Author's response to reviews

**Title:** Linkage Analysis of HLA and Candidate Genes for Celiac Disease in a North American Family-Based Study

**Authors:**

Susan L Neuhausen (susan@genepi.med.utah.edu)
Michael Feolo (feolo@ncbi.nlm.nih.gov)
James Farnham (jfarnham@genepi.med.utah.edu)
Linda Book (pclbook@ihc.com)
John J Zone (zone@ultraderm.med.utah.edu)

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Re: Linkage Analysis of HLA and Candidate Genes for Celiac Disease in a North American Family-Based Study

Dear Editor:

We have carefully reviewed the comments from the two reviewers and have edited the manuscript accordingly. Our response to each of the reviewer's comments are listed below.

Reviewer Dr. Greco

Response to general comments:

a. "Only 65 families, parents apparently not available." The parents were available. I have changed the methods to clarify this. (The 65 in the text was a typographical error and should be 62.) Sixty-two families should provide sufficient power for investigating candidate genes. The candidate gene tests have a higher prior probability of linkage and generally a higher density of markers than the genome-wide search, therefore, a smaller resource is needed.

b. "They explore only a small set of markers". We explored only a small set of markers as we were specifically looking at candidate regions while we were accruing our resource.

c. "The conclusions are more a show of Type II error in the study design than of negative results." Power is an issue in all studies. However, we were able to generate a two-point HLOD of 3.1 and a multipoint HLOD of 5.0 at HLA, showing that this resource does have sufficient power to detect significant evidence for linkage. Thus, even though other genes for celiac disease likely account for a lower proportion of sibling risk than HLA, we likely have reasonable power to detect at least nominal evidence for linkage at another locus of at least moderate effect.

d. "There are by now 5 genome screening studies..." We have now included information on previous studies, including genomic search studies, in a sentence in the introduction and in a more complete summary in the discussion.

Response to specific comments:
1. "Introduction: redundant on celiac disease description, but no mention on previous studies relevant to the aims." Dr. Greco has great expertise in celiac disease, so the introduction may appear too detailed. However, as this is a genetics journal with a general readership, we thought it was important to educate them on what celiac disease is. We have added the previous studies to the introduction and discuss them in more detail in the discussion.

2. "Methods should be shortened by 2/3." We have shortened the methods.

3. "The choice of IDDM markers is outdated, since many have not been replicated." We chose the set of candidates based on genes with related functions or to other loci implicated in autoimmune disease such as IDDM. Some have not been replicated for diabetes, but they were reasonable candidates based on function, so they were examined.

4. "The results do not support with sufficient power a completely negative statement." We have said that we did not find evidence for linkage in our families, which is a true statement. We do not state that we have excluded linkage, which would be a completely negative statement. Based on our HLODs at HLA, this was a powerful enough resource to identify linkage for a candidate gene or region.

Response to Dr. Partenen.

1. "as only one or a few markers at best were typed at some of these candidate regions, can this low level of information cause some of the negative results? " Dr. Partenen is correct that we may not have detected linkage because of the choice of marker when only 1 or 2 were examined. Therefore, we have added the sentence "For those regions where we examined only 1 marker, it may be that one marker was insufficient in order to detect linkage even if it existed' to the second paragraph in the discussion.

2. "the HLA DQ alleles were not typed; as virtually...". We do have HLA typing on the affecteds and have included a footnote to Table 1 and a reference to the methodology in the Methods section. Ninety percent of the affecteds have the high risk DQ2 heterodimer, with the majority of the remainder being DQ8. For those affecteds diagnosed by serology with no biopsy, 94% had the DQ2 genotype (45/48) and the remaining 3 affecteds were DQ8. Thus, those diagnosed by serology have the same HLA type as those diagnosed by biopsy.

3. "only 1/2 of families showed linkage to HLA... this is surprising". The alpha for the HLOD is only a rough approximation and thus is not exact. The estimate of the proportion of linked families was approximately 65%, which would be expected based on estimates that HLA accounts for less than half of the sibling risk. Secondly, as Dr. Partenen points out, affected individuals can be DR5/DR7 such that the overall linkage evidence in those families would be negative.

4. "A minor point in Introduction". We have changed the DQA1 0501 DQB1 0201 to DQA1*05 and DQB1*02. "

In summary, we have shortened the Methods section, as well as clarified which family members were genotyped. In the introduction and discussion, we have added text relating to the previous genomic search results that have been in the literature. In the discussion, we have also added a sentence stating that we may not have had sufficient coverage to find linkage at a particular region. We have added data pertaining to the HLA high-risk genotypes in affecteds diagnosed by biopsy or serology.