Author's response to reviews

Title: Impact of HFE genetic testing on clinical presentation of hereditary hemochromatosis: new epidemiological data

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Author's response to reviews:

Please find enclosed a revised version of our manuscript that we revised according to the reviewers' commentaries (Manuscript number: 1568666994748701 - New epidemiological data on hereditary hemochromatosis)

Answer to the reviewers

Reviewer 1: Diane C Tucker

The format of Methods section being at the end of the paper is recommended in the instructions to the authors.

Major compulsory revisions:

1.- The statistical tests were performed two-sided (difference in either direction, and not in only one direction). We mentioned that, in men, only two signs tended to be less frequently observed following the introduction of genetic testing, and not that they were significantly less frequently observed.

2.- As suggested by the reviewer, we shortened the discussion section. We reduced the length of the paragraphs on diagnosis strategies and on penetrance (page 8).
3.- We clarified what it means for the diagnosis of HH to be made in front of signs of fatigue in Result section (page 5). This means that the disease can be diagnosed earlier before non specific signs, such as an unexplained and persistent fatigue. This earlier detection is the result of a better medical education of physicians who can now more often suspect a diagnosis of HH before non specific signs. We mentioned this in the text (Results section - page 5).

Minor essential revisions: none.

Discretionary revisions: none.

Reviewer 2: Robert Britton

Major compulsory revisions:

We agree with the reviewer but the aim of our study, which is not a population screening study, was to compare the clinical data of patients diagnosed with HH before and after the development of HFE genetic testing. It is commonly admitted that the better iron parameter used in the strategy for HH diagnosis and reflecting body iron overload is serum transferrin saturation, which should be greater than 45%. Once the disease is suspected (before clinical signs), the second step of the diagnosis strategy is to determine the serum transferrin saturation concentration. If this value is elevated, molecular analysis of the main HFE mutations must be done to confirm the diagnosis. It is the reason why we included patients whose serum transferrin saturation was greater than 45%.

As suggested by the reviewer, we changed the manuscript title and entitled it Impact of HFE genetic testing on clinical presentation of hereditary hemochromatosis: new epidemiological data.

Minor essential revisions:

We performed all the minor essential revisions proposed by the reviewer. We thank him for his help.

Method section - Page 11 (Study population): We mentioned in the text that the last date of patient entry for inclusion in this study (December 31st 2003). For most of patients, HFE genetic testing is generally requested by the referring physician and performed before entry in the phlebotomy program.

Method section - Page 11 (Clinical questionnaire): The age of onset means the age of diagnosis. We changed this term throughout the manuscript. The clinical signs mentioned in the questionnaire were determined by a clinical exam performed at the entry in phlebotomy program (exam made by the physicians of the phlebotomy centre). We mentioned this in the text (Method section - Clinical questionnaire - page 11).
Discretionary revisions:

We also performed all the discretionary revisions proposed by the reviewer.

Reviewer 3: Claus Hellerbrand

Major compulsory revisions:

1.- The results were adjusted for alcohol consumption, as in a previous study, we showed that excessive alcohol consumption increased the disease expressivity among a cohort of patients homozygous for the C282Y mutation (Scotet et al. Am J Epidemiol 2003;158:129-134). Only a little proportion of patients had excessive alcohol consumption (8.0% - n=33). Obviously, this was not clear and we clarified this in the text.

As requested by the reviewer, we mentioned, in the Method section (Statistical analysis - page 12), how data concerning alcohol consumption were collected. The definition of excessive alcohol consumption was already presented (Statistical analysis - page 12). We reported, in the Result section (page 6), that 8.0% of the 415 patients declared having excessive alcohol consumption (n=33). This concerned 9 of the 91 patients diagnosed before 1996 (9.9%) and 24 of the 324 patients diagnosed after 1996 (7.4%).

2.- As we mentioned in the Method section (Clinical questionnaire - page 11), the questionnaire was completed at the time of diagnosis. The clinical signs reported are those associated at the time of diagnosis. It is not a study on outcome and survey of patients. When we speak from fatigue, it means an unexplained and persistent fatigue. We clarified this in the text (Results - page 5).

3.- It is clear that iron transferrin saturation is the critical parameter reflecting body iron overload. As we mentioned in the Result section (page 6), we did not observe decrease for transferrin saturation after 1996 as it was a parameter used for the inclusion of patients (concentration greater than 45%).

4.- The identification of the HFE gene has lead to a better understanding of the disease and has enabled a better medical education of physicians, who can now more often suspect a diagnosis of HH before non specific signs. This has conduced physicians to suspect more often HH (and not only anaemia) in front of an unexplained and persistent fatigue in women, in whom clinical manifestations appear later (around 50 y. versus 40 y. in men). This has lead to the diagnosis of a higher proportion of females at an earlier stage.

5.- As suggested by the reviewer, instead of distinguishing patients included before and after 1996 in their phlebotomy program, we used the subgroup diagnosis based on HFE genotyping or not. We made this change throughout the manuscript and in the table and figures.
Minor essential revisions:

1. Precise information on the methods of genotyping and on the origin of the genomic material used were described in detail in some of our previous articles, that we referenced in the text (Determination of HFE genotype - page 11/12). In order to avoid making the text cumbersome, we preferred mentioning the references in the text.

2. The serum iron concentrations were determined by standard biochemical methods, including collection of serum after 12h starving, realisation of at least two measurements, .... The collection of the serum and the measurement of serum iron parameters were standardised. We mentioned this in the text (Method section - Determination of biochemical parameter levels - page 11).

3. There was no systematic information on liver iron content. This data was only available among the patients who had a liver biopsy.

4. In our cohort of patients enrolled in a phlebotomy program, 15 revealed no HFE mutation (among whom four were diagnosed before the implementation of the HFE gene testing). The proportion of homozygous C282Y patients before and after the implementation of the genetic test has not changed.

5. All the statistical tests were performed two-sided); consequently the p-value do not have to be correct. We mentioned this in the text (Method section - Statistical analysis - page 12).

Discretionary revisions: none.