Author’s response to reviews

Title: Multihormonal pituitary adenoma concomitant with Pit-1 and Tpit lineage cells causing acromegaly associated with subclinical Cushing’s disease: a case report

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Author’s response to reviews:

BMC Endocrine Disorders,

Dear Dr. Louise Symmons,

We are very glad to hear that our manuscript is potentially acceptable for publication in BMC Endocrine Disorders, once some essential revisions are achieved. We would also like to thank you and the reviewers of our paper for many insightful remarks as well as thoughtful suggestions. Please find for your consideration of a revised version of our manuscript: BEND-D-17-00032R1, entitled "Multihormonal pituitary adenoma concomitant with Pit-1 and Tpit lineage cells causing acromegaly associated with subclinical Cushing's disease: a case report” by Tomoko Takiguchi et al.

On the following pages please find our point-by-point responses to each valuable comments. Please note that our revised manuscript is submitted as a clean copy without any tracked changes, colored or highlighted text according to your decision letter.

We now believe that the new revisions to the manuscript will be enough to address reviewers’ concerns in an appropriate and satisfactory manner, and hope that our revised paper will be acceptable for publication in BMC Endocrine Disorders.

Sincerely yours,

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1. We note that the reviewers commented on low dose DEX suppression. Please could you clarify whether this is and all other treatments the patient received are considered standard care for their condition.

Reply: Thank you so much for your constructive advice. As described in text, Oki et al. recommend 0.5 mg DST with a cortisol cut-off concentration of 3 μg/dL to be used as the initial step in diagnosing ACTH-dependent CS, because the most sensitive and specific cut-off value of plasma cortisol concentration using 0.5 mg DST was 3.05 μg/dL with 99.1% sensitivity and 98.4% specificity (Endocrine J. 56(7)897-904). And this is one of the common method to screen the autonomous secretion of ACTH and clinically diagnose subclinical Cushing disease. It is also noted that octreotide treatment was subjected to our patient prior to transsphenoidal surgery. Thus, we believe that these and all other treatments are considered standard care according to the medical guidelines for the treatment of acromegaly by American association of clinical endocrinologists 2011.

2. We note that you used a non-functioning adenoma and Cushing's adenoma for comparison. Please can you clarify in the Ethics approval and consent to participate section whether you obtained informed consent, written or verbal, from all patients for use of their cells including the 45 year old patient in this case report. If verbal, please state the reason and whether the ethics committee approved this procedure.

Reply: Thank you very much for your important notice. We obtained written informed consents from all patients of GH-producing adenoma, Non-functioning adenoma, and Cushing adenoma. It should also be noted that this study was approved by the Committee on Ethics in Human Research of Chiba University (approval number: No. 373). Therefore, we added the following sentence in the text “this study was approved by the Committee on Ethics in Human Research of Chiba University (No. 373). Written informed consents were obtained from the patients of GH-producing adenoma, Non-functioning adenoma, and Cushing adenoma for publication of this case report.” in Ethics approval and consent to participate section.
3. The Availability of data and materials section refers to the raw data used in your study and presenting tables and figures is not sufficient to state that all data is contained within the manuscript. Please only use this statement if you have indeed provided all raw data on which your study is based. We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

- The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

- The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- All data generated or analysed during this study are included in this published article [and its supplementary information files].

- The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

- Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

- The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].
• Not applicable. If your manuscript does not contain any data, please state 'Not applicable' in this section.

Please note that if you do wish to share your raw data and do not have consent from all patients to publish this data it will need to be de-identified.

Please also note that if you include your raw data as a supplementary file you will need to provide, after the References, a section titled “Additional files” where you list the following information about each of your supplementary files: * File name (e.g. Additional file 1), * Title of data, * Description of data. All additional files will also need to have been cited in the main manuscript.

Reply: Thank you so much for your suggestions. In terms of privacy protection, we would like to carefully handle the availability of our data, since original excel file regarding the raw data of the real time PCR in Figure 3E includes personal information of the patients. Therefore, we would like to choose that the datasets used and/or analysed during the current study are available from the corresponding author in response to reasonable request. In addition, we found that the figure titles were mistakenly embedded within all figures as pointed out below (6), and that in Figure 3E, NF was mistakenly not calculated as control. Taken together, we added the following sentence “the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.” in The Availability of data and materials section with providing new Figure 3.

4. We note that you have not included the contributions of author Yui Miyabayashi (YM) in the Authors’ contributions section. The contributions of all authors must be listed in this section.

Please note that an 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. According to the ICMJE guidelines, to qualify as an author one should have:
a) made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; AND

b) been involved in drafting the manuscript or revising it critically for important intellectual content; AND

c) given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; AND

d) agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Reply: We apologize for our forgetting to include “YM” in the Authors’ contributions section although YM was added as a new author. We wrote “TT1, HK, and YM participated in the endocrinological treatment and collected the data.” in the Authors’ contributions section. All authors made substantive intellectual contributions to a published study according to the ICMJE guidelines.

5. We note that you mention an FK in the Authors’ contributions section who is not present in the author list. If these initials refer to Eri Komai please change them to EK for consistency.

Reply: We apologize for our mistake. We corrected “EK” in the Authors’ contributions section.
6. Please remove the figure titles embedded within the figure files. All figure titles/legends should be placed at the end of the main manuscript, after the References, and not within any of the figure files.

Reply: We removed the figure titles within the figure files.

7. We note that Table 3 is called ‘Table 2’ in the title and has not been referenced in the manuscript text. Please amend this.

Reply: We apologize for our mistake. We corrected Table 2 as the oligonucleotide primers and Table 3 as summary of past reports. We added the following sentence “Quantitative PCR was conducted using primers for GH, Pit-1, POMC, Tpit, and NeuroD1, as noted in Table 2.” in the RNA extraction and Real-time RT-PCR section (as shown line 204). We corrected “Table 3” in the discussion section (as shown line 260).

8. We note that at the bottom of Table 1 it states that “Abbreviations are show in the Appendix” however, you have not included an appendix. Please amend this.

Reply: We apologize for our mistake. We wrote “Abbreviations: ACTH, adrenocorticotropic hormone; GH, growth hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; TSH, thyroid-stimulating hormone; GH, growth hormone” in Table 1.

9. Please submit your revised manuscript as a clean copy without any tracked changes, colored or highlighted text, as these are no longer required at this stage of the editorial process.

Reply: We prepared a clean copy without any tracked changes, colored or highlighted text.