Author’s response to reviews

Title: HLA-A and -B alleles and haplotypes in hemochromatosis probands with HFE C282Y homozygosity in central Alabama

Authors:

James C. Barton (ironmd@dnailmail.com)
Ronald T. Acton (acton@uab.edu)

Version: 2 Date: 2 Oct 2002

PDF covering letter
re: revision of original manuscript HLA-A and -B alleles and haplotypes in hemochromatosis probands with HFE C282Y homozygosity in central Alabama by J.C. Barton and R.T. Acton

Dear Dr. Goodlee and Colleagues:

Thanks you very much for the opportunity to revise our manuscript according to the suggestions and criticism of the Reviewers. We chose to do so for improvement of the paper, although this was not required by the journal. The pertinent comments of the Reviewers are shown below in italics, and our suggested responses are indicated in standard font.

**Reviewer 1 (Dr. DeSousa)**

“. . .The present study uncovers new associations of the mutation with haplotypes HLA A2B39, A3B13, and A3B44 not previously reported. I could not find a reference to the frequencies upon which this conclusion is based; something the authors can easily add strengthening the validity of the statistical analysis (page 10).”

These observations represent some of the novel findings of the present study, and therefore cannot be referenced otherwise. As we stated in the Discussion: “The absolute frequencies of haplotypes A*02-B*39, A*03-B*13 and A*03-B*44 (in addition to A*03-B*07 and A*03-B*14 haplotypes) were significantly greater in Alabama probands than in control subjects. A significantly increased frequency of the haplotype A*03-B*44 was observed in the present Alabama hemochromatosis probands, whereas a relative increase in this haplotype was detected in hemochromatosis cohorts in Sweden, Brittany, and Utah only after “correction” of the data for the preponderance of other haplotypes [2, 8, 19]. Haplotypes A*02-B*39 and A*03-B*13 have not been reported to occur with increased frequency in any hemochromatosis cohort from locations other than Alabama.” Accordingly, we believe that no change in the manuscript is indicated to clarify this. Please see insertion of haplotype frequency table below, and addition of several haplotypes to the above list as described in detail below.

*In the Materials section, the authors also overlooked to mention the ethnic origin of the probands although it becomes apparent later that they are white.*
In the first paragraph of the Subjects and Methods, Selection of Subjects, Hemochromatosis Probands, we indicated that all probands were white and all were adults. To clarify this point, we have inserted this sentence in the same paragraph: “All probands in the present study were white adults (> 18 years of age).” Minor changes were made to accommodate this revision.

*On flaw that perhaps cannot be overcome is the use of different methods for HLA typing in probands (DNA based) and controls (serology).*

We inserted the following sentence in the Laboratory Methods, second paragraph: “Because the levels of resolution of the DNA-based and serological typing methods we used are similar, alleles detected by these respective methods provide concordant allele assignments, with the exception of B*70 and B*72 that were not detected by serological methods.” A related statement was inserted in first paragraph of the HLA-A and -B Haplotypes section of the Results and in the footnote of Table 3 (revised manuscript). A related statement was omitted from the first paragraph of the HLA-A and -B Haplotypes section of the Results.

**Reviewer 2 (Dr. Porto)**

*Table 4 is not self explanatory. It is not indicated the difference between x and [x]. It is presumed from the text that is ‘relative increase’*.

We previously indicated the difference in x and [x] in the footnote to the Table. To emphasize this for the reader, we have changed the table footnote such that this sentence appears first: “Entries that correspond to absolute data are displayed without brackets; “corrected” data are displayed in brackets.”

*Table 4 would be more informative is the actual frequencies were indicated. This is specially the case for A2B39, A3B44, and A3B13 in central Alabama, which are shown to be significantly increased but the frequencies are not indicated anywhere in the text.*

The Reviewer is correct; we inadvertently failed to state the frequencies for these haplotypes. Accordingly, we have inserted a table of HLA haplotypes for all hemochromatosis probands and control subjects that becomes Table 3 in the revised manuscript (please see). This permits readers to reference our work for any and all haplotype data of interest.

*Showing haplotype frequencies would help to interpret Table 3, since the expected frequencies of two-haplotype matches in this patient population could be estimated (for this purpose it would also be valuable to indicate the frequencies of A2B60, A3B57, A3B62, A28B44, A29B44, and A3B47).*

In our response to the previous criticism, we inserted a new table of haplotype data for hemochromatosis probands and control subjects in this study. The new table (now Table 3) will permit readers to compute predicted frequencies of haplotype combinations as they desire.
In inserting this new Table, we re-computed frequencies and values of p for all data. In doing so, we discovered that a computer we used originally had a corrupted statistical software file. Accordingly, we re-analyzed all data for the paper on two additional computers. No change in the major observations of the paper was indicated. However, we did detect several other haplotypes that occurred with significantly greater frequency in probands than in control subjects. In addition to A*03-B*07 and A*03-B*14, we observed that the haplotypes A*01-B*60, A*02-B*39, A*02-B*62, A*03-B*13, A*03-B*15, A*03-B*27, A*03-B*35, A*03-B*44, A*03-B*47, and A*03-B*57 were also significantly more frequent in Alabama probands. Only three of these haplotypes had been determined to be increased in the present cohort at the time of our original submission. These added findings and corresponding notations are indicated in appropriate areas of the text, including Abstract, Results, Statistics, Discussion, and Conclusions.

In the third column heading of the original Table 3 (now Table 4). HLA Haploidentical Alabama Hemochromatosis Probands, we have inserted the word “Observed” to indicate that these data are not “predicted.”

Since it is referred in the Discussion the possibility that differences in the number of associated haplotypes may be explained by differences in the numbers of patients analyzed it would be helpful to indicate on Table 4 the number of haplotypes (n) analyzed in each region.

This sentence was added to the footnote of the Table: “The respective numbers of hemochromatosis and control haplotypes analyzed in these studies were: Germany 76, 1784; Denmark 39, 1791; Sweden 100, 190; Brittany 609, 475; Portugal 16, 203; Italy 42, 5638; Australia 98, 63; Utah 345, 835; and Alabama 118, 1210.”

Minor changes were made to accommodate these revisions and to update the Reference section. Altogether, we believe that the paper has been significantly improved. Thanks in advance for your further review of our work.

Yours truly,

James C. Barton, M.D.

cc: Ronald T. Acton, M.D.