Author's response to reviews

Title: Hereditary Hemochromatosis (HFE) genotypes in heart failure: Relation to etiology and prognosis

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Author's response to reviews: see over
Referee 1
Minor
1) Abstract: I prefer that the aim is clearly stated in the abstract.

Our reply
We agree and the aim of the study has been incorporated in the abstract

New version
(under background) In a large cohort of HF patients, we sought to determine the etiological role and the prognostic significance of HFE genotypes.

2) Introduction, 1st paragraph: You write that the prevalence of HH is 0.37-0.46: is that the clinical prevalence, the biochemical or the genetic prevalence?

Our reply
To specify the numbers, it has been stated that it is the biochemical prevalence

Old version
The prevalence of HH in Denmark has....

New version
The biochemical prevalence of HH in Denmark....

3) I prefer using “polymorphisms” instead of “variants”

Our reply
Accordingly, polymorphisms has been incorporated in the manuscript instead of variants

4) In the results, you discuss a result in relation to reference 17. Do not discuss results in the Results section. Furthermore, I wonder if the reference 17 is correct. Is it reference 24 instead?

Our reply
We agree with the concerns of discussing results in the Result section, why we rephrased the paragraph. Furthermore the reference has been corrected accordingly.

Old version
The HFE genotype distribution in this heart failure population was consistent with newly published results obtained in a large Danish general population cohort17

New version
The HFE genotype distribution is displayed in table 2 in conjunction with a newly published result obtained in a large Danish general population cohort[19] and is consistent with the Hardy....

5) Table 3: please make a footnote to “other”, “unknown” and “deceased”.

Our reply
We agree and have put a footnote with regards to Other, Unknown and Deceased

6) Please, put the log-rank p-values on the fig 1a-d. It seems as though C282Y carriers differ significantly from non-carriers.

Our reply
In accordance with referee 3, the figure has been deleted

7) Concerning genotyping, please also refer to the dbSNP rs-numbers for C282Y, H63D, and S65C in the Methods.

Our reply
The dbSNP rs-numbers have been added

Old version
we examined the frequency of the most common HFE variants C282Y, H63D and S65C in a large HF..

New version
we examined the frequency of the most common HFE polymorphisms C282Y (dbSNP rs 1800562), H63D (dbSNP rs 1799945) and S65C (dbSNP rs 1800730) in a large HF...

Major:
1) 2nd paragraph of Introduction and the whole discussion:
a) Danesh has written a meta-analysis in 1999 in Circulation on risk of IHD according to different iron parameters and van der A et al (Circ Cardiovasc Genet. 2008 Oct;1(1):43-50) has written the most comprehensive IPD meta-analysis on risk of HFE genotypes and IHD. Both of the studies conclude that increased iron parameters (Danesh) and HFE genotype (specifically C282Y/C282Y) do not associated with IHD (or MI). Please, correct the introduction and Discussion according to these studies.

Our reply
We agree that the above mentioned studies excludes HFE genotypes to IHD, but as referee 3 points out, the literature is conflicting as we have already stated in the introduction. The paper by Van der A et al, has been incorporated in the introduction as a powerful statement towards HFE-polymorphisms not playing a role in IHD. In the discussion we accordingly removed the passage stating that HFE genotypes associates with IHD (see also response to concern no 4).

Old version
The HFE variants also associates with ischemic heart disease (IHD),[11,15] neurodegenerative disorders[20] and cerebral stroke[21] among others. The association with IHD is somewhat conflicting as recent publications found none.[22]

New version
The HFE H63D polymorphism also associates with neurodegenerative disorders[22] and cerebral stroke[23] among others.
b) Furthermore, please distinguish between the heart disease associated with the juvenile hemochromatosis, in which heart involvement is far more evident than in the adult-onset form (e.g. see Pietrangelo NEJM). This might also be the reason for you not finding the hemochromatosis gene mutations associated with HF in your population as this is far older.

Our reply
We agree that cardiac involvement is far more evident in juvenile hemochromatosis (JHH) but this condition is associated with alterations in HJV and HAMP why we would not expect to find HFE alterations in these cases which has onset of symptoms in their second and third decade of life. As HH was an exclusion criterion we would not expect to have any JHH cases in our cohort why the distinction seems less needed in our manuscript. Nevertheless we emphasized in the introduction that the cardiac symptoms related to HH especially correlates to JHH.

Old version
Clinically, HH manifests as increased erythropoiesis, liver cirrhosis, diabetes mellitus, hepatocellular carcinoma and potentially heart disease (heart failure, ischemia and arrhythmia)

New version
Clinically, HH manifests as increased erythropoiesis, liver cirrhosis, diabetes mellitus, hepatocellular carcinoma and potentially heart disease (heart failure, ischemia and arrhythmia, especially in juvenile hemochromatosis)

2) You should sell the paper on the much more interesting finding that in a population of HF patients with HF not caused not by HH-variants, HFE-variants may protect against mortality due to the fact that the patients do not get anemia.

