Author’s response to reviews

Title: Novel PYGL mutations in Chinese children leading to glycogen storage disease type VI: two case reports

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Author’s response to reviews:

Letter to the editor and reviewers

We are grateful for the comment of the editor and reviewers. We addressed all the comments. The revised manuscript using track changes has been submitted.

Response to reviewer 1

1. The authors present the cases as having different severity, but the primary difference in the cases is the liver size. The liver size could be a reflection of the diet and carbohydrate intake prior to diagnosis. Otherwise, the patients clinically were similar. In fact, patient 1 (the milder case without hepatomegaly) had worse laboratory studies and growth. The response to treatment was also better for patient 2 in terms of growth. Why did the authors conclude that these children had different phenotypes? Could other factors like diet contributed to some of the differences seen?

A: We thank the reviewer for the comment. Patient 1 (17 months old) was younger than patient 2 (26 months old) when they were referred to our hospital. Children who are younger are supposed to have a greater need for glucose but patient 1 never showed fasting hypoglycemia for several times during hospitalization. The fasting blood glucose value of patient 1 (above 4 mmol/L for
many times) was higher than patient 2 (3.43mmol/L and 2.85mmol/L for twice) even though patient 1 was younger.

Liver size reflects the degree of glycogen storage, although it could also be affected by the diet and carbohydrate intake. It is hard to explain the difference of the AST and ALT between the two patients. The level of AST and ALT could not only be affected the extent of glycogen storage, but also by many other factors, like age, carbohydrate intake, feeding frequency, infection and so on.

In case presentation section, we have clarified the different genetic background of stature. For patient 1, the height of her father is 155 cm (-2.90 SD) and that of her mother is 157 cm (-0.67 SD). The height of patient 1 before treatment was -2.87SD, which was only 0.81SD lower than her targeted height (149.5cm, -2.06SD). While for patient 2, the height of her father is 176 cm (+0.54 SD) and that of the mother is 164 cm (+0.63 SD). The height of patient 2 before treatment was -1.67SD, which was 2.21SD lower than her targeted height (163.5cm, +0.54SD). Therefore, patient 1 was milder than patient 2 in aspect of growth which reflected the long term effect of the glucose level.

As for treatment response, the time length of treatment was different. Patient 1 improved her growth from -2.87 SD to -2.31 SD after 3 months treatment. Patient 2 increased from -1.67 SD to -0.93 SD after 7 months treatment, and the swelling of her liver was relieved. All the biochemical parameters including liver transaminases turned to be normal. We contacted patient 1 in November 2019 and found that her growth increased to -1.81 SD after 7 months treatment. Therefore, we updated the data in this manuscript.

2. The goal of any case report is to provide information that will benefit the field. The novel parts of this article are the lack of hepatomegaly and the new mutations. The lack of hepatomegaly is focused on by the authors, but it is of less significance since GSD VI has always been in the differential diagnosis of GSD VI. Therefore, the authors should but more focus on the significance of the new mutations. Do these mutations have significance for screening? Do these mutations have implications for future interventions like gene editing, gene therapy, or mRNA therapy?

A: We thank the reviewer for the comment. GSD VI is a very rare and usually a relatively mild disorder that presents in infancy and childhood. The variants c.1621-258_2178-23del and c.1832C>T have never been reported in database or in literature. We don’t suggest screening these rare variants of PYGL for their low frequencies.

We agree with the reviewer that mutations like the gross in-frame deletion have implications for further interventions like gene therapy. For example, antisense oligonucleotide-mediated exon skipping is an emerging therapy for DMD patients. Exon skipping can restore the reading frame by removing the mutant exon and/or its flanking exon(s) from the DMD pre-mRNA, leading to truncated, yet functional protein expression as seen in the milder disorder(1).
3. Is type VI rare or under diagnosed in the Chinese population? Were these patients from different locations? How can this help to get other children diagnosed?

A: We thank the reviewer for the comment. GSD VI is a very rare disorder in China. GSD VI patients were under diagnosed due to the relatively mild phenotype and the difficulty of enzymatic assay with liver tissue. These two patients were from different areas in China. Patient 1 was from Guizhou province, which located in the southwest of China. Patient 2 was from Anhui province, which located in the east of China. The charge of WES for a patient in our hospital is ¥3600 (≈500 USD dollars), which is a relatively cost-effective method that could avoid invasive liver biopsy and liver PYGL enzymatic assay.

