Peer Review File

Article information: https://dx.doi.org/10.21037/tp-23-164

Reviewer A

This is an interesting study describing the cases of cerebral creatine deficiency syndrome in children in China. Overall, the study is important and raises the importance of detecting cerebral creatine deficiency in children, and the need to manage syndrome with creatine diet (with exception to children with genetic mutation in SLC6A). The manuscript would be stronger if the following questions were addressed or discussed:

1. The authors stated, "The brain MRI showed that 3 patients with suspected CCDS had a thin corpus callosum, 3 had abnormalities in the white matter, 2 had a large regional brain volume, and 1 had cerebral atrophy." However, it was unclear how the investigators made the conclusion. It would be helpful to show MRI images of the corpus callosum or WM abnormalities.
Reply 1: Thank you for your advice and it has been modified as to your advices. The MRI results of these 8 patients are based on the cranial MRI diagnosis results from department of Radiology. Diagnosis of CCDS patients is mainly based on the very low creatine peak by MRS and gene and metabolites results. MRI results are for reference. Some CCDS patients have normal MRI.

2. Children treated with creatine diet displayed variable improvement. Can the investigators speculate why this might be the case? Does age receiving the treatment, and the duration or amount of creatine treatment have a role?
Reply 2: Thank you for your advice and it has been added as to your advices. Yes, you're right, it depends on the age they were treated, how long they've been treated, and how well they've followed the advice. Creatine may be absorbed differently by each patient. We recommend to be followed up regularly to adjust the dose of creatine.

3. Although progress was observed after creatine treatment, it was unclear how progress was defined. For example, what is the difference between progress in movement vs. significant progress in movement. Do the children who show significant improvement after creatine diet perform as well intellectually as children who don't have CCDS?
Reply 3: Thank you for your advice and it has been modified as to your advices. The progress is mainly observed in three aspects: 1 movement, 2 comprehension and communication ability, 3 language ability. Generally, after 2 ~ 4 weeks, there is significant progress in movement, such as strength, ability to walk up stairs, ability to run, significant progress in comprehension and interaction after 1 ~2 months, significant progress in language after 1 ~3 months. The first child with CCDS2 was followed up for 10 years, she performed as well intellectually normal children and went to school normally. The other children with CCDS2 could not be judged now because the follow-up time was not long, but the progress in all aspects was obvious.

Reviewer B

This study by Sun et al relates the first description of a cohort of CCDS patients in China (14 diagnosed CCDS patients out of 3568 children with development delay; on these 14 patients, 6 GAMT-deficient, 8 SLC6A8-deficient, and 0 AGAT-deficient).

This is a nice and useful paper as it extends our knowledge on the ourcome of CCDS and the actual efficacy (or non-efficacy) of their available treatments. The paper is well written and will become acceptable for publication, provided the authors address the following minor points:

- In the abstract, it is written that 3568 children with developmental delay were screened for CCDS, while later in the article (introduction, results) the number of 1586 is written. Which one is correct?
Reply: Thanks for pointing out the error. It is 3568. We have modified our text as advised.

- The prevalence of CCDS in this study is said to be 0.25%. However, 14/3568 leads to 0.39%. => ? ... and it would be even more with 1586 (0.88%).
Reply: Thanks for pointing out the error. Yes, the prevalence is about 1/255, or 0.39%. We have modified our text as advised.

- Results, lines 192-194: which brain abnormalities, for which patient with which diagnostic? => Are there specific abnormalities for GAMT and for SLC6A8 deficiencies?
Reply: Thank you, we added. …The brain MRI showed that 3 patients (patient 3,8,13) with suspected CCDS had a thin corpus callosum, 3 (patient 1,4,13) had abnormalities in the white matter, 3 (patient 2,8,10) had wider gap in outer brain, and 1 had cerebral atrophy (patient 11) …. There are no specific abnormalities for GAMT and for SLC6A8 deficiencies The diagnosis of CCDS is based on the abnormal creatine level by MRS, MRI results are only for reference, many CCDS patients have normal MRI. ….

- Discussion, lines 226-227: Authors write "CCDS ... lead to low Cr levels in the blood, urine and brain." This is not always true. Decreased Cr in blood is true for AGAT and GAMT deficiencies, but is normal generally in SLC6A8 deficiency. In urine, Cr is increased under SLC6A8 deficiency.
Reply: Thanks for pointing it out. You're right. It's been reported in articles that patients with SLC6A8 deficiency have normal creatine level in blood. However, we found there were also decrease in our patients with SLC6A8 deficiency in blood by LC-MS/MS, and also often a significant decrease creatinine in blood.

- Discussion, line 284: authors may quote the review by Fernandes-Pires (2022, Molecular Genetics and Metabolism), which discusses the actual and possible future treatments for CCDS.
Reply: Thank you so much for your advice. It's a good reference and has been added.

