Author's response to reviews

Title: Clinical features and gene mutational spectrum of CDKL5-related diseases in a cohort of Chinese patients

Authors:

Zhao Ying (zhaoying2008renji@163.com)
Zhang Xiaoying (zxy269163@163.com)
Bao Xinhua (zwhang@pku.edu.cn)
Zhang Qingping (zhangqp1988@126.com)
Zhang Jingjing (zjj25952@163.com)
Cao Guangna (dudu.laile@163.com)
Zhang Jie (bingling8766@126.com)
Li Jiarui (lijr@mail.cbi.pku.edu.cn)
Wei Liping (weilp@mail.cbi.pku.edu.cn)
Pan Hong (Panmuren@263.com)
Wu Xiru (wwning@bbn.cn)

Version: 2 Date: 26 December 2013

Author's response to reviews:

Dear editor:

Thank you very much for receiving our paper (MS: 1732881438110922). Here is a cover letter giving a point-by-point response to the reviewer’s suggestions.

Comments from Reviewer: nicoletta landsberger
1. The introduction describing symptoms so far associated to CDKL5 is quite poor
   We have added more symptoms in the introduction section.
2. They have to better explain the aim of the work that was to analyze for the first time a cohort of Chinese patients
   We have further explained the aim of the work in the article.
3. The gene/protein nomenclature is not respected (human gene goes in italics and capital letter, the human protein is capital but not in italics)
   We have corrected the spelling mistake of CDKL5 gene and protein
4. The all text contains a huge amount of misspelling and wrong sentences
   We have asked an English speaking person to corrected and modified the languages

Comments from Reviewer: Renzo Guerrini
1. The paper is too long and should be shortened. In particular, the results
section “clinical manifestation” should be made available as online supplemental material. A brief clinical synopsis of the patients should then be added to the same section.

A brief clinical synopsis of the patients was added and put the detail information of the patients as an online supplemental material.

2. Authors use the terms early-seizure of RTT or Hanefeld variant of RTT. However, recent publications have clarified that CDKL5-related disorder should be considered as separate from RTT, rather than another variant.

When the patients were enrolled in this study, they meet the criteria of early-seizure of RTT or Hanefeld variant of RTT. At that time we did not know if they had the CDKL5 gene mutation. Only after the mutations were found in seven of these patients, can the term CDKL5-related disorder be diagnosed in these patients with CDKL5 mutation, and the others still be diagnosed as early-seizure of RTT or Hanefeld variant of RTT. So in the section of patients, we used the term "early-seizure of RTT or Hanefeld variant of RTT".

3. Please add a sentence in the methods section indicating which CDKL5 gene accession numbers have been used to describe the mutations.

We have add the CDKL5 gene accession numbers in the paragraph.

4. “Non-syndrome” should be changed in “non-syndromic”

We have changed the “Non-syndrome” into “unknown epileptic syndrome” as suggested by another reviewer Dr. Alessandra Renieri.

5. The text should be revised for typos and grammatical errors

We have tried to revised them.

6. Patient 7 had two XCI values. Is this correct?

The two XCI values were for Patient 6 and 7, a pair of twins.

Comments from Reviewer: Alessandra Renieri

Major Compulsory Revisions

1. The definition of "non-syndromic EP" was incorrectly used throughout the manuscript. In fact, from a technical point of view, “non syndromic EP” means isolated epilepsy without other signs such as developmental delay o other (stereotypies, etc). I believe that using this term the authors intended to include patients having a complex clinical phenotype including epilepsy and other signs, in which the clinician did not recognized a known syndrome. Therefore, the term "non-syndromic EP" should be replaced with "unknown epileptic syndrome".

1. We have replaced "non-syndromic EP" with “unknown epileptic syndrome”

2. Out of 9 females, six were “a priori” diagnosed as early-seizure variant and three as “non-syndromic epilepsy” (meaning “unknown epileptic syndrome”). I have analyzed very carefully the phenotype of these last 3 patients (Patient n.1, n.3, and n.10). From the description, these 3 patients seem to have a clinical diagnosis of early-onset seizure variant of Rett syndrome as well. Therefore, the authors should be emphasized in the manuscript that, although the clinician who saw the 3 patients did not think of this diagnosis “a priori”, they can be classified
in this type of diagnosis “a posteriori”.

2. Patient n.1, n.3, and n.10 were diagnosed as early onset epileptic encephalopathy when they were first referred to our clinician. Due to their young age, features of early-seizure variant RTT was not so obvious. In the following up assessment, some features of RTT presented. Due to the CDKL5 gene mutations were found in these patients, CDKL5 –related disorder was diagnosed thereafter, so early-seizure variant RTT was not diagnosed.

3. Authors should highlight in the abstract and throughout the manuscript the main results that are:

a) mutations in CDKL5 were found in females with early-onset seizure variant of Rett syndrome diagnosis;

b) mutations in CDKL5 were found in males with early-onset epileptic encephalopathies different from Rett syndrome (this is only one male but the trend is evident).

Additional evidence of this difference is seen in Table 1 in which all females have stereotypes that are instead absent in the only one CDKL5 mutated male.

