Author’s response to reviews

Title: The haplotype of UBE2L3 gene is associated with Hashimoto's thyroiditis in a Chinese Han population

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Version: 1 Date: 05 Jan 2016

Author’s response to reviews:

Dear editor Amar Agha and reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript (BEND-D-15-00053). Those comments are very valuable and helpful for revising and improving our paper. We have tried our best to revise our manuscript according to the comments. Revised portion are marked in red in the manuscript.

Thank you very much for your consideration of publishing this manuscript in BMC Endocrine Disorders. Please feel free to contact us if you have any other questions and need any further information about our manuscript.

Best wishes!

Yours sincerely,

Jin An Zhang (M.D. & ph.D)
Professor and director,
The ubiquitin-proteasome system (UPS) is a key player in maintaining cellular homeostasis by protein ubiquitination, a multi step enzymatic process regulating stability, function and localization of modified proteins. This system is highly regulated by post-translational modification and covalent binding.

Little information so far is available regarding the transcriptional regulation of the genes involved in the process. Ube2l3 is an E2 ubiquitin-conjugating enzyme, that participates in ubiquitination of substrates leading to the degradation of target molecules.

Genetic variants in Ube213 have been reported in various inflammatory diseases such as rheumatoid arthritis, SLE and Crohn’s gastroenteropathy. The authors investigated several SNPs in Ube213 in a cohort of 1028 patients with autoimmune thyreopathies such as graves and Hashimoto thyreopathy as well as healthy controls including subphenotypes. The present study reports an association between one Ube213 haplotype and Hashimoto thyreopathy (HT) in the Chinese population. The haplotype TCGGC was increased in frequency in subjects with HT (OR 1.44, P=0.031)

The authors conclude that the Ube3L2 is likely a susceptibility locus for HT.
The study is interesting. The authors implicate an important cellular protein degradation and metabolic pathway with autoimmune endocrine thyroid diseases.

However, the manuscript is lengthy and appears blown up given the degree of novelty added to the existing body of literature.

Referee: 1

Major comments:

1. The selection of SNPs is somehow conceivable, but is at least arbitrarily enough that correction for multiple testing must be performed. This exercise is especially difficult when the variations are in close proximity to each other as shown by the haplotype block. This reviewer has severe doubts that the significant increased frequency of the TCGGC haplotype stays significant when corrected for multiple testing. A conservative procedure for that would be dividing the original p value by the 4 existing haplotypes to obtain the corrected p value: 0.05/4= 0.0125.

Response:

Thank you for your constructive suggestion. These SNPs were chosen because of their strong association with the other autoimmune diseases such as SLE, RA, Crohn’s disease, and Sjögren’s syndrome. The changes were highlighted in blue in line 7 and 8 on page 4 in the main text.

We have used “haplovie 4.2” software and “hapmap” online query tool to give a figure which show the location of these 5 SNPs within UBE2L3 are in the same block and the distances between them, unfortunately, the location of the five loci were too scattered to display in the same picture, so we instead made a simple block picture using “haplovie 4.2” software only.

In haplotype frequencies analysis, we compared the frequencies of each haplotype between each case group (AITD, GD or HT) and healthy controls instead of mutual comparison among the four haplotypes. Taking an example of TCGGC, the original data as shown in Table 1, 2, and 3. We therefore think that correction for multiple testing is not required. This kind of statistical method was also used in several genes analysis in our published papers (1. Polymorphism of IL-37 gene as a protective factor for autoimmune thyroid disease. Journal of Molecular Endocrinology. 2015; 55 (3) : 209-218; 2. Variants in IRAK1-MECP2 region confer susceptibility to autoimmune thyroid diseases. Molecular and Cellular Endocrinology. 2015 ; 399:244-249; 3. Polymorphisms of CLEC16A Region and Autoimmune Thyroid Diseases. G3-Genes-Genomes-Genetics,2014;4:973-977)
Table 1

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Control(%)</th>
<th>AITDs(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGGC</td>
<td>105(5.899)</td>
<td>149(7.311)</td>
</tr>
<tr>
<td>Others</td>
<td>1675(94.101)</td>
<td>1889(92.689)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Control(%)</th>
<th>GD(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGGC</td>
<td>105(5.899)</td>
<td>91(6.811)</td>
</tr>
<tr>
<td>Others</td>
<td>1675(94.101)</td>
<td>1245(93.189)</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Control(%)</th>
<th>HT(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCGGC</td>
<td>105(5.899)</td>
<td>58(8.286)</td>
</tr>
<tr>
<td>Others</td>
<td>1675(94.101)</td>
<td>642(91.714)</td>
</tr>
</tbody>
</table>

2. The methods section could be shortened by 1/3.

Response:

Thank you for your suggestion. The method section was shortened as suggested and all the changes were highlighted in blue in line 21-23 on page 4 in the revised manuscript.

