Author's response to reviews

Title: Association between novel TARDBP mutations and Chinese patients with amyotrophic lateral sclerosis

Authors:

Hui-Ling Xiong (chocolatexhl@163.com)
Jin-Yang Wang (wangjinyanghs@163.com)
Yi-Min Sun (moqu4922@hotmail.com)
Jian-Jun Wu (jungliw@gmail.com)
Yan Chen (chhyann@gmail.com)
Kai Qiao (kateqiao@hotmail.com)
Qiao-Juan Zheng (zqjyrh@yahoo.com.cn)
Gui-Xian Zhao (zhaogx5639@yahoo.com.cn)
Zhi-Ying Wu (zhiyingwu67@yahoo.com)

Version: 2 Date: 27 November 2009

Author's response to reviews: see over
A point-by-point response to the concerns

In response to the reviewer Janine Kirby:

Minor essential revisions:
1) Please provide the sex/ethnic group (Han?) of the controls.
The controls consisted of 100 men and 100 women and all of them are Han people from Southern China. This content has been added in *Subjects* section.

2) Although the data suggests p.Ser292Asn is pathogenic, without functional data, you cannot state unequivocally. Thus, the abstract should be adjusted to "p.Ser292Asn is predicted to be a pathogenic mutation" and the second paragraph of the results section should have "to this pathogenic mutation" removed. We have revised them according to your suggestion.

3) What do you mean by "the patient reused BiPAP"? Do you mean "refused"?
We were very sorry for writing “refused” as “reused” by mistake. We have corrected it.

4) What did the asymptomatic father (proposed to carry the mutation) die of, and at what age?
The asymptomatic father died of stroke at the age of 43.

5) Please clarify what you mean in the discussion by "None of them is homogenous"? Do you mean "homozygous"?
We have corrected “homogenous” to “homozygous”.

6) Minor corrections in the language
a) BACKGROUND - "To date, totally 30 TARDBP" would be better as "To date, a total of 30 TARDBP"
We have revised it.

b) Clinical features - "neurological examination showed progress of disease" would be better as "neurological examination showed progression of disease"
We have revised it.

c) Pedigree analysis - "..II5 carried Ser292Asn though his sample is unavailable to the detection" would be better as "..II5 carried the p.Ser292Asn mutation, although his sample is unavailable for confirmation"
We have revised it.

In response to the reviewer Roberto Del Bo:

1) The demonstration of the causative role of the TARDBP mutation in ALS pathogenesis is very weak within the family. Are the authors able to perform functional experiments to support the pathological role of p.292Asn mutation?
Thank you for your concern. Our further study is to perform functional experiments to
support the pathological role of Ser292Asn mutation. Professor Christopher E. Shaw of King’s College London has kindly provided us the plasmid encoding wild-type TDP-43.

2) The cohort of ALS patients analyzed is small compared to other studies.
Yes, we have to admit that our cohort of ALS patients analyzed is small compared to other studies (this has been discussed in the Discussion section). In the further study, we will try our best to recruit more subjects.

3) Have the authors analyzed FUS/TLS gene in their cohort of patients (at least in all FALS patients)? I think that it must be performed.
Thank you for your advice. Actually we have screened FUS mutations in our cohort of patients. However, no mutation has been identified. Thus we didn’t show this negative result.

In respondent to reviewer Rita Guerreiro:

Major compulsory revisions:

1. Although SIFT is a very easy to use in silico prediction software, it is not perfect. Authors should not assume the definite pathogenicity of a mutation based on the prediction form SIFT. Authors can say that the mutation found is most probably a pathogenic one, but it is incorrect to say that since SIFT predicted the variation to be deleterious to the protein function, this is a pathogenic mutation.

We have revised according to your advice.

2. The authors should provide the correct nomenclature for all the mutations and polymorphisms at the coding and/or gene level, besides the protein level (for instance: 239-18t>C and Tyr374Term).

Thank you for your advice. We have revised them.

3. It is briefly referred in the abstract and methods sections that the studied patients do not have mutations in SOD1. Was this data published in a previous study? If so, a reference should be added.

This data has not been published in a previous study. Screening SOD1 mutations in FALS patients is our routine work.

