Author’s response to reviews

Title: Case reports: three Novel variants in PCCA and PCCB Genes in Chinese Patients with propionic acidemia

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Author’s response to reviews:

Dear Editor and Reviewers:

We greatly appreciate this opportunity to revise our manuscript. We would like to thank the reviewers for their kind comments regarding our work and for their efforts in reviewing this paper. We have carefully read the editor’s and the two reviewers’ comments regarding our submission (MGTC-D-19-00561R1), and have responded point by point to each of the reviewers’ comments as listed below, and the revised portions are marked in red in the paper. We feel that the reviewers’ comments have greatly helped us to strengthen our manuscript and we hope that the revised version will be acceptable for publication in BMC Medical Genetics.

Sincerely yours,

QI Yang.

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Response to Reviewer 1:

Abstract section:

1. Line 34 (new sentence) - 38 is better to be divided into 2 sentences: 1st sentence describing the mutations in PCCA gene, 2nd sentence in PCCB gene.

Response: Thanks for the excellent suggestion. We have divided it into 2 sentences: 1st sentence describing the mutations in PCCA gene, 2nd sentence in PCCB gene.

2. Line 38 (new sentence), suggested revision: "Among those mutations, three mutations (…please mention) are novel."

Response: We have revised it in the manuscript.

Case presentation:

3. How were the patients' condition after the specific diets had been given? Did they show improvement on clinical parameters?

Response: After the specific diets, patient2 experienced a few metabolic crises; and the result of MS/MS and GC/MS detection showed an improvement in metabolic level (Table 1). He underwent fundoplication and gastrostomy tube placement at age of 1.5 years for caloric intake. He is now three years old and has shown mild mental retardation, and his weight has increased from the 5th percentile to the 20th percentile. Patient 3 had several episodes of infections, diarrhea, metabolic acidosis; and the result of MS/MS and GC/MS detection showed an improvement in metabolic level (Table 1). She is now five years old with moderate mental retardation. In the past few years, she has been admitted to the hospital several times due to episodes of infections, diarrhea, metabolic acidosis and generalized tonic-clonic seizures. She also had poor weight gain (6th percentile). Her recent heart assessment is normal.

Methods:

4. The reference sequence for PCCA and PCCB gene should be mentioned.
Response: We have revised it in the manuscript.

5.Line 115: Direct Sanger sequencing? For which parts were the sequence performed (coding exons and exon-intron boundaries?) Did the authors already mentioned all the primers being used (including the primers to detect the mutation in the introns)? The primer-design software must also be mentioned.

Response:

**Mutation analysis**

A peripheral blood sample (2 ml) was obtained from the patients and patients' parents. Genomic DNA was extracted from peripheral blood using Lab-Aid DNA kit (Zeesan Biotech Co., Ltd., Xiamen, China). DNA concentration was determined using a NanoDrop ND-2000 spectrophotometer and software (NanoDrop 2000; NanoDrop Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Polymerase chain reaction (PCR) primers were designed using an online tool Primer version 3 (frodo.wi.mit.edu) to include all exons and exon-intron boundaries of the PCCA (NM_000282.3) and PCCB genes (NM_000532.4) (Tables 3 and 4). PCR was conducted in a 50 µl reaction volume consisting of: 10X buffer, 1.5 mmol/l magnesium chloride, 200 µmol/l dNTPs, 400 nmol/l of each primer, 200 ng/µl DNA and 2 U Taq DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China). Initial denaturation occurred for 5 min at 95˚C, followed by 35 cycles of 30 sec at 95˚C, 30 sec at 56~60˚C and 60 sec at 72˚C. Final extension occurred for 10 min at 72˚C. PCR products were stained with ethidium bromide and visualized on a UV transilluminator following 1.0% agarose gel electrophoresis and were sequenced directly in an ABI 3500 genetic analyzer (Thermo Fisher Scientific, Inc.). Potential novel mutations were considered following alignment of the patient's genome sequence against the ClinVar (www.ncbi.nlm.nih.gov), HGMD (www.hgmd.cf.ac.uk/ac/), and the dbSNP (www.ncbi.nlm.nih.gov/SNP) databases. Sorting Intolerant From Intolerant, PolyPhen 2.0 and Mutation Taster tools were used to evaluate the pathology of the novel mutations. Classification of variants was performed according to the ACMG standards and guidelines.

6.Line 168 - 171: please explain the sentence, in which it is mentioned that the frameshift alteration after codon 120 leading to PTC which is located at codon 160.

Response: The variant c.359_360delAT(p.Y120Cfs*40) caused a frameshift alteration after codon 120 leading to a premature termination codon (PTC) which is located at codon 160, resulting in truncation of the PCCB protein, thus, leading to a loss of function.

7.The compound heterozygous mutation in Patient 3 sound to cause severe consequence on PCC enzyme, but apparently Patient 3 has better phenotype (especially related with her cardiac condition). Do the authors have any explanation/opinion on this?

