Peer Review File

Article Information: http://dx.doi.org/10.21037/tp-21-26

Reviewer Comments

The study reported in your manuscript addresses an interesting topic, but the manner of presentation raises objections as follows.

1. **First of all, the genotype notation of the patient in this article is inaccurate. The mutation of *FGD1* gene in a male patient cannot be homozygous or heterozygous because *FGD1* is located on X chromosome (Male has only one X chromosome.). "Hemizygous" is correct. Please check your article: "heterozygous mutation c.500delA" (line 46), "homozygous mutation c.500delA" (line 89), "homozygous mutation of c.500delA" (line 151) and "homozygous" (line 178).**

Reply 1: Thank you for your correction. We have modified our text as advised (see line 46, 89, 159 and 188).

2. **Second, please use the words "*FGD1*" and "FGD1" properly. *FGD1* represents gene and FGD1 represents protein. For example, FGD -> *FGD1* (line 50). *FGD1* -> FGD1 (line 79). *FGD1* -> FGD1 (Line 84). *FGD1* -> FGD1 (Line 195). FGD1 -> *FGD1* (Line 207). FGD1 -> *FGD1* (Line 229).**

Reply 2: Thank you for your correction. We have modified our text as advised: *FGD1* -> FGD1 (see line 79, 84, 174, 200 and 205), FGD1 -> *FGD1* (see line 63, 217 and 239).

3. **Line 63-64: Please quote the sentence from the reference accurately, or the meaning will be different from the original paper. "Approximately 2 to 3 new AAS patients are diagnosed each year" -> "2–3 new patients with a proven mutation in the *FGD1* gene per year."**

Reply 3: We have modified the sentence according to the reference (see line 63).

Changes in the text: 2–3 new patients with a proven mutation in the *FGD1* gene are diagnosed each year, with a maximum incidence estimated around 1/25000.

4. **Line 64-67: The authors describe the growth defects (from fetal period) with AAS in the introduction. Please show the patient' s birth weight, birth height and growth curve in the case presentation.**

Reply 4: In the introduction section of our article, we did learn that some children with AAS showed growth defects from fetal period by reading the references. In our case, we reported that the birth height and weight of this AAS patient were slightly lower than the normal average range. However, the patient's growth curve was not obtained because the patient's parents did not record his height and weight annually. We added some data about the patient' s birth weight and birth height (see line 97-98).

Changes in the text: He was born with full-term delivery, birth weight 2500g, birth length 48cm, and he had no suffocation after birth.

5. **Line 86-87: "AAS can also be inherited in an autosomal dominant or an autosomal recessive mode (8)." …There is no description in Reference No.8.**

Reply 5: We have added new references (reference 7-8).

Changes in the text:

7. van de Vooren MJ, Niermeijer MF, Hoogeboom AJ. The Aarskog syndrome in a large family, suggestive for autosomal dominant inheritance. Clinical genetics 1983;24:439-45.
8. Teebi AS, Naguib KK, Al-Awadi S, et al. New autosomal recessive faciodigitogenital syndrome. Journal of medical genetics 1988;25:400-6.

6. **Line 87-88: "different modes of inheritance have been reported even with one pedigree." …Please write the source (reference).**

Reply 6: We have added a new reference (reference 9).

Changes in the text:

9. Xu M, Qi M, Zhou H, et al. Familial syndrome resembling Aarskog syndrome. American journal of medical genetics Part A 2010;152A:2017-22.

7. **Line 96: What are the complications associated with a previous orchiopexy? Please describe specifically.**

Reply 7: We have added the complications associated with the previous orchiopexy (see line 100-103).

Changes in the text: Five years ago, the child was found to have empty scrotum on both sides and testicles were not touched. Orchiopexy for bilateral cryptorchidism was performed at the Second Hospital of Qinghai Province. Prior to the second surgery, the child was found the both testicles retreated and the left testicular atrophy.

8. Line 103-104: I cannot see the joint laxity from the figure 2. Please describe the assessment of joint laxity (such as Beighton score or other method).

Reply 8: Thank you for your comments and corrections. We have removed the description of joint laxity under figure 2 and corrected it with the stubby phalange. In addition, we took your suggestion and added a separate Beighton score to evaluate the laxity of joints (see Table 3).

Changes in the text:

Table 3. The Beighton score of hypermobility.

Description	Scoring
Passive dorsiflexion of the fifth metacarpophalangeal joint to ≥ 90 degrees	2
Passive hyperextension of the elbow ≥ 10 degrees	2
Passive hyperextension of the knee ≥ 10 degrees	2
Passive apposition of the thumb to the flexor side of the forearm, while shoulder is flexed 90 degrees, elbow is extended, and hand is pronated	0
Forward flexion of the trunk, with the knees straight, so that the hand palms rest easily on the floor	1
Total	7

Not hypermobile (0-4); Increased mobility (5-6); Hypermobile (7-9).

