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Peer Review File

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Reviewer Comments

This is an interesting case of the proband and her aunt, the first with a proven KCNJ5 somatic mutation APA, and the second with (apparently) unilateral nodular adrenal hyperplasia. The latter appears not to have undergone adrenal venous sampling, so the decision for unilateral surgery was undertaken on the evidence of CT - probably reasonably if it applied to the proband, less so in her aunt.
In a revised version the authors should address the following points, minor first and then major:

Comment 1: The English of the paper is often non-idiomaytic.ungrammatical, and occasionally not understandable. Before submission thee aurhors need to have a native English-speaking colleague copy edit the draft to address this issue;

Reply 1: We thank the reviewer for this important comment. Now, we have re-read the whole manuscript carefully and corrected the typographical and grammatical errors. In addition, the manuscript was also revised with the assistance of medical writing service (Am J Expert).

Comment 2: Lines 34-35 are not easy to understand; need recasting;

Reply 2: We thank for the reviewer's comment. We have modified the previous sentence "Multiple-gene panel analysis was performed on DNA samples from resected adrenal lesion and peripheral blood of the proband. The suspected variant was verified by Sanger sequencing in parallel DNA samples of her maternal aunt." into follows: "Multiple-gene panel analysis was applied in both resected adrenal lesions and peripheral blood of the proband to screen potential genetic variants. Then, the detected variant was verified by Sanger sequencing in her maternal aunt." (page 2, lines 31-34)
Changes in the text has been marked with yellow. (page 2, lines 31-34)

Comment 3: On immunohistochemistry, what did the aunt's adrenal show?

Reply 3: The HE staining of the aunt showed nodular hyperplasia of the adrenal cortex. We have added the following information in the legend of figure 3: "Hematoxylin and eosin (H&E) stain of paraffin embedded resected adrenal tissue showing nodular hyperplasia of the adrenal cortex." (page 14, lines 290-292)
Changes in the text has been marked with yellow. (page 14, lines 290-292)

Comment 4: Line 53 - this sentence is absolutely wrong. Both resistant hypertension and hypokalemia account for only ~10% of primary aldosteronism;

Reply 4: We agree with the reviewer that this information needs correction. We have modified the previous sentence "Its major clinical manifestations include resistant hypertension and hypokalemia." into follows: "Its typical clinical features include resistant hypertension and hypokalaemia" (page 3, lines 52-53)
Changes in the text has been marked with yellow. (page 3, lines 52-53)

Comment 5: Line 62 - this is not true of all cases of FH-III; some are mild and responsive to MRAs.

Reply 5: We agree with the reviewer's comment. Therefore we have modified our manuscript as follows:" The phenotype of FH-III shows variability due to different KCNJ5 mutations. A severe type of FH-III usually has childhood onset, displays severe hypertension and hypokalaemia, and is resistant to antihypertensives, including aldosterone antagonists, while some cases of FH-III exhibit a mild phenotype and even lack radiologic findings in adrenals" (page 4, lines 65-69)
Changes in the text has been marked with yellow. (page 4, lines 65-69)

Comment 6: Lines 65- 67 - the group of PA patients not identified as bearing a somatic mutaton in CNCL2 includes bot pateints with APA and with BAH; recast.

Reply 6: We have modified this sentence as follows: "There is another group of inherited PA, which has been referred to as FH type II (FH-II). Unlike FH-I, they are nonglucocorticoid-remediable, and negative for GRA gene mutation testing. FH-II families may display as APA and adrenal hyperplasia, which is clinically indistinguishable from sporadic PAs." (page 4, lines 71-76)
Changes in the text has been marked with yellow. (page 4, lines 71-76)

Comment 7: Lines 70-71 - this is not the case. There remain the non-CNCL2 cases of FH-II; it is not ..."still largely uncovered".

Reply 7: We thank for the reviewer's correction. We have modified the previous sentence "Currently, although some progress has been made, the etiology of the familial PAs is still largely uncovered and needs further exploration." into follows:" The molecular basis of FH-II has been studied for decades." (page 4, line 76) and " However, there still remains a group of inherited cases without the CLCN2 mutation and that cannot be explained by known genetic causes. Therefore, the aetiology of familial PAs still needs further exploration" (page 4, lines 79-82)
Changes in the text has been marked with yellow. (page 4, lines 76 and 79-82)

Comment 8: Line 96 - 72, not four significant figures.

