Author’s response to reviews

Title: Growth Data and Tumour Risk of 32 Chinese children and adolescents with 45, X/46, XY mosaicism

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Author’s response to reviews:

Reply to the reviewer’s comments,

Reviewer 1:

1. The first sentence in the paper is not correct: 'The 45, X/46, XY mosaicism is considered to be a rare congenital malformation' - abnormal karyotype is not a 'malformation' (some patients with low percentage aneuploidy do not have malformations).

   Reply: Thank you for your comment; we have made the sentence more rigorous. This sentence was changed to: 'The 45, X/46, XY disorder of sex development (DSD) is a rare congenital malformation'.

   Background section, line 2, page 4, we replaced mosaicism with disorder of sex development (DSD).

2. The authors state in the 'Patients' section that they included patients with 'aberration of their Y-chromosome'. I see in Table 1 only one patient with i(Y) in the karyotype. Were there any other aberrations? There is no description how these 'aberrations' were diagnosed. Was a
molecular screening for Y-chromosome deletions performed? This information should be added to the method description.

Reply: Thank you for your comment. We performed only karyotyping. We apologize for the lack of further information about Y-chromosome deletions.

3. The authors stated on page 5 that the pubertal staging was performed according to the criteria of Tanner but cited a paper (ref. 4) which describes pubertal changes in boys. A reference to puberty staging in girls or a general reference to both genders should be added.

Reply: Thank you for your comment. We changed the reference to one describing pubertal stages in both genders. The reference is Emmanuel M, Bokor BR. Tanner Stages. Statpearls[Internet]. Treasure Island (FL): StatPearls Publishing; 2019-2019 Jan 19.


4. In the description of immunohistochemistry (page 6) positive and negative controls ought to be mentioned.

Reply: Thank you for your comment. We have added the description of positive and negative controls: 'a positive result evidenced by a brown, negative lack of brown staining.'

Surgery and histology section, line 1, page 6: We have removed 'a positive result evidenced by a brown nuclear signal', and added 'a positive result evidenced by a brown, negative lack of brown staining.'

5. Table 1 should list the height SDS and info on the tissue source - have there been biopsies or gonadectomies (or both) performed in each patient?

Reply: Thank you for your comment. Table 1 lists the height SDS and information on the tissue source; please see new Table 1.

6. Table 1 lists only gonadoblastoma (GB) as pathology, but in Table 2, in the summary of the findings included as the last study, PTL+GB is listed in 7/24 patients. Please explain what it is meant here?
Reply: Thank you for your comment. We now show only GB calculations.

7. The legend to Figure 2 should describe what is the histology of each image, especially that some pictures are pale. I can see that G and H show gonadoblastoma, but it is not clear what is shown in other images, especially A and B. In addition, the legend states that 'negative for OCT3/4 have 'blue nuclear signal' - blue colour is counterstaining and not a 'signal'. Correct to 'lack of red staining' or just remove the phrase.

Reply: Thank you for your comment. Figure 2 shows only the results of OCT3/4 immunohistochemistry. A detailed description of gonadoblastoma is shown in Figure 3. A and B are the images of OCT3/4 immunohistochemistry of two patients’ (No. 21 and 31) gonadal tissues; they lack red staining, indicating negative staining. Thank you for your guidance on the interpretation of the OCT3/4 results. Thank you for your suggestion on OCT3/4, we chose to remove the 'blue nuclear signal'.

In the legend of Figure 2, we removed 'blue nuclear signal'.

8. In Figure 3, image A supposedly shows 'normal testicular tissue'. What was the age of the patients who had this specimen. It looks a bit like germ cell neoplasia in situ (GCNIS) previously known as CIS testis. Was this specimen stained for OCT3/4? The last sentence of the figure legend contains errors: 'The nests were encapsulated by granular cells /Sertoli cells'. The nests were not 'encapsulated (there is no capsule) and granular cells = granulosa cells.

Reply: Thank you for your comment. We have corrected the figure and the legend. Please see new Figure 3.

