Tracheal motile cilia in mice require CAMSAP3 for formation of central microtubule pair and coordinated beating

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Corresponding author(s): Masatoshi Takeichi, RIKEN

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Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E21-06-0303
TITLE: "Tracheal motile cilia in mice require CAMSAP3 for formation of central microtubule pair and coordinated beating"

Dear Masatoshi,

Thank you for submitting your manuscript for our consideration at MBoC. It has now been seen again by the same reviewers who saw it for Review Commons. They are broadly supportive, but you will note that Reviewers 2 and 3 have some final points of clarification, which I am confident you and your colleagues can address. So, I'd invite you to submit a final revision that addresses these points. If possible, I'll endeavour to render the decision myself without needing to consult the reviewers again. It would help me if the revised MS could highlight your revisions in a font of a different colour.

I look forward to seeing your revision soon.

Best wishes,

Alpha

Alpha Yap
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Takeichi,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager

MBoC Editorial Office
mbc@ascb.org

Reviewer #2 (Remarks to the Author):

The authors have addressed most of my comments satisfactorily. The following are some minor points that the authors should clean up to improve the manuscript.

1) P4. "Recent studies identified "basalin' localized at the basal plate, and its depletion resulted in disruption of the basal plate as well as collapse of CP (Dean et al., 2019)"

Maybe a few words to explain basalin would be beneficial.

P5. "epithelial cells of mice through the action of the CC1 domain of the protein, tethering non-centrosomal microtubules to these sites, and this results in the assembly of"

What is the CC1 domain? Is it equivalent to the C-terminal CKK domain? Maybe a bit explanation would help.

2) P5. "Superresolution microscopy revealed that a fraction of CAMSAP3 accumulates around the sites where the CP is supposed to be initiated."

Super-resolution microscopy

3) P6. "Camsap3dc/dc (Camsap3 mutant) mice frequently produced clicking or chattering sounds(Audio 1), suggesting that they might have respiratory problems."

Characterization of respiratory problems is far from sufficient. PCD mouse usually develop rhinitis, sinusitis and otitis media. The paper spends much time discussing Camsap3 and PCD, however the phenotype characterization is not sufficient.

4) P8. "These observations suggest the possibility that an outer microtubule doublet was abnormally internalized at upper regions of the axoneme, as observed in PCD (Burgoyne et al., 2014)."

"as observed in certain PCD cases"

5) P8. "but was often sticking out of this BP structure by 20 nm or so"

It is unclear how the 20 nm is obtained. What is the standard deviation? Sample size?

6) P8. "Axonemes in Camsap3-mutated cells appeared to have a similar zone, except that microtubules were not always detectable in the BP of mutant samples, as seen in upper axonemal zones (Figure 2D right, dark arrow)."

Where is the dark arrow? Is this referring to the black arrow or another arrow?

7) P10. "which can yield a 140 nm lateral resolution."

Which can yield around (or approximately) 140 nm lateral resolution

8) P11. "The distances (mean {plus minus}
SD) between #1 and #2 and between #2 and #3 along the longitudinal axis were 476 {plus minus}
126 nm (n = 30) nm and 364 {plus minus}
47 nm (n = 34), respectively."

Delete second nm "(n=30) nm..."

9) P11. "the distances (mean {plus minus}
SD) between g-tubulin and the #1, #2 or #3 CAMSAP3 were estimated at 962 {plus minus}
130 (n = 25), 592 {plus minus}
89 nm (n = 18) and 240 {plus minus}
37 nm (n = 19), respectively"

insert nm after 130

10) P13 "we overlaid their positions on its ultrastructural image,"

Add "TEM" before "ultrastructural image"

Reviewer #3 (Remarks to the Author):

The authors have clearly made extensive efforts to address my concerns, particularly those concerning quantitation and statistical support. It seems that this has given rise to some minor changes to the conclusions, but overall still supports their main conclusions.

