Author's response to reviews

Title: Screening for inborn errors of metabolism in symptomatic children: a three-year study in Zhejiang Province, China

Authors:

Xinwen Huang Dr (xinwenhuang@126.com)
Lili Yang Dr. (lilyang_001@gmail.com)
Fan Tong Dr. (451446720@qq.com)
Rulai Yang Dr. (nesily_yang@yahoo.com.cn)
Zhengyan Zhao Prof. (zhaozy@zju.edu.cn)

Version: 3 Date: 18 October 2011

Author's response to reviews: see over
Dear Dr. Simon Harold,

Thank you for your decision letter concerning our manuscript (Ms: 1713577757577736). The comments from you and the reviewers on our manuscript have been most helpful. We dealt fully with the criticisms in the new revised version, which is now re-submitted. In the following we specify the changes made in the manuscript, replying point-by-point to the comments.

With these changes, we hope that our present manuscript is suitable for publication in BMC Pediatrics.

With best regards,

Yours sincerely

Zhengyan Zhao and Xinwen Huang
Response to Prof. Veronica WILEY’s comments:

Reviewer's report:
This is a valuable addition to the literature on screening for inborn errors of metabolism. There are some suggestions which may improve the paper

Thank you very much for your valuable comments. We have incorporated your comments into the revised paper and the point to point response given below. Your comments have given us more deeper insight on the related topic of this study.

Major Compulsory Revisions
1. The authors indicate in "Cut-off value" that cut-off values were set on full term newborns. What was the age of the baby for the cut off values. There are significant differences of cut-offs with age. The subjects ranged from 0.04 to 168.2 months, therefore the authors need to comment and discuss the likelihood that disorders could be missed with the cut-offs for newborns.
   Response: Thank you very much for your points. The range of the age of the baby for cut-off values is 5-7 days after birth. We agree with your comments about the significant differences of cut-offs with age. Due to the cut-off values have been set up based on the newborns, some cases may be missed when using these cut-offs to detect IEM in those symptomatic ones. Actually we are now setting up age specific cut-off values for IEM disorders which will be used for the screening of symptomatic ones later. As you suggested, we discussed the likelihood of missed cases with the cut-offs for newborns in the revised paper.

2. The authors need to detail the timing of collection in relation to symptoms. FAO defects may be missed depending on the timing of sample collection. This should be included in the discussion
   Response: We agreed with your point. Some FAO defects may be missed with the incorrect collection timing. In newborns, sample collection is required to be taken before feeding. However, sample collection in the symptomatic infants cannot be performed so strictly due to the patients' conditions. Therefore, some cases may be missed. This point has been added in the discussion as well.

3. The papers of Mohamed Rashed should be referenced in the discussion as this work was pivotal in the introduction of MSMS for screening
Response: Yes, you are right. “Dr. Mohamed Rashed and his team introduced automated electrospray MS/MS technology to the field and proved that it can be used for organic acids, amino acids and fatty acids oxidation defects. The modification on the method they done have paved the way for the wide acceptance of the ms/ms technology in expanded newborn screening programs across the globe.” We have added his papers in the discussion.

4. There needs to be rewriting of some sections for clarity
a. Methods; MS instrument and analytes: Is citrulline included twice?
b. the acylcarnitines could be moved ...and 20 acylcarnitines (C0, C2, C3 ....), were evaluated individually and in specific combinations. What combinations were used?
Response: a: I am sorry for the writing mistakes, and I have changed it in the revision. b: The specific combinations are shown in Table 1. The related disorders are also indicated in Table 1.

5. Methods are not detailed and authors state "All procedures of sample preparation and MS analysis were performed according to the protocol provided by the manufacturers". The manufacturers provide alternate procedures and therefore a brief description of the solvents used and sample preparation for example whether the samples are derivatised would enhance understanding. Most laboratories performing this testing use LC MSMS with ESI. Was API used?
Response: We provide a brief description of the procedures and the solvents used and sample preparation in the revision. Our laboratory also performed the test with LC MSMS with ESI. Waters Quattro API was used.

6. Discussion: The discussion requires rewriting to avoid repetition and improve clarity. Especially paragraph 5 about organic acidaemia, and paragraph 6
Response: The discussion has been rewritten as you suggested.

7. Discussion, paragraph 4: The authors state "All the PCD patients had good outcome if detected at early age with timely life-long treatment." As this is a three year study, is this phrased correctly?
Response: The description is incorrect and we have changed it. Thank you!

Minor Essential Revisions
1. Abstract; Methods: the authors indicate that diagnoses were further confirmed through ... and molecular studies. I was unable to find molecular results in the paper. Which disorders were confirmed with DNA analysis?