9. Table 2 lists among 'Nations' both 'America' (not a nation) and 'USA'. Please explain and correct.

Reply: Thank you for your comment. We have corrected the table.

Rafael Fabiano Machado Hado Rosa (Reviewer 2): The study is quite interesting and shows important results, which add to the knowledge about this patients with 45,X/46,XY mosaicism. I have only a few suggestions to make. I suggest removing the space that exists in the description of the karyotypes, that is, all numbers and letters must be together: 45,X/46,XY. In the text, I suggest removing ",0" from the values that describe it. For example, remove the ",0" from 50.0% and leave 50%. Although the text is generally well written, it could have an improvement on
some points. In some places some word or article seems to be missing. In Table 1, I suggest removing the % of the described values from the cell lines and placing it as the top of the column - Karyotype (%/%). I think the article should be accepted and published after these minor corrections.

Reply: Thank you very much for your comment. We removed the space that exists in the description of the karyotypes; all numbers and letters are together. We removed ".0" from the values that described percentages. We removed the % and placed it at the top of the column [Karyotype (%/%)].

Mona Mekkawy, M.D, PhD (Reviewer 3): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Reviewer Comments:

1- In introduction section, the second line, the authors wrote:

[So far, more than 500 mosaicism patients have been reported].

I think this is not a precise estimate, the actual number could be much more than 500.

Reply: Thank you for your comment. We have removed this sentence.

2- In the methods, the statement: (Karyotyping was performed in lymphocytes isolated from peripheral blood, using routine G-banding) is very brief. It should be described in a little more details.

Reply: Thank you for your comment. We added the detail that Karyotyping was performed on cultured blood lymphocytes arrested in the mitosis phase and stained with Giemsa dye. We performed only karyotyping. Sorry about no further information about Y-chromosome deletions can be added.

Karyotyping section, line 41, page 4: We have removed 'Karyotyping was performed in lymphocytes isolated from peripheral blood', added 'Blood sample (0.3-0.5 ml, heparin anticoagulation) was added into cell culture medium (Dubai Biomedical Co., Ltd., Guangzhou, China). Dolchicine was added into the culture medium at 69 hours. Culture was carried out for
72 hours. Karyotyping were performed on cultured blood lymphocytes arrested in the mitosis phase and stained with Giemsa dye.'

3- Kindly follow the ISCN recommendations in writing the karyotypes.
Reply: Thank you for your comment. We have made some changes accordingly.

4- (ISCN 2016) reference should be added to reference list.
Reply: Thank you for your comment. We have added the reference.

1. Karyotyping section, line 4, page 5: We have added the reference: [4].


5- Language editing is required.
Reply: Thank you for your comment. We had the language edited by American Journal Experts.

6- The writing of patients' results should be written in a more clear concise manner.
Reply: Thank you for your comment. We have rewritten the results in a more clear and concise manner.

7- In the section of pubertal development and HPG axis evaluation, the authors divided the patients into two groups of 9 patients and 13 patients, please clarify the cause of this division.
Reply: Thank you for your comment. We apologize for not stating this clearly. The 9 patients could be diagnosed with hypergonadotropic hypogonadism according to basal FSH levels (>40 IU/L). Evaluations of the 13 patients were performed according to GnRH and HCG stimulation assays.

8- In the discussion: the sentence:
(Tumor risk in patients with female phenotype was reported to be much less than ambiguous phenotype) lacks a reference

Reply: Thank you for your comment. This reference for this sentence is 31, and it was added after the sentence.

9- In discussion, I think there is no need to write the sentence: (Gonadal tumor risk of children and adolescents may be higher than we imaged). As stated by Looijenga et al. (2007), the application of OCT3/4 and TSPY markers, although informative in diagnosis of adult male patients, it might result in over diagnosis of tumor risk as it cannot easily distinguish premalignant germ cells from germ cells showing delayed maturation which is frequently found in gonads of DSD patients.

Reply: Thank you for your comment. We have removed the sentence.

10- The reference writing inside text should be revised.

Reply: Thank you for your comment. We have revised the references one by one.