Author's response to reviews

Title: HLA HAPLOTYPES ASSOCIATED WITH HEMOCHROMATOSIS MUTATIONS IN THE SPANISH POPULATION

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Author's response to reviews: see over
Dear Editor,

enclosed please find the second version of the paper entitled: “HLA HAPLOTYPES ASSOCIATED WITH HEMOCHROMATOSIS MUTATIONS IN THE SPANISH POPULATION” by Pacho et al. (Manuscript ID 1402390333713256).

Thank you for your help,
Best regards

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POINT-BY-POINT DESCRIPTION OF THE CHANGES MADE

Answers to reviewer 1 (Ronald Acton)

Question
Although the authors indicated that in the revised manuscript HFE was in italics this has not been done.HFE is not in italics throughout the manuscript.

Answer
It has been done.
Question
In the background section first paragraph lines 11 and 12 amino acid should be two words not as one word (aminoacid). In line 13 of this paragraph, "the risk of disease in heterozygous", ahould be “risk of disease in persons heterozygous”.

Answer
It has been changed.

Question
Under methods first paragraph the authors indicate that, "a presumptive diagnosis of hemochromatosis using an elevated saturation criterion". The diagnosis of hemochromatosis is usually based on elevated transferrin saturation greater than 60% on at least 2 occasions in the absence of other known causes of elevated transferrin saturation, or other clinical criteria (Witte, DL, Clinica Chimica Acta 245:139-200, 1996).

Answer
“using an elevated saturation criterion” has been deleted.

Question
The title of Table 1 would be clearer if, "the C282Y group" was changed to the C282Y homozygous group. In addition the Table columns labelled, "C282Y Haplotypes" would be clearer if changed to C282Y Homozygotes.

Answer
It has been changed.

Question
Under the results section, first paragraph, line 5, change, "haplotypes in comparison" to haplotypes in the homozygous group in comparison.

Answer
It has been changed.

Question
Table 3 is out of order and should be Table 2. As presented now the authors jump from data on HLA antigens in the C282Y homozygous group to haplotypes and then back to data on HLA antigens in the H63D homozygous group.

Answer
As the reviewer suggested in the first revision, the two tables have been renumbered to be presented in the same order as data are discussed.
Question

The title in the present table 3 should be changed to ...in the H63D homozygous group... and the columns from H63D Haplotypes to H63D Homozygotes.

Answer

It has been changed.

Question

In the second paragraph under the results, line 1, the authors refer to H63D carriers. However, they state in the methods section that the subjects are H63D homozygous. This need to be clarified.

Answer

“carriers” has been changed to: “homozygous”.

Question

Under the Discussion section, second paragraph, line 2, the following sentence is confusing. “This finding supports the founder hypothesis of Simon et al [5]: the ancestral haplotype where the C282Y mutation occurred was HLA-A3/B7.....” The sentence might be clearer if changed to: ...where the C282Y mutation occurred on the ancestral haplotype HLA-A3/B7...

Answer

It has been changed.

Answers to reviewer 3 (Graca Porto)

Question

1.I understand that the authors have used blood samples which have been probably referred for diagnosis (probands?) but from whom they do not have much clinical information. For the purpose of this study, whose objective is to test a large sample of homozygous for the mutations, it is not essential to have a full clinical description, however, a clear definition of the sample is needed. I would suggest to change this paragraph as follows: “A total of 100 unrelated individuals homozygous for the C282Y mutation, 138 unrelated individuals homozygous for the H63D mutation and 17 individuals heterozygous for the S65C mutation were selected for this study. These were subjects in whom HFE genotyping has been previously performed on a medical care basis because of a presumptive diagnosis of hemochromatosis. In addition, 1113 unrelated, apparently healthy subjects were used as controls for the study. HFE genotyping was performed in 230 individuals from the control group.” (cut this phrase from the next section).

Answer

It has been changed.

Question

At this point I am confused. The authors refer that they genotyped 230 controls. In the results section they refer to 230 individuals without HFE mutations. Were all the genotyped subjects wild type? This is not possible given the frequency of the mutations in the normal Caucasian population. Please clarify.
**Answer**

The reviewer is right. “HFE genotyping was performed in 230 individuals from the control group” has been changed to “In addition, HLA typing was performed in 230 individuals whom HFE genotyping was negative for the three mutations”.

**Question**

2. The first paragraph of the discussion on: H63D and HLA does not provide a correct interpretation of the cited papers. I would suggest, instead of “and between HLA-B44 and the same clinical form”: “and a strong linkage disequilibrium between H63D and all A29 containing haplotypes assigned in a large population of normal Portuguese families(22)”. I think this point is relevant. First because reference 22 is not a study of individual associations but it is a study in 587 normal subjects whose haplotypes were assigned by family segregation and not by linkage disequilibrium. Secondly, as already pointed out by M.D. de Juan, this finding is confirmed with success with this study, and not contradicted. The major difference between the two studies is the significance of the association with the haplotype A29-B44, but this could be due to differences in the method of assigning haplotypes. In conclusion, I would also suggest modifying the discussion in this chapter on line 5 of the second paragraph. Instead of: “with lesser levels of significance” to substitute for: “confirming the results reported by Cardoso et al (22) in the normal Portuguese population.”

**Answer**

It has been changed.

**Final consideration**

The English language has been checked by a native speaker.