Reviewer's report

Title: A new 500kb haplotype associated with high CD8+ T-lymphocyte numbers predicts a less severe expression of hereditary hemochromatosis

Version: 1 Date: 29 July 2008

Reviewer: K Sigvard Olsson

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The present authors have in a series of studies from Portugal, reported that the number of lymphocytes may predict the severity of iron loading in patients with hereditary hemochromatosis (ref 16-19,21-23, 26). These patients have a North European genetic background in common, being homozygous for the C282Y mutation. However the clinical expression in these carriers is very variable. Large genotype studies have shown that only a fraction of C282Y homozygotes will develop severe organ damage and also that most symptoms usually associated with hemochromatosis are not different from that in controls, with the exception of liver problems (ref 10,11). It is therefore of importance as the authors point out to identify factors that affect the penetrance of this disorder.

In the present study the authors report that they have identified such a modifying factor in a haplotype situated between HLA-A and HFE on chromosome 6, band 23.1. Those who inherits the AAT haplotype from both parents will develop a severe iron loading and low lymphocyte counts, while those who inherit the GGG haplotype will develop a “much less severe expression” and escape symptoms. They also will have high lymphocyte counts.

In the introduction the authors report that one additional study (ref 20) has supported their previous findings indicating an inverse relationship between lymphocyte counts and severity of iron loading. However, it should be noted that this relationship was significant only for those who had inherited a certain haplotype (HLA-A1-B8) and also that those with the most severe phenotype = cirrhosis, in this larger patient series, had lymphocyte counts not different from those without cirrhosis.

Study population:

From their previously reported patient series of 64 adult hemochromatosis patients of whom 22 were detected at family investigation (ref 26) they have now selected 56 patients, 45 probands and 11 family members for their study. They have also included 10 homozygous HH patients from Vancouver, Canada, because of “different ethnicity”, ignoring the view that they most probably are descendants to the same common north European ancestor as their own patients from Portugal.

Clinical characterization of subjects.

The patients were divided in two groups according to the presence or absence of
symptoms.
From the experience gained in the above mentioned studies (ref 10,11), this is hardly recommendable. A liver affection was the only symptom or sign that was more common in hemochromatosis subjects than in controls. What the authors wanted to study is the influence of genetic factors upon iron loading, not the development of symptoms!. The authors are well familiar with previous studies where a cut off value for a major iron overload has been set at serum ferritin of 1000µg/L or 5 grams of total body iron stores (TBIS) (ref 9).. This is because a severe liver damage (fibrosis, cirrhosis) is a rare complication below this cut off.
In the result section page 18 the authors report that all patients with severe iron overload = >5 g are homozygous for AAT. Why not use such a cut off value or another where the influence of age is considered?
Page 9 middle (95% confidence interval) check the figure 0.419!
Results
Table 1 shows data of 56 subjects, table 2 only 52?
Discussion:
The authors now claim that they have identified a new haplotype, GGT, that will predict a milder phenotypic expression in its carriers. But they have also identified another haplotype (AAT) that in homozygous form will result in severe iron overload.
These findings are indeed very interesting.
But which factor is the modifier? AAT or GGT? The authors do not discuss the possibility that their patients represent the tip of the iceberg. The presence of the AAT in homozygous form therefore may be the major modifier of the phenotype, rather than the GGT haplotype.
When the authors present “the global impact” of their findings in Figure 2, the GGT/GGT carrier seems deviating. However to most workers dealing with hemochromatosis this 39 year old male homozygote would be recognized as perfectly “normal” with his serum ferritin of 695 µg/L and iron stores of 2.1 g. I would not look for a modifier in this person.
The weakness of the study is that it includes so few mildly affected homozygotes, which constitutes the major fraction of hemochromatosis (Ref 10-11).
The statistics (Table 2) showing a difference between AAT/AAT and GGT/AAT is very weak. Serum Ferritin values were not different between the groups
Mildly affected homozygotes are often found among family members. In their study from 2006 (ref 26) 22 homozygous family members were included but only 11 is mentioned in the present study. Perhaps mildly affected subjects are present here?
The present study has to be extended to include also more mildly affected patients before firm conclusions can be made.
Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.