Response: Neonatal intrahepatic cholestasis caused by citrin deficiency, ornithine transcarbamylase deficiency and primary carnitine deficiency were confirmed with DNA analysis.

2. Abstract; Conclusions: Subjects are not "old age" the median was 28.8 months. Old age should be rephrased throughout the paper

Response: We have rephrased the words.

3. Abstract; Conclusions: Suggested rephrasing: Poor medication compliance could reduce the efficacy of treatment

Response: We have changed as you suggested.

4. Background Para 1: morbidity in children with an IEM.

Response: Changed as you suggested

5. Methods; Cut-off value: should be: The borderline cut-off

Response: Changed as you suggested

6. There should be no space in the number of newborns ie 12,720

Response: Changed as you suggested

7. The patients were referred for confirmatory tests if the second analysis was above the cut-off value.

Response: Changed as you suggested

8. Confirmatory tests: there may be inappropriate spaces: between included and repeat; between biotin and ,

Response: Changed.

9. .... test were performed to subgroup hyperphenylalaninaemia ...

Response: Changed.

10. Results: HMG shoud be HMG CoA lyase

Response: Changed as you suggested
11. Results; aminoacidaemias: the authors state "Ten PKU patients had persistent developmental delay after treatment. How long were they treated? Was there any improvement with treatment? 
Response: Symptoms improved remarkably in these patients after treatment with 11, 13, 16 months separately. However, mental retardation of various degrees persisted in these patients.

12. FAO disorders: typos: The one patient detected with MCAD ...
...her blood C6, C8 and C10 levets were elevated at screening.
Response: Changed.

13. Organic acidaemias; could be redrafted: Patients with MMA and PA presented with hypoglycemia, metabolic acidosis, convulsions and developmental delay. Two MMA patients died and the parents of the other two discontinued treatment. Two of the 4 PA patients died, one from metabolic acidosis and the other due to respiratory failure. The symptoms in individuals with GA I varied. Two presented with recurrent convulsions and motor development delay at 1 year of age, while the other had macrocephaly and hypotonia at 4 months of age. The cranial MRI showed extensive abnormal signals in the white matter and basal ganglia.... All three patients improved remarkably after treatment. (Comment: the authors could indicate how the improvement was determined)
Response: Changed as you suggested. No recurrent epilepsy occurred in two patients after treatment and three had normal motor and mental development in the last follow-up.

14. Discussion; paragraph 3; line 4: median age
Changes done

15. Discussion; paragraph 4: Is there a reference for the incidence of FAO in China?
Response: Newborn screening for IEM has only been adopted in several cities in China. Therefore, no incidence of FAO in China has been reported yet. In 2007, Shanghai carried out a study of screening for IEM in symptomatic children. They reported an incidence of 0.5% (14/2941) in these children. The reference was added.

16. Discussion; paragraph 4: I do not understand the sentence about MCAD
Changes done as following “MCAD was reported to be the most common type in Europe and USA, and its incidence even was higher than that of the aminoacidemias. However, only one case of MCAD was found during this three-year screening in our study. The other types of fatty acid oxidation disorders are also rarely found in the Chinese population.”

17. Discussion; paragraph 5: All 4 MCD patients recovered dramatically ...
Motor development delay
Did the authors mean: The baby with HMG CoA lyase deteriorated rapidly and died before diagnosis?
Our study indicated that if patients with an organic acidaemia were not diagnosed and treated at an early stage of disease, there is likely to be irreversible neurological sequelae?
Response: Changed as you suggested.

18. Discussion; paragraph 6: About ten patients became asymptomatic... How many?
Response: Ten patients became asymptomatic. Changes done.

19. Table 1: Cut-off values for ratios should be included
Response: Cut-off values for ratios included.

20. Table 2,3 and 4: Spacing should be corrected for clarity
Discretionary Revisions
1. Results; line 1: A total of 11,060 symptomatic patients (6720 boys, 4340 girls)
with a median age...
Response: Changed as you suggested.

Quality of written English: Needs some language corrections before being published
Response: We have asked a medical editor to have an extensive language editing for the revised paper.
Prof. Rita Christopher’s comments:

*Thank you very much for your valuable comments. We have incorporated your comments into the revised paper and the point to point response given below. Your comments has given us more deeper insight on the related topic of this study.*

Major Compulsory Revisions

Q1: The authors have mentioned that the MS-MS analysis has been performed according to the protocol provided by the manufacturers. The name of the manufacturer and some details of the assay procedure could be provided.

*Response: Details of the assay procedure are provided in the revised edition.*

Q2: Blood acylcarnitine and amino acid concentrations are known to alter with age. The authors have established the cutoff values by a study on full-term newborns. The median age of the patients in their study is 28.8 months. Since age-specific cut-off values have not been used, this could have resulted in errors during interpretation. The authors need to address this issue suitably.

