Hypomorphic and hypermorphic mouse models of *Fsip2* indicate its dosage-dependent roles in sperm tail and acrosome formation

Xiang Fang, Yaser Gamallat, Zhiheng Chen, Hanran Mai, Pei Zhou, Chuanbo Sun, Xiaoliang Li, Hong Li, Shuxin Zheng, Caihua Liao, Miaomiao Yang, Yan Li, Zeyu Yang, Caiqi Ma, Dingding Han, Liandong Zuo, Wenming Xu, Hao Hu, Ling Sun and Na Li
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Original submission

First decision letter

MS ID#: DEVELOP/2020/199216

MS TITLE: Hypomorphic and hypermorphic mice models of Fsip2 indicate its dosage-dependent roles in sperm tail and acrosome formation

AUTHORS: Xiang Fang, yaser gamallat, Zhiheng Chen, Hanran Mai, Pei Zhou, Chuanbo Sun, Xiaoliang Li, Hong Li, Shuxin Zheng, Caihua Liao, Miaomiao Yang, Yan Li, Zeyu Yang, Caiqi Ma, Dingding Han, Liandong Zuo, Wenming Xu, Hao Hu, Ling Sun, and Na Li

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some concerns which need to be addressed prior to consideration of the manuscript for publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost

in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors identified mutation in FSIP2 in infertile men. They created a KI mice model with this mutation and show that these KI mine presented the typical MMAF phenotype. It was also shown altered expression of genes related to sperm tail, acrosome and spermatide developement. Proteomic analysis revealed changes of many proteins at fibrous sheath,mitochondria and the acrosome. These new information will lead us to understand the role and mechanism of action of FSIP2 in the future.

Comments for the author

1. Fig.1A and 5A: 3-5 cells should be shown in each picture.
2. Fig.5D : A new WB should be presented since the beta-actin band is also higher than the control.

Reviewer 2

Advance summary and potential significance to field

The present study reports findings regarding a homozygous truncating mutation of the FSIP2 gene in a Multiple Morphological Abnormalities of Sperm Flagella (MMAF) patient through whole-exome sequencing (WES). The author generate a knock-in mice model, Fsip2-KI that resembles MMAF phenotype mutations. In addition, they generate mutation the Fsip2-OE (overexpression) mice model which exhibits a longer sperm flagella when compared to wild type (WT) mice. The authors show that proteins associated with fibrous sheath, mitochondrial sheath and acrosomal vesicle are affected by truncation and overexpression of Fsip2. Notably, the authors find that only two C-terminal motifs of Fsip2 are conserved in species with internal fertilization. They conclude their results contribute to explain how the Fsip2 protein regulates sperm flagella generation and acrosome formation.

Overall this work is carried out in a careful and adequate manner and is a very interesting and relevant to the field of sperm physiology and reproduction. It contributes to a better understanding of the complex architecture and workings of the sperm flagella.

Comments for the author

There are some issues that must be dealt with before the paper is published that are detailed bellow. In addition, though I include the pdf with many suggested corrections in color, the paper should be read by somebody that is very familiar with English.

Detailed comments:
1) On page 4, around line 28; should it be 10 mM?

2) In the beginning of the Results section it is mentioned that the pH of the patient’s semen is altered but it is not included in the table and not found. I guess it is the external pH.

3) The title after Table 2 is not clear regarding longer, longer than what?

4) The rewrite the phrase “The differentially expressed genes between the 4 subclusters of Round spermatid of the OE and WT mice were identified, which consisted of 257 (156 upregulated and 101 downregulated), 1111 (512 upregulated and 599 downregulated), 626 (365 upregulated and 261 downregulated) and 1062 (622 upregulated and 440 downregulated) genes, respectively. Fsip2 was

upregulated in all subclusters of Round spermatids. Gene Ontology (GO) enrichment analysis indicated that the upregulated genes were involved in sperm flagellum and motile cilium (Figure 4E), in agreement with the longer principal pieces and midpieces observed in the OE mice sperm (Figure 3C).”; it Is not clear at all.