3. The results section should be shortened by 1/3.

Response:

We appreciate for your suggestion. The results section was shortened as suggested and changes were highlighted in blue in line 25-28 on page 5 and line 1-3 on page 6 in the revised manuscript.

4. The discussion is lengthy and it very speculative. The authors cannot make any assumption as to what the mechanism of this finding might by, it is even unclear whether
it holds when tested in other cohorts, thus the conclusion drawn by the authors is not supported by the data shown.

Response:

Your suggestion is very helpful for improving our manuscript. The discussion is now concise to avoid long and complex translations. The changes were highlighted in blue in line 2-9, 16-17 and 29 on page 7 and line 1-4 and 6-8 on page 8 in the revised manuscript.

5. The manuscript is, when proofread by an English language speaker, and revised (see above) potentially well suited for a short report.

Response:

Thank you for your kind advice. We have our manuscript rechecked by a native English speaker named Fatuma-said Muhali who was graduated as a Master member from our research group last year and works as a physician in Tanzania. Whose Email is muhalifatma@yahoo.co.uk. And revised portions are marked in blue in the new version of the paper.

6. Table 1 may be in supplement.

Response:

Your advice is useful. The changes were highlighted in blue in supplementary table.

7. Table 3-5 can be omitted and summarized in the text by 1-2 sentences.

Response:

Table 3 is about genotype and allele distributions of AITDs which merged HT and GD together and table 5 is about genotype and allele distributions after GO stratification. They are both negative results thus we deleted table 3 and 5. However table 2 and table 4 which were genotype and allele distributions of HT and GD results respectively may give readers a better understanding of the results thus we decide to keep those two tables. The changes were highlighted in blue in line 25-28 on page 5 and page 14 in the main text.

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Editorial Requests

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Please note that all submissions to BMC Endocrine Disorders must comply with our editorial policies. Please read the following information and revise your manuscript as
necessary. If your manuscript does not adhere to our editorial requirements this will cause a delay whilst the issue is addressed. Failure to adhere to our policies may result in rejection of your manuscript.

Ethics:

If your study involves humans, human data or animals, then your article should contain an ethics statement which includes the name of the committee that approved your study.

If ethics was not required for your study, then this should be clearly stated and a rationale provided.

Response:

The name of the Ethics Committee in line 2-4 on page 4 in the main text.

Consent:

If your article is a prospective study involving human participants then your article should include a statement detailing consent for participation.

If individual clinical data is presented in your article, then you must clarify whether consent for publication of these data was obtained.

Response:

Our article is not a prospective study and no individual clinical data in our article

Availability of supporting data:

BioMed Central strongly encourages all data sets on which the conclusions of the paper rely be either deposited in publicly available repositories (where available and appropriate) or presented in the main papers or additional supporting files, in machine-readable format whenever possible. Authors must include an Availability of Data and Materials section in their article detailing where the data supporting their findings can be found. The Accession Numbers of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript must be provided and include the corresponding database name.

Response:

Corresponding database name of Primer 3 online and Haploview 4.2 in line 24 and 25 on page 11.

Authors Contributions:
Your 'Authors Contributions' section must detail the individual contribution for each individual author listed on your manuscript.

Response:

Authors Contributions: YW conceived of the study and participated in its design, data collection, manuscript writing. YFZ participated in patients recruitment and data collection. QW, JX(Jing Xu) and NY carried out laboratory tests. LFS, JX(Jian Xu) and STH performed statistical analysis and helped to draft the manuscript. JAZ conceived of the study and participated in its design, statistical analysis and revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. These are in line 9-15 on page 9 in the main text.