Peer Review File

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Reviewer A

Comment 1: The syndromes of RTH beta and RTH alpha are being confused – the discussion does not clearly separate these. An example is line 183 to 185; "in the present study….." I am not sure what the authors are referring to; their study, or the current literature regarding RTH alpha? It is clear from the biochemistry that the patients do not have RTHalpha; the diagnostic difficulty here is distinguishing RTHbeta from TSHoma, once assay interference has been excluded.
Reply 1: Thanks for reminding me. Our original writing did cause ambiguity, now we modify it as follows according to the comments of reviewers.
Changes in the text: Lines 183-187, we change "In the present study, serum T4 and T3 levels were in the low to normal range, T3 was in the normal to high range, and TSH levels were normal. None of the five cases had clinical manifestations, such as constipation, bradycardia, cognitive development disorder, severe short stature, or skeletal deformity, so this *THRA* mutation can be excluded in our study" to " In our study, none of the four patients had any clinical manifestations such as constipation, bradycardia, cognitive dysplasia, severe short stature, skeletal deformity, and thyroid function examination results did not conform to the characteristics of RTHalpha laboratory, so this *THRA* mutation can be excluded in our study".

Comment 2: Title misleading – only 4 of the 5 cases had a THRB mutation
Reply 2: Reply 1: Thanks for reminding me. Based on comments from Viewers, we reviewed the history, clinical manifestations and laboratory results of the fifth patient again and concluded that the patient was difficult to be diagnosed as RTHbeta. Finally, with the agreement of all authors, we decided to delete the fifth patient's profile.
Changes in the text： We change "five" in the title to "four" .

Comment 3: Table 4- the mutations are not listed correctly; the amino acids are not correct Use correct nomenclature for RTH – see Refetoff et al, Thyroid 2014, PMID24588711.
Reply 3: We are very sorry for our incorrect writing, and it is rectified in Table 4.
Changes in the text： We change A338T to R338W and "G460L" to "E460K" in Table 4.

Comment4: The discussion around TSH inhibition and use of somatostatin analogues to differentiate between RTHbeta vs TSHoma needs to be strengthened, using the available literature
Reply 4: We are sorry for our negligence, and we add the discussion in line 204.
Changes in the text： We add " Somatostatin can negatively regulate TSH, and somatostatin receptor is expressed on the cell surface of pituitary TSH tumor. Octreotide, a somatostatin analogue, can inhibit TSH secretion and even shrink tumors(14). It can be used for preoperative preparation and postoperative recurrence or treatment of TSH tumor. RTH patients have low response to somatostatin, and the mechanism is unclear." in line 204.

Comment 5: Change throb to palpitations

Reply 5: We appreciate it very much for this good suggestion, and we have done it according to your ideas.
Changes in the text: We change throb to palpitations on Line 32, 106, 110, 197.

Comment 6: Did the THRB negative case have an MRI? This is the case where MRI findings are most relevant.
Reply 6: Thanks for reminding me.
Changes in the text: Based on comments from Viewers, we reviewed the history, clinical manifestations and laboratory results of the fifth patient again, and concluded that the patient was difficult to be diagnosed as RTHbeta. Finally, with the agreement of all authors, we decided to delete the fifth patient's profile.

Comment 7: "heterozygous mutations in THRB are common" needs to be rephrased – these are overall rare
Reply 7: Thanks for reminding me. Our original writing did cause ambiguity, now we modify it as follows according to the comments of reviewers.
Changes in the text: Lines 45-48, we change " With the development of gene sequencing technology, heterozygous mutations in THRB are common, but not all children with thyroid hormone resistance syndrome have mutations" to "Heterozygous mutations in THRB are overall rare, even if with the advanced development of next-generation sequencing, not all the children with thyroid hormone resistance syndrome has mutations".

Comment 8: Change "thyroid receptor A" to "thyroid hormone receptor alpha", similarly for beta too
Reply 8: We appreciate it very much for this good suggestion, and we have done it according to your ideas.
Changes in the text:
In line 52, we change "thyroid receptor A" to "thyroid hormone receptor alpha".
In line 53, we change "thyroid receptor B" to "thyroid hormone receptor beta".

Comment 9: Line 82, change "autoantibody test" to "laboratory tests"
Reply 9: We appreciate it very much for this good suggestion, and we have done it according to your ideas.
C**hanges in the text**: Line 82, we change "autoantibody test" to "laboratory tests".

Comment 10: The method of calculation of TSH inhibition should be listed in methods. Would it not be better to calculate this as basal TSH – minimum TSH/basal TSH x 100%? I could not access paper ref 15 – are the methods used to determine TSH changes post octreotide identical to those described here?
Reply 10: We appreciate it very much for this good suggestion, and we have done it according to your ideas.
Changes in the text: Line 92, we added the method of calculation of TSH inhibition to methods and made corresponding modifications in Table 3.

Comment 11: Additional references (Mannavola, Clin Endocrinology 2005, PMID 15670193 & Beck-Peccoz, JCEM 1989, PMID 2491862) should be added and referred to.
Reply 11: We appreciate it very much for this good suggestion, and we have added reference (PMID 15670193) in line 212 and reference (PMID 2491862) in line 225.
Changes in the text： We added " Mannavola et al. have demonstrated that acute administration of somatostatin analogues leads to a similar reduction in TSH in patients with TSH-omas or PRTH ". in line 210-212.
We added "thus confirming previously reported data." in line 225.

