**Peer Review File**

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**Reviewer A**

**Comment 1**: Spelling errors: Line 362: Pedersen instead of Pederson; Line 507: Steig T instead of Steig Ta

**Reply 1:** These have now been corrected. Lines 365 and 524 in the revised manuscript.

**Reviewer B**

**Comment 1:** In Page 6, Line 96, authors state "…young people with HFE mutations rarely show overt symptoms". This is true but the concept could be better explored. Clinical manifestations are indeed very rare in young C282Y homozygous subjects, but evidence of increased transferrin saturation is reported in HH starting in childhood and predicting the later development of iron overload in young adult life (Porto et al. Pharmaceuticals (Basel). 2019 Aug 22;12(3):122). In spite of being apparently uncommon (I mean "apparently" because in fact, no populations studies have so far addressed its frequency), this phenotype should not be ignored, particularly in terms of disease prevention.

**Reply 1:** This is an important point and we thank the reviewer for drawing it to our attention. The following text has now been inserted into the manuscript (Lines 96-99 of the revision: "Nevertheless, even in childhood, evidence of iron overload may be apparent in the form of increased transferrin saturation (TSAT) in genetically predisposed individuals, and this correlates with the development of iron overload in young adult life (Porto 2019)."

**Comment 2:** About other genetic or non-genetic conditions that may alter disease expression in HH, information is missing about the influence of the immune system or inflammation and I invite the authors to include this topic. Regarding the immune system, there is a large body of evidence that the severity of iron loading is associated with immunogenetic markers (including HLA and other micro or extended MHC Class-I haplotypes) and with abnormally low peripheral blood CD8+ T lymphocyte counts. These associations have been shown in different HH populations from worldwide spread countries including Norway, Portugal, Canada and US (1-5, etc.). There has been in general some confusion about this topic in terms of defining the cause or effect nature of the immune abnormalities in HH. Solid evidence exists, however, demonstrating that primary defects in CD8+ T lymphocytes will cause iron overload in animal

models (6-10) and that low CD8+ T lymphocyte numbers in HH are genetically determined and not caused by the iron overload (11).

**Reply 2:** We thank the reviewer for bring up this point. We have added a paragraph to the revised manuscript to address this. We have used some of the references the reviewer has suggested, along with several others. The new wording (Lines 404-419) reads: "Another physiological modulator to iron loading is the immune system. Interest in this area was piqued soon after the cloning of the HFE gene with the recognition that HFE was a non-classical MHC class I-like protein (5), and is consistent with the earlier association of HH with the HLA system (49). However, even before HFE was identified, it was recognized that HH patients with more severe iron loading had abnormally high CD4/CD8 lymphocyte ratios (92) and that this reflected constitutively low CD8+ lymphocyte numbers (93-96). Although the mechanisms are not fully understood, HFE may act as a suppressor of CD8+ T cell activation and differentiation (97,98). In addition, primary defects in the immune system per se can lead to iron loading, and this could influence the hemochromatosis phenotype. Not only do mice lacking both HFE and □2-microglobulin have more severe iron overload than mice lacking only HFE (99), but mice lacking classical MHC class I proteins also accumulate excess iron (100,101). Indeed, extended HLA haplotypes have been associated with variations in iron loading in HH (95,96,102). The mechanisms linking both the adaptive and innate immune systems to iron homeostasis are only partly understood and this represents a fruitful area for further investigation." The influence of the immune system is also now acknowledged in the Abstract (Line 45).

**Comment 3:** Regarding the impact of inflammation, it is interesting to note the putative impact of hepcidin upregulation on reversing the iron overload phenotype in HH. This is well demonstrated in the report of a time-course analysis of serum hepcidin, iron and cytokines in a C282Y homozygous patient with Schnitzler's syndrome treated with IL-1 receptor antagonist (van Deuren et al. Haematologica. 2009 Sep;94(9):1297-300).

**Reply 3:** We have now added this paper along with an earlier study of our own using mice where we demonstrated that the inflammation-induced upregulation of hepcidin was effective in both the presence or absence of the HFE. The added text (Lines 238-240) reads: "Interestingly, hepcidin expression can be increased by inflammation by a pathway that is independent of HFE (64), and thus chronic inflammation has the potential to suppress the iron loading phenotype (65)."

**Comment 4:** Minor correction: Anytime in the text it is referred “C282Y mutation”, it should be written “C282Y variant” to comply with the current genetic nomenclature.

**Reply 4:** This has now been corrected throughout the manuscript. The relevant line numbers where corrections have been made are 217, 220, 221, 295 and 348