Author’s response to reviews

Title: Proteinuria as a presenting sign of combined methylmalonic acidemia and homocysteinemia: case report

Authors:
Ruyue CHEN (781135340@qq.com)
Xiao-zhong LI (xiaozhonglicn@yeah.net)
Qiang LIN (wslinqiang@aliyun.com)
Yun ZHU (ruyuechen@foxmail.com)
Yun-yan SHEN (syunyan@sohu.com)
Qin-ying XU (listenway_li@163.com)
Xue-ming ZHU (xueming_zhu@aliyun.com)
Lin-Qi CHEN (clq631203@aliyun.com)
Hai-Ying WU (hainewhy@126.com)
Xu-Qin CHEN (1136051418@qq.com)

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Author’s response to reviews:

Dear Editor,

We are grateful for the thoughtful suggestions provided by you and reviewers for our manuscript entitled “Proteinuria as a presenting sign of combined methylmalonic acidemia and homocysteinemia: case report”. Based on these comments, we have made careful modifications to the original manuscript. We hope that the new manuscript will meet the high standards of your excellent journal. Shown below are our point-by-point responses to the reviewer’s comments/questions.

Respond to reviewer 1:
1. Abstract Background: Combined methylmalonic acidemia (MMA) and homocysteinemia is an inherited metabolic disease related to vitamin B12. Disorders of the metabolism and absorption of vitamin B12 can lead to decrease in activity of methionine synthetase and methylmalonate coenzyme A mutase (MCM), which results in increased levels of methylmalonic acid and homocysteine in blood and urine. Delete the first sentence so it will be (Disorders of the metabolism and absorption of vitamin B12 can lead to decrease in activity of methionine
synthetase and methylmalonate coenzyme A mutase (MCM), which results in increased levels of methylmalonic acid and homocysteine in blood and urine.

Answer: We have deleted the first sentence (Combined methylmalonic acidemia (MMA) and homocysteinemia is an inherited metabolic disease related to vitamin B12.). (Abstract, line 2, page 2)

2. Discussion

MMA is an inherited metabolic disease. Various factors lead to the deficiency of MCM or coenzyme Cbl, which results in abnormal accumulation of metabolites. The latter can also cause homocysteinemia. Change it to Various factors lead to elevated MMA, where it is biomarker for some inherited metabolic disorders such as the deficiency of MCM or coenzyme Cbl, which results in abnormal accumulation of metabolites however the latter can also cause homocysteinemia. As I mention in my previous review the discussion is too long for a case report. author can move some parts of discussion to the background, and just leave the renal lesion part in discussion, also the treatment that he/she mention in discussion should be in backgroud too after shorten it all. so they will remain only with a nice discussion about why elevated MMA and homocysteine effect the renal and cause proteinuria.

Answer: We have modified the sentences above, moved some parts of discussion to the background and reorganized the discussion section. (Background, page 4; discussion, page 8-12)

Respond to reviewer 3:

1. Line 5 please refer to combined MMA and homocysteinemia as a group of disorders.
Answer: We have modified MMA below to combined methylmalonic acidemia (MMA) and homocysteinemia because we have deleted the first sentence (Combined methylmalonic acidemia (MMA) and homocysteinemia is an inherited metabolic disease related to vitamin B12.) according to the suggestion of reviewer 1. (Abstract, line 2, page 2)

2. Line 14 replace MMA with "these disorders".
Answer: We have modified MMA below to combined methylmalonic acidemia (MMA) and homocysteinemia because we have deleted the first sentence according to the suggestion of reviewer 1. (Abstract, line 5, page 2)

3. Line 53 replace gene detection with "molecular analysis".
Answer: We have replaced gene detection to molecular analysis. (Abstract, line 22, page 2)

4. Line 56 replace mutations with "genetic causes".
Answer: We have replaced mutations to genetic causes. (Abstract, line 23, page 2)

5. The background it still way to short and needs more introduction about the cobalamin disorders. Much of this text is already in the discussion and can be moved to the introduction. At minimum there needs to be a few sentence describing all the cobalamin defects, their genes and complementation groups.
Answer: We have moved some parts of discussion to the background and reorganized the discussion section. (Background, page 4; discussion, page 8-12).