Our reply
We agree that it is an interesting finding, but due to the small number of patients with HH-variants we do not find substantial evidence to pursue it in this manuscript.

3) Therefore I also suggest that you present a table of the results from the Cox-regression analyses: crude and adjusted.

Our reply
Please see the above reply

4) Clinically and bichemically H63D/H63D is usually not related to HH. In the Discussion, I would prefer that you write C282Y/C282Y is not associated with IHD (van der A et al (se above)) but H63D/H63D may be associated with neurodegenerative disease and stroke (maybe due to a different mechanism other than iron overload).

Our reply
In accordance we have rephrased the paragraph (please see reply to 1a)

5) In the Discussion you should point out the limitation on the number of patients making you unable to stratify on the more interesting genotype status for
C282Y/C282Y homozygotes than the clinically less interesting and less useful carrier-status. Due to this limitation it would be helpful to have a power-calculation in the results.

Our reply
We have elaborated the limitation paragraph accordingly. Concerning the need for power calculation we feel that 3 carriers are not enough to substantiate any conclusions regardless of power calculations.

Old version
Univariate analyses were significant, but due to the small sample size (27 patients), we did not have enough statistical power to make any definite conclusions.

New version
Univariate analyses were significant, but due to the small sample size (27 patients) especially with the C282Y homozygous polymorphism (3 patients) we did not have enough statistical power to make any definite conclusions.

Referee 2

Major Compulsory Revisions
1. HFE genotypes did not significantly affect all-cause mortality in HF. How about the cardiovascular death? The authors should add the table of causes of death.

Our Reply:
We think it is a good point by the referee, but it was not possible to retrieve this kind of information in our material. All of the patients suffered from severe heart failure (NYHA III-IV episode within a month prior to inclusion) why the main cause of death should be expected to be due to cardiovascular disorders and fits well with investigations reported prior to our study with mortality rates of about 50 % over a 5-year period in heart failure populations. These considerations have been implemented in the manuscript.

New version (under discussion)
Although we did not specify the cause of death to be cardiac or non-cardiac related as the study by nature is observational, all patients suffered from severe heart failure (NYHA class III-IV episode within a month prior to inclusion) why the main cause of death should be expected to be due to cardiovascular disorders. This fits well with published data where cardiac mortality rates of about 50 % over a 5-year period in HF-patients is seen.[30]

2. In this study, patients with atrioventricular block or severe liver dysfunction were not included. The criteria of ‘severe’ liver cirrhosis should be added in the manuscript. As the authors mentioned, some patients with “classical” hereditary hemochromatosis were possibly excluded. If possible, the authors should show the frequency of the patients with hereditary hemochromatosis in the patients not included in this study criteria.
Our reply:
Point taken, the criterion has been included in the manuscript and furthermore we have commented on the frequency of HH in the excluded group.

Old version
Patients with severe liver affection, i.e. cirrhosis of the liver....

New version
Patients with severe liver affection, i.e. biochemical suspicion of cirrhosis of the liver..

Furthermore, none of the excluded patients was diagnosed with HH during the follow up period assessed by the Danish Patient Register.

Discretionary Revisions
1. Plasma B-type natriuretic peptide (BNP) is an important and objective indicator in patients with HF. If possible, plasma BNP levels should be added in table 1.

Our reply
Unfortunately, it was not possible to perform BNP-analysis and incorporate it in the manuscript.

Referee 3
Major Compulsory Revisions
1 I do not see the need for figure 1. If anything, it’s confusing as it gives equal visual impact to the very small number of S65C carrier individuals who died during the 4 year period compared with those with C282Y or H63D. The same data has been adequately presented in the text and on Figure 3.

Our reply
Accordingly, figure 1 has been removed

2 I would change the wording of the last paragraph from "therefore it seems that performing HFE genotype...to "Therefore it seems that performing HFE genotype screening in heart failure patients has no value unless of course they display clear clinical signs of HH." And I think the last sentence should be omitted. If a doctor wants to know the effect hemoglobin levels on heart failure mortality it will be far better to study this connection directly.

Our reply
We rephrased the last paragraph and also emphasized that measurement of hemoglobin levels in conjunction with hepcidin seems interesting in the setting of heart failure.

Old version
Therefore, it seems that performing HFE genotype screening in heart failure patients is still not necessary, unless they…..

Future studies should evaluate if certain HFE genotypes could have a modifying effect on heart failure mortality due to the effect on hemoglobin levels.

New version
Therefore, it seems that performing HFE genotype screening in heart failure patients has no value, unless they…….

Future studies should evaluate if certain HFE genotypes could have a modifying effect on heart failure mortality due to the effect on hepcidin and subsequently hemoglobin levels.

Minor Essential Revisions
3 Table 2 Distribution of HFE genotypes. To avoid confusion between cultures who use a comma and those who use a full stop to delineate four figure numbers, I suggest removing the punctuation in the column corresponding to the numbers for Pederson et al. 3.871 can something quite different from 3,871 but I think 3871 is understood by all.

Our reply
We agree and the table has been corrected accordingly