28 September, 2024

Editor-in-Chief

*World Journal of Clinical Pediatrics*

Submission ID: 100493

Manuscript title: Molecular profiles and long-term outcomes of Thai children with hepatic glycogen storage disease in Thailand

**Dear Editor,**

Thank you for your letter dated 19 September 2024. We are pleased to know that our manuscript has been rated as potentially acceptable for publication in *World Journal of Clinical Pediatrics,* subject to revisions and response to the Reviewer comments.

We would like to submit a revised manuscript that has been modified according to the instructions provided in the decision letter and reviewer comments. All changes made during the revision process are appropriately marked and highlighted in yellow. Also appended to this letter are point-by-point responses to the comments raised by the reviewers.

We would like to take this opportunity to express our sincere thanks to the reviewers who have identified areas of the manuscript that needed corrections or modifications. Their suggestions have indeed improved the quality of our manuscript. We would also like to thank you for allowing us to submit a revised copy of the manuscript.

We hope that the revised manuscript is in order and that it can be accepted for publication in the *World Journal of Clinical Pediatrics*.

Sincerely yours,

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**Response to Editor's comments:**

We appreciate the editor's and reviewers' recognition of the value of our data and their constructive feedback. We have carefully addressed the major revisions suggested and have made the necessary improvements to our manuscript. Thank you for your thoughtful consideration.

**Point-by-point responses to Reviewer comments**

**Response to reviewer**
**Review 1:**

The current study entitled "Molecular profiles and long-term outcomes of Thai children with hepatic glycogen storage disease in Thailand " is meaningful. To further improve the quality of the manuscript, only small modifications are suggested: Pathological section images need to add a scale bar to increase readability.

**Answer:** Thank you very much for the comment. We added the magnification power of these pictures in the figure legend already.

**Reviewer 2:**

This study provides valuable insights into the clinical, biochemical, and molecular features of glycogen storage diseases (GSD) in Thai children, an understudied population. The authors' efforts in conducting whole-exome sequencing (WES) to identify causative genetic variants and assessing long-term outcomes are commendable. However, a few aspects of the study

design, data interpretation, and presentation could be strengthened to enhance the rigor and impact of the findings.

1. The study includes only 8 patients, which limits the statistical power and generalizability of the findings. Consider discussing the limitations associated with the small sample size and strategies for future studies to increase the cohort size.
**Answer:** We appreciate the reviewers' comments on this aspect. Then we added the limitation of our study at the end of discussion section (page 17-18).

2. Provide more detailed clinical descriptions of the patients, including age at onset, family history, and any comorbidities.
**Answer:** Thank you very much for this helpful guidance. We added more detailed clinical descriptions including median age at diagnosis and treatment initiation from the first presentation (page 11). We added that 25% of our participants had history of consanguinity in family (page 11) and the comorbidities at the last follow-up (page 13).

3. Clarify the criteria used for assessing developmental delay and the specific biochemical markers of hypoglycemia, transaminase, and lipid abnormalities.
**Answer:** Thank you very much for your dedication to our manuscript. The definition of each biochemical profiles including hypoglycemia, hyperuricemia, transaminase, dyslipidemia, anemia and hyperbilirubinemia were added into the material and method section (page

8 and 9). For developmental assessment, Denver II Developmental Milestones was used to evaluate all children with suspected developmental delay in our hospital. However, there was no record of this assessment in the medical record of all participants. Then we added this point as our limitation in the discussion section (page 17; Additionally, hypoglycemia, a hallmark of the disease, may be present from birth and can go undetected, potentially impacting child development—a factor not deeply explored in this study.)

4. Describe the bioinformatic pipeline used for WES data analysis, including variant filtering criteria and validation methods.

**Answer:** Thank you very much for your insight comments. The bioinformatic pipeline employed for WES data analysis involved a multi-step filtering process to identify potentially pathogenic variants. All single nucleotide variants (SNVs) and indels were initially filtered based on their location within exons or flanking introns of genes relevant to the disease of interest. Only non-synonymous variants were considered, and those with a minor allele frequency less than 1% in the 1000 Genomes Project and 0.1% in the Genome Aggregation Database (GnomAD) were retained. Additionally, variants were excluded if they were identified in more than 10 alleles in a control cohort of 5,432 Thai exomes. For missense variants, predictions from SIFT and Polyphen were used to assess their potential impact on protein function. Finally, variants were prioritized if they were associated with the patient's phenotype or known to be involved in the disease under investigation. This rigorous filtering approach ensured the

identification of the most likely pathogenic variants for further validation. Hence, this content was added in material and method sections (page 10).

5. For the two novel variants identified in AGL, provide additional information such as allele frequency in control databases, potential functional impacts, and whether they have been reported in other GSD cases.

**Answer:** Thank you very much for this suggestion. We provided a comprehensive comparison of two novel AGL variants, including their allele frequencies in control databases, potential functional impacts, and ACMG classifications in Table 2.

6. Provide more details on the specific dietary interventions (e.g., dosage, frequency, and compliance) and their impact on biochemical markers and clinical symptoms. Discuss potential confounding factors that may have influenced the observed improvements in height, liver enzymes, blood sugar, and lipid profiles.

**Answer:** Thank you for your insightful comment. We have now included more detailed information on the specific dietary interventions, patient compliance, and the impact of these factors on clinical and biochemical profiles in the results section (pages 11-13). Additionally, we have addressed potential confounding factors that may have influenced the final outcomes in the Limitations section of the discussion (page 17-18).

7. Compare the clinical and molecular findings with those reported in other populations to highlight any unique features of Thai GSD patients. Discuss

how the long-term outcomes compare to similar studies with larger sample sizes and different treatment protocols.

**Answer:** Thank you again for your constructive comment. We added more content in this important aspect with more references in the discussion section (page 16 and 17).

8. Acknowledge the limitations of the study, including sample size, potential biases, and lack of genetic counseling information.

**Answer:** Thank you very much for your comment. We have now included the main limitations of our study in the discussion section (page 17). Additionally, we provided genetic counseling to all participants and their guardians prior to blood sampling for genetic analysis. This information has been added to the materials and methods section (page 7).

9. Suggest future research directions, such as expanding the cohort size, investigating genotype-phenotype correlations, and exploring novel therapeutic approaches.

**Answer:** Thank you very much for your insight suggestion. The further research directions was added in the discussion section already (page 17).

10. In summary, this study represents an important step forward in understanding GSD in Thai children. With some modifications to address the above recommendations, the authors have the potential to produce a more impactful manuscript that will contribute significantly to the field of pediatric metabolic disorders. I recommend this study for publication pending the authors' incorporation of the suggested revisions.

**Answer:** Thank you very much for your positive comment and support. We hope that our study will contribute to larger future research and provide new insights into glycogen storage diseases in Thai children.

We sincerely appreciate the time and effort you dedicated to reviewing our manuscript. Your insightful comments and constructive feedback have significantly improved the quality of our work. We are grateful for your thorough evaluation and thoughtful suggestions, which have guided us in refining our research and enhancing its clarity. Your expertise has been invaluable in helping us present our findings more effectively. Thank you once again for your contributions and for supporting the advancement of our research.

Warm regards,

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