Author's response to reviews

Title: Increased expression of ACTH (MC2R) and Androgen (AR) receptors in giant bilateral myelolipomas from patients with congenital adrenal hyperplasia

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Response to Reviewers:

Editor comments:

1. Name of ethics committee: Please update your ethics statement to include the name of the ethics committee that approved your study.

We updated our ethics statement to include the name of the ethics committee that approved your study (page 5, line 11).

2. Consent statement: Please state in the Methods section whether written informed consent for participation in the study was obtained from participants or, where participants are children, a parent or guardian.

We stated in the Methods section whether written informed consent for participation in the study was obtained from participants (page 5, line 12).

3. Copyediting: We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional language editing service.

We improved the style of written English as suggested.

4. Line Numbering: Please revise your manuscript to include line and page numbers.

We revised our manuscript to include line and page numbers.

Reviewer 1:

We have improved our manuscript according the suggestions and comments.
Minor remarks:

1. Title. The current title is misrepresentative. In the study, one only patient with CAH had evidence of MC2R and AR overexpression. Suggest editing the title. We edited the title to better represent our findings: “Increased expression of ACTH (MC2R) and androgen (AR) receptors in giant bilateral myelolipomas from patients with congenital adrenal hyperplasia.”

2. Abstract: Expand abbreviations - ACTH, MC2R, SF1. Conclusion section needs to be reworded as only one CAH patient had evidence of overexpression of MC2R and AR. We expanded the abbreviations in the abstract, as requested. Conclusion section was reworded as suggested.

3. Background: Recommend including a sentence about MC2R and ACTH in adrenal cortex in this section. MC2R needs to be expanded. We included a sentence about MC2R in the background as requested (page 4, line 19).

4. Paragraph 2: last line - the authors are interested in the role of ACTH and Androgens hyperstimulation in the pathogenesis. This sentence needs minor correction. We corrected the following sentence: “we hypothesized that ACTH and AR might have a role in the pathogenesis of myelolipomas” (page 4, line 23).

5. Expand abbreviations- ACTB, GAPDH. We expanded ACTB and GAPDH abbreviations (page 6, line 5).

6. Suggest including Hounsfield units from CT studies for all patients. Abdominal masses were identified with computed tomography (CT) in the two patients with bilateral myelolipomas and CAH, or magnetic resonance imaging (MRI) in the two sporadic ones. CT scan revealed giant bilateral adrenal myelolipomas with fat component density in both cases. Although the density was suggestive of myelolipoma, the measurement of HU is not available, because CT imaging was not digitalized at that time. MRI demonstrated fat tissue signal in both sporadic cases. These information were included in the case report (page 7, line 12 and 18; page 8, line 4).

7. Need a space after description of patient 1 and 2. We included a space as requested between patient 1 and 2 as requested.

8. Suggest including ACTH and androgen values for patient 3 and 4 (if available) as MC2R expression was high in these 2 individuals. Also suggest including their ages. We have added all requested data about patients 3 and 4: “Patient 3 (a 48-yr-old female) and patient 4 (a 40-yr-old female) presented with incidental findings of
sporadic unilateral adrenal myelolipoma … The patients did not present any clinical presentation of hyperandrogenism and non-classical CAH was rule-out by clinical evaluation.” (page 8, paragraph 1).

9. Please clarify if CAH (was ruled out by clinical and/or genetic testing) and or other cause of ACTH and androgen excess was ruled out in patients 3 and 4. The patients did not present any clinical presentation of hyperandrogenism and non-classical CAH was rule-out by clinical evaluation.” (page 8, paragraph 1).

10. Sentence on accepted mechanism- needs minor grammatical correction.
We made minor changes in the following sentence as requested: “One of the hypotheses is that hematopoietic and fat elements could derive from common progenitor cells secondary to stimulatory factors” (page 9, line 9).

11. Discussion: This manuscript provides evidence supporting the hypothesis of chronic ACTH stimulation and myelolipoma pathogenesis, but given that MC2R overexpression was documented in only one CAH patient, it is premature to conclude that the evidence in fact proves it. This sentence needs to be reworded.
We reworded the following sentence as requested: “In conclusion, we first demonstrated here MC2R or AR overexpression in giant bilateral myelolipomas from poor-compliance CAH patients. Therefore, we can speculate that chronic ACTH and androgen stimulation may play a causative role in myelolipomas of poorly controlled CAH patients.” (page 10, paragraph 2).

12. It is interesting that patient 1 with CAH did not show MC2R and AR overexpression inspite of elevated lab findings. ACTH exposure was not long enough? Authors to discuss this finding.
We discussed the topic of ACTH exposure: “Despite of chronic ACTH hyperstimulation, myelolipoma from patient 1 did not present MC2R overexpression” (page 9, line 19).

13. Inclusion of a histopathology figure highlight the components of myelolipoma will be a nice addition to this manuscript.
We regret that it was not possible to include histopathology figure of myelolipomas associated with CAH. Unfortunately, the blocks/slides were not localized in the archives of the Pathology section.

14. Spacing error: Throughout the manuscript
All spacing erros were corrected.

Reviewer 2:
We have improved our manuscript according the suggestions and comments.

Minor remarks:
1. The first sentence in the item “methods” in the Abstract should be rephrased. The way it is write makes the sample number unclear: “We analyzed...
...expression using real time qRT-PCR in two giant bilateral adrenal myelolipomas from 4 female patients:....”.

We rephrased the sentence in the Methods from the Abstract, as requested: “in two giant bilateral adrenal myelolipomas from two untreated simple virilizing CAH cases and in two sporadic adrenal myelolipomas.”

2. The authors should say in the first paragraph of the item MATERIAL AND METHODS the total number and general characteristics of the analyzed samples.

The sentence about the sample number was better described as requested: “Four patients with myelolipomas were evaluated in this study: two giant bilateral adrenal myelolipomas from two untreated simple virilizing CAH cases and two sporadic adrenal myelolipomas.” (page 5, line 14).

3. Correct name for the Leptin gene (LEP).

We corrected the name for the Leptin gene (LEP) in all manuscript.

Discretionary Revisions:

There is, in the item X-CHROMOSOME INACTIVATION AND CAG REPEAT NUMBERS OF THE AR GENE, an extra space before the last base in the first primer sequence and the punctuation mark “apostrophe” is missing after the number “5” and number “3” in the same primer.

We corrected the extra space before the last base in the first primer sequence and included the apostrophe after the number 5 and 3 (page 6, line 15).