5) In general, it is not helpful for the reviewer not to have the Figures numerated.

6) In Fig. 5B which is which? The results stated by the authors in Fig. 5C are not clear at all and not quantified.

7) Figure 6C should be described in more detail.

8) Immunostaining looks clear in Fig 7 but the authors should show quantitative data, one cell is not enough.

First revision

Author response to reviewers' comments

Reviewer 1 Comments for the author
1. Fig.1A and 5A: 3-5 cells should be shown in each picture.
Reply: Thanks for this suggestion. We showed more cells in Fig.1A and 5A.

2. Fig.5D: A new WB should be presented since the beta-actin band is also higher than the control.
Reply: Thanks for this suggestion. We presented a new WB result in Fig. 5D.

Reviewer 2 Comments for the author
There are some issues that must be dealt with before the paper is published that are detailed bellow. In addition, though I include the pdf with many suggested corrections in color, the paper should be read by somebody that is very familiar with English.
Reply: We have made the modification accordingly to the corrections in color. The paper was also edited for proper English language at LetPub. Thank you very much for your help.

Detailed comments:
1) On page 4, around line 28; should it be 10 mM?
Reply: Thanks for this suggestion. Yes, it should be 10 mM.

2) In the beginning of the Results section it is mentioned that the pH of the patient’s semen is altered but it is not included in the table and not found. I guess it is the external pH.
Reply: Thanks for this suggestion. We added the pH values of semen in the Table 1.

3) The title after Table 2 is not clear regarding longer, longer than what?
Reply: Thanks for this suggestion. We rephrased this sentence: "The flagella of the OE mice sperm show longer principal pieces and midpieces relative to those of the WT mice".

4) The rewrite the phrase “The differentially expressed genes between the 4 subclusters of Round spermatid of the OE and WT mice were identified, which consisted of 257 (156 upregulated and 101 downregulated), 1111 (512 upregulated and 599 downregulated), 626 (365 upregulated and 261 downregulated) and 1062 (622 upregulated and 440 downregulated) genes, respectively. Fsip2 was upregulated in all subclusters of Round spermatids. Gene Ontology (GO) enrichment analysis indicated that the upregulated genes were involved in sperm flagellum and motile cilium (Figure 4E), in agreement with the longer principal pieces and midpieces observed in the OE mice sperm (Figure 3C).”; it Is not clear at all.
Reply: Thanks for this suggestion. We revised Fig. 4E, which showing the GO enrichment of the 4 subclusters of Round spermatid of the upregulated genes of the OE mice compared with the WT mice.

We also rephrased the sentences: “We identified the differentially expressed genes between round spermatids of the OE and WT mice. Round spermatid 1 consisted of 156 upregulated and 101 downregulated genes; round spermatid 2 consisted of 512 upregulated and 599 downregulated genes; round spermatid 3 consisted of 365 upregulated and 261 downregulated genes; round spermatid 4 consisted of 622 upregulated and 440 downregulated genes. Fsip2 was upregulated in all subclusters of Round spermatids. Gene Ontology (GO) enrichment analysis indicated that the upregulated genes of the Round spermatid 1, 2, 3, and 4 of the OE mice were involved in sperm flagellum and motile cilium (Fig. 4E), in agreement with the longer principal pieces and midpieces observed in the sperm from the OE mice sperm (Fig. 3C).”

5) In general, it is not helpful for the reviewer not to have the Figures numerated.
Reply: Thanks for this suggestion. We numerated all the Figures.

