Author's response to reviews

Title: Autosomal dominant pseudohypoaldosteronism type 1 with a novel splice site mutation in MR gene

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Author's response to reviews: see over
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Dear Dr Danielle Talbot
Senior Scientific Editor
BMC-series journals

We highly appreciate the very good criticisms of our manuscript # Manuscript ID: 1031306878282480 entitled “Autosomal dominant pseudohypoaldosteronism type 1 with a novel splice site mutation in MR gene”
I was very much impressed with the earnest and very kind criticisms in details of all reviewers. This revised version is very much improved with the correction refer to reviewers’ recommendations.
Enclosed please find our revised manuscript. We hope that the changes made in the manuscript are satisfactory. Our replies and related revisions are listed below.

Specific answers to criticisms of the reviewer

Reviewer1
Reviewer: Adam Whaley-Connell
Reviewer's report:
The only concern is informed consent from the subjects. I do not see in the methods section where the authors obtained informed consent from the subjects or from an institutional review board. This needs to be obtained and or disclosed before reported in the literature.

Answer
Thank you very much for your detecting my severe error. Of course we already get IRB approval and got written informed consent from the parents. I added the sentence at the last paragraph of the case report. I also faxed the consent form to the editor.

Reviewer2
Reviewer: Maria-Christina Zennaro
Thank you very much for your earnest review and good comments.

Reviewer's report:

Major compulsory revisions:
Introduction: the authors should also distinguish PHA1 according to the clinical feature of renal or generalized mineralocorticoid resistance, according to Hanukoglu et al, J Clin Endocrinol Metab 1991.

Reviewer 3 also gave me the same comment. I added several sentences about the classification and background of PHA1 in the background section.

Also, they should correct, introduction, line 6: ‘patients with renal, autosomal dominant PHA1………….’:.

Corrected.

Introduction, second paragraph, line 7; please correct: ‘conserved DNA binding domain encoded by exon 3-4 and a ligand binding domain responsible for ligand binding and ligand-dependent transactivation encoded by exons 5-9

Corrected.

Discussion:
Page 6: the references concerning the cited work of Sartorato et al are missing.

I added the reference 6 to several sentences of the discussion. Thank you very much.

Discretionary Revisions:
Page 7: Concerning the suggestion that there might be incomplete NMD in the patients’ tissues, it is remarkable that the authors had to use nested PCR and 35 cycles of amplification to detect the exon-skipped mRNA. Is this also true for the mRNA deriving
from the wild type allele? It would be certainly relevant to perform qPCR in order to
determine the ratio of one transcript vs the other.
A complete characterization of the underlying mechanism would of course require
detection of the truncated protein in vivo and functional analysis in vitro. Without this
information, one has to hypothesize that, although not mediated by NMD,
haploinsufficiency given to the loss of major functional domains of the receptor, is the
pathogenic mechanism of PHA1 in this patient.

I completely agree with your opinion. For more refined study, quantitative PCR
and abnormal protein detection is needed. However, this time it was difficult to
detect mRNA with one-step PCR both in the wild type and patient’s samples
because there exists several non specific PCR products. Nested-PCR could in
success to detect the normal and exon skipped band clearly. In addition, to detect
abnormal protein is much laborious. I think reviewer 2 knows the situation very
well and include this comment to the discretionary comment. Thank you very much.
I added this limitation of our study to the manuscript as bellow.
“This study includes two limitations. First, we have not conducted quantitative PCR
because we could not amplify clear PCR products with the one-step PCR, therefore,
conducted nested PCR. Second, we have not determined the mutant peptide
expression analysis because kidney tissues were unavailable in this disease. We must
also hypothesize that, although not mediated by NMD, haploinsufficiency given to
the loss of major functional domains of the receptor, may be the pathogenic
mechanism of PHA1 in this patient. However, we can say, at least, some amount of
mutant transcripts are expressed in the kidney tissues and the onset mechanism of
haploinsufficiency because of complete NMD was denied in our patient.
”

Conclusions
Genotype-phenotype correlations are very difficult in PHA1 patients, given the highly
variable phenotypic expression. However, the authors should consider that salt intake in
the diet may modulate the phenotype after weaning and that some patients may
compensate their phenotype without treatment just by a high salt diet.
We added this sentence in the discussion, page 6. Thank you very much.

We also added comments about genotype-phenotype correlations in the discussion section following reviewer 3’s comment.

Minor:
The manuscript needs some English editing.
Introduction, line 9: please correct: ‘In most cases, renal PHA1 is the………’
Methods, DNA amplification: Please correct: ‘all eight coding exons…’
Results: Only the mutation nomenclature according to the guidelines of the Human Genome Variation Society should be indicated; authors please delete (IVS 6-2 A>C).
Fig. 1: please correct mutation nomenclature
Abbreviations: plasma renin activity

All my errors were corrected. Thank you very much.

Reviewer 3
Reviewer: Gilberta Giancchetti

Reviewer's report:
The background is poor and the authors could add more information about the classification of the pseudohypoaldosteronism and about the genetic alterations of this disease.

Reviewer 2 also gave me the same comment. I added several sentences about the classification and background of PHA1.

Is not really clear the relationship between mutation and phenotype. Are sure the authors that the genetic alteration involved only the MR receptor and not other genes? Discuss this point Recent papers have been published on this topic.

We added the sentence as bellow citing Riepe’s recent review. Thank you very much for your good information.

“About genotype-phenotype correlations, Riepe describes in his review article that no association could be drawn from the available clinical data and MR gene
mutations. Furthermore, distinct differences of the disease severity between various affected members within one family or between unrelated individuals carrying the same mutation could be observed. These facts may suggest the existence of other factors such as modifier gene, environmental factors or infections. “

We greatly appreciate the reviewers helpful criticisms of our work. I believe that the manuscript has benefited substantially from the revision, and hope that it is now suitable for publication

Yours sincerely,

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