Author's response to reviews

Title: Identification of novel biomarker candidates by proteomic analysis of cerebrospinal fluid from patients with moyamoya disease using SELDI-TOF-MS

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Author's response to reviews: see over
September 3, 2010

Dear Professor Erwin Stolz:

Please find enclosed the revised version of our manuscript (MS: 1548332595400712) entitled “Identification of novel biomarker candidates by proteomic analysis of cerebrospinal fluid from patients with moyamoya disease using SELDI-TOF-MS”.

We would like to thank you and the reviewers for the helpful comments regarding our manuscript. We have addressed all the comments and hope that our explanations and revisions are satisfactory. Our responses to reviewers are enclosed and all corresponding parts are highlighted in red in the manuscript.

We hope that the revised version of our manuscript is now suitable for publication in *BMC Neurology*.

Yours sincerely,

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Response to the reviewers

Reviewer: Peter Berlit

Thank you for your comments and useful suggestions which have helped us to improve our manuscript. As indicated in the responses that follow, we have taken all these comments and suggestions into account in the revised version of our manuscript.

Comment #1
The patient group is rather heterogenous (age 1 to 54 years! and interestingly more men than women).

Response
As described in the guidelines set by the Research Committee on Moyamoya Disease (Spontaneous Occlusion of Circle of Willis) of the Ministry of Health and Welfare of Japan (Surg Cereb Stroke 2009, 37:321-337 (in Japanese)), the distribution of age of onset is expanding (one peak is found under 10 years of age and the other peak is found between 20 and 30 years of age). Both age groups are considered one group according to the guidelines in Japan. Therefore, we believe that our MMD patient population ranging from 1 to 54 years of age is acceptable.

On the other hand, the same guidelines states the gender ratio of patients with MMD is 1:1.8-1.9 for men to female. We believe that the ratio of our MMD patient population (11 men and 9 women) is not critically problematic.

Comment #2
Whether the biomarker candidate proteins found in this study really play a role in angioneogenesis remains speculative and needs to be confirmed in a larger and better defined population.

Response
We fully agree with your comment, and think this study would be positioned as a pilot study on the use of SELDI-TOF-MS for MMD. This point mentioned by the reviewer could be one of the important issues to be resolved in subsequent studies.

Comment #3
The paper should be clearly shortened and interpretations that are pure speculation should be removed.

Response
Following the reviewer’s comment, we have rewritten and removed text throughout the manuscript, especially in the Discussion section. New text has been highlighted in the manuscript.

Reviewer: William CS Cho

Thank you for your comments and useful suggestions which have helped us to improve our manuscript. As indicated in the responses that follow, we have taken all these comments and suggestions into account in the revised version of our manuscript.

Comment #1
Some more SELDI and CSF proteome references are needed to be introduced in the text, e.g.

Response
Unfortunately, the second manuscript (Cho WC. Research progress in SELDI-TOF-MS and its clinical applications. ChinJ Biotech (Sheng Wu Gong Cheng Xue Bao) 2006, 22:871-876.) is written in Chinese, so we could not adapt it into our manuscript. However, we have cited the other suggested references regarding SELDI and CSF proteome in our revised manuscript.

Comment #2
Why there are only three pHs being done as it is quite a routine practice of doing more fractions (e.g. 6 fractions)?

Response
As this comment indicates that our examination using three pH conditions should be divided into more fractions, we readdressed our study with respect to this point. The protein profiles generated using Q10 Chip showed peaks that were not completely different among the three pHs with some duplicate peaks, while peak size differed. Therefore, we believe that our results using three pH conditions did not missed any major detectable proteins (peptides) in this study.

**Comment #3**

Did any experiment done in parallel on IMAC chip?

**Response**

Based on the fact that all proteins have positive or negative charge outside their isoelectric point, we used Q10 and CM10 Chips as ion-exchangers with suitable buffers to detect a wide range of proteins (peptides). Therefore, in this study, we did not analyze using IMAC chip.

**Comment #4**

The sample size for the experiments is small.

**Response**

The population with MMD is quite small (page 5, lines 3-5). The total 32 CSF samples (20 for MMD and 12 for controls) comprised the limit of sampling over about a 2-year period, February 2008 to December 2009, in our department. We think this study should be positioned as a pilot study on the use of SELDI-TOF-MS for MMD. Your point is important and further studies with a larger cohort will be needed to confirm our results.

**Comment #5**

Inferred proteins related to MMD pathophysiology were found at ExPASy protein database.

**Response**

We apologize for the incorrect information. First of all, we had no intention on assuming that the peptide detected in this study was related to the pathophysiology of MMD. Actually, we applied the TagIdent tool (http://au.expasy.org/tools/tagident.html) to identify the proteins (peptides) detected in this study from the UniProt Knowledgebase (Swiss-Prot and TrEMBL) databases based on their molecular mass.

We have corrected the following text in the revised manuscript:
“Candidate protein (peptide) biomarkers were inferred using ExPASy protein database (http://www.expasy.org) the TagIdent tool (http://au.expasy.org/tools/tagident.html) from the UniProt Knowledgebase (Swiss-Prot and TrEMBL) databases based on the definite molecular mass.”

We have also corrected the footnote for Table 3:

“The number in parentheses is the serial number of the protein in ExPASy the UniProt Knowledgebase (Swiss-Prot and TrEMBL) databases.”