My key concern about novelty has been somewhat alleviated by the improved superresolution microscopy analysis. The improvement of these areas adds significant value. My concerns about quantitation have largely been addressed, with more clarity about sample sizes, more quantitation and more statistical support.

I have two lingering concerns:

Re. CAMSAP3 antibody. The authors did not answer my question: I could not see any evidence (eg. uncropped Western blot) that the CAMSAP3 antibody is specific, which is critical when being used for immunofluorescence. Tanaka et al. 2012 claims in the methods that it is specific, but I could not find the evidence for this. Similarly, in this manuscript, I can't find any evidence. This is a key control important to justify the conclusions.

Re. Cytoplasmic microtubule distribution in the CAMSAP3 mutant. The authors state that "this is a negative data" so did not do any "further analysis". To me, this is a key control which is necessary to disentangle any cytoplasmic vs. ciliary function of CAMSAP3. Hiding data in the supplementary and claiming that a result is negative is not the same as analysing it properly to _show_ that a key control is negative.

Authors' response to the reviews for the manuscript #E21-06-0303

Our point-by-point responses to each comment (in italic) are explained below.

Reviewer #2:

The authors have addressed most of my comments satisfactorily. The following are some minor points that the authors should clean up to improve the manuscript.

Response: We appreciate the comments from this reviewer, which are very helpful in improving the manuscript.

1) P4. "Recent studies identified "basalin' localized at the basal plate, and its depletion resulted in disruption of the basal plate as well as collapse of CP (Dean et al., 2019)"

Maybe a few words to explain basalin would be beneficial.

Response: The sentence was re-written as below:

"Recent studies identified a protein named "basalin' through the analysis of a trypanosome TZ proteome, which is localized at the basal plate, and its depletion resulted in disruption of the basal plate as well as collapse of CP (Dean et al., 2016; Dean et al., 2019), suggesting that this structure is important for CP nucleation."

P5. "epithelial cells of mice through the action of the CC1 domain of the protein, tethering non-centrosomal microtubules to these sites, and this results in the assembly of"

What is the CC1 domain? Is it equivalent to the C-terminal CKK domain? Maybe a bit explanation would help.

Response: "CC" represents "coiled-coiled". The sentence was re-written as below:

"epithelial cells of mice through the action of the CC1 domain, a coiled-coiled region of the protein, tethering non-centrosomal microtubules to these sites, and this results in the assembly of"

2) P5. "Superresolution microscopy revealed that a fraction of CAMSAP3 accumulates around the sites where the CP is supposed to be initiated."

Super-resolution microscopy

Response: Corrected.

3) P6. "Camsap3dc/dc (Camsap3 mutant) mice frequently produced clicking or chattering sounds(Audio 1), suggesting that they might have respiratory problems."

Characterization of respiratory problems is far from sufficient. PCD mouse usually develop

rhinitis, sinusitis and otitis media. The paper spends much time discussing Camsap3 and PCD, however the phenotype characterization is not sufficient.

Response: We had no particular intention of relating the *Camsap3* mutation phenotypes to PCD. This sentence was rewritten as below:

"Camsap3$^{dc/dc}$ (*Camsap3* mutant) mice frequently produced clicking or chattering sounds (Audio 1), prompting us to examine whether the respiratory system has any problem."

4) P8. "These observations suggest the possibility that an outer microtubule doublet was abnormally internalized at upper regions of the axoneme, as observed in PCD (Burgoyne et al., 2014)."

"as observed in certain PCD cases"

Response: Corrected as suggested.

5) P8. "but was often sticking out of this BP structure by 20 nm or so"

It is unclear how the 20 nm is obtained. What is the standard deviation? Sample size?

