Author’s response to reviews

Title: Clinical and whole-exome sequencing findings in two siblings from Hani ethnic minority with congenital glycosylation disorders

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Author’s response to reviews:

Dear Dr. Kent Lai,

Thank you for the letter regarding your suggestions concerning our manuscript entitled “Clinical and whole-exome sequencing findings in two siblings from Hani ethnic minority with congenital glycosylation disorders (MGTC-D-19-00057R2)”. Suggestions are valuable to us and we have revised the manuscript accordingly.

Here, we clearly mark and reply to the queries in a note where applicable (See Attachment). But the deleted part can’t clearly mark, should we provide the original manuscript?

We hope this revised manuscript is now suitable for publication.

Your advice is greatly appreciated. Especially “Another major concern is that PMM2-CDG is a well-documented autosomal recessive disorder and therefore, even if the c241-242 del is "pathogenic by prediction", it will not make much sense a heterozygous change identified could result in disease manifestation. In fact, if this were the case, why is the mother "unaffected"? “, this gave very good advice. According to this comment, we revised our manuscript.

Sincerely,
Zhen Zhang

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Below is our response to suggestions.
1. “Even through number of reads from WES were mentioned, more detail information of how they perform the whole-exome sequencing followed by analytical strategy should be provided including quality and quantity measurement used such as the Q30 Score, filtering parameters, as well as software and its criteria used for bioinformatic analysis.”

Response: We offer a Q30 guarantee; The bioinformatic analysis: using Bcl2Fastq software (Bcl2Fastq 2.18.0.12, Illumina, Inc.), raw image files were processed for base calling and raw data generation. Then, Short Oligonucleotide Analysis Package (SOAP) aligner software (SOAP2.21,soap.genomics.org.cn/soapsnp.html) was used to align the clean reads to the reference human genome (UCSC hg19, http://genome.ucsc.edu/). Polymerase chain reaction (PCR) duplicates were removed by the Picard programme. The single nucleotide polymorphisms (SNPs) were determined by the SOAPsnp programme. The reads were realigned by Burrows-Wheeler Aligner (BWA) software 0.7.15, and the deletions and insertions (indels) were detected by Genome Analysis Toolkit software 3.7. In addition, the identified indel SNPs were annotated using the Exome-assistant programme (http://122.228.158.106/exomeassistant). To determine their pathogenicity, non-synonymous variants were evaluated by four algorithms, namely, PolyPhen (http://genetics.bwh.harvard.edu/pph2/), Protein Analysis Through Evolutionary Relationships (PANTHER, www.pantherdb.org), Sorting Intolerant from Tolerant [SIFT, (http://sift.jcvi.org/) and Pathogenic Mutation Prediction (Pmut; http://mmb.pcb.ub.es/PMut/). (The bioinformatic analysis:clearly mark with yellow)

2. “A functional assay, such as an enzymatic measurement of PMM2 gene, could be interesting to show the deficiency of enzymatic activity after mutation, provided that patient samples are available.”

Response: According to Editor's comments of MGTC-D-19-00057 which is “After reading your novel and interesting work carefully, I believe it will make a more valuable contribution as Case Report rather than Research Article. In order for your manuscript to be considered as a Research Article, it is this Editor's opinion that at a minimum, expression analysis of the nucleotide changes, especially for the MYH 9 c.679 G>A, are required to confirm the loss of the enzyme activity. “, we hoped that our manuscript to be considered as a Research Article and tried to do enzymatic activity with RT-PCR, according to the methods of “ Next generation sequencing identified two novel mutations in NIPBL and a frame shift mutation in CREBBP in three Chinese children, Orphanet Journal of Rare Diseases, (2019) 14:45”. Unfortunately, we failed because samples are unavailable to get “RNA”. This may be that the samples are kept at -80 ° for about 2 years.

3. “Despite the authors claimed that both patients suffer from CDG, the clinical presentation shown in Table 1 offered little support for such diagnosis. At the very least, the authors should show the glycosylation status of serum biomarkers such as transferrin to support the diagnosis. “

Response: We supplemented the clinical of CDG in our manuscript (clearly mark with yellow). About transferrin testing, blood samples were collected in tubes containing 0.2 M EDTA on February 7th, 2017. So, we are not available to get good quality serum to do transferrin. We only can found that the result of ferritin is 220.3ug/L(Done on 6th, May, 2017).

4. The sequencing results shown in Fig. 3 are confusing. In the text, the authors claimed "The heterozygous PMM2 c.241-242 del was identified in the proband's affected brother, the proband's unaffected mother (Fig. 3)…… But in Fig. 3, the PMM2 c.241-242 del does NOT appear to be present in the "affected" brother. The brother's sequence is the same as the father's.

Response: In Sanger sequencing, the brother's sequence is forward sequencing while the proband and their parents are reverse sequencing.
5. Another major concern is that PMM2-CDG is a well-documented autosomal recessive disorder and therefore, even if the c241-242 del is "pathogenic by prediction", it will not make much sense a heterozygous change identified could result in disease manifestation. In fact, if this were the case, why is the mother "unaffected"? This reviewer appreciates the authors' mentioning that total absence of PMM2 activity is incompatible with life, but most patients do have homozygous hypomorphic mutations (see https://www.ncbi.nlm.nih.gov/books/NBK1110/). Were there any PMM2 enzyme activities measurement done on patient cells?

Response: This is a good comment of “In fact, if this were the case, why is the mother "unaffected"? “Thank you very much! In this family, the proband (IV6) and her younger sibling (IV7) were affected while their elder half-brother is normal (see Fig.1 IV1). Why the proband and her younger sibling are affected? According that there are some literature which reported compound heterozygosity in PMM2, such as“Three families with mild PMM2-CDG and normal cognitive development.Am J Med Genet A. 2017 Jun;173(6):1620-1624. doi: 10.1002/ajmg.a.38235. Epub 2017 Apr 19.Vals MA1,2,3, Morava E4,5, Teeäär K6, Zordania R1, Pajusalu S1,2, Lefeber DJ7, Õunap K1,2, there is compound heterozygosity mutation (c.241-242 del variant and c.395 T>C in gene of PMM2) in this family.

<table>
<thead>
<tr>
<th>Member of family</th>
<th>Allele state c.241-242 del variant in gene of PMM2</th>
<th>Allele state in c.395 T&gt;C in gene of PMM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 The proband</td>
<td>het</td>
<td>het</td>
</tr>
<tr>
<td>2 The younger sibling</td>
<td>het</td>
<td>het</td>
</tr>
<tr>
<td>3 The mother</td>
<td>het</td>
<td>Wild</td>
</tr>
<tr>
<td>4 The father</td>
<td>Wild</td>
<td>het</td>
</tr>
</tbody>
</table>

There were not PMM2 enzyme activities measurement done on patient cells because patient samples are unavailable and samples of this family are kept at -80 ° for about 2 years.

We apologize again for our mistake done in last manuscript.