Reviewer's report

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

Version: 1 Date: 27 January 2009

Reviewer: Louise Simard

Reviewer's report:

Synopsis:

The novelty of the work presented in Chen et al. is the development and validation of a new assay that could potentially be used in the diagnosis of SMA, a severe autosomal recessive lower motor neuron disease that affects 1 in 6000 live births. DNA diagnosis of spinal muscular atrophy (SMA) is offered in most routine diagnostic laboratories due to its frequency and the robustness of mutation screening which detects over 95% of affected individuals. The use of high-resolution melting analysis (HRMA) has gained in popularity since first introduced in the early 2000’s and, as demonstrated by the authors, it is capable of detecting SMN1 (as well as SMN2) exon 7 deletions, the major cause of SMA. The development and validation of the SMN HRMA assay is clearly presented and the application of such a sensitive and specific assay for diagnostic purposes is of interest.

Major Compulsory Revisions:

The manuscript cannot be accepted in its current state and requires significant, but easily manageable, revisions. It is highly and respectfully recommended that the text be revised by an individual who specializes in editing work produced by authors where English is a second language. I will try to provide a scanned PDF of the document with some corrections but further work would be required.

Aside from English grammar, there are a few additional points that must be addressed.

Paragraph 2, Background: There are four assays that have been described for SMN1 exon 7 deletions. The additional diagnostic test for the SMN1 gene involved liquid microbead arrays using xMAP technology from Luminex described in Pyatt et al. (2007) Clin Chem 53(11):1879-1885. While this is a sensitive, high-throughput approach that can be used to detect SMN1 exon 7 deletions from blood spots, the cost may prohibit its application in many laboratories. This assay should be listed in the possible approaches; however, it does not detract from the novelty and potential usefulness of the HRMA approached presented by Chen et al.

Methods: Given the primers quoted I come up with a PCR product of 241 bp, but I could be wrong. I was also surprised that the SMN1 sequence-specific probe
was directed against the G/A difference in intron 6 (i.e. intron6(-45)) instead of
the diagnostic T/C difference at nucleotide position +6 of exon 7. Thus far, the
intrinsic polymorphism appears to be gene specific but many fewer genotypes
have been tested for this polymorphism. It would be important to comment on
why this rationale was chosen and the confidence level for the G nucleotide
always being associated with SMN1. It might be necessary to state that this
assay should be replicated in other cohorts to provide a better estimate of the
specificity, i.e. able to detect 100% of all known SMN1 homozygous deletions. I
have given an example that could be used also in the Conclusion section of the
manuscript.

In the 1st paragraph of the results section, it is stated that the 55 SMA patients
were presumed to be homozygous mutation; however, in the methods section it
is stated that SMA diagnosis was confirmed by RFLP and DHPLC. Thus, this
should read “with a confirmed diagnosis of SMN1 exon 7 homozygous deletion”.
Of note, the use of “exon 7 homozygous deletion” should be consistent
throughout the text.

It appears that Figure 1A is mislabeled: the (a) profile should correspond to
SMN1 deleted SMA patients, the (b) profile to the SMN2 deleted normal controls
(n=3) and the (c) profile to normal controls with both SMN1 and SMN2. If this is
not so, then there is something wrong with the assay or with my understanding of
the assay. In Figure 1B, it appears that the figure legend is correct.

In the discussion, it is recommended that the nomenclature used to describe the
nucleotide differences between SMN1 and SMN2 should be as described in
Lorson et al., 1999, PNAS 96:6307-6311. This nomenclature is more familiar to
the SMA community. Thus the 114(G/A) difference refers to position intron 6(-45)
with the G associated with SMN1 and the A with SMN2 and the 163(C/T) refers
to nucleotide exon 7(+6) with the C associated with SMN1 and the T with SMN2.
The latter is the mutation used in SMA diagnostics.

Given that the unlabeled probe in this study is to intron6(-45) and not exon 7(+6),
it would be prudent to include the following in the conclusion:

"This study has provided “proof of principle” 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 its specificity.
This would provide an empirical estimate of whether the G in intron 6(-45) is
indeed always associated with the diagnostic C in exon 7(+6) of the SMN1
gene."

This is critical as gene conversion events between SMN1 and SMN2 are well
documented and this feature of the SMA locus could affect the specificity of the
HRMA assay as described.

Level of interest: An article whose findings are important to those with closely
related research interests
Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I have absolutely no competing interests.