Response: We added a sample number (n = 25). In this observation, it was difficult to measure the real length of the structure, as the sections were randomly tilted. Therefore, we did not perform statistical analysis to obtain a mean value and SD. Instead, we presented the maximum value as the most reliable information. To make this point clearer, the sentence was re-written as below:

"but was often sticking out of this BP structure by approximately 20 nm at maximum"

6) P8. "Axonemes in Camsap3-mutated cells appeared to have a similar zone, except that microtubules were not always detectable in the BP of mutant samples, as seen in upper axonemal zones (Figure 2D right, dark arrow)."

Where is the dark arrow? Is this referring to the black arrow or another arrow?

Response: We meant "dark" as "black". This was corrected.

7) P10. "which can yield a 140 nm lateral resolution."

Which can yield around (or approximately) 140 nm lateral resolution

Response: Corrected as suggested.

8) P11. "The distances (mean {plus minus}SD) between #1 and #2 and between #2 and #3 along the longitudinal axis were 476 {plus minus}126 nm (n = 30) nm and 364 {plus minus}47 nm (n = 34), respectively."

Delete second nm "(n=30) nm..."

Response: Deleted.

9) P11. "the distances (mean {plus minus}SD) between g-tubulin and the #1, #2 or #3 CAMSAP3 were estimated at 962 {plus minus}130 (n = 25), 592 {plus minus}89 nm (n = 18) and 240 {plus minus}37 nm (n = 19), respectively"

insert nm after 130

Response: Inserted.

10) P13 "we overlaid their positions on its ultrastructural image,"

Add "TEM" before "ultrastructural image"

Response: Added.

Reviewer #3:

I have two lingering concerns:

Re. CAMSAP3 antibody. The authors did not answer my question: I could not see any evidence (eg. uncropped Western blot) that the CAMSAP3 antibody is specific, which is critical when being used for immunofluorescence. Tanaka et al. 2012 claims in the methods that it is specific, but I could not find the evidence for this. Similarly, in this manuscript, I can't find any evidence. This is a key control important to justify the conclusions.

Response: In our past papers, we showed that the same anti-CAMSAP3 antibody as used in the current study did not detect any significant immunofluorescence signals in cultured cells whose CAMSAP3 was depleted by siRNA (Figure S2B, Tanaka *et al.* 2012, PNAS, 109:20029-34), as well as in the cryosections of epithelial tissues derived from *Camsap3*-null mutant mice (Figure S2d, Mitsuhara *et al.* 2021. Sci Rep, 11:5857), whereas this antibody consistently detected CAMSAP3 clusters in untreated cells or wild-type tissues. Such previous results warrant the specificity of this antibody in recognizing CAMSAP3 proteins at least when it is used for immunofluorescence staining. These are cited at the section of “Antibodies” in Materials and Methods in the revised manuscript.

Re. Cytoplasmic microtubule distribution in the CAMSAP3 mutant. The authors state that "this is a negative data" so did not do any "further analysis". To me, this is a key control which is necessary to disentangle any cytoplasmic vs. ciliary function of CAMSAP3. Hiding data in the supplementary and claiming that a result is negative is not the same as analysing it properly to _show_ that a key control is negative.

Response: We are thankful for this reasonable remark. In the present study, we judged that the observations on cytoplasmic microtubules are not crucial in making our conclusion that

CAMSAP3 is important for CP formation, therefore having moved the data to supplemental information in response to the Reviewer #2's recommendation. For addressing the point raised by the reviewer, we would like to find another opportunity to statistically confirm our observations on cytoplasmic microtubules, particularly when data of such analysis are of critical importance in exploring the potential role of CAMSAP3 in the cytoplasm.

RE: Manuscript #E21-06-0303R
TITLE: "Tracheal motile cilia in mice require CAMSAP3 for formation of central microtubule pair and coordinated beating"

Dear Masatoshi,

Thank you for addressing these final comments of the reviewers. I think that everything is in reasonable order, so am delighted to accept your manuscript for MBoC.

Best wishes,
Alpha

Alpha Yap
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Takeichi:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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