Reviewer’s report

Title: PCR amplification of a triple-repeat genetic target directly from whole blood in 15 minutes.

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Reviewer: Jonathan J. J Magaña

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This is an interesting study that demonstrated the successful amplification of the normal CTG tract of DMPK gene, directly from whole blood, using a rapid 15-minute PCR protocol that includes a hot-start/cell lysis step. This methodology could be used as a rapid PCR assay for the DM1 exclusion. The final discussion fails however to comment on some critical aspects of the work described in order to evaluate the sensitivity of the technique. Moreover, it is essential to validate the method of analysis proposed. There are many considerations.

Major comments:

- DNA test for DM1 is highly relevant because, it confirms the diagnosis in patients with familiar history or in cases with clinically uncertain symptoms, eliminating the need for invasive muscle biopsy. Currently, molecular diagnosis identifies the DM1 mutations in 100% of affected subjects, therefore it is necessary identified the presence of abnormal expanded allele. In this case, the authors should be discuss and fortify the utility of this rapid PCR assay for the identification of possible CTG carriers in non-affected relatives, as a fast DM1 exclusionary screening (not for DM1 diagnosis).

- The author asserts “In concordance with current guidelines of reporting only normal, pre-mutation, or expanded alleles, exact repeat sizing of the assay is not a necessity”. This asseveration is dangerous because trinucleotide repeats are highly polymorphic in human population and might present large normal alleles near of the threshold between the normal and pre-mutation status or alleles between pre-mutation and pathological state, for that reason in these cases is necessary the quantification of the CTG repeats. The asseveration is valid only for the clinical report.

- The PCR assay using direct blood was successful. Nevertheless the analysis of PCR reaction was adjusted for the Agilent bioanalyzer system. The authors note that this method may be useful for the diagnosis of DM1, however there are technical obstacles that are currently impeding this objective. This analysis system must be validated:

  a) The correlation between the molecular size of each peak with the number of CTG repeats is important for determine the expansion range (normal, premutation or mutation)

  b) Sometimes the electrophoretic shift in Agilent Systems in comparison to
analysis on ABI PRISM sequencer, may have electrophoretic displacements. How could ensure that this phenomenon does not happen?

c) The authors mention “However, for two donors (#13 and #36; Figure 2), characterized as homozygotes by gel electrophoresis data, the bioanalyzer began to resolve two peaks. Based on our manual interpretation of the data and the resolution sensitivity of the assay, it is possible that the DM1 alleles for these donors are 2-3 repeats apart (Figure 4E and supplemental data); a size difference outside the resolution limits of our agarose gel data”

i) How many CTG repeats represents approximately every band?

ii) Could be a contamination?

iii) These peaks appears in the same molecular size of an abnormal peak in diverse heterozygous patients (i.e. 22p, 23p, 17p, 38p); Could be considered an artefact of the technique?

According to these comments, is necessary to analyze these samples (at least some samples) with a fluorescent PCR method in combination with capillary electrophoresis analysis of the amplified products and TP-PCR or by CTG tract sequencing, for elucidate these questions.

Minor Essential Revisions:
- The references of DM1 heterozygous frequencies in the global population average are unclear.

- The research does not mention the detection limit of the analysis by Agilent bioanalyzer, in several reports have been identified the detection limit of other techniques such as fluorescent PCR and further capillary electrophoresis analysis (<100 CTG repeats).

- The authors should clearly state importance of rapid PCR assay and its use with other diagnostic systems analysis (Southern blot, fluorescent capillary electrophoresis and others).

- Minor typographic errors throughout the manuscript

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

'I declare that I have no competing interests'