Comment 12: What were the genes tested on the panel used?
Reply 12: We appreciate it very much for this good suggestion. Gene panel includes DU0X2*, DU0XA2, FOXE1, GNAS, HESX1, IGSF1, NKX2-1, NKX2-5, PAX8, P0U1F1, PROP1, SECISBP2, SLC16A2, SLC26A4, SLC5A5, TBL1X, TG, THRA, THRB, TPO, TSHB and TSHR genes. We have provided these genes in line 96.
Changes in the text: We add "Gene panel includes DU0X2*, DU0XA2, FOXE1, GNAS, HESX1, IGSF1, NKX2-1, NKX2-5, PAX8, P0U1F1, PROP1, SECISBP2, SLC16A2, SLC26A4, SLC5A5, TBL1X, TG, THRA, THRB, TPO, TSHB and TSHR genes" in line 96.

Comment 13: "course of disease" should be changed to "duration of symptoms".
Reply 13: We appreciate it very much for this good suggestion, and we have done it according to your ideas.
Changes in the text: Line 100, we change "course of disease" to "duration of symptoms".

Comment 14: Lines 35-36 and line 118 contradict each other.
Reply 14: Thanks for reminding me. Our original writing did cause ambiguity, now we modify it as follows according to the comments of reviewers.
Changes in the text: Line 118, we change "The FT3 and FT4 levels were elevated in all cases, while TSH was increased in two patients" to " The FT3 levels were elevated in all cases, FT4 levels were elevated in three patients, while TSH was increased in one patient".

Comment 15: Lines 153 to 154 need to be rephrased; PRTH vs GRTH is not analogous to Graves' disease vs Hashimoto's thyroiditis.
Reply 15: Thanks for reminding me. Our original writing did cause ambiguity, now we modify it as follows according to the comments of reviewers.
Changes in the text： Lines 153-154, we delete "similar to other thyroid disorders, such as Hashimoto's thyroiditis and Graves' hyperthyroidism".

Comment 16: Ref 11 is not an appropriate reference for line 174.
Reply 16: We are sorry for our negligence, and it has been corrected.
Changes in the text： We have corrected the references in line 174.

Comment 17: Line 159 should mention that RTHbeta only occurs due to THRB deletion if both copies of the gene are deleted.
Reply 17: Thanks for reminding me. Our original writing did cause ambiguity, now we modify it

as follows according to the comments of reviewers.
Changes in the text: Lines 159-160, we change "deletion of the whole *THRB*" to " deletion of the both copies of the *THRB*".

Comment 18: Was assay interference excluded? Especially important for the THRB gene mutation negative case.
R**eply 18:** Thanks for reminding me. We modify it as follows according to the comments of reviewers.
Changes in the text: Based on comments from Viewers, we reviewed the history, clinical manifestations and laboratory results of the fifth patient again, and concluded that the patient was difficult to be diagnosed as RTHbeta. Finally, with the agreement of all authors, we decided to delete the fifth patient's profile.

Comment 19: Line 202-203, I suspect a word is missing; "can be used to differentiate from RTH".
Reply 19: We are very sorry for our incorrect writing, and it was added to line 203.
Changes in the text： Line 203, we change " can be used to differentiate from RTH" to " can be used to differentiate TSHoma from RTH".

Reviewer B

Comment 1: Whilst their observation that the octreotide inhibition test is not useful in making a diagnosis agrees with the published literature (Mannavola et al Clinical Endocrinology 2005 62, 176-181), the authors fail to mention that in the Mannavola paper, chronic administration of a long-acting somatostatin analogue was a useful dynamic investigation. This is a significant omission, and the authors should consider including this in their discussion and citing this previous publication.
R**eply 1:** We appreciate it very much for this good suggestion, and we add this reference to our study. The method of chronic administration of a long-acting analogs was not mentioned in our study, because in China, long-acting somatostatin analogs are only approved for the treatment of adult TSHomas and have no indications for children.
Changes in the text: We added " Mannavola et al. have demonstrated that acute administration of somatostatin analogues leads to a similar reduction in TSH in patients with TSH-omas or PRTH ". in line 210-212.

Comment 2: Baseline TSH was raised (27mU/L) and circulating TG (>500) very elevated in case 3. Do the authors have an explanation for this?
Reply 2: Thank you for your question. We think that patients with RTH have TSH resistance, and some patients have elevated serum TSH, which promotes thyroid hormone synthesis. The results of thyroid function examination still suggest RTH features.
C**hanges in the text：** ---

Comment 3: Case 5, in whom a THRB mutation was not found, exhibited raised FT3 but normal FT4/Total T4 which is atypical for RTHbeta. Have the authors considered an

alternative diagnosis (e.g., assay interference or a genetic variant in thyroid hormone binding proteins).

Reply 3: Thanks for reminding me. We modify it as follows according to the comments of reviewers.

Changes in the text: Based on comments from Viewers, we reviewed the history, clinical manifestations and laboratory results of the fifth patient again, and concluded that the patient was difficult to be diagnosed as RTHbeta. Finally, with the agreement of all authors, we decided to delete the fifth patient's profile.

Comment 4: In Table 4, some mutations (A338T, G460L) are listed with an incorrect one-letter amino acid code.

Reply 4: We are very sorry for our incorrect writing, and it is rectified in Table 4.

Changes in the text： We change A338T to R338W and "G460L" to "E460K" in Table 4.