4. Table 1 requires some explanation. If the liver disease was mild in patient 1, why did the patient have high lactate? Why was the patient with "no hepatomegaly" have higher ALT/AST and triglycerides compared with patient 2. The lab abnormalities have not been addressed. Were total protein and prealbumin concentrations checked? What were the beta-OH-butyrate concentrations?

A: We thank the reviewer for the comment. Usually individuals need to be fasting for over 12h for blood test, which may result in metabolic abnormalities like hyperlactic acidemia and hyperlipidemia. The TG values of the two patients were almost the same (4.39 VS 4.37 mmol/L). The level of AST and ALT could not only be affected the extent of glycogen storage, but also by many other factors, like age, carbohydrate intake, feeding frequency, infection and so on. Total protein and prealbumin were normal and the values were listed in table 1. Beta-OH-butyrate was not tested, but urine ketone of the two patients were negative.

5. The authors state in the background and discussion that the clinical manifestations of GSD VI are similar to GSD I, III, IXa, and XI. I respectively disagree with this statement. GSD I and XI look vastly different from GSD VI. GSD I is much more severe, and does not have ketosis. GSD XI has malabsorption, renal abnormalities, acidosis, and a very different clinical picture.

A: Specialists of GSD can diagnose different types based on laboratory biochemical test and clinical phenotype. GSD I patients are more severe without ketosis. GSD XI patients have malabsorption, renal abnormalities and acidosis. But there are also some similarities among these types in clinical manifestations and differential diagnose can be difficult for general physicians in local hospitals. Patient 1 had transferred to several hospitals and remained undiagnosed before she came to our hospital.

Response to reviewer 3

1. Patient 1 with the deletion displays the typical growth delay and laboratory abnormalities. While the authors claim that the otherwise typical symptom of hepatomegaly was not present, this observation does not justify to call it a mild expression of GSDVI. Neither ultrasound data
are presented nor liver biopsy was performed to assess the extend of changes in the liver (a procedure not necessary after successful genetic and clinical diagnosis).

A: We thank the reviewer for the comment. The liver size reflects the degree of glycose storage, although it could also be affected by the diet and carbohydrate intake. Patient 1 in this manuscript is the only one that suffer from GSD VI without obvious hepatomegaly in our hospital, and she had not been diagnosed before. Besides, hypoglycemia was absent in patient 1. Precisely, the liver size should be measured by MRI which had not been conducted before treatment.

2. WES is compared in the manuscript to TS. However, no details on TS are provided, and it remains unexplained how this form of testing could have missed a homozygous multi-exon deletion. All addressing of this comparison is therefore unnecessary.

A: We thank the reviewer for the comment. We have edited the manuscript in this version.

3. The authors emphasize that they did a SNV and CNV analysis of their WES data sets. This is state-of-the-art and needs no emphasizing, briefly mention variant calling software.

A: We thank the reviewer for the advice. We have edited the manuscript in this version.

4. WES or Clinical ES is in my opinion justified to perform in the case of children presenting with unclear hepatopathies.

A: We thank the reviewer for the comment. The cost of WES here is ¥3600 (≈500 USD dollars), which is a relatively cost-effective method that could avoid invasive liver biopsy and liver PYGL enzymatic assay.

5. The biochemical laboratory parameters were determined at what patient age, and earlier to causal treatment?

A: Patient 1 was 17 months old when she was sent to our hospital and the biochemical laboratory test was determined 3 months earlier (14 months old) in the local hospital. Patient 2 was 26 months old when she was sent to our hospital and the biochemical laboratory test was determined a month earlier (25 months old). The biochemical laboratory parameters were determined before the causal treatment.

6. The manuscript is much too long and there is a great redundancy of data and sentences. A most recent and comprehensive review on the topic should be cited and can replace older references, Prishnani KS et al. Genet Med 2019.
A: We thank the reviewer for the advice. We have cited the review and edited the manuscript in this version.