doc).

**Title:** Susceptibility to ulcerative colitis in Hungarian patients determined by gene-gene interactions

**Authors:** Patricia Sarlos, Dalma Varszegi, Veronika Csongei, Lili Magyari, Luca Jaromi, Lajos Nagy, and Bela Meleg

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 5317

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

2 In Tables 2 and 4 smaller character sizes have been applied for optimal display (9 pt).

3 Revision has been made according to the suggestions of the reviewer:

**Answers to the Reviewer 00009530:**

1. *Data that would complete the analysis and also increase the interest of the paper are those addressing possible genotypic-phenotypic interactions. In any case, information on the different types of disease in patients enrolled should be provided.*

Unfortunately, data collection was insufficient for the majority of the patients due to the high number of the involved centers; detailed clinical information was available for only 67 (20.9%) UC patients. Since in the present study we focused on gene-gene interaction, therefore the analyses of genotypic-phenotypic correlation could be neglected.

2. *Also, some data regarding the enrollment of controls should be provided (volunteers? blood donors? patients affected by other diseases?)*

The control subjects were healthy blood donors and did not have any gastrointestinal or other autoimmune disorders. The origin of DNA samples was the central Biobank governed by the University of Pecs, as part of the National Biobank Network of Hungary ([www.biobank.hu](http://www.biobank.hu)).

3. *Page 1, line 20: analyses instead of analyzes.*

Typing mistake was corrected.

4. *Page 2, line 7: and IL23 (...) is repeated.*  
Duplicated items were deleted.
5. *Page 3, lines 11-13: differentiation, maintenance, coordination should be written without article... and other.*  
Corrected.
6. *The title is too generic, I believe that there should be a precise reference to the studied genes.*  
An alternative title was created, although the new title exceeds the given limit:  
'Interaction between interleukin-23 receptor gene and IBD5 locus in Hungarian ulcerative colitis patients'.

#### **Answers to the Reviewer 00646257:**

1. *Since multiple tests are involved, the authors need to consider multiple comparisons.*  
Correction for multiple comparisons related to gene-gene interactions has been missed in other published studies too (Roberts et al. (2007), American Journal of Gastroenterology; Kring et al. (2009), PLOS One; Infante et al. (2005), Journal of Neurological Sciences; Marrakchi et al. (2009), Inflammatory Research; Vural et al. (2009), Acta Neurologica Scandinavica; Wang et al. (2009) Journal of Clinical Immunology, Jaromi et al. (2010), Journal of Molecular Neuroscience). Our results lose their significance after correction for multiple comparisons (either Bonferroni correction or Benjamini-Hochberg procedure), if it is required, we can emphasize this in the results section discussing gene interaction data: 'Our results do not retain significant association after correcting for multiple comparisons'.
2. *I wonder if it might be necessary to try other interaction analysis such as multifactor dimensionality reduction (MDR) analysis.*  
MDR analysis is considered a nonparametric alternative to traditional statistical methods such as logistic regression, and generally used in studies analyzing large volumes of data and multiple gene-gene interactions. For that very reason the results of the present study did not seem to require verification by other statistical approaches.
3. *The authors need to present some replication results for their findings.*  
Relevant previous interaction analysis results were discussed in detail in the 'Discussion' section.

#### **Answers to the Reviewer 00073423:**

1. *Abstract 1. Part of the sentence " and IL23R (rs1004819, rs2201841)" replicated twice.*  
Replicated items were deleted.

2. *Methods: 2. There is no detailed phenotypic data and data for the sample collection (years, participating centers etc.).*

Sample collection started in 2003 in collaboration of the following participating Hungarian centers: 1st and 3rd Department of Internal Medicine, University of Pecs; Department of Medicine and Gastroenterology, Markusovszky Hospital, Szombathely; Department of Medicine and Gastroenterology, Rethy Pal Hospital, Bekescsaba; 2nd Department of Medicine, Semmelweis University, Budapest.

3. *Selection of SNPs under analysis has to be justified. Why study didn't include rs11209026 SNP in the IL23R region, as it is the most significant hit in the IL23R locus and it has been replicated in the number of GWAS and GWAS meta-analysis?*

SNPs were selected based on the susceptibility (not protective) effect and allele frequency values reported in recent publications. The *IL23R* rs1004819 and rs2201841 variants were proved to confer increased risk to IBD (CD and UC) pathogenesis. The primary aim of our study was to analyse susceptibility gene variants of the *IL23R* gene, therefore the rs2201841 and rs1004819 variants were selected and tested for the first time in Hungarian UC population. Besides the *IL23R* rs11209026 variant was found to be protective in Hungarian UC population (OR: 0.55; Lakatos et al. 2008, Digestive and Liver Disease), it represents very low allele frequency (2.68% in Hungarian UC; Lakatos et al. 2008, Digestive and Liver Disease).

4. *Has positive control (with already known genotypes for the tested SNPs) been included in the PCR-RFLP? Have the primers used been already verified by sequencing?*

The specificity of PCR primers and the genotype of positive controls used for the PCR-RFLP method were verified by sequencing before genotyping.

5. *Results: Stratification is the method helping to assess the presence of systematic differences in allele frequencies between subpopulations, i.e. in your case between different IBD5 loci SNP carriers and non-carriers and this doesn't show possible gene-gene interaction. Stratification is mainly applied for SNPs in very complex regions with high LD in order to help to verify the other signals. In your analysis the IBD5 loci wasn't associated with the disease and by stratifying (in other words "eliminating" the effect) by IBD5 loci just showed the same results as your single SNP association analysis, meaning your results were driven by the association of UC with IL23R, but not with co-association with IBD5. I would recommend to remove this analysis from the study, as it doesn't give any new knowledge.*

However stratification is generally applied in studies observing complex gene regions with high LD, it is an often used method for confirming the independence of gene effects irrespective of LD features. Among others Hampe et al. (2007, Nature Genetics) used stratification for testing the independence of CD susceptibility genes. Latter study revealed that the *ATG16L1* rs2241880 variant is a risk factor for CD pathogenesis even in the absence of high-risk *CARD15* mutations. Roberts et al (2007, American Journal of Gastroenterology) also used stratification for the analyses of gene-

interactions between *IL23R*, *ATG16L1* and *CARD15* variants. Although in our study the *IBD5* variants did not show significant associations with UC risk, whereas the *IL23R* variants did; the stratification analyses suggested that the *IL23R* effect was not totally independent of the *IBD5* genotypes (or other unknown factors correlated to the *IBD5* locus), since the *IL23R* rs1004819 variant did not show association with the disease on wild type *IBD5* background. In our opinion these results representing the 'modifier' effect of *IBD5* (or other unknown factors correlated to the *IBD5* locus) supplement the results of the single gene analyses.

**Answers to the Reviewer 00033055:**

No major or minor comments have been made by the reviewer.

4 References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Patricia Sarlos MD  
1st Dept. of Internal Medicine  
University of Pecs  
Pecs, Rakoczi 2.  
H-7623, Hungary  
Telephone/Fax (+36)-72-536-427  
E-mail: [sarlosp@gmail.com](mailto:sarlosp@gmail.com)