Author’s response to reviews

Title: Prune Belly Syndrome in Surviving Males can be caused by Hemizygous Missense Mutations in the X-linked Filamin A Gene

Authors:

Nida Iqbal (nida.iqbal3@gmail.com)
Thomas Jascur (tjascur@yahoo.com)
Steven Harrison (steven@broadinstitute.org)
Angelena Edwards (Angelena.edwards@utsouthwestern.edu)
Luke Smith (Luke.Smith@utsouthwestern.edu)
Erin Choi (Erinchoi123@utexas.edu)
Michelle Arevalo (Mkarvelol@gmail.com)
Catherine Chen (Catherinej.chen@utsouthwestern.edu)
Shaohua Zhang (shaohua.zhang@utsouthwestern.edu)
Adam Kern (adamjkern@gmail.com)
Angela Scheuerle (angela.scheuerle@utsouthwestern.edu)
Emma Sanchez (emma.sanchez@childrens.com)
Chao Xing (chao.xing@utsouthwestern.edu)
Linda Baker (linda.baker@childrens.com)

Version: 1 Date: 24 Oct 2019

Author’s response to reviews:

October 8, 2019

BMC Medical Genetics
Dear Sir/Madam:

We would like to thank the reviewers for their thoughtful reviews and helpful points to improve our manuscript. We have submitted a revised manuscript and below address the individual reviewer’s comments in red.

Reviewer 1 (Lyuba Dineva Miteva, PhD)

METHODS:

1. Since X-chromosome inactivation results are given in Supplementary Figure 1 I recommend the subheadings X-chromosome inactivation to give briefly in the figure's legends and erase From Methods section. We agree and have made this change, moving to Supp Fig 1.

2. The subheading "FLNA expression studies" should be changed as gene expression studies, with respect to ITGB1 expression analysis. We agree and have made this change (p4, line 137). How many samples /biological repeats/ were included in FLNA expression studies? We purchased from Biochain. Each tissue type includes samples from multiple adult donors (pooled) and was run in triplicate. This was added to the manuscript (p4, line 138).

3. Please, explain why you perform gene expression assays across normal adult human RNA samples (to corroborate with the mouse fetal expression patterns) and Immunohistochemistry on bladder tissue isolated from normal pediatric PBS-unaffected individuals? Don't you expect the age-related or disease-related differences? Yes, we wanted to investigate this possibility. Do you have information about FLNA expression across PBS cases? Unfortunately, we do not have bladder tissue samples from any of the four males with FLNA missense mutations for FLNA IHC testing. We have performed IHC on 3 other PBS human bladders without FLNA mutations and have detected no significant change in FLNA protein immunostaining, which is not unexpected as our FLNA mutations biologically are gain of function and thus might not disturb FLNA protein quantity.

We have added to page 6, line 263-266:
“Although we did not have bladder biopsies from the four PBS males described in this report, FLNA bladder IHC on 3 other PBS cases of undiagnosed molecular cause revealed no significant change in FLNA protein immunostaining (data not shown), which is consistent with gain of function FLNA mutations that do not disturb FLNA protein quantity (see discussion).”

RESULTS:

4. Please, arrange the results section to achieve logical and easier reading. Figures and Tables should be given in consequent order. Changed on (p4-5, multiple lines).

5. Please, limit the discussion elements in the results. Agree and done (p4-7, multiple lines).

DISCUSSION:

6. The discussion should be shortened and focused. Discussion has been shortened and focused(p7-9, multiple lines).

7. The limitations of the study should be included. Done and thank you (p9, lines 403-407).

8. A reference list should be shortened and in the order that they occur in the text. Done- thank you (p11-16).

9. List of abbreviations should be in alphabetical order. Done- thank you (p9-10, multiple lines).

* Minor point: Use double line spacing in preparing main manuscript text. Will do in the future- thank you.

Kentaro Mizuno, Ph.D., M.D. (Reviewer 2): General comments:

The genetic cause of Prune Belly Syndrome (PBS) remains unknown, and authors focused on candidate genes fit an X-linked recessive mode of inheritance. In this study, authors have identified three hemizygous mutations (p.Cys2160Arg, p.Ala1448Val, p.Gly2236Glu) in FLNA gene in one multiplex kindred and two sporadic PBS males using whole exome sequencing. Beside, authors have also shown distribution of FLNA protein and disruption of binding to integrin. This is the first evidence for an X-linked cause of PBS in multiple unrelated individuals and expands the phenotypic spectrum associated with FLNA in males surviving even into adulthood.
Specific comments:

This paper is well-written, structured article. Although PBS is rare disease, authors enrolled individuals with PBS and their family. Authors' efforts brought the excellent result, and this article would be one of the most important in the research field of PBS hereafter. Thank you for the kind words. No changes to the manuscript.

Kirsten Y. Renkema (Reviewer 3): Iqbal and coworkers report on the identification of hemizygous FLNA gene variants in male Prune Belly Syndrome (PBS) patients who survive into adulthood. The evidence shown for a causal relationship between PBS and FLNA gene variants includes 1) the identification of overlapping FLNA gene variants in two half-brothers with PBS by whole exome sequencing, 2) the identification of two additional PBS patients with FLNA gene variants, 3) functional characterization of the variants that shows enhanced binding of FLNA to ITGβ1. I recommend a more in-depth discussion on genotype-phenotype correlation by connecting what is known from patients described previously to the results of the current study. Done (p7-8, lines 324-329)

Minor comments

1. Discussion: Please, elaborate more on whether you see a clear genotype-phenotype correlation between FLNA variants and the different related phenotypes. How could the diversity in phenotypes related to FLNA variants be explained? The discussion section contains a lot of information on patients previously described. How does this information connect to your findings specifically? Please, connect the literature to you own findings in male PBS patients who survive into adulthood. Added (p7-8, lines 324-329):

Overall, there is not a clear genotype-phenotype correlation when these old published cases are added to our series, other than the fact that most MNS have FLNA exon 22 mutations within Ig10. As FLNA Ig10 is quite remote from the FLNA Ig19-21 domain, it is biologically unclear how missense mutations in Ig10 yield the obstructive uropathy phenotype. As these genotypic differences exist and since the PBS phenotype due to deleterious FLNA mutations is seen with or without OPDSD, we prefer to segregate our PBS phenotype from the MNS phenotype, as depicted in Figure 5A.

2. Page 8, line 9: 'Not only does our work add to the population of surviving males with (...)'. What is meant here? What does the work add exactly? Please, clarify. We have rephrased this section (p7, lines 313-317).

3. Methods: Page 4, line 20: please add units (e.g. degrees Celsius) to indicated 37 and 65. This was shortened and moved to Supplemental Figure as recommended by Reviewer 1.
4. Methods: Page 4, line 42: explain how the normal pediatric PBS-unaffected individuals were recruited for bladder tissue isolation. Added thank you

Bladder biopsy was obtained from consenting normal pediatric individuals at the time of bladder surgery to correct refluxing ureters (p 4, lines 146-147).

5. Page 6, line 60: the authors indicated Figure 3. Please, be more precise on the contents of Figure 3A and/or 3B? Corrected, thank you (p 6, line 260).

Thank you for the secondary review and for the opportunity to improve our manuscript.

Sincerely,

Nida Iqbal Karra, PhD

Linda A. Baker, MD
Professor of Urology
Director of Pediatric Urology Research
University of Texas Southwestern Medical Center
Pediatric Urologist, Children’s Health