Author’s response to reviews

Title: Rapid detection of PAH gene mutations in Chinese people

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Author’s response to reviews:

Technical Comments:

General comments

The authors would benefit from taking the time to check the format of the manuscript. The numbering of paragraphs must be corrected as there is no consistency. All formatting marks should be removed from the images. In addition, it would be of value for the manuscript to be edited so as to amend the language structure of certain sentences and paragraphs. The manuscript requires significant improvement before it can be accepted for publication.

Reply: Thanks for your comments, we have checked the format of the manuscript. And we also amend the language structure of some sentences and paragraphs.
Major comments

The authors use samples for which a genetic diagnosis has already been obtained which is good for the proof of concept of a novel technique. They do not, however apply the newly developed technique to a new band of patient samples or test the technique on the general population, thereby identifying novel mutations and consequently population specific variants. It is suggested that this be conducted as not all genetic variants will cause disease. In addition, it is suggested that more individuals are tested to ensure that variants identified may only be attributed to disease. Moreover, it would be beneficial to include the prevalence and incidence of PKU in the Chinese population in demonstrate the usefulness of this analytical technique.

Reply: Thank you for your comments, the objective of this study is to establish a novel technique. So we didn’t consider testing on the general population and including the prevalence and incidence of PKU in the Chinese population. And there are researches have reported that more than 70 species mutations of PKU have been detected in the China, mostly on exon 6th, 7th, 11th, and 12th exon. Among them, the 9 mutations R111X, R176X, Ex6-96A>G, R241C, R243Q, R252Q, Y356X, V399V, R413P are the most common types of PAH mutations in Chinese population

Minor Comments

Page 3:
Line 6-7: Correct the formatting of the sentence.

Line 46: "have been detected in the country, mostly on exon 6th, 7th, 11th, and 12th exon."

* Please correct the sentence.

Line 57: What is the full term of "G6PD"?

Page 4:
Line 5: 1 MATERIAGALS AND METHODS

* Please correct this sentence.
Page 5:

Line 2: FIG. 1

* Please be consistent. Use "Figure" or "Fig.".

Line 12-27: Manufacturer's details. Please include these as completely as possible.

Paragraph 1.2.4 Molecular Hybridization Analysis

* It would be beneficial to the authors to describe in this section what is meant by "Liquid A, Liquid B" etc.

Page 6:

Amend the formatting of the paragraphs.

Line 26: 2 RESULT

* Please correct this to "Results"

Page 8:

Line 26: 4.CONCLUSION :

* Please correct the formatting of this paragraph

Reply: thank you for your comments, we have revised the corresponding places in manuscript
Guy Lenk (Reviewer 2): The paper is concerning the detection of PAH mutations utilizing a multiplex PCR system followed by reverse dot hybridization. It employs a panel of 9 mutations that are described as being the most common in the Chinese population. The motivation for this research was to develop a sensitive, specific and cost-effective technique to detect these changes.

Although this seems to be a sound scientific undertaking, the language in the paper struggles to convey the ideas presented. There are many minor examples throughout that need to be addressed to meet the rigor and quality of this journal, but additionally the technical descriptions of the experiments and results themselves is lacking. Two specific examples follow:

P5; line 55 - "If all or most of the wild-type control spots are not colored, the test fails, and it needs to be retested after the analysis of the cause." All or most is very vague. What is the number? If half the control spots were to fail would that be considered a valid test?

Reply: Thank you for your suggestion. We have try to response or amend our expression in manuscript

Section 2.3 (Evaluation of Methodology) - This section states that of the 200 test samples with known mutations this method detected all with "100% specificity and accuracy". That is an impressive result, and should be expanded upon. How many of each variant were tested? How was the "blinding analysis" mentioned carried out? Since these tests require the ability to determine is a spots intensity is sufficient, were these assessed by multiple individuals? What was the state of the control spots in these assays.

In general much more data and more description of the actual analysis of the hybridization technology needs to be included. This paper also could benefit from a more extensive statistical analysis of the results.

Reply: Thank you for your suggestion. We have try to response or amend our expression in manuscript.