Response:
Response: The variant c.359_360delAT(p.Y120Cfs*40) caused a frameshift alteration after codon 120 leading to a premature termination codon (PTC) which is located at codon 160, resulting in truncation of the PCCB protein, thus, leading to a loss of function. A similar splicing mutation (c.1398 + 1G>T) has been reported to be associated with PA. The splicing mutation c.1398 + 1G>T in the PCCB gene results in exons 13-14 being skipped, leading to a novel aberrant aberrant transcripts [17]. We propose that our novel variant (c.1398+1G>A) acts in the same manner. The splice site mutation (c.1398+1G>A) is expected to eliminate splicing following exon 13, and causes exons 13–14 skipping, leading to aberrant splicing of the transcript. The CoA carboxyl transferase N-terminal and C-terminal form the active site of the PCCB subunit where c.359_360delAT(p.Y120Cfs*40) and c.1398+1G>T are part of the active site and thus predicted to affect the catalysis or substrate binding. Therefore, according to the ACMG standards and guidelines for the interpretation of sequence variants, the mutations of c.359_360delAT(p.Y120Cfs*40) and c.1398+1G>T are pathogenic. To date, of all mutations in PCCB described in patients, phenotypic severity has been observed to differ in patients with biallelic nonsense, deletion, or null mutations, which may be related to differences between individuals in NMD activity and NMD efficiency. Splice site variants are also seen, and, in general, result in milder disease [18]. Therefore, our patients exhibit a varying degrees of phenotype, which may be due to differences in protein activity, expression, and timely treatment.

8. Minor corrections:

- Please revise all typos, bad sentences/grammar, the wrong use of capital and italic, and space on some parts

- Please be consistent on how to write the references.

Response: We thank reviewer for pointing out the numerous errors which we regret. We have revised the manuscript accordingly.

Response to Reviewer 2:

Background should explain what is the importance of genetics examination (PCCA and PCCB) for the case management or diagnosis.

1. the technique, material (reagents), software or sequence reference used should be explained more detail in methods section.

Response: Thanks very much for the valuable comments.

Mutation analysis
A peripheral blood sample (2 ml) was obtained from the patients and patients' parents. Genomic DNA was extracted from peripheral blood using Lab-Aid DNA kit (Zeesan Biotech Co., Ltd., Xiamen, China). DNA concentration was determined using a NanoDrop ND-2000 spectrophotometer and software (NanoDrop 2000; NanoDrop Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Polymerase chain reaction (PCR) primers were designed using an online tool Primer version 3 (frodo.wi.mit.edu) to include all exons and exon-intron boundaries of the PCCA (NM_000282.3) and PCCB genes (NM_000532.4) (Tables 3 and 4). PCR was conducted in a 50 µl reaction volume consisting of: 10X buffer, 1.5 mmol/l magnesium chloride, 200 µmol/l dNTPs, 400 nmol/l of each primer, 200 ng/µl DNA and 2 U Taq DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China). Initial denaturation occurred for 5 min at 95˚C, followed by 35 cycles of 30 sec at 95˚C, 30 sec at 56~60˚C and 60 sec at 72˚C. Final extension occurred for 10 min at 72˚C. PCR products were stained with ethidium bromide and visualized on a UV transilluminator following 1.0% agarose gel electrophoresis and were sequenced directly in an ABI 3500 genetic analyzer (Thermo Fisher Scientific, Inc.). Potential novel mutations were considered following alignment of the patient's genome sequence against the ClinVar (www.ncbi.nlm.nih.gov), HGMD (www.hgmd.cf.ac.uk/ac/), and the dbSNP (www.ncbi.nlm.nih.gov/SNP) databases. Sorting Intolerant From Intolerant, PolyPhen 2.0 and Mutation Taster tools were used to evaluate the pathology of the novel mutations. Classification of variants was performed according to the ACMG standards and guidelines.

2. the discussion:

a. how the author predict the effect of C.359-360 del AT and c.1398+1G>T mutation in PCCB gene (line 163-169)? please explain or cite the relevant article!

b. c.1288C>T (p.R430X ) mutation in the PCCA gene, C.359-360 del AT and c.1398+1G>T A mutation in PCCB gene also found in either mother ther of the patient. Is there any data regarding clinical or laboratory finding of the parents? if the mutation is related to PA, you might find something from their parent. please explain.

Response:

a.

Thanks for the advice.

The variant c.359_360delAT(p.Y120Cfs*40) caused a frameshift alteration after codon 120 leading to a premature termination codon (PTC) which is located at codon 160, resulting in truncation of the PCCB protein, thus, leading to a loss of function. A similar splicing mutation (c.1398 + 1G>T) has been reported to be associated with PA. The splicing mutation c.1398 + 1G>T in the PCCB gene results in exons 13-14 being skipped, leading to a novel aberrant transcript [17]. We propose that our novel variant (c.1398+1G>T;A ) acts in the same manner. The splice site mutation (c.1398+1G>T;A ) is expected to eliminate splicing following exon 13, and causes exons 13–14 skipping, leading to aberrant splicing of the transcript. The CoA carboxyl transferase N-terminal and C-terminal form the active site of the PCCB subunit
where c.359_360delAT(p.Y120Cfs*40) and c.1398+1G&T are part of the active site and thus predicted to affect the catalysis or substrate binding. Therefore, according to the ACMG standards and guidelines for the interpretation of sequence variants, the mutations of c.359_360delAT(p.Y120Cfs*40) and c.1398+1G&A are pathogenic. To date, of all mutations in PCCB described in patients, phenotypic severity has been observed to differ in patients with biallelic nonsense, deletion, or null mutations, which may be related to differences between individuals in NMD activity and NMD efficiency. Splice site variants are also seen, and, in general, result in milder disease [18]. Therefore, our patients exhibit a varying degrees of phenotype, which may be due to differences in protein activity, expression, and timely treatment.

b.

Thanks very much for the valuable advice. We have performed a detailed assessment of the phenotypes of the parents of the three patients, all of whom were normal. Propionaemia is an autosomal recessive genetic disease. The parents of the patients were only the carriers of c.1288C&T (p.R430X), c.359-360del AT and c.1398 + 1G&A, and therefore did not exhibit a propionate phenotype phenotype.