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:

May 23, 2017

Tim Shipley, Ph.D.
Editor,
BMC Endocrine Disorders

Dear Dr. Shipley,

We thank you and the reviewers of our paper for many insightful remarks as well as thoughtful suggestions of our manuscript. Please find for your consideration of a revised version of our manuscript 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 raised by the reviewers. Edited parts of manuscript are highlighted in yellow. In addition, we have added Yui Miyabayashi as co-author since she contributed to the revision step of this manuscript.

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,

Tomoaki Tanaka
Reviewer reports:

Chris Yedinak (Reviewer 1): Multihormonal pituitary adenoma concomitant with Pit-1 1 and Tpit lineage cells causing acromegaly associated with subclinical Cushing's disease: a case report

Very interesting case study demonstrating the coexistence of ACTH positive (GH neg) tumor cells and GH positive cells in a single tumor along with subclinical presentation of Cushing's disease in an acromegalic patient.

This is a fascinating analysis supporting the need for detailed immunohistochemical analysis of pituitary tumors for the determination of appropriate post-operative treatment. This is particularly pertinent given the difficulty identifying these cases clinically at presentation.

Few recommendations for clarity:

1. Line 121 Awkward description of an elevated 24 hour urine free cortisol level.

Reply: Thank you so much for your carefully reading and pointing out our awkward description. We agreed and, then rewrote the word “/day” and the sentence “indicating the increased cortisol secretion”.

Line 122. The low dose Dex suppression - specify overnight testing. 0.5mg may not be adequate to differentiate iatrogenic or etiologies other than pituitary dependent CD
Reply: Thank you so much for your constructive advice. We understand the reviewer’s question. Oki et al. recommend 0.5 mg DST with a cortisol cut-off concentration of 3 μg/dL to be used as initial screening for ACTH-dependent Cushing syndrome, 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). In addition, it is proposed that people from East and Southeast Asia are leaner than those in Western countries, thus it has been suggested that the 1 mg low dose dexamethasone suppression test may be too strong in suppressing plasma cortisol concentration in Japanese patients. We wrote “as described previously (Oki et al. Endocrine J. 56(7)897-904)” (as shown in new line 123).

Line 126 To 8mg Dex/CRH testing was cortisol also elevated at 15mins post stim and was this >1.8ug/dl?

Reply: Thank you very much for your thoughtful comment. We performed 8 mg dexamethasone suppression test and CRH test separately. CRH showed that ACTH responded (vor: 38.9 pg/ml, 15 min: 113.0 pg/ml) and cortisol responded (vor: 15.0 μg/dL, 30 min 21.0 μg/dL). We wrote these data. (as shown in new line 127)

Line 137. 'thrice' is not commonly used now. Oct/LAR was this OctLAR(®) or Sandostatin® LAR Depot administered monthly for 3 months?

Reply: Thank you very much for constructive advice. Yes, you are right and we agree. We deleted “thrice” and rewrote “Sandostatin® LAR Depot was administered monthly for 3 months” (as shown in new line 139).

Line 165. Given treatment with octreotide post op the 'necessity' for treatment may be better expressed as a 'potential' associated with lack of GH or tumor control.

Reply: Thank you so much for your comment. According to reviewer’s suggestion we substituted “the necessity” to “potential” (as shown in new line 168).

Line 199-206 use a table or a diagram
Reply: Thank you so much for your suggestion. We made the Table 3 for the primer sequences and it looks better for broad readers.

Line 223 add ‘referential’ before non-functioning to clarify

Reply: Thank you so much for your suggestion. Please see the reply for the comment of the reviewer 2 “Please give details of the non-functioning adenoma and Cushing’s adenoma used for comparison”.

Line 239. Can authors speak to Tpit expression in these cells.


Reply: Thank you so much for your question and suggestion. We wrote “Considering the fact that Tpit determines alternate fates during pituitary cell differentiation (Pulichino et al. Genes Dev), Tpit would be involved in the pathophysiology of ACTH positive cells in this tumor of presented case.”

Rachel Crowley (Reviewer 2): The authors have described a case of a patient with clinical evidence of acromegaly and some evidence of autonomous hypercortisolism (in line with recent guidelines this might be a more appropriate term to use than SCD).

The manuscript would benefit from additional information:

What was the frequency and dosage of OCT-LAR?

Reply: Thank you so much for your constructive question and we apologize for our unclear description. The patient was given with 40 mg OCT-LAR monthly.
What was the peri-operative management with regard to hydrocortisone / cortisol status assessment?

Reply: Thank you so much for your question. Yes, as reviewer speculated, hydrocortisone was administered to this patient as the peri-operative management to avoid the risk of post-operative adrenal insufficiency.

Was the patient assessed for a germline genetic disorder?

Reply: Thank you so much for very thoughtful questions. While germline genetic analyses have not been examined in this case, it would be very interesting for broad readers to examine the involvement of germline and/or somatic mutations including AIP/USP8/Rb/p21 for the future study.

Is the 0.5mg dexamethasone a 48 hour test with dex every 6 hours, or a single dose of 0.5mg?

Thank you so much for your constructive advice. We applied a single dose of 0.5 mg dexamethasone suppression test as described previously. 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).

What is meant by pseudopapillema on histology?

Reply: Thank you so much for your question. We apologize for this mistake and deleted this expression.

What is meant by ACTH stained cell in the cytoplasm and Pit1 stained cell in the nucleus?
Reply: Thank you so much for your questions. The positive staining by ACTH antibody in the cytoplasm suggested that ACTH secretions can be mediated mainly from the cytoplasm as secretory granules, thereby its staining was observed in cytoplasm of the cells. On the other hand, the positive staining by Pit1 antibody in the nucleus meant that Pit1 functions as a key transcriptional factor for the differentiation of Pit1-lineage through transactivating its target genes, and therefore it is mainly localized in the nucleus in the other cells.

Please give details of the non-functioning adenoma and Cushing's adenoma used for comparison

Reply: Thank you so much for your advice. Two patients having pituitary tumors were clinically diagnosed with non-functioning and Cushing disease respectively in our hospital. We wrote the following sentences. “Each surgical obtained pituitary-tumor samples, whose patients diagnosed with non-functioning and Cushing disease respectively, showed null cell type and ACTH-positive densely-granulated type pathologically, which were consistent with non-functioning and ACTH-producing adenoma respectively. These samples were used for comparison.”

Note reduction of 11bHSD1 alone should not lead to failed dexamethasone suppression unless there is co-existing Cushing's disease

(see Tomlinson paper JCEM 2002)

Reply: Thank you very much for your good suggestion. We added the following sentences and the paper. “However, it has been reported that a patient with Cushing’s disease was spared cushingoid features because of a defect in a peripheral conversion of cortisone to cortisol. If GH and IGF-1 reduce 11β-HSD activity, co-existence of Cushing disease cannot be avoided (Tomlinson et al. JCEM 2002).”

Suggest change line 278 to read HPA axis in acromegaly

Reply: Thank you so much for your useful suggestion. We deleted “evaluate the ACTH/cortisol hormone dynamics on diagnosis of SCD” and rewrote “read HPA axis”.
Correct spelling transsphenoidal line 90 and change to ‘with SCD’ in line 136

Reply: Thank you so much for your carefully reading and pointing out our expression. We corrected the word “transsphenoidal” and changed to “with SCD”.