3. The above points were highlighted in the abstract and manuscript

Minor Essential Revisions

1- paragraph of “Object”: the phrase “have been associated with early-onset epileptic encephalopathies such as infantile spasms, early-onset intractable epilepsy and the Hanefeld variant of Rett syndrome.” should be reworded as follows: “have been associated with early-onset seizure variant of Rett syndrome, also named Hanefeld variant, or early-onset epileptic encephalopathies”.

1. revised.

2- paragraph of “Conclusion”: the phrase “Mutations in CDKL5 gene account for 2.6% “9/71” of 71 girls and 3.2% “1/31” of 31 boys with early-onset epileptic encephalopathies or the Hanefeld variant of Rett syndrome.” should be reworded as follows “Mutations in CDKL5 gene is responsible of early-onset seizure variant of Rett syndrome in girls and of early-onset epileptic encephalopathies such as infantile spasms in males.”

2. revised.

3- paragraph of “Methods”: authors should replace “MRI” and “EEG” with “magnetic resonance imaging” and “electroencephalogram”. The use of abbreviations in abstract is not appropriate.

3. We have replaced “MRI” and “EEG” with “magnetic resonance imaging” and “electroencephalogram”

INTRODUCTION

1. Reference n.1 should be replace with a paper describing clinical criteria of the variant such as Artuso R. et al. Brain Dev. 2010 Jan;32(1):17-24.

2- Reference n.2 is mentioned in inappropriate manner with respect to the sentence to which it refers, therefore it is recommended replacing it with reference n.15.

2- As we added more descriptions of syndromes associated to CDKL5-related disorder as suggested by another reviewer nicoletta landsberger, Reference n.2 is another reference in accord with the added content. 

3- The phrase “The early-seizure of RTT, initially described by Hanefeld in 1985, showed overlapping phenotype with early-onset epileptic encephalopathies.” should have as reference the Hanefeld’s paper.

3- We have reworded the phrase “The early-seizure of RTT, initially described by Hanefeld in 1985, showed overlapping phenotype with early-onset epileptic encephalopathies.” with “Accumulated evidence showed that phenotype of CDKL5-related disorders overlaps with early-onset seizure variant of RTT (RTT, OMIM 312750) and early-onset epileptic encephalopathies with X-linked infantile spasms (ISSX, OMIM 308350)”, so references are changed.

4- Please enter appropriate citations to the following sentence “The initial genetic screening of CDKL5 mutations were performed in a cohort of patients with early-onset seizure variant of RTT without MECP2 mutation and infantile spasms with some Rett-like features.”, such as Scala E. et al. J Med Genet. 2005 Feb;42(2):103-7, and Mari F. et al. Hum Mol Genet. 2005 Jul 15;14(14):1935-46.

4- We have cut out the sentence mentioned above

5- last sentence of first paragraph: “drug-resietant” should be changed in “drug-resistant”.

5- We have cut out the sentence containing the phrase “drug-resistant”.

6- last sentence of first paragraph: add a comma after “months of life”.

6- we have modified the content of the first paragraph

METHODS
1- Describe the used sequencing method
1. We have described the used sequencing method in this section.

RESULTS
1. In “CDKL5 gene mutations”: “one (2.77 %, 1/36) cases were diagnosed with infantile spasms” should be changed in “one case was diagnosed with infantile spasms”.

1- In “CDKL5 gene mutations”: “one (2.77 %, 1/36) cases were diagnosed with infantile spasms” have been changed in “one case was diagnosed with infantile spasms”.

2- In “Clinical manifestation”, the authors should describe for each female patient what kind of stereotypes is present.

2- In “Clinical manifestation”, we have described for each female patient what kind of stereotypes is present.
3- In “Clinical manifestation”, add the actual head circumference percentiles for each patient (female or male). This should also be added in Table1.

3- In “Clinical manifestation”, we have added the actual head circumference percentiles for each patient (female or male). Due to it is measured at different age, we did not put them in the table so as to avoid misunderstanding.

4- In “Clinical manifestation”: the authors should describe for each patient the mutations found.

4- In “Clinical manifestation”: we have described for each patient the mutations found.

5. The authors should collect additional Rett signs such as feeding difficulties, sphincter control, IQ, gastrointestinal disturbances, bruxism, eye pointing capability, and breathing dysfunction. This may help readers in understanding the difference between females and male.

5- We have put some additional Rett signs such as swallowing difficulties, bruxism, eye pointing capability, and breathing dysfunction in the clinical manifestation.

6- In “Patient 6 and Patient 7”, it is not clear to me what means “elder” and “younger” in twin sisters.

6- As a pair of twins, the first one who were born was the elder, the second one was the younger.

DISCUSSION and CONCLUSIONS

1- Discussion and Conclusions should be completely rewrite highlighting the fact that CDKL5 mutations are responsible of a variant of Rett syndrome in females and a slightly different condition in males, where Rett syndrome characteristics are more difficult to recognize.

We have revised the discussion and conclusion section and highlighted the fact that CDKL5 mutations are responsible of a variant of Rett syndrome in females and a slightly different condition in males.