Author's response to reviews

Title: Rapid diagnosis of spinal muscular atrophy using High-Resolution Melting Analysis

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Author's response to reviews: see over
Cover Letter

Revised Content:

1. According to Professor Louise Simard,
   (1) The manuscript has been revised according to the advice of Professor Louise Simard, it has been also sent to the American Journal Experts(AJE) and was edited by native English speakers.
   (2) In Paragraph 2 of Background section, we have added the comments on liquid microbead arrays.
   (3) In Methods: the length of the PCR product should be 241 bp which we had made a mistake in the original manuscript.
   (4) The G nucleotide in intron 6 (-45) being associated with SMN1 is generally accepted (NCBI sequence Viewer AH006635; Lorson et al., 1999, PNAS 96:6307-6311). On the other hand, in our previous study, we had sequenced these area for more than 100 SMA patients and 100 normal controls, the results showed that G nucleotide is always associated with SMN1(datum not published). In the preliminary experiment, we use two probes targeting the differences at nucleotide position +6 of exon 7 and intron6(-45), the result of latter is much better. So we use the probe directed against the G/A difference at intron6(-45) finally.
   According to the advice of Professor Louise Simard, we have added the following contents to the conclusion: “This study has provided “proof of principle” data indicating the utility and sensitivity of HRMA when applied to diagnostic testing for SMA. However, our findings should be replicated in a much larger sampling of SMA patients to assess the specificity.”
   (5) In the 1st paragraph of the results section, we have replaced “55 SMA patients were presumed to be homozygous mutation” with “55 SMA patients with a confirmed diagnosis of SMN1 exon 7 homozygous deletion” following Professor Louise Simard’s advice.
(6) Figure 1A was mislabeled in original manuscript, we have exchange the “a” and “b” in the revised figure.

(7) In the 1st paragraph of the discussion section, the nucleotide differences between SMN1 and SMN2 had been corrected according to Lorson et al., 1999, PNAS 96:6307-6311 (reference 17).

2. According to professor christina brahe,

(1) We have deleted the sentence “the system applied in molecular analysis of SMA is not perfect yet” in the abstract.

(2) We have revised the 1st paragraph of the background section according to the advice of professor christina brahe.

(3) In the second paragraph of the background, we have added the references of van der Steege et al. for the RFLP analysis.

(4) The references written in Chinese have provided important bases for this study, we think they are necessary for this paper.

(5) In the 1st paragraph of the discussion section, the “phosphorylated” does not stands for “dephosphorylated”.

(6) As professor christina brahe mentioned, as well as DHPLC, HRMA has a more extensive use for mutation screening rather than SNP analysis, but a instrumentation of LightScanner (Idaho Technology) is required, which confine its application in many laboratories. In this study, we present HRMA as an alternative technique for the diagnosis of SMA, we have revised the conclusion in order to make our opinion more neutral.

3. We have revised the author’s name and their affiliations, have change the order of some authors, marked Zhi Ying Wu as the Equal corresponding author, and have changed the e-mail of Wan Juan Dong.