Author’s response to reviews

Title: Quality of life scores differs between genotypic groups of patients with suspected hereditary hemochromatosis

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Dear Editor Prof. Matteo Pasini

Medical Genetics

Please find attached the revised version of manuscript. We have made the suggested changes and the inserted modifications are in blue.

We also thank reviewer’s suggestions which have improved our study.
We hope this revised version has reached the high standards of Medical Genetics and that it will be interesting for its readers.

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Answers to reviewer

Annick Vancllooster (Reviewer 1):

Dear Editor, Fonseca et al. wrote an interesting manuscript about the quality of life (QoL) in patients with hereditary haemochromatosis (HH). It is important that the QoL in patients with HH is studied, but I have some major methodological remarks about this article, that need to be addressed.

1. 79 patients with hyperferritinemia are included in this study of which 29 are homozygous for c.C282Y. Group 2 is a combination of patient with primary iron overload with or without HFE mutations. Patients with only one gene mutation as included in Group 2 in this study are carriers and do not have HH. Also patients without the presence of any HFE gene defect are included in group 2, although this is not the reason for their iron overload and so they are not HH patients. In Table 1, there is already a significant difference between group 1 and 2 in demographic data. You would expect there are more men than women if you have a mean age of 45 years, but there are far more men in group

We can not claim that patients are not HH patients. We used “suspected HH patients” because we tested the main HFE gene mutations. We know that other HFE and non-HFE are present in this group of patients with primary iron overload.

We understand the important preoccupation of the reviewer. However, we had rigorous parameters for the exclusion criteria, including secondary iron overload (methods section). The term “suspected” is the most adequate in this patient group, according some experts in HH.

We observed more men in group 2, probably, due to the lower penetrance of the genetic alterations of non-HFE p.C282Y homozygous genotype, which may involve the causes in this group.
2. The serum ferritin (SF) and transferrin saturation (TS) values differ significantly at time of inclusion. In principle, you need to have two groups with comparable demographic data, to allow meaningful statistical conclusions. In particular in this study, where the genotype is associated with QoL, there should be no statistical difference in ferritin. Is the perceived difference in QoL associated with the genotype, to the gender of the patients in each group, to the transferrin saturation? It is methodologically impossible to reach any conclusions on this matter. It would be meaningful to plot QoL against ferritin for both genetic groups into one pool. That way the researchers could at least get an indication whether the ferritin level correlates with QoL or not. The problem that then remains, is the absence of formal proof of iron accumulation (by biopsy or MRI). Hyperferritinemia can occur as a biochemical aberrancy in the absence of actual iron overload. 2. Although there are no significant differences between the two groups in the supplementary table, table 2 shows differences on four domains: physical functioning, bodily pain, vitality, social functioning. Since the groups are not comparable, the scores should be given graphically with rationale of the SF value and the different SF-36 items.

Yes, it is. The dependent variable is QoL and the independent variable is “groups 1 and 2” (genotypes). In an analyzes with covariates, we added gender, age, serum ferritin, and transferrin saturation as covariates for adjustment of QoL domains. The data are shown in Statistical section.

We were not able to perform the patients' MRI or biopsy. This is a limitation of the study, which was cited in the limitation paragraph, but we excluded secondary causes.

We agree with the reviewer’s sentence about SF and SF-36. However, the rational independent variable is “genotypes”, which leads to different SF values (this variable is a consequence, especially in our selection excluding secondary iron overload). In addition, similarly to selection of HH patients over the world, the patient’s iron status values were related to different periods, as cited in the Methods.

3. There is no information on medical data of the patients. Do they have diabetes, osteoporosis, liver failure, … All these items will probably decrease the QoL and are more likely in c.C282Y homozygous patients.

We did a comparison of the frequency of common diseases (diabetes, hypercholesterolemia, liver diseases, bone and thyroid diseases, etc) among groups. We did not observe significant difference.

We also included this data in the manuscript.

4. The SF-36 questionnaire was filled in after knowledge of the genotype (p6). Patients were treated already with phlebotomies? Literature describes patients benefit from treatment, which can influence the outcome of the SF-36 questionnaire. What about the 'additional question' that was asked to evaluate the transition of health status within a year.
Patients answered the questionnaire at the diagnosis, during or after the treatment (phlebotomies). The distributions among genotype groups were similar. We have included this information in the Results section.

The questionnaire is based on 36 questions. There is an additional question that evaluates the transition of health status within a year, but we did not use because suspected HH patient were selected in different period. Thus, we excluded the sentence about this “additional question”.

John Waye (Reviewer 2):

The primary goal of this research was to evaluate whether domains of QL are different according to genotype groups in patients suspected of hereditary hemochromatosis. QL differences were identified between the two groups, Group 1 being C282Y homozygotes and Group 2 being other HFE genotypes. The results are as expected. The C282Y homozygotes had worse QL scores compared to other genotypes. The homozygous C282Y genotype has higher penetrance than the other genotypes and these patients are more likely to have physical symptoms of hemochromatosis, and consequently worse QL indicators. A second factor that might come into play is that the SF-36 questionnaire was administered after the participants were made aware of their genotypes. The participants were highly educated (>60% university educated) and would have no trouble finding out which genotype is most likely to be associated with severe health problems. For some presymptomatic individuals, this might be enough for C282Y homozygotes to feel "affected" and have worst QL scores.

We agreed that the finding could be expected. But there are few studies, with different approaches, using SF-36 questionnaire in HH patients. The present studied was able to conclude that patients with p.Cys282Tyr homozygosis had worse QL scenario assessed by SF-36, compared with patients with primary iron overload that do not carry the same genotype. Being aware of this relationship between genotypes and QL might be helpful in the overall management of patients suspected of hereditary hemochromatosis.

We were not able to apply the questionnaire before and after of their genotype result.

We agree that pre-symptomatic patients may have been influenced by their genotype information, thus affecting their QoL. This is “implicit” in the association analysis of the genotype with QoL.

Previous studies of our group showed Brazilian HH patients being highly educated. We did not observe difference of education level among 1 and 2 groups (table 1).