Author's response to reviews

Title: CD209 in inflammatory bowel disease: a case-control study in the Spanish population

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Author's response to reviews: see over
CD209 IN INFLAMMATORY BOWEL DISEASE: A CASE-CONTROL STUDY IN THE SPANISH POPULATION

Dear editor,

We have read carefully the reviewers’ comments and modified our manuscript accordingly to them. In the following point by point reply we detail the changes performed (underlined in the new version of the manuscript).

Reviewer Tooru Shimosegawa:

MAJOR POINTS

1. The reviewer suggests that we must be very cautious in interpreting our results because of the low number of DR3-positive ulcerative colitis (UC) patients, the Hardy-Weinberg analysis resulting from DR3-positive individuals (p=0.051) and the weak significance observed (p=0.04).

We would like to point out that there are very few rs4804803_GG homozygous individuals in our UC DR3-positive patients and consequently statistical power to detect a significant difference between GG homozygous and heterozygous individuals is very low. Then, although we observed a significant increase of CD209 rs4804803_G carriers, our study is not powered enough to distinguish between the susceptibility caused by heterozygous or homozygous. Independently of which genotype(s) could cause susceptibility, that association would alter Hardy-Weinberg proportions. In fact, Hardy-Weinberg departures have been proposed as indicative of the presence of a susceptibility factor on the disease group. Thus, deviation from Hardy-Weinberg proportions could not be indicating a bias in our data as suggested by the referee but could be due to the role of CD209 in that group of patients. It must be also underlined that the UC DR3-positive group is low due to the protective role of DR3 in the Spanish population. Nevertheless, the weak significance obtained in the UC DR3-positive vs. DR3-negative requires further studies and a sentence has been added in the abstract and discussion.

We agree that a p-value 0.04 is borderline significance, but would it be preferable to consider it a negative result? We prefer to deem it as an interesting result but needing further confirmation.

MINOR POINTS
1. Ulcerative colitis patients with proctitis have been included in the group of patients showing left-sided disease. This has been included in the Table 1.

**DISCRETIONARY REVISIONS**

As the referee points out, case-control study stratifying by clinical characteristics would be very interesting but we consider that stratification by genetic factors (HLA-DR3 in UC patients and CARD15 in CD patients) probably offer more homogenous groups of patients than when clinical patterns are considered, which are sometimes affected by the subjectivity of the clinician and by duration of disease. Moreover, due to power issues and to avoid multiple testing without “a priori” hypotheses, the aim of our work was limited to the study of the CD209 influence on susceptibility to IBD and we believe that the study of clinical characteristics could be detrimental to overall statistical power. However, following the reviewer’s suggestion, we studied the influence of CD209 on IBD patients after stratifying by clinical forms (behaviour and location in Crohn’s disease, and extension in ulcerative colitis). A significant association emerged only when CD patients were stratified by behaviour (58% of B2 CD patients carry the CD209_G allele vs. 41% of B2+B3 CD patients, p=0.006 OR=1.99 95% CI 1.18-3.35).

However, it is known that behaviour of CD changes over the course of the disease, and its use in phenotype-genotype analysis has been discouraged (E Louis et al., 2001. Behaviour of Crohn’s disease according to the Vienna classification: changing pattern over the course of the disease. Gut 49:777). Although this kind of analysis could be performed considering disease duration, we have no data from all patients about the duration of disease (data shown in Table 1 represent the values corresponding to a high number of the IBD patients studied but not to all of them; we understand that mean values from all patients will be essentially the same.

**Reviewer Hitoshi Asakura:**

**MAJOR POINTS**

As we answered to the other reviewer, case-control study after stratifying by clinical characteristics would be very interesting but we considered that stratification by genetic factors (HLA-DR3 in UC patients and CARD15 in CD patients) probably offer more homogenous groups of patients than when clinical patterns are considered, which are sometimes affected by the subjectivity of the clinician and by duration of disease.
Moreover, because due to power issues and to avoid multiple testing without “a priori” hypotheses, the aim of our work was limited to the study of the $CD209$ influence on susceptibility to IBD, we think that the study of clinical characteristics could be detrimental to overall statistical power. However, following reviewer’s suggestions, we studied the influence of $CD209$ on IBD patients after stratifying by clinical forms (behaviour and location in Crohn’s disease, and extension in ulcerative colitis). A significant association emerged only when CD patients were stratified by behaviour (58% of B2 CD patients carry the $CD209_G$ allele vs. 41% of B2+B3 CD patients, $p=0.006$ OR=1.99 95% CI 1.18-3.35). However, it is known that behaviour of CD changes over the course of the disease, and its use in phenotype-genotype analysis has been discouraged (E Louis et al., 2001. Behaviour of Crohn’s disease according to the Vienna classification: changing pattern over the course of the disease. Gut 49:777). Although this kind of analysis could be performed considering disease duration, we have no data from all patients about the duration of disease (data shown in Table 1 of the new manuscript represent the values corresponding to a high number of the IBD patients studied but not to all of them, we understand that mean values from all patients will be essentially the same). For this same reason it is not possible to perform stratified analysis considering age at onset, we have not enough data. Nevertheless, the lack of association after stratification by location is now included in the new version of the manuscript.

MINOR POINTS

A foot note has been added to Table 1 to indicate that Crohn’s disease patients were classified following Vienna classification.

Yours sincerely,

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