9. **Line 105-106: The authors describe that the data of the patient were consistent with male hypergonadotropin hypogonadism's characteristics. Did you check the testis size, texture (hard or soft), LHRH test, or hCG test?**

Reply 9: We have added the results of bilateral testicular palpation and B-ultrasound (see line 104-106). Because the patient's parents did not cooperate, LHRH test and hCG test were not performed.

Changes in the text: The right testicle can be touched on the right groin, with 2.7×0.9×0.8cm approximately. The left atrophied testicle was on the left groin, which was difficulted to touch. The texture of the testicles on both sides was hard.

10. **Methods: Do the authors always perform Sanger sequencing after WES? Please explain why you perform Sanger sequencing after WES.**

Reply 10: Thanks for your question. Sanger sequencing has low pollution, intuitive results, and extremely low false results. Sanger sequencing is the gold standard including common PCR, fluorescent quantitative PCR, second-generation sequencing, mass spectrometry and other methods. The positive results obtained by second-generation sequencing must be verified by Sanger sequencing.

11. **Results: It would be easy for the readers to understand, to describe "results of WES" and "results of Sanger sequencing".**

Reply 11: We have rewrite the results as "results of WES" and "results of Sanger sequencing" (see line 158-166).

Changes in the text:

Results of WES.

The patient's *FGD1* gene (transcript number: NM_004463.2) has a hemizygous mutation of c.500delA in exon 3 (the 500th nucleotide A in the coding region of the *FGD1* gene is deleted), which makes the frameshift mutation occurred after tyrosine at position 167 (p.Y167fs).

Results of Sanger sequencing

The subsequent Sanger sequencing confirmed the presence of this variant in the patient. Besides, Sanger sequencing results revealed the patient's mother to be heterozygous for the same variant. This mutation does not exist in the *FGD1* gene of the subject's father (Fig. 3).

12. **Line 154-155: Please use the same terminology, not "Sanger sequencing" and "direct sequencing", for the readers easy to understand.**

Reply 12: We have changed "direct sequencing" to "Sanger sequencing" (see line 165).

13. **Line 173: Please show source (reference) for the sentence "only 20% of patients have been determined to carry pathogenic mutations in *FGD1*."**

Reply 13: We have modified the sentence (see line 182-184) and added a new reference (reference).

Changes in the text:

Mutations in the *FGD1* gene are currently the only known genetic cause of AAS, although only about 20% of Aarskog families have been determined to carry pathogenic mutations in *FGD1*

located in Xp11.21 (14).

14. Verhoeven WMA, Egger JIM, Hoogeboom AJM. X-linked Aarskog syndrome: report on a novel FGD1 gene mutation. Executive dysfunction as part of the behavioural phenotype. Genetic counseling (Geneva, Switzerland) 2012;23:157-67.

14. Line 180-181: The reference No.13 does not represent "similar frameshift mutations in *FGD1* are recorded in the clinVar database". Please attach the proper reference.

Reply 14: Thanks for your suggestion. We have removed the reference No.13. For more specific answers, please see Reply 15.

15. Line 182: I cannot find the reference for pathogenic mutations at amino acid 705 and 713. Please show the reference.

Reply 15: Thank you for your opinion. First of all, I would like to describe the steps I used to get the results using the clinVar database. We entered the NCBI database's home page, select the subpage of the clinVar database. Then we searched for "*FGD1*(gene)" and selected the frameshift mutation type in all search results. The clinVar database contains a total of 12 reported frameshift mutations in the *FGD1* gene. It could be clearly seen that similar frameshift mutations in *FGD1* are recorded in the clinVar database, including the pathogenic or likely pathogenic mutations at amino acid residues 176, 177, 298, 316, 705, and 713 (all of which occur after Tyr167). Regarding the reference for pathogenic mutations at amino acid 705 and 713, please check the following two references. (From the reference link of the cinVar database)

705: National Center for Biotechnology Information. ClinVar; [VCV000095087.2], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000095087.2 (accessed Feb. 28, 2021).
713: National Center for Biotechnology Information. ClinVar; [VCV000095088.2], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000095088.2 (accessed Feb. 28, 2021).

16. Line 197: The mutation of the patient was maternally inherited. Did the mother show some clinical signs such as short stature, delayed puberty or mental disorder?

Reply 18: We have added content about family history (line 98-100). The patient's parents are short in stature and have no mental illness. There is no record of the parents' puberty.
Changes in the text: There is no family history of genetic disease. The parents are in good health and have no special facial features. The patient's father was 168cm in height and the mother was 155cm.

17. Line 247-249: Please delete the sentence "The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved." because the sentences are duplicated.

Reply 17: Thank you. We have removed the repeated sentences.

18. Line 249: Did the authors acted a trial? If not, delate.

Reply 19: We have not conducted a clinical trial and the related statement has been deleted.