Reviewer C

Overall: This manuscript defines broad objectives to comprehensively describe the pediatric presentation of creatine deficiency syndromes in China, However, the diffuse nature is presented with vague details. It is inaccurate in several points and poorly cited. It does not have adequate details about assessment methodologies. The article reads like someone has reviewed patient records and the literature and is having a

conversation with a student highlighting the features instead of producing a well-cited, accurate assessment and description of novel findings of patients with these disorders.

Abstract:
1. The abstract uses the abbreviation of CCDS2. Most readers would not know that this represents guanidinoacetate methyltransferase (GAMT) deficiency. It would be better to define the three distinct diseases that constitute cerebral creatine deficiency syndromes in the manuscript as: 1) creatine transporter deficiency (CTD) or SLC6A8 deficiency, and the biosynthesis disorders: 2) guanidinoacetate methyltransferase (GAMT) deficiency and 3) L-arginine: glycine amidinotransferase (AGAT) deficiency.
Reply: Thanks for your advice. It has been modified as to your advices.

2. In the methods, the cohort is listed as 3,568. Is this a typographical error and should be 1,568?
Reply: Thanks for pointing out the error. It is 3568, have modified.

3. The role of magnetic resonance spectroscopy is not to "diagnose" the patients. It is just one the steps in making the diagnosis. It can confirm that a variant is pathogenic as a functional test. It can also note the creatine deficiency in the brain when a patient presents with developmental delays.
Reply: Thanks for your advices. It has been modified as to your advices.

Highlight Box:
1. The key finding (lines 69-70) should be changed to "While CCDS are rare conditions, our findings may improve understanding of children with developmental delay.
2. Line 77 use "including" instead of "by" proton magnetic resonance spectroscopy.
Reply: Thanks for your advices. It has been modified.

Introduction:
1. The citations selected for reports of CCDS are not appropriate. The first international instances of GAMT, AGAT and SLC6A8 deficiencies should be cited along with large case series. This would include current citations from the bibliography #1, and #26 plus Item CB, Stöckler-Ipsiroglu S, Stromberger C, et al. Arginine: glycine amidinotransferase deficiency: the third inborn error of creatine metabolism in humans. Am J Hum Genet 2001; 69:1127–33; Cecil KM, Salomons GS, Ball WS Jr, et al. Irreversible brain creatine deficiency with elevated serum and urine creatine: a creatine transporter defect? Ann Neurol 2001; 49:401–4.
Reply: Thanks for very much. We have modified and added as your very accurate advices.

2. Line 98. The phrasing of the sentence is awkward. Consider rephrasing to indicate the concept that development delays in CCDS and other disorders are nonspecific and similar in presentation.
Reply: Thanks for your advices. It has been modified.

3. The concept of three CCDS conditions described in lines 101-105 should be presented earlier in the paragraph after either the second or third paragraph. The abbreviations of CCDS1, CCDS2 and CCDS3 should not be used, as describing the condition by the defect makes it more understandable to the reader.
Reply: Thanks for your advices. It has been modified.

4. Line 113: “findings” should be replaced by “analyses”. Our analyses revealed 7 novel genetic variants that expand the knowledge of CCDS.
Reply: Thanks for your advices. It has been modified.

5. Lines 107-114 read as results. The introduction should summarize the known findings of the CCDS as a good amount of work has been published internationally since 2001 for these three conditions. The introduction should then identify the unique aspects of this work. Is there a reason why CCDS are only now being reported in China? Are the Chinese variants distinct from other known variants?
Reply: Thanks for your advices and has been modified. We have introduced the large number of cases in the previous paragraph (>300cases). The last paragraph describes the cases we found and the results of treatment.

6. Lines 114-116 read as conclusions instead of introduction.
Reply: Thanks for your advices. It has been modified.

7. The introduction should define the scope of the work. Why is important to know the incidence, the novel variants, the means for diagnosing the condition, how diagnosis matters, limitations of treatments, etc. Define the goals of the study and why is this work important to report?
Reply: Thanks for a lot. We have modified our text as advised.

Methods:
1. Lines 131-132. Were the methods for assessing Cr and GAA novel or was the analyses based on prior published works? If based on known methods, please cite the methods. Are the methods for dried blood spots and urine the same? If not, describe. Where do normative reference values come from?
Reply: The detection methods of Cr and GAA are based on previously published articles. References have been added. Like other amino acid, all reference values are obtained by our laboratory from a large number of normal samples. Urine is different from dry blood spots, reference has been added, thank you very much!

2. The methods should separate information about patients and their families from that of analytical methods for assessing biochemical specimens, genetics and imaging.
Reply: Thanks for a lot. We have modified our text as advised.

