Author’s response to reviews

Title: Association of Nrf2-encoding NFE2L2 haplotypes with Parkinson's disease

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Version: 2 Date: 2 November 2009

Author’s response to reviews: see over
Dear editor

Many thanks for your e-mail regarding our recent submission to your journal (Manuscript ID: 2135084987286277). We think the criticism by the reviewers is fair and have tried to meet all of their comments as specified below. In our mind, this has improved the manuscript significantly.

Reviewer #1

We thank the reviewer for his positive comments on our paper.

"In the abstract the authors forgot to mention in the results section that the protective haplotype is in the NFE2L2 gene. Besides they should also include the decreased risk of PD observed in the Swedish population."

We have specified that the haplotype is an NFE2L2 haplotype in the abstract (page 3, line 11). However, while there is a global significant association to risk for the haplotype window, we would prefer not to report the decreased risk of PD conferred by this particular haplotype in the Swedish material as this possible association was not statistically significant.

"Although shortly mentioned in the conclusions, the authors should comment on the negative result with respect to the KEAP1 gene (possibly not enough SNPs?)."

The discussion on KEAP1 has been extended and data from the publicly available data from Maraganore et al. (2005) Am. J. Hum. Genet. 77:685–693 has been added as a support for lack of association (page 13’s last two lines lines and page 14 first two lines).

Reviewer #2

“The authors have performed a SNP and haplotype study of 2 genes (NFE2L2 and KEAP1) involved in oxidative stress to evaluate association to Parkinson Disease (PD). The association studies were performed in two independent case/control samples to evaluate replication of any observed effect. The authors identified a protective haplotype that was associated with older age of onset in the Swedish sample and decreased risk of PD in the Polish sample."

We thank the reviewer for very valuable comments, especially on the statistics, and her overall interpretation of our study.

“The haplotype analysis is a critical result for the study, but not enough information is provided in the methods. Specifically, how were haplotype probabilities handled in the logistic/linear models? One would expect an EM algorithm to provide all possible haplotype configurations with their associated probabilities. Was only the highest probability haplotype used? How were two haplotypes per person handled in the models? Was software designed for haplotype association analysis used and therefore could be referenced (such as PLINK or Haplo.stats)."

We thank the reviewer for this comment and have included a more detailed description on how haplotype probabilities were handled in the methods section (page 8, lines 4-9). Indeed we have used software (Helix Tree 6.3) dedicated for proper haplotype analysis.
The main finding of the manuscript is a haplotype allele associated with onset age in one sample and risk in the other. This does not meet the "same allele, same phenotype" standard for replication. While the global haplotype p-values appear to be significant for risk in both samples, the haplotype-specific results do not replicate. The Swedish result appears to be driven by haplotypes increasing risk, which are not significant in the Polish sample, or in one analysis significantly having the opposite direction of effect. Thus, that they have demonstrated replication is not convincing and statements (bottom of page 12) that "haplotype GAGCAAAA showed strong protective association with PD in two independent European populations" is not accurate when the association was observed only to onset in one of the populations. The authors should address this issue in the discussion and provide an argument for why association to onset and risk constitutes replication.

We agree that the replication with regards to specific haplotypes does not follow the “same allele, same phenotype” standard for replication and we have reworded the phrase "haplotype GAGCAAAA showed strong protective association with PD in two independent European populations" to “haplotype GAGCAAAA was associated with decreased risk of PD in the Polish material and older age at onset of PD in the Swedish material.” (page 13, lines 6-8). We also discuss the issues raised by the reviewer in the Discussion section (page 13 lines 9-22).

“There is a wealth of publicly available genome-wide association data for PD that could possibly be used (depending on the availability of these exact SNPs) to address replication of the finding.”

We have reviewed the publicly available data from Maraganore et al. (2005) Am. J. Hum. Genet. 77:685–693 and, indeed, one NFE2L2 SNP showed association with PD in one out of two study groups and data from this study are now included and discussed in the Discussion section (page 12, lines 11-18).

We hope that you find these answers satisfying and that you are willing to consider this revised paper for publication in your journal.

Yours sincerely,

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