Author's response to reviews

Title: Evidence for Synergistic Effects of PRNP and ATP7B Mutations in Severe Neuropsychiatric Deterioration.

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Dr. Tim Sands, Executive Editor
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Biomed Central
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Dear Dr. Sands:

Thank you for allowing us to revise our manuscript (MS:1876839145932681) along the lines of the comments provided by the referees.

We agree with the comments from the reviewers and have revised the manuscript as a consequence. We will address each of the reviewer’s comments in turn.

Yours sincerely,

Mark Tarnopolsky, MD, PhD, FRCPC
Professor
McMaster University
Reviewer #1:

We thank this reviewer for her excellent comments on our paper. We have made the suggested changes in the manuscript to acknowledge more clearly the limitations of the study from the standpoint of its small size (p. 8, paragraph 1).

Reviewer #2:

We thank this reviewer for his excellent comments and we shall address them in the order in which they were presented:

1. Page numbers and references converted.
2. Literature citations:
   a. Please note that both of the previously published papers referred to by this reviewer concerning the possible interactions of variations in PRNP with variants in ATPB involve a genetic polymorphism at codon 129, 75 codons downstream in the PRNP gene. This genetic polymorphism in turn affects a completely different domain of the PrP protein (beta sheet 1) from the one discussed here (octapeptide repeat). We have nevertheless included citations of these two papers (p. 8, paragraph 1).
   b. We have also included a citation as suggested by the reviewer, to the paper that first described pathogenic mutations in the ATPB gene in association with Wilson’s disease (p 3, paragraph 1).
   c. Regarding the citation of early investigations of the binding of copper to PrP, we originally chose to cite a more recent review (Millhauser 2007, Ref 8) in which the interested reader would find considerable background discussion, as well as a landmark paper (Kramer et al 2001, Ref 16) in which it was demonstrated that PrP binds copper in vivo at a range of concentrations considered to be physiologically relevant. We have now added a citation of the seminal paper by Aronoff et al. (2000) that first demonstrated the relevance of the octapeptide-repeat domain of PrP to copper binding (p 9, paragraph 2).
   d. We were unfortunately unable to discern the context in which Reviewer #2 recommended that we should cite the paper by Boesenberg et al. Ann Neurol 2005 Oct;58(4):533-43. The patient we describe does not apparently have CJD, and we consider such a discussion to be tangential to the key messages of our
paper. Thus, although we are open to further discussion of this point, we prefer not to include such a citation, which would also require new supporting discussion.

3. Regarding our having possibly overstated our conclusions on the basis of one case report, we had indeed attempted to be very clear that our suggestion of a possible functional linkage between ATPB and PRNP gene variants via copper metabolism was merely suggestive and hypothetical, and worthy of further exploration rather than a definitive conclusion. However, we agree with the reviewer that this should be made even clearer, and have added wording on p 8 (paragraph 1) to accomplish this attenuation.

4. We do not agree that the discussion offered in the Conclusion is entirely speculative – rather, we attempted to base it on as detailed a consideration as possible of the structure and function of both the ATPB and the PrP proteins, with the intention of offering a testable hypothesis. In addition, regarding our supposed proposal of “remote effects” of the G54S variant on copper binding, as mentioned in the manuscript this codon is located within a segment of the octapeptide-repeat domain of PrP with unknown function but nevertheless a high degree of evolutionary conservation. In paragraph 4 on p 9 (continuing to p 10), we attempted to make the nature of our hypothesis very clear, including several strong qualifications. We were also unable to determine the precise relationship between paragraphs 3-5 of the Conclusion and the pure speculation alleged by this reviewer. For these reasons, although we of course remain open to further discussion we would prefer not to remove paragraphs 3-5 of the Conclusion.

5. Please note that the only publication we cite that refers to G54S is Beck JA et al. Hum Mutat 2010, 31(7):E1551-1563. The paper by Beck JA et al. Neurology 2001, 57(2):354-356 makes no reference to this genetic variant. However, we found that we had inadvertently mis-numbered the citations of these two papers in the text, and we have corrected this.