3. How are children diagnosed with developmental delay, intellectual disability, epilepsy, behavior and movement disorders and speech and language disorders, in general? What is the process in China at the authors institution? What assessments are performed?
Reply: Children with developmental delay were diagnosed by the Gesell Developmental Scale, including adaptive behavior, gross motor behavior, fine motor behavior, verbal behavior and individual-social behavior. We have modified our text as advised.

4. Descriptions about genetic analyses generally provide more detail about how specimen collection, storage, materials, approach, etc.
Reply: Thanks for a lot. We have modified our text as advised.

5. Lines 143-148: The description regarding the MRI and MRS examination is completely inadequate. There is no "manufacturer's standard protocol" for MRI and MRS. How were images and spectra acquired, where were spectra obtained in the brain and how were spectra quantified? The inclusion of results in this section is inappropriate. This section should describe how images were reviewed for abnormalities. It should also define how normative spectral levels were obtained.
Reply: Thanks for a lot. We have modified our text as advised. Spectra were obtained mainly from the basal ganglia, thalamus, and white matter. We consider that the main focus of this paper is to summarize and introduce the pathogenesis, diagnosis and treatment of CCDS disease. The well-established detection methods are briefly introduced and will not be described in detail. Spectra were quantified by MRS and had data, all these figures and pictures are not showed, only the most important creatine peak in MRS are showed, considering the length of this paper.

6. Lines 151-160: The treatment includes numbers of patients for two of the three CCDS conditions. It would be appropriate to describe how these protocols were designed and cite published sources of reference for these therapies.
Reply: Thanks for a lot. We have modified our text as advised and add 3 references.

7. How is CCDS incidence determined for your study?
Reply: Thanks for pointing out the error. Yes, the prevalence is about 1/255, or 0.39% (14/3568). We have modified as advised

Results:
1. Lines 172-177: How were the 1,585 narrowed down to 148 and then to 14? The decision algorithm needs to be clearly stated. Was genetic testing performed on all 1,585? How many had MRI with MRS collected?
Reply: Thanks for very much. We have modified and added as your very accurate advices. Yes, genetic testing performed more than on 1585 patients. Some of the 3586 children with developmental delays were first performed by LC-MS/MS, and others were performed as by genetic testing according to the test sheet prescribed by the clinician. They would be verified by genetic testing or LC-MS/MS later, and finally confirmed by MRS.

2. Table 1 was cut off in the pdf making it difficult to read and review. The column with Cr and GAA; can the authors make it clearer which are from the dried blood spots and which are from urine?
Reply: We have modified and added as advices.

3. Line 183: All patients with suspected CCDS had a Cr level below <85 micromole per liter? Even those with SLC6A8 deficiency?
Reply: yes. We know that there are many reports that the Cr levels in blood of CCDS1 patients is generally not abnormal. However, we found that our patients did also decrease in DBS, or it may be a transient decrease, which may just be measured.

4. Line 184: how were Cr, GAA assessed during followup? Which method?
Reply: We have modified and added as advices. At the early of follow-up, blood and urine samples were followed up once in 2-3 months, and brain creatine was followed

up once in half a year. One year later, blood and urine samples were assessed 4 times /year, and the brain creatine was assessed once a year.

5. Which patients were identified for genetic testing?
Reply: All patients with metabolic abnormalities were identified for genetic testing

6. Line 200: GAMT patients had dried blood spots evaluated for follow-up?
Reply: Yes, blood creatine and GAA level of GAMT patients were evaluated for followed up, and also with cerebral creatine by MRS, but less frequently because it's not as convenient and expensive.

7. How were seizures/epilepsy evaluated?
Reply: All patients were evaluated by clinicians (department of Child Care and Neurology) in our hospital. The diagnosis of epilepsy is evaluated by a neurologist with clinical symptoms and abnormal electroencephalogram

8. How were neurodevelopmental outcomes evaluated? Line 206
Reply: evaluated by Gesell Developmental Scale in department of Child Care and Neurology

9. Which anti-epileptic drugs were used? Line 210
Reply: Sodium Valproate.

10. How were motor and cognitive skills evaluated? Line 211
Reply: Motor skills: obvious strength, ability to walk or run and climb stairs, gross motor and fine motor progress. Cognitive skills: significant progress in understanding and communication, able to read children's books, start to speak, etc.

11. Table 2 was cut off in the pdf making it difficult to read.
Reply: We have added as advices.

Discussion:
1. Citations should be included for known biochemistry of creatine and transport.
Reply: We have added as advices.

2. Line 242: N-acetyl asparate instead of N-acetate aspartate
Reply: Thanks, you very much. We have modified and added as advices

3. Line 245: Should you include confounds associated with LC-MS/MS? Dietary issues?
Reply: Thanks, you very much. We have modified as advices

4. Line 275-276: citation for known incidence?
Reply: yes. References 31-33.