Minor essential revisions:

Title:

1. The term “mutations” (plural) implies that more than one mutation was found and associated with ALS.

In the present study, we have identified one novel mutation (Ser292Asn) in a FALS family and 2 silent mutations (p.Gly40Gly and p.Ala366Ala) in SALS patients.

Abstract:

2. “A homology search of the TDP-43 protein in different species demonstrated that it was highly conserved.” – is this sentence referring to the protein or to the mutation found?
It refers to the mutation found.

**Background:**

3. Reference numbers should be provided for all the genes cited.
   We have cited the reference numbers [2-5] after the whole sentence.

4. “These mutations affect the C-terminal region of TDP-43 may influence protein-protein interaction, exon skipping and splicing inhibitory activity, thus, may influence the proper function or transport of TDP-43.” should read: “…region of TDP-43 and may influence protein…”?
   We have corrected it.

5. “Here, we first screened for TARDBP mutations in Chinese patients with ALS, and have identified one novel missense mutation, two silent mutations and one polymorphism.” – this sentence should be reformulated (does this mean that TARDBP was a first screen and something else was screened after?)
   Thanks for your concern. We mean that our study is the first screen for TARDBP mutations in Chinese patients with ALS. It is the first TARDBP mutation data about Chinese patients with ALS. We have revised it according to the reviewer’s advice.

**Methods:**

6. “The sequence of the primers and the annealing temperatures are shown in the table.” – the table number should be provided in the text.
   There is only one table in this manuscript.

**Results:**

7. “One year later, he began to have some difficulty in using chopsticks and lifting his right arms…” should read “…his right arm”?
   We are sorry about this mistake. We have corrected it.

8. “Neurological examination showed muscle weakness involved all the extremities…” should read “…weakness involving all the extremities…”?
   Sorry about that. We have corrected it.

9. “…we speculated that Ser292Asn was from the dead and asymptomatic father.” – sentence should be reformulated for clarity.
   We have reformulated it according to the reviewer’s advice.

**Discussion:**

10. Authors should further discuss the segregation data on the family: the mutation was found in four asymptomatic members beside the proband. The age at onset of the disease of the proband and the ages of these family members should be compared.
    Thanks you for your advice. We have added the content in the text.

11. Authors should specify that studies by Gijselinck I et al and Guerreiro RJ et al failed to find mutations in sporadic ALS series.
We have revised it according to the reviewer’s advice.

12. “None of them is homogeneous” – this sentence is not clear within its context. We mean that all of the TARDBP mutations identified are heterozygous. We have revised it in the context.

13. “The frequency of TARDBP mutations is 3.6% in FALS and 1.0% in SALS” – the authors should specify which population(s) these numbers refer to and provide a reference. We have added them according to the reviewer’s advice.

14. “Here, we have first screened the TARDBP mutations in Chinese patients...” – sentence should be reformulated for clarity. We have reformulated this sentence in the context.

15. “This frequency is much lower than those of previous studies, which may be due to the small sample size and the different races” – authors should re-write this sentence in order to clarify the following: 1) the frequency of mutations in a population does not arise from the size of the series studied (the probability of finding mutations does); 2) different races refer to different genetic backgrounds? We have re-written this sentence according to your advice.

Discretionary revisions:

1. To avoid any misunderstanding, authors could use the term “mutation” when referring to the Ser292Asn and the term “variant” when referring to the other non-pathogenic changes found. Thank you for your advice.

2. In the results section, subsection “Clinical features of the FALS patient carrying Ser292Asn mutation”, the number of the proband in the pedigree (II.2) could be included. Thank you for your advice. We have added it.

3. Assumptions are made for the genetic status of individuals I.1 and II.5. The same could be done for individual II.3. Thank you for your advice. However, since we didn’t find the mutation (Ser292Asn) in any children of individual II.3, it is hard for us to assume the genetic status of individual II.3.

4. “The authors also thank the anonymous reviewers for improving the manuscript.” – BMC Medical Genetics reviewers are not anonymous – this suggests the manuscript was previously submitted elsewhere. To our knowledge, we think that BMC Medical Genetics reviewers are anonymous. We have revised it.