Reviewer's report

Title: The CTLA4 variants may interact with the IL23R- and NOD2-conferred risk in development of Crohn's disease

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Reviewer: Kazuhiro Tsukamoto

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COMMENTS:

1. The characteristics, e.g., age and gender, of 482 healthy control subjects were not described in this manuscript. Thus, authors should indicate the characteristics of CD patients and control subjects in the same Table 1. In addition, the authors should compare the characteristics between CD patients and control subjects before the genetic analysis, because it is important to evaluate environmental backgrounds between patients and control subjects on an association study by case-control study.

2. Why did the authors select rs736611 SNP, rs231775 SNP (+49A/G), rs3087243 (CT60), rs1427676 SNP, rs11571302 SNP (JO31), and rs11571297 SNP (JO27-1) in CTLA4 as genotyped tag SNPs for the genetic analysis? There are at least 80 SNPs within this gene in the database on the International HapMap Web site. In particular, JO31 and JO27-1 SNPs in the 3' untranslated region of CTLA4 are rare polymorphic markers and their frequencies are not described in data on the International HapMap. Thus, the authors should explain how to select six genotyped tag SNPs from ~80 SNPs in CTLA4 in this study in the Methods section.

3. Although the frequencies of CT60, JO31, and JO27-1 SNPs in the Czech population showed the weakly significant association with those of NOD2 or IL23R, which are recognized as CD-susceptibility genes, I cannot agree on how to construct the haplotypes composed of CT60, JO31, and JO27-1 in CTLA4, because all genotyped SNPs, including CT60, JO31, and JO27-1, in this study indicated the lack of susceptibility to the onset and development of CD. Moreover, the authors did not evaluate the polymorphisms of CTLA4, NOD2, and IL23R as the independent genetic factors, which contribute to the onset and/or development of CD, for one another by multivariate regression analysis.

4. It is very difficult to understand the results of the haplotype analysis in CTLA4 in Figures 2 and 3. Moreover, these figures do not make a sense. Thus, the authors should describe the numbers, frequencies, and distributions of both haplotypes and diplotypes of CTLA4 in a new Table after removal of Figures 2 and 3. In particular, the number of the CD patients possessing the "ATG" minor haplotype as well as the number of the control subjects should be described in this study. From the point of view of genetic analysis, diplotype analysis is more
important for association study in comparison to haplotype analysis, because diplotypes reveal the individuals, whereas haplotypes show the chromosomes. Of course, the disorders including CD imply the individuals as phenotypes.

5. The "ATG" minor haplotype of CTLA4 can be not associated with the ileal-only involvement (L1) of CD in the Results section, because statistical analysis revealed that 95% confidence interval (CI) was 0.462 - 1.03, implying no statistically significant difference. Thus, the result and its evaluation are wrong.

6. The references following below should be cited in this study.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.