6) In Fig. 5B which is which? The results stated by the authors in Fig. 5C are not clear at all and not quantified.
Reply: Thanks for this suggestion. We rephrased the figure legends for Fig. 5B: “(B) Duolink In situ assessment reveals the physical interaction between Fsip2 and Acrv1. The left panel and right panel represent two WT sperm. The DAPI (the top panel), the PLA signal (the middle panel) and the merged view (the bottom panel) are shown. Nuclear marker DAPI is in blue and proximity ligation assay (PLA) signal is in red (scale bar, 5 μm).” In addition, a diagram showing the Proximity Ligation Assay was added below the results.
We also revised the Fig. 5C. The top panel showed the tissue immunostaining of the WT, Fsip2-KI and Fsip2-OE mice; the bottom panel showed the corresponding enlarged views. The white arrows marked the Acrv1 staining. The quantitative data of Acrv1 was indicated by the western blot results in Fig. 5D.

7) Figure 6C should be described in more detail.
Reply: Thanks for this suggestion. We rephrased this sentence: “Twelve fibrous sheath-associated proteins, 9 mitochondrial sheath-associated proteins and 32 acrosomal vesicle-associated proteins, which were downregulated in the KI mice and upregulated in the OE mice simultaneously when compared with the WT mice are indicated in Fig. 6C, accounting for 46.2% (12/26), 34.6% (9/26), and 43.8% (32/73) of known fibrous sheath-, mitochondrial sheath-, and acrosomal vesicle-associated proteins, respectively.”

8) Immunostaining looks clear in Fig 7 but the authors should show quantitative data, one cell is not enough.
Reply: Thanks for this suggestion. We revised Fig. 7, which showed multiple cells. Fig.7 was incorporated into Fig. 6 (as Fig. 6E); the quantitative data were indicated by the western blot results in Fig. 6D.

Second decision letter

MS ID#: DEVELOP/2020/199216

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My apologies on the delay in the review time. I have received the review from one reviewer, who continues to be in support of the study. However, there are two issues that need to be addressed before I can take a decision to accept the manuscript for publication. First, the data in Fig. 2 is qualitative and needs to be supported by quantitative analysis, and second, Acrv1 in Fig. 5A is not

visible, and a much better image (or more the one) need to be presented. In addition, the reviewer has made extensive textual suggestions (attached) to better clarify the study.

Reviewer 2

Advance summary and potential significance to field

In general the authors have answered most questions and carried out the suggestios. The manuscripts was significantly corrected. There are a few points I will mention but after correcting them the paper could be published.
Though the pictures in Fig. 2 are fine, they show a single sperm, it certainly would be good to show a summary either in bars or box plots.
Frankly speaking I ccould not see Acrv1 in Fig. 5A. The authors have to supply a better image. I tried in my computer and my Ipad and failed. Neither PLA in B.
On page 17 please see the change "5463 aa truncation". This sentence is not clear to me: is the truncation of 5463 aa, or at that site?
There are some suggested corrections in blue, I will attach the pdf file.

Comments for the author

Notes made directly on attached manuscript PDF file.

Second revision

Author response to reviewers' comments

Reviewer 2 Advance summary and potential significance to field...

In general the authors have answered most questions and carried out the suggestions. The manuscripts was significantly corrected. There are a few points I will mention but after correcting them the paper could be published. Though the pictures in Fig. 2 are fine, they show a single sperm, it certainly would be good to show a summary either in bars or box plots. Frankly speaking I could not see Acrv1 in Fig. 5A. The authors have to supply a better image. I tried in my computer and my ipad and failed. Neither PLA in B. On page 17 please see the change "5463 aa truncation". This sentence is not clear to me: is the truncation of 5463 aa, or at that site?
There are some suggested corrections in blue, I will attach the pdf file.
Reply: Thank you very much for all these suggestions.
(1) We showed a bar plot (Fig. S1) to indicate the fluorescence intensity of Fsip2 of the WT, KI and OE mice sperm in Fig. 2D.

(2) Some details of the Figure. 5A and 5B were lost when converted to a single pdf file; the signals of Acrv1 and PLA were visible in the Fig 5. tif. For Fig 5A, we also provided a close-up view to indicate the Acrv1 signal.

(3) The patient has a non-sense mutation p.E5463Efs*7, which means the effective length of FSIP2 after truncation is 5463 aa.

(4) We revised the manuscript according to the corrections in blue.

Third decision letter

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.