Author's response to reviews

Title: Rapid and reliable detection of alpha-globin copy number variations by quantitative real-time PCR

Authors:

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Version: 4 Date: 22 November 2013

Author's response to reviews: see over
Author's response to reviews

Title:
Rapid and reliable detection of α-globin copy number variations by quantitative real-time PCR

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Version: 2 Date: 22 November 2013

Author's response to reviews: see below
Reviewer's report

Title: Rapid and reliable detection of alpha-globin copy number variations by quantitative real-time PCR

Version: 3 Date: 9 October 2013

Reviewer: Sirous Zeinali

Reviewer's report:

1-Major Compulsory Revisions

2-The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

3-Minor Essential Revisions:

A-The paper is well written and the authors have tried to show that their method is more useful for detection alpha globin gene CNVs than other methods or at least is of use.

There may be few grammatical mistakes which should be corrected like the last sentence before Conclusion "then" should be "than".

Thanks, the grammatical mistakes have now been corrected

A-In the methods section for NanaoDrop the company is not Nano Drop but is Thermo Scientific Inc. USA

The company has been changed to Thermo Scientific Inc. USA

B-The title may need consideration since this method is best to be used for unknown deletions and also duplications, otherwise multiplex gap-PCR is reliable and quick, yet much less expensive for detecting known deletions. The title and even the abstract does not show this and may be misleading.

We agree with the reviewer that traditional multiplex gap-PCR is a reliable and a less expensive method to detect known deletions. Our new method is a good supplement in cases where thalassemia is suspected but cannot be confirmed by traditional multiplex gap-PCR. We have added this to the abstract.

We have considered the title, but prefer it to be left as it is, as we believe that the title primarily describes the method and not the intended use of the method.

With few modifications the paper can be accepted for publication.

Thank you for your constructive and positive comments.

4-The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

5-Discretionary Revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

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

Declaration of competing interests:

No competing interest
Reviewer's report

Title: Rapid and reliable detection of alpha-globin copy number variations by quantitative real-time PCR

Version: 3 Date: 9 October 2013

Reviewer: Deborah Rund

Reviewer's report:

I have several minor essential revisions to suggest.

1. The authors do not completely clarify how they integrate this new methodology in terms of any particular patient sample. They discuss this in general terms, and say if they don't find the mutation using the other methods they try this. My question is whether all samples are first analyzed using CNV. If so then there is a possibility that they will miss something. For instance there are a fair number of individuals who are compound heterozygotes for a deletion and a point mutation. If they find deletion(s), do they continue to search using other methodologies if the phenotype is not fully explained?

   We agree with the reviewer that this is an important issue. We use the HBA-CNV method together with direct sequencing in cases where the patients show typical, hematological changes indicating thalassemia (increased erythrocyte count, low MCV and MCH), but normal HPLC plot and negative gap-PCR are found (described in Discussion). We do agree that it is important to be able to explain the hematological phenotype and we do search for compound heterozygosity in cases where regular heterozygosity does not fully explain hematology.

2. The authors should explain what they mean by: (paragraph entitled: Detection of copy number variation in patients) "the 170 kb spanning duplication" (line 8), I do not understand this term, perhaps it is novel? Should it not say what the 170 kb spans?

   The sentence has been changed. The breaking points of the duplication are not characterized; it might be a novel duplication. The duplications spans the genes NPRL3, HBZ, HBM, HBA2, HBA1, LUC7 and ITFG3.

   In the same paragraph line 4 they have an apparent grammatical error: "As example: instead of saying "As an example".

   The grammatical mistake has been corrected.

3. Are there any limitations to this assay? Nothing is perfect! Can the authors discuss at least one pitfall or possibility of error?

   We thank the reviewer for bringing up the issue of limitations. We have discussed the possibility of misinterpretation in patients with \(\alpha_a^{anti3.7}\)-triplication on one allele and \(\alpha_a^{3.7}\)-deletion on the other allele (\(\alpha_a^{anti3.7}/-a^{3.7}\)) in the revised manuscript.

   Along those lines, what is the reason for the deviations from whole numbers in the actual assay results? Is it from pipetting errors?

   The CopyCaller software provides both calculated copy numbers and predicted copy numbers. The calculated number of copies of the target sequence in each test sample is determined by relative quantification using the \(\Delta\Delta Cq\) method. The whole numbers are the predicted copy numbers based on the calculated numbers.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare I have no competing interests.