6. MeCbl deficiency does not result in MMA combined with homocysteinemia, please correct this sentence line 17.
Answer: We have deleted this sentence (In addition, homocysteine cannot be demethylated to methionine if methylcobalamin (MeCbl) is deficient, resulting in MMA combined with homocysteinemia).

7. Please discuss and reference the literature on renal disease in cobalamin disorders and IGS. In cblC there is significant literature on atypical hemolytic uremic syndrome HUS, which may be going on in Case 1. There is some information about this in the discussion already and should be included briefly in the introduction as well.
Answer: The renal diseases in cblC and IGS have been described and discussed in discussion section and have been introduced briefly in background section. (Background, line 19, page 4; Discussion, page 10)

8. Why is methylmalonic acid referred to as methylmalonic acid -2 and methylcitrate -4? Please remove the numbers.
Answer: We have removed the numbers. (Case 1, line 23, page 5)

9. Line 41 should read "compound heterozygous variants".
Answer: We have modified a heterozygous variant to compound heterozygous variants. (Case 1, line 2, page 6)

10. What type of B12 was used to treat both cases? This is very important as cyanocobalamin will not work for cblC patients. Hydroxocobalamin? Methylcobalamin? Please provide the details.
Answer: Both cases had the same type of Vitamin B12 (cyanocobalamin) in our hospital. After the application of Vitamin B12 and other treatments, the level of homocysteine and methylmalonic acid in blood and urine decreased and the hemoglobin level in two patients increased gradually.

11. Please comment on the spinal cord abnormalities. Did they look similar to subacute combined degeneration of the cord? This is a known complication in B12 disorders, often late onset presentation of cblC.
Answer: The pity was that the relevant examination was not done due to financial reasons. What we have known was the imaging changes in terms of the spinal cord abnormalities, which were different from subacutecombined degeneration of the cord.

12. It is unclear what genes were tested in both cases. Were all the cobalamin genes sequenced by NGS? Please add to the paper the gene list.
Answer: Both cobalamin genes were sequenced by NGS. The detailed gene list were seen in table 1, 2 and figure 3.

13. Line 12 should read compound heterozygous variants.
Answer: We have modified a heterozygous variant to compound heterozygous variants. (Case 2, line 20, page 7)

14. First sentence edit to state "disorders of inborn errors of cobalamin metabolism" instead of MMA.
Answer: We have replaced MMA to "Disorders of inborn errors of cobalamin metabolism". (Discussion, line 8, page 8)

15. Line 59 morbidity is not the correct term - are they referring to incidence?
Answer: We have replaced morbidity to incidence. (Discussion, line 12, page 8)

16. Most of the first and second paragraph could be moved to the introduction.
Answer: We have moved some parts of discussion to the background and reorganized the discussion section. (Background, page 4; discussion, page 8-12)

17. This section is referring to MUT type MMA not cobalamin disorders and should not be discussed because it is a completely different pathophysiology. Please remove references 21 and 22 and focus discussion of cblC and atypical HUS. (However, most renal damage occurs in the non-infantile period; it is a late-onset presentation and thought to be related to abnormal accumulation of aminoacids. The accumulation of methylmalonic acid and its intermediate metabolites, as nephrotoxic substances, participates in the inflammatory response of renal tissue, and leads to kidney injury (mainly renal tubulointerstitial damage). The clinical manifestations include renal tubular dysfunction, renal tubular acidosis or renal hypertension.)
Answer: We have deleted these sentences above according to the suggestions.
18. Figure 3 can you add the variants to the Sanger figure.
Answer: We have modified the two pictures in Figure 3.

19. Table 1 and 2 please harmonize the variants, they are written in different formats between case 1 and 2 and should be described as here https://varnomen.hgvs.org/bg-material/simple/.
Answer: We have modified the Table 1 and 2 according to the website above.

20. Please refer to cblC using its official name: https://www.omim.org/entry/277400.
Answer: We have modified the cblC on the basis of the website above.

21. Also the preferred term is variants vs mutations.
Answer: We have replaced mutations to variants according to the suggestions.

22. The preferred term for methylmalonyl CoA mutase enzyme is MMUT not MCM.
Answer: We have replaced MCM to MMUT according to the suggestions.

We hope that these revisions are satisfactory, and that the revised version will be acceptable for publication in BMC Medical Genetics. We look forward to hearing from you at your earliest convenience.

Yours faithfully
Corresponding author: Xiao-Zhong Li.