Author's response to reviews

Title: Polymorphisms of interleukin-21 and interleukin-21-receptor genes confer risk for autoimmune thyroid diseases

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Author's response to reviews: see over
To: Ms Eloisa Nolasco, The BioMed Central Editorial Team

From: Jin-An Zhang

Re: 1525384091852. Response to review of “Polymorphisms of interleukin-21 and interleukin-21-receptor genes confer risk for autoimmune thyroid diseases”

Date: February 22, 2013

We appreciate the thoughtful comments from the reviewers and have made the following revisions based on their suggestions. And the modified parts were expressed by boldfaced words. Meanwhile, after manuscript checked by a native English speaker, some grammatical and spelling mistakes was replaced by red font sections.

Editorial comments:
1. Please revise your methods section to provide the name of the ethics committee that approved your study.
Response: It was revised.

2. Please include an ‘Authors’ contributions’ section before the Acknowledgements and Reference list.
Response: The 'Authors' contributions' section was added.

Reviewer #1: Matt Simmonds

Major comments:
1. Abstract and throughout the manuscript, the authors have put IL-21 and IL-21R in italic to represent they are genes and then written the word gene after them. If you put a gene name in italics you then do not need to write the word gene after. Can the authors please correct this throughout the entire manuscript?
Response: Abstract, objective, line 3, we replaced “IL-21 and IL-21R gene” by ‘IL-21 and IL-21R’

2. Abstract and throughout the manuscript, Can the authors please present all OR and 95% CI to only two decimal places. Also through the manuscript the authors swap between having OR = XX and OR=XXX and as such can the authors please choose one style and stick to it.
Response: They were changed.

3. Abstract, Results, line 1, can the following be added the start of the sentence ‘For IL-21’ to help put the results in context.
Response: It was added.

4. Abstract, Conclusion, Can the authors please state the names of the Il-21 and Il-21R SNPs they found associated with AITD.
Response: We have changed. It was showed as ‘Our study confirmed the contribution of rs2221903 in IL-21 gene to GD susceptibility. And we may be the first to disclose the impact of rs4833837 and rs2221903 of IL-21 gene on the risk of HT, as well as rs3093301 and rs2285452 in IL-21R gene’

5. Introduction, paragraph 2, can the authors please provide some more detail on what they mean when they say ‘In autoimmune diseases, such as systemic lupus erythematosus (SLE) [3], rheumatoid arthritis (RA) [4] and Sjogren’s syndrome (SS) [5], animal and clinical researches disclosed the dysregulations of IL-21 and IL-21R.’ such as how are they dysregulated?
Response: The sentence was changed and explained as follows,”Animal and clinical researches disclosed the dysregulations of IL-21 and IL-21R in autoimmune diseases. For example, compared with IL-21R-competent BXSB-Yaa mice for multiple parameters of SLE, the IL-21R-deficient Yaa mice showed none of the abnormalities characteristic of systemic lupus erythematosus (SLE) [3]. IL-21R is overexpressed in the inflamed synovial membrane and in peripheral blood or synovial fluid leukocytes of rheumatoid arthritis (RA) patients [4]. And the evident increase of serum IL-21 level in primary Sjogren’s syndrome (pSS) patients has positive correlation with the levels of gamma-globulin and erythrocyte sedimentation rate[5]. These suggests that IL-21 and IL-21R may play a critical role in the pathogenesis of autoimmune disease.”

6. Introduction, paragraph 3, the authors’ state ‘In HapMap-CEU population, IL-21 included in a large block (480kb) of linkage disequilibrium which showed genetic associations with type 1 diabetes mellitus (T1DM) [7,8], RA [8], juvenile idiopathic arthritis (JIA) [9], psoriasis and psoriatic arthritis (PA) [10.]’ Can the authors please explain a little more what they mean by this point, such as have other genes in the region been associated with these diseases and if so can the authors give an idea of how many genes are encompassed within this 480kb block.
Response: It was replaced by “In HapMap-CEU population, a large block (480kb) of linkage disequilibrium encompassing KIAA1109/Tentr/IL2/IL21 showed genetic associations with type 1 diabetes mellitus (T1DM) [7,8], RA [8], juvenile idiopathic arthritis (JIA) [9], psoriasis and psoriatic arthritis (PA) [10].”

7. Introduction, paragraph 4, the authors state ‘Vincent Plagnol [17] reported that the SNPs in chromosome 4q27 were associated with the GD susceptibility.’. Can the authors please state which SNPs were associated with GD and also state that this was a Caucasian dataset.
Response: Replaced “Vincent Plagnol [17] reported that the SNPs in chromosome 4q27 were associated with the GD susceptibility” with “Plagnol [17] reported that the rs2069763 in chromosome 4q27 were associated with the GD susceptibility in a Caucasian cohort”
8. Materials and Methods, 2.2 Genotyping, paragraph 2, Why did the authors select 4 SNPs in IL-21 and 2 SNPs in IL