Peer Review File

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Reviewer A

Comment 1: lines 23-24: non motile cilia can be mobile (in this case the movement is passive). Such is the case for the great majority of primary cilia.
Reply 1: We agree with the reviewer that the movement of the great majority of primary cilia is passive.
Changes in the text: We have modified our text as advised (see line 21-22).

Comment 2: The cerebral cortex is composed of the most superficial region of grey matter located underneath the pia. So, stricto sensu, progenitors are not located in the cerebral cortex but in the telencephalon.
Reply 2: We are very sorry for our incorrect writing of localization of progenitors. Our purpose is to show that the distribution of primary cilia is broad.
Changes in the text: We have deleted “in the human cortex” in our text as advised (see line 142).

Comment 3: In the case of mutation of CPAP, it seems that the salient problem is a problem of mitosis due to an impairment of centrioles and not a problem of cilia. Such mechanisms should be distinguished otherwise confusion will generate controversies.
Reply 3: We said that "CPAP is required for centriole biogenesis, necessary for the symmetric and asymmetric divisions in the cerebral cortex" is to illustrate the background of CPAP. Actually, CPAP provides a scaffold for the cilium disassembly complex (CDC), playing roles in the neurogenesis and brain size control. (PMID: 26929011)
Changes in the text: So as to avoid confusion, we have re-written this part according to the review's suggestions (see line 162-163).

Comment 4: The problem of mutant mice (Ftm, Inpp5…) is due to a defect of ventro-dorsal polarization (explaining that Gli3R is able to correct the phenotype). Such an impairment is easily explained by the role of signal transduction played by the cilia. This is not discussed by the authors.
Reply 4: In this part, we aimed to show that the mutation of different ciliopahty gene cause different phenotypes. And we will discuss the signal transduction in detail later in the article.
Changes in the text: We have changed our text as advised (line 190-192).

Comment 5: I have never seen OFD1 patients with cerebellar agenesis. Cerebellar hypoplasia, vermian dysplasia are commonly observed but not cerebellar agenesis. What are authors’ references?

Reply 5: We are very sorry for we make the mistake as confusing the conception of cerebellar agenesis and cerebellar hypoplasia.
Changes in the text: We have corrected our text as advised (see line 127).

Comment 6: I think that this paper should be re-written to analyse the complex subject of this problem. So, I recommend major revision before accepting this review.
Reply 6: We would like to thank the reviewer for your insightful comments. And we have redesigned some framework of this review. We moved the description of ciliopathies to the section after "Ciliary systems" as it provides evidence of the vital function of primary cilia in biological process that present with brain specific phenotypes (see line 112-138). We have reduced the section of "Ciliopathies" to keep this part short and concise (see line 239-293). In the part of "the roles of primary cilia in cortical development", we have made the data cited to be explained clearly and cohesively by stating how the gene/protein normally works then how disruption (mutation/ knockout/etc) to the gene/protein causes dysfunction (see line 169-171, 180-181, 185-188, 204-207, 248-250, 253-254, 263-264, 268-269). Since a major goal of the review is to describe the cilia related genes/proteins that have an impact on cortical development, we have added a column to Table 2 that briefly describes the impact of the gene/protein on cortical development.

Reviewer B
Comment 1: It is better to highlight and emphasize the recent progresses more that have been achieved since following review articles had been published: Youn and Han, Am J Pathol 188,11-22, 2018; Gabriel et al., Front Cell Neurosci, 14, 115, 2020.Otherwise, readers can learn a few things from this review.
Reply 1: We have searched the relevant literatures for the past 2 years, and combined them with the content already in this paper. Actually, there are only several relative researches discovered recently. Thus, we try to connect new progresses with what we have introduced.
Changes in the text: We have added some recent progresses in the paper (see line 143-148, 174-179, 235-237, 285-289)