Reply 8: We thank for the reviewer's kind remind. We have changed the figures from 72.08 to 72 in the manuscript. (page 6, line 114)

Comment 9: Line 98 - middle mm value missing.

Reply 9: We thank for the reviewer's kind remind. We have added the missing value in the manuscript. (page 6, line 116)

Comment 10: Line 100 - one normal value missing.

Reply 10: We thank for the reviewer's kind remind. We have added the missing value in the manuscript. (page 6, line 119-120)

Comment 11: Suggest lines 134-152 be omitted.

Reply 11: We thank for the reviewer's suggestion and feel grateful to explain our discussion here: In original lines 134-141, we made discussions for our observation that no pathogenic germline mutation was detected in these two family members. It could be possible that these two patients are sporadic PAs and coincidently found in one family. Except coincidence, there remains other possibility to explain the link between those two patients, since inheritance is contributed by both genetic and epigenetic factors. We therefore discussed the relevant epigenetic evidence related to

PA. In the revised version, we have weakened the conclusion and shortened this part of discussion as follows:" Nevertheless, genetic factors cannot fully explain the inheritance of PA. Previous studies have shown aberrant DNA methylation status in adrenocortical adenoma and APA (11-14), indicating epigenetic factors could be taken into consideration during the etiological investigations." (pages 8-9, lines 168-171)
In accordance with the reviewer's suggestion, we have removed the original lines 142-152.
Changes in the text has been marked with yellow. (pages 8-9, lines 168-171)

Comment 12: Lines 154-156 - concurrence on an N=1 is a gross overstatement; delete.

Reply 12: We have removed this statement "our report provides with new clinical evidence for the concurrence of those two diseases" in our manuscript.

Comment 13: Lines 156-176 - speculation, and not supportable; omit.

Reply 13: We agree with the reviewer that the link between craniopharyngioma and PA is based on speculation. We have followed the reviewer's suggestion and removed this part (the original lines 156-162) and modified as follows:" Currently, no evident connection has been made between craniopharyngioma with PA, further evidences will be needed to explore the possible link between those two diseases." (page 9, lines 174-176) For the proband, she firstly suffered with hypopituitarism and subsequently diagnosed with APA. Several pituitary hormones are indicated to be involved in the coordination of aldosterone secretion. Concerning of the unusual occurrence, and potential link of these two endocrine disorders, in original lines 163-176, we briefly reviewed the relevant evidence and made discussion based on the reported case. We have simplified this part and weakened our statement as follows: "Moreover, hypopituitarism may worth consideration in the reported case, as several pituitary hormones have been shown to be involved in the coordination of aldosterone secretion." (page 9, lines 176-178)
Changes in the text has been marked with yellow. (page 9, lines 174-178)

More general issues, as follows.
Comment 14: There are now data that the prevalence of PA is 3-5 fold higher than previously recognized. There is absolutely no evidence for these two cases being familial, and if PA indeed is 30% of hypertension, there is a just over one in three

chance of any hypertensive relative of the APA bearing proband having a non-familial form of PA, largely idiopathic hyperaldosteronism given that it is a much more common cause of PA than APA.

Reply 14: We totally agree with the reviewer that the evidences for these two cases being familial are insufficient. To avoid misconception, we have used the term "family-clustered PA" instead of "familial PA" to describe these two patients in the manuscript. We also added the following discussion in the manuscript:" In our report, there is no sufficient evidence for these two cases being familial. Thus, they could possibly be sporadic PAs.". (page 8, lines166-168)
Changes in the text has been marked with yellow. (page 8, lines166-168)

Comment 15: Was the probands cousin (the Aunt's son) investigated? If so, what did he show? Was he hypertensive? If not, why not? In recent studies ~12% of normotensives tested PA-positive on the basis of 24-hour urinary excretion of aldosterone, so he would be worth investigating even if he is not hypertensive. Who knows - if he has the same profile as his mother, between them they might be the missing link for non-CNCL2 FH-II....

Reply 15: We share the same concern with the reviewer. We have contacted the son of the Aunt at the beginning of the study, he was found hypertensive during his annual health examination and claimed no discomfort symptoms such as weakness. Unfortunately, he was not willing to visit the hospital and take the endocrine tests. We hope could get more information from him in the future.