*Response: We agree with your comments about the significant differences of cut-offs with age. Due to the cut-off values have been set up based on the newborns, some cases may be missed when using these cut-offs to detect IEM in those symptomatic ones. We are now Age specific cut-off values for IEM disorders. As you suggested we discussed the likelihood of missed cases with the cut-offs for newborns in the revised paper.*

Q3: Adequate data to support the method by which the positive cases have been confirmed should be provided.

*Response: Detailed confirmatory tests have been given in the revised paper.*

Q4: The authors have reported 3 cases with ornithine transcarbamylase (OTC) deficiency, a proximal urea cycle defect. One of the notable deficiencies in the use of tandem mass spectrometry is the inability to screen proximal urea cycle disorders, including OTC defects. The criteria used for identifying OTC cases and methods used for confirming the diagnosis should be mentioned.

*Response: We found the patients had low cit with or without increased ORN level and increased blood ammonia at screening. Genetic analysis confirmed this diagnosis. The criteria used for identifying OTC cases and methods used for confirming the diagnosis have been added.*
The limitations of the study should be stated clearly.

Response: The limitations of the study have been added in the revised edition.

The text of the manuscript has grammatical and spelling mistakes and should be extensively edited.

Response: We have asked a medical editor to have an extensive language editing for the revised paper.
Prof. Bradford L Therrell's comments:

Thank you very much for your valuable comments. We have added your comments into the revised paper and the point to point response given below. Your comments have given us a more deeper insight on the related topic of this study.

Q1: Some of the English wording used throughout is a bit awkward primarily due to English not being the first language of the authors. For example, the screened patients were referred to as being “in old age” rather than simply noting that they were not newborns and some were a few years old when screened. At the end of the “Conclusions,” the next to last sentence is also awkward. It might be better stated that, “Lack of compliance with recommended treatments and medication dosage lead to poorer outcomes.” As part of the argument for more screening, it would be helpful to know what portion of the screened cohort were actually screened as part of the routine newborn screening experience (if any). Later in the manuscript, there is discussion of better "cover" in China. This should be 'coverage' instead of 'cover'. The term 'high risk' and the term 'symptomatic' are used interchangeably. I suggest using only 'symptomatic' rather than 'high risk' since high risk is much more vague and may be confused with having older siblings with a condition being screened, for example.

Response: Changes done as you suggested.

Q2: MS/MS newborn screening is noted to have been a part of hospital services since 2009, but few details are given. Are all newborns given the opportunity for screening?

Response: Not all newborns were given for the opportunity for screening. The screening for IEM in newborn is still a pilot study in Zhejiang Province.

Q3: How many of the 11,060 were actually screened as part of their routine newborn screen? How many of these actually required follow-up testing for confirmation and how many were cleared on follow-up? A cost of about $60 is noted for this testing on high risk patients, which would be considered high in most MS/MS newborn screening settings. Hopefully this cost would decrease with higher volumes of testing, but no mention of this possibility is made.

Response: None of these 11,060 children in newborn participated in newborn screening for IEM. A total of 81 children required confirmatory tests and 19 were cleared on follow-up. The cost may decrease the volume of testing, however, cost of newborn screening for IEM will be free soon later.
Q4: Also, while it is noted that MS/MS screening is not routinely covered by the insurance program, no further comments are made regarding the possibilities for inclusion or a possible mechanism by which a change might be made. **Response:** We hope to promote the insurance coverage by reporting this pilot study results. We will use our data as a part of the evidence for the local government to implement expanded newborn screening into free healthcare items.

Q5: An interesting short description of the MS/MS and confirmatory techniques used is given, along with information about the analyses and cutoff values in the “Methods.” It would be interesting to at least mention, if not correlate with, the recently published international study of target cutoff values for MS/MS newborn screening attached Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genetic Med. 2011 Mar;13(3):230-54.). **Response:** I have read the paper published in Genetic Med and included it in our references.

Q6: Likewise, when indicating the confirmatory testing that was done, mention is made that not all tests were available in the authors’ laboratory, and other laboratories are named. It would be useful to know which tests were done in which labs. While I found the accompanying tables interesting, the column listed as “Cutoff ” in Table 1 is actually a range and not a single value. This needs some clarification.  
**Response:** This part of description on confirmatory testing has been rephrased to a much detailed one. The Cut-offs used is a range of 99% CI of the value.

The “Results” section comprehensively reviews the case findings for each group of conditions. Each group is included in the “Discussion” section as well. The “Discussion” could probably be shortened and made more compact. It would be interesting to speculate as to what the findings might be in the general population. **Response:** Discussion has been rewritten as you suggested.

Quality of written English: Needs some language corrections before being Published  
**Response:** We have asked a medical editor to have an extensive language editing for the revised paper.