Comment 2: A part of "3.3 Mediating signaling pathways" and "3. Ciliopathies" are better to presented as general information between the section 2 about primary cilia structure and the section 3 about primary cilia in cortical development. An idea is that line 216-235 and the majority of line 269-304 can be moved. In the section about primary cilia roles in cortical development, the authors focus on the nervous system that includes the brain as well as the cortex.
Reply 2: Considering the reviewer's suggestion, we have moved the description of ciliopathies up to the section after "Ciliary systems" as it provides evidence of the vital function of primary cilia in biological process that present with brain

specific phenotypes. We have reduced the section of "Ciliopathies" to keep this part short and concise. And we think the part of "3.3 Mediating signaling pathways" is one of the most important parts in "the roles of primary cilia in the cortical development". Thus, we keep the whole part still there to maintain continuity of content.
Changes in the text: We have modified the part of "ciliopathies" and presented it between the section 2 about ciliary system and the section 3 about primary cilia in cortical development (see line 112-138). We also have added some new literatures associated with this part (see line 285-289) and some background of cilia-related genes/proteins. (see line 248-250, 253-254, 263-264, 268-269).

Comment 3: The first part of the introduction is confusing and, actually, not precise. Not all cilia are called "anntennae". Ependymal cilia do not remove dust; they propel CSF. If the authors present "general cilia", they also mention fallopian tube. To be frank, the authors recommend the authors to focus on only primary cilia from the beginning to prevent readers from "getting lost".
Reply 3: It is really true as the reviewer suggested that we should focus on only primary cilia from the beginning to highlight our topic.
Changes in the text: We have deleted the section about the motile cilia (see line 21-23).

Comment 4: Figures 1 and 5 should show already published data. The photographs are the authors' original data, aren't they?. If so, the data are not guaranteed at all especially because the authors have NO publication about the field. If the authors want to show photographs of fluorescent immunostaining, they need to reprint published data of the others with appropriate approval from the publishers. The reviewer, though, thinks that the photographs are not necessarily required for this review.
Reply 4: Yes, these photographs are our original data. As suggested by the reviewer, we will delete the original data.

Comment 5: The transition zone is does not lie "between" the axoneme and the basal body. The axoneme and the basal body are fully contiguous. There are no gap between the axoneme and the basal body. The TZ should be a "zone" around the bottom of the axoneme that is just above the basal body.
Reply 5: We thought wrongly that the TZ lies between the axoneme and the basal body. We have made corrections according to the reviewer's comments.
Changes in the text: We corrected the text (see line 47-48).

Comment 6: The subtitle of the section 2 (in the manuscript, labeled as 1 in a wrong manner) is not appropriate. The section describes not only the structure but also IFTs and dynamic behaviors of primary cilia. Another phrase is better.
Reply 6: It is really true as the reviewer suggested that the subtitle of the section 2 is not appropriate.

Changes in the text: We changed another subtitle (see line 42).

Comment 7: The statement "The ciliary membrane is a lipid bilayer derived from Golgi-associated vesicles and connects with the cell membrane" (line 56-67) requires references.
Reply 7: We apologize for our omission, and we have added the reference.
Changes in the text: We added reference in line 54.

Comment 8: The description "rich in multiple signaling receptors, such as hedgehog (Hh), and Wnt" (line 59) is not correct, and should be rephrased. Hh and Wnt are not receptors. They are ligands.
Reply 8: Tanks for correcting our wrong expression. We have had rephrased the sentence.
Changes in the text: see line 241-243.

Comment 9: In line 95, the phrase "in a stress state" is not clear. No literature is cited. No example is presented.
Reply 9: "in a stress state" means stimulation with serum.
Changes in the text: We have modified our text as advised (see line 91).

Comment 10: In line 145, "2-dimensional (2D) result" is difficult for readers. The authors probably intend to state "results acquired in 2-D culture system".
Reply 10: We are very sorry for our poor expression. That is what we mean.
Changes in the text: We have modified our text as advised (see line 167).

Comment 11: The numbering of sections and subsections are often mistakenly labeled.
> line 46: 1 -> 2
> line 115: 2 -> 3
> line 269: 3 -> 4
> line 306: 4 -> 5
Reply 11: We are very sorry for our negligence of numbering.
Changes in the text: We have modified our text as advised (see line 42, 66, 112, 140, 295).

Comment 12: The manuscript requires English editing. Followings are some errors or mistakes the reviewer found out.
> line 75: interesting -> interestingly
> line 171: In the experiment that knocked down 30 cilia-related genes -> in the experiment where 30 cilia-related genes were knocked down
Reply 12: We are very sorry for our poor English expression. We have had our paper professionally edited by a service. We hope this manuscription can give you a different feeling.
Changes in the text: We have modified our text as advised (see line 71 and 202).

