Author’s response to reviews

Title: A Novel Germline ARMC5 Mutation in a Patient with Bilateral Macronodular Adrenal Hyperplasia: a case report

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Version: 2 Date: 28 Feb 2018

Author's response to reviews:

Dear reviewers:

In response to the reviewer’s comments to the manuscript (Paper #: MGTC-D-18-00001R1) titled “A Novel Germline ARMC5 Mutation in a Patient with Bilateral Macronodular Adrenal Hyperplasia: a case report”, we have made the revision of the manuscript with the following itemized and point-by-point response to each reviewer’s comments.
Reviewer reports:

Amrik Sahota, PhD (Reviewer 1): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format. Please overwrite this text when adding your comments to the authors.

1. This case report describes the identification by whole exome sequencing (WES) of an inactivating germline mutation (c. 517C>T, p. Arg173*) in one allele of armadillo repeat containing 5 (ARMC5), a putative tumor suppressor gene, in a 51-year-old female with bilateral macronodular adrenal hyperplasia (BMAH), which is a rare cause of Cushing syndrome (CS). Subsequent WES of the excised tumor tissue identified a wide range of variants, including single nucleotide polymorphisms, indels, and loss of function mutations, but none of them were related to CS. Thus, the authors state that the germline mutation alone was sufficient to induce BMAH in this patient. The inability to detect a somatic mutation in tumor tissue may be due to limitations of WES rather than to the absence of the mutation per se. Structural alterations that cannot be detected by WES include inversions and copy number changes.

Answer: Many thanks to the reviewer’s comments. We agree with that the inability to detect a somatic mutation in tumor tissue may be due to limitations of WES, and we have discussed this limitation in the discussion (Page 10, first paragraph).

2. What the author describe, but do not call it by name, is haploinsufficiency, a well-known mechanism of disease causation. Thus, BMAH in this patient may be due to haploinsufficiency rather than to inactivation of ARMC5 through a two-hit mechanism. While the two-hit model explains many cases of the inactivation of tumor suppressor genes, there are reports of alternative mechanisms of tumor initiation. These include haploinsufficiency and epigenetic changes (for review, see Paige AJ (2003). Cel Molec Life Sci 60: 2147-2163).

Answer: Many thanks to the reviewer’s comments. Indeed, the combination of high-frequency LOH and rare point mutations has led to the suggestion that ARMC5 might demonstrate
haploinsufficiency (absent or reduced function due to the loss or inactivation of a single allele), just as CDKN1B described by A. J. W. Paige (Reference: Paige AJ (2003). Cel Molec Life Sci 60: 2147-2163). Although it needs to be further substantiated, we have added this in our discussion (Page 9, last paragraph).

Sulman Basit, PhD (Reviewer 2):

Comments to the Author

Subject: A Novel Germline ARMC5 Mutation in a Patient with Bilateral Macronodular Adrenal Hyperplasia: a case report

1. Authors have reported a novel sequence variant in the ARMC5 gene. Generally the manuscript is well written. Following minor corrections/suggestions may be incorporated to facilitate acceptance of the manuscript for publication.

Answer: Many thanks to the reviewer’s comments.

2. DNA extraction and sample collection from the tumor tissue has not been described. Benign tissue from tumor containing organ may contain un-mutated genome. What procedure was used to avoid this?

Answer: Many thanks to the reviewer’s comments. We have noted that benign tissue from tumor containing organ may contain un-mutated genome. Therefore, to avoid this, we have conducted precise isolation of the tumor specimen during the sample collection with the help of the H&E staining. And we have added the descriptions of the DNA extraction and sample collections in the manuscript (Page 8).

3. I would suggest Sanger sequencing of all exons of ARMC5 gene in DNA isolated from carefully selected tumor. Please note that ARMC5 is a small gene with only 4 exons.

Answer: Many thanks to the reviewer’s suggestion. We did perform the sequencing of all coding exons of ARMC5 in the patient. Moreover, this sequence was analyzed using Mutation Surveyor version 2.51 (SoftGenetics LLC) with comparison to the reference sequence in GenBank.
(http://www.ncbi.nlm.nih.gov/genbank/). And we have added this information in our manuscript (Page 8).

4. MAH in this case is corticotropin dependent or corticotropin independent? Please elaborate.

Answer: MAH in this case is corticotropin independent. Because the results of low- and high-dose dexamethasone suppression tests were negative, described in our manuscript (Page 7); additionally, the pituitary magnetic resonance imaging did not reveal any masses (Page 7).

5. Several typographic errors are present throughout the text.

For example;

* page 6, line 1, change "wide-type" to "wild-type".

Answer: We have corrected the typographic error.

* Page 9, last paragraph, 4th line, change "AMC5" to "ARMC5".

Answer: We have corrected the typographic error.

6. The authors need to show the genomic position of the variant and also give the frequency of the variant in normal population from ExAc/gnomAD database.

Answer: Thanks for this suggestion. We have added the position information in Page 8 Line 8. As a novel heterozygous germline mutation, it was first reported in this study and not recorded in ExAc/gnomAD database.