Reviewer's report

Title: HLA haplotypes associated to the hemochromatosis mutations in the Spanish population

Version: 1 Date: 23 June 2004

Reviewer: Graca Porto

Reviewer's report:

General

This paper addresses the question of the HLA antigens and haplotypes associated with the three more common HFE mutations, C282Y, H63D and S65C in a sample of the Spanish population. Based on the results presented the authors confirm the linkage disequilibria between the C282Y and H63D HFE mutations respectively with the HLA A3 and A29 alleles, as previously described, and report a new association of the S65C HFE mutation with the HLA-A26 allele. Moreover, on the basis of an analysis of HLA haplotypes, they assign the haplotypes associated with the mutations, respectively A3/B7 with C282Y and A29/B44 with H63D, and speculate about the ancestral haplotypes carrying the HFE mutations in the population, and estimate the relative age of the mutations. While the study design is interesting and is intended to provide relevant genetic information about the origins of the HFE mutations in the Spanish population, there are major concerns about the lack of information on the methods' description which may seriously compromise the interpretation derived from the study (see below).

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

a) For allele associations.
To perform their study, the authors have selected a large population of individuals homozygous for the mutations (except for S65C given the low frequency of this mutation). In the Methods section, the description of individuals should be more complete. One may assume that the 100 unrelated individuals homozygous for the C282Y mutation are hemochromatosis patients but nothing is referred about the clinical status of the H63D homozygous, who are not included in the "unrelated normal subjects". Are they patients with iron overload? Are they taken from the normal population? Who are the controls? Were they HFE genotyped?

b) For haplotype associations
Another question in the Methods section is about the haplotype assignment. How were haplotypes defined? There is no reference to family segregation studies. This is quite relevant because if haplotypes are defined not by family segregation but on the basis of the assumed linkage disequilibria, there may be an important bias towards the results because we are facing a round argument, i.e., we conclude that there is a linkage to the haplotype that we “a priori” assume that is in linkage disequilibrium. Of course it is very plausible that the A29B44 is the ancestral haplotype but the fact is that, in terms of haplotype frequencies, in a previous study where H63D carrying haplotypes were defined in the Portuguese population on the basis of family segregation (reference 19 in the text) there were more H63D-A29 carrying haplotypes without B44 (60%) than H63D-A29-B44 haplotypes (40%), in contrast with the results presented here in a Spanish population where 65% of all H63D-A29 carrying haplotypes are also defined as B44. This point should be clarified and discussed if, indeed the haplotypes were assigned by linkage disequilibrium.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Discussion, H63D and HLA, second paragraph, line 3: do the authors really mean Danish population or Australian? (ref.18).

Discretionary Revisions (which the author can choose to ignore)


What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

None