Answers to Reviewers for Manuscript Code: 37600

REVIEWER 1 (Code: 02486710)

Reviewer Comments: • A novel paper evaluating the SNPs in both PTPN2/22 together along with correlation with gene expression and MAP susceptibility in CD. • Introduction is too long. It includes too much information which looks confusing. • Have you evaluated severity of CD on the outcomes? Does extend of the CD effect outcomes?

Authors Comments: Thank you for your comments. We have revised the introduction to be briefer and less confusing. We have addressed the severity of the CD patients in the manuscript as well in the methods section under the heading Clinical Samples. We are only given the information that the CD patients have moderate to severe symptoms and we do not know the extent of the CD symptoms from the patients. Please see revisions highlighted and commented as Reviewer #1 (02486710) changes.

REVIEWER 2 (Code: 02440884)

Reviewer Comments: In the experimental study the role of PTPN2/22 polymorphisms in the negative regulation of the immune response in CD patients and their susceptibility to mycobacteria is addressed. The nice study is well performed and illustrated. Comments 1. A characterization of the blood T-cells could be helpful. Is there any FACS data available? 2. The authors describe an impaired T-cell proliferation in blood. Evidence for an impaired mucosal immune system is not fully given. This point should be addressed in the Discussion section.

Authors Comments: Thank you for your comments. We did not do FACS studies because we wanted to look at the whole T-cell population together instead of segregating out the different subpopulations. Since *PTPN2/22* is found in all T-cell types, we believe it was not necessary to separate out the T-cells by groups. Although we only looked into T-cells from blood and not parts of the mucosal intestinal tissues in the CD patients, we believe that the T-cells will react the same, regardless of the origin. We have added this statement in the discussion as requested. Please see revisions highlighted and commented as Reviewer #2 (02440884) changes.

REVIEWER 3 (No Code Given, From Email)

Reviewer Comments: The authors provide several related experiments assessing the role of single nucleotide polymorphisms (SNPs) in PTPN2/22 in interferon gamma gene, MAP DNA and T cell activity for subjects with Crohn's disease and healthy subjects. The authors found that heterozygous and minor alleles in PTPN2:rs478582 and PTPN22:rs2476601 were more common in Crohn's disease and associated with IFN gamma expression and MAP bacteremia and Crohn's subjects had greater T cell activity following treatment with MAP PPD-like and PHA. The authors conclude that the SNPs alter the immune response and lead to MAP susceptibility. Overall I think this is an interesting paper with relevant findings but there are several areas that need to be addressed:

1. Further details are required about the control population and their demographics to confirm that they are similar. As suggested in Figure 1 there are multiple predisposing factors that may increase the risk of development of Crohn's disease and affect immune function, thus information on the healthy subjects and how they were selected is important in this study.

2. With the gene associations, what has been shown is a correlation between SNPs in PTPN2/22 an increase in IFN gamma and a higher proportion with MAP DNA and increase T cell activity. The authors conclude that the genetic differences are the cause of the immune changes although this has not been entirely substantiated. To suggest that it is these SNPs that have caused the abnormality, other potential confounder genes need to be evaluated and accounted for, such as IL-10. Do the authors have any evidence to support this?

3. The authors have not provided any potential limitations with the techniques used in their discussion, please elaborate.

4. A significant p value by convention is <0.05 while the authors have used < or = 0.05. Is there a reason for this? If not this should be changed.

Authors Comments: Thank you for your comments. Please see revisions of manuscript that are highlighted and commented with Reviewer #3 (From Email) for changes. Please see below for each answer for the comments you have suggested:

1. During the recruitment of the control populations' samples, we required not only a written consent, but a survey as well in determining if the person in question had any type of medical abnormality (CD, T1D, RA, and "other diseases" were the choices). Healthy control patients that we recruited did not have any type of medical condition to

the best of their knowledge. We have added this statement in the Materials and Methods section under the Clinical Samples headline.

2. Although we only examined the negative regulators PTPN2/22 in our samples along with the production of IFN-γ, you are correct that we need to look into other genes that influence IFN-γ production. However, since CD and the other autoimmune inflammatory disorders we talk about in the manuscript are T-cell mediated disorders, we focused on T-cell regulation mostly. Since PTPN2/22 are found in all T-cells and are negative regulators of said T-cells, this intrigued us more than looking into the other factors of IFN-γ production. Since PTPN2/22 will ultimately regulate IFN-γ produced from T-cells, we believed that looking into these regulators was sufficient. The next steps in this project would be to look into the genes that help promote IFN-γ production (cytokines such as TNF-α and IL-12 for example) and that help inhibits IFN-γ production (cytokines such as IL-6 and IL-10 for example) and their effect on subjects with SNPS in *PTPN2/22*. Also, the next step would be looking into how other IFN-γ producers (macrophages or NK cells for example) could affect CD subjects with T-cell dysregulation. We have added this statement in the discussion section.

3. Limitations for each technique is as follows and added to manuscript:

a. ***PTPN2/22* genotyping**: Since we used a very diverse population (no restriction on race, place of origin, age, or gender), alterations in the allele distribution of SNPs in *PTPN2/22* could occur. SNPs overall have been shown to fluctuate between different races and countries of origin, thus this could possibly make a difference in determining what *PTPN2/22* SNPs are considered significant. Further isolated population studies on *PTPN2/22* SNPs in CD patients are needed.

b. ***PTPN2/22* and *IFN-γ* gene expression**: Please see above in authors comment #2 on the limitation of examining only *PTPN2/22* effect on *IFN-γ* expression.

c. **Detection of MAP *IS900* DNA:** MAP *IS900* DNA can be detected in a subjects' sample regardless of the bacteria being alive or dead, thus is difficult to determine if the subject at time of blood drawn had live MAP or dead MAP. To alleviate this limitation, culturing of blood from the subject is necessary to determine if the subject has live MAP or dead MAP from a previous infection.

e. **T-cell proliferation**: All T-cells were isolated out and were not divided by subpopulations, thus determination of overall T-cell activity after proliferation is examined. Doing this technique this way limits the ability to examine individual T-cell populations, thus in future experiments, T-cell populations will be sorted out before being proliferated. This will determine which T-cell population is more active in subjects with SNPs in *PTPN2/22*.

4. All P-values that say "≤" have been changed to "<" in the revised manuscript since all significant P-values were lower than 0.05.