Reviewer C

Comment:

• Line 21 delete “vividly” and change “looks like” to “resembles”
• Line 26 change “;” to “, while”
• Line 40 delete “relevant”
• Line 46: “1. The structure of primary cilia” should be “2.” The numbering of the subheadings should be corrected throughout the manuscript.
• Line 61-62 change “we have not known its ultrastructure clearly until recently” to “its ultrastructure was unknown until recently”
• Line 62-66 I think should be broken up into two sentences – too confusing
• Line 72 add “and” before “motor proteins”
• Line 89 replace “accompanied” with “regulated”
• Line 92 change “participants” to “participates”
• Line 109 change wording of “more and larger”
• Line 110 delete “like”
• Line 139 add “that” before leads
• Line 151 delete “that”
• Line 173 add and “s” to “disrupt”
• Line 175 change “their cell bodies strangely distributing” to “abnormal distribution of their cell bodies”
• Line 183 add “s” to “lead”
• Line 184 change “fail” to “failure”
• Line 188 delete “show”
• Line 194 change “basic” to “necessary”
• Line 211 delete “maybe” and change to “these methods can possibly support”
• Line 222 add “and is” between “family,” and “extensively”
• Line 294 change “which” to “and”
• Line 306 change “concluding” to “conclusion”
• Line 314 add an “s” to “pathway” change “locate” to “are located”
• Lines 319-320 reword “are required to further investigation”

Reply: We are very sorry for our poor English expression. We have already had our paper professionally edited by a service. And we have redesigned some framework of this review. We moved the description of ciliopathies to the section after “Ciliary systems” as it provides evidence of the vital function of primary cilia in biological process that present with brain specific phenotypes (see line 112-138). We have reduced the section of "Ciliopathies" to keep this part short and concise (see line 239-293). In the part of “the roles of primary cilia in cortical development”, we have made the data cited to be explained clearly and cohesively by stating how the gene/protein normally works then how disruption (mutation/ knockout/etc) to the gene/protein causes dysfunction (see line 169-171, 180-181, 185-188, 204-207, 248-250, 253-254, 263-264, 268-269). Since a major goal of the review is to describe the cilia related genes/proteins that have an impact on cortical development, we have added a column to Table 2 that briefly describes the impact of the gene/protein on cortical development. We

hope this manuscription can give you a different feeling. Again, we are very sorry about that.

Reviewer D

Comment 1: Despite being a review on the cortex, some of the most compelling evidence for the role of cilia in the cortex is left to the end when ciliopathies are discussed. Indeed, EM work was critical but so was the identification of cilia-associated genes in causing human disease. The whole review would make more sense if the description of ciliopathies were moved up to the section after "the structure of primary cilia" as it provides evidence of the vital function of primary cilia in biological process that present with brain specific phenotypes. Reference to Table 2 should be made in this section also. Finally, this would set the authors up as they discuss the mouse data to explain why the organoid system would be useful.

Reply 1: It is really true as the reviewer suggested that we should move the description of ciliopathies to the section after "the structure of primary cilia". Severe brain phenotypes in human diseases show the indispensable functions of primary cilia in the developing cortex to us. It is natural for the roles of cilia to be introduced in the next part. Reference to Table 2 is made in this section also. We put the discussion of organoid system later.

Changes in the text: We have modified our text as advised (see line 112-138). And we also explain the reasons why organoid systems would be useful later in this text (see line 174-179).

Comment 2: The authors should expand the section on "the roles of primary cilia in cortical development" and include a "roadmap", a general description of what will be described in the following sections and refer to Table 1 which summarizes the data discussed.

Reply 2: We have tried our best to expand the section on "the roles of primary cilia in cortical development". We added some new literatures related to this part. we have made the data cited to be explained clearly and cohesively by stating how the gene/protein normally works then how disruption (mutation/ knockout/etc) to the gene/protein causes dysfunction. We also added a "roadmap" in the first paragraph in this part and refer to Table1.

Changes in the text: We have modified this part as reviewer suggested. Some new literatures (see line 143-146, 235-237), a "roadmap" (see line 146-148), refer to Table 1 (see line 148-149).

Comment 3: At present, too many facts are stated more like a list, as a series of sentences, than as a fact supported by data. The data cited should be explained clearly and cohesively by stating how the gene/protein normally works then how disruption (mutation/ knockout/etc) to the gene/protein causes dysfunction. There are too many examples where the authors attempt to explain

BOTH at once or explain the later without the former. There are exceptions; lines 140-143 provides a clear and cohesive explanation of the data. In contrast, lines 158-161 does not provide a clear explanation of the data, also see lines 228-230. This is a pervasive issue for which we cannot compile all examples. The authors should revise so that the data cited throughout the manuscript support a fact or assertion.
Reply 3: The reviewer is correct. We should provide both the gene/protein normally works and how disruption to the gene/protein causes dysfunction to make text clear.
Changes in the text: Considering the reviewer's suggestion, we have added clear explanation of the data (see line 169-171, 180-181, 185-188, 204-207, 248-250, 253-254, 263-264, 268-269).

Comment 4: Since a major goal of the review is to describe the cilia related genes/proteins that have an impact on cortical development, the authors should add a column to Table 1 that briefly describes the impact of the gene/protein on cortical development. Furthermore, this table needs to accurately cite the relevant references. At present, it is unclear whether the references are in regards to the function (as listed in the Table) or the cortical phenotype so a column stating the cortical phenotype will clarify.
Reply 4: We have added a column to Table 1 and accurately cited the relevant references according to the reviewer's comments.
Changes in the text: Please see Table 2 Summary of the genes/proteins associated with ciliopathies discussed in this review, with a short description of their main known functions and impact on cortical development.

Comment 5: There are a few points that are not factually accurate. For example, in line 249-251 the author acknowledges the controversy surrounding the relationship between primary cilia and Wnt signal transduction. However, in line 216-218 the authors state that Wnt receptors are on the ciliary membrane. The authors assert that the ciliary membrane is derived from Golgi-associated vesicles (line 57) with no citation.
Reply 5: Wnt receptors are actually on the ciliary membrane. Wnt signaling pathway contains canonical Wnt (Wnt/β-catenin) and non-canonical Wnt (planar cell polarity). The relationship between primary cilia and canonical Wnt signal transduction is controversial. Because some found that defective cilia do not affect it, while ohters found a passive association between cilia and canonical Wnt. And non-canonical Wnt has been reported to have a positive relationship with cilia. We aimed to point out the controversy between cilia and canonical Wnt, deeper researches needed to be done.
Changes in the text: We have made a few other additions to clear up misunderstandings (see line 274) and added citation about ciliary membrane (see line 54).

Comment 6: In the conclusion, the authors express that studies of brain organoids will be a powerful approach to get answers to pertinent next questions. Throughout the manuscript the data described primarily utilized mouse models. Can the authors elaborate on why organoid systems would supersede mouse models?
Reply 6: Recent studies revealed the outer subventricular zone (oSVZ) which contain IPCs and outer radial glia cells (oRGs). Interestingly, oRGs abundantly occur in human cerebral cortex with limited presence in rodent cortex. Zhang et al used brain organoids to model human brain and identified oRGs in week 12 organoids (PMID: 31197141). And we explain why organoid systems are superior to mouse models (see line 174-179). We also think cerebral organoids bridge the gap between mouse models and human diseases to investigate disease mechanisms. But organoid systems are expensive and still being explored. Conversely, mouse models are well known to us and easier to obtain than the former. We can combine both of them to discover next questions.
Changes in the text: We have explained why organoid systems supersede mouse models in model human diseases (see line 174-179).

Comment 7: The manuscript requires careful editing for syntax and odd word choice. For example, "destroyed, destruction, damaged" are used many times to describe abnormal or aberrant situations.
Reply 7: We are very sorry for our poor English expression. We have tried our best to modify this text.

Comment 8: Hnn was referred to as a gene in line 183. hnn is the null phenotype of the gene Arl13b.
Reply 8: It is an important comment. We made the mistake. Hnn is an ENU-induced mutation that responsible for the hnn phenotype disrupts Arl13b.
Changes in the text: We have improved our description as advised (see line 214).

Comment 9: Line 110, delete "In a word", no single word is used thereafter
Reply 9: We feel sorry for our poor English expression.
Changes in the text: We have modified our text as advised (see line 107).