Author's response to reviews

Title: Association of HFE common mutations with Parkinsons disease, Alzheimers disease and Mild Cognitive Impairment in a Portuguese cohort

Authors:

Rita J Guerreiro (ritajoao@gmail.com)
Jose M Bras (brasjm@gmail.com)
Isabel Santana (isabelsantana@huc.min-saude.pt)
Cristina Januario (cristinajanuario@gmail.com)
Beatriz Santiago (ubaldo2500@hotmail.com)
Ana S Morgadinho (anamorgadinho@portugalmail.pt)
Maria H Ribeiro (mhgarrucho@huc.min-saude.pt)
John Hardy (hardyj@mail.nih.gov)
Andrew Singleton (singleta@mail.nih.gov)
Catarina R Oliveira (catarina@cnc.cj.uc.pt)

Version: 2 Date: 2 May 2006

Author's response to reviews:

May 2, 2006

Dear editor:

RE: Manuscript MS: 4960593595744065 - Association of HFE common mutations with Parkinsons disease, Alzheimers disease and Mild Cognitive Impairment in a Portuguese cohort

We are grateful for the reviews of our paper, which are fundamentally constructive and complete. We have altered the manuscript according to the reviewer's suggestions and hope that you find these modifications satisfactory. The changes made are shown on the following page. Additionally we have removed figures from the main text and submitted them as separate image files.

If you have any questions please do not hesitate to contact me.

Kind regards,

Rita Guerreiro

Reviewer: 1

1) In the introduction, there is no literature review or reference to why the authors looked at MCI.

The following sentence was included in the introduction chapter:

"In this sense HFE mutations have been associated with different stages of dementia (Braak stages) and increased oxidative stress, thus a study including MCI patients is of relevance"

The following sentences were included in the discussion:

"...and data from Pulliam et al. that suggested HFE mutations were associated with increased oxidative stress and Braak AD stage [7]. The latter study was the primary impetus behind us studying these variants in MCI, a recognised prodromal stage of AD."

2) In the results section, the authors report testing the CORRELATION. They probably meant association or relationship. Statistical correlation is a different kind of test.
Changes were made accordingly, substituting the word correlation by association.

3) What is the interpretation of the difference in allele frequency between AD and PD patients that the authors report?

The following statement was added in the discussion:

The observation that the 282Y allele is overrepresented in the PD cohort compared to the AD cohort demonstrates explicitly the main findings of this paper; that this variant may infer risk for PD, but not AD in the Portuguese population. While it is tempting to speculate that differences in iron handling may differentiate the molecular underpinnings of these two disorders, the current data is too far removed from this mechanismically and too preliminary to make this a convincing argument.

4) The explanation given to the difference in results between various studies by the authors is linkage disequilibrium in the same gene or linkage disequilibrium in diverse populations. Their explanations do not follow and it is hard to accept this since all studies mentioned studied the same gene variants.

We have clarified this in the discussion:

"The discordant results may be explained by several factors; first, the results of the current study and those of Dekker and colleagues represent false positive findings; second the results of Buchannan and colleagues represent false positive findings; third, 282Y is not a causal variant, but in linkage disequilibrium with another variant that underlies disease risk, the degree and direction of a disease association when genotyping what is in effect a tagging SNP are both sensitive to the structure and content of a given block of linkage disequilibrium; these factors are both potentially different between populations."

5) In the Introduction, the authors refer to HFE as a disease and a gene at the same time... "HFE is most often caused by mutations in the gene HFE...." Also, compound heterozygosity for these mutations appear to be at risk for HFE... They mean hemochromatosis?

HFE is commonly used to designate both the disease hemochromatosis and the gene, as can be seen in OMIM (http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=235200)

However, we have changed HFE to hemochromatosis, when the disease was being referred to.

6) In the discussion, the authors refer to the paper of Dekker et al as reporting no association between HFE and PD but points to reference No 20 which is another paper. The sentence following refers to Dekker et al paper.

A correction in the text was made in accordance.

7) There is a need for an improvement in the English used throughout the manuscript.

The manuscript has been carefully proof read and changes made accordingly.

8) The sample size is not large and powerful enough to reach definitive conclusions.

This work is ongoing as part of a larger collaborative study.

9) The selection of patients is not clearly explained. What do the author mean by AD patients were selected from a larger sample selected in a consecutive manner. What was selected and from what is not clear from this sentence. What were the selection criteria and from what sample, what source?

We have included this sentence in the Methods section:

"AD patients were selected from a consecutive clinic case series of those who gave permission for sampling..."
(over 90% of cases consent for blood sampling), collected by neurologists at the University of Coimbra Hospital. Selection was performed to include patients with a negative familial history and a late age at onset for the disease ([greater than or equal to] 65 years). This group included 130 patients (79 females and 51 males)

10) Is it possible that relatives living in the same area and coming to the same clinic were included?

Although a detailed genealogical history outside of the nuclear family was not taken, there's no suggestion that any of the subjects were related to each other.

11) The author did not specify how the control group was recruited.

The following sentence has been included in the Methods section:

"This group comprised mainly spouses of patients and caregivers that were accompanying patients to the clinic."

Reviewer: 2

1) The writing is acceptable, but in the Tables comma should be used.

We have changed the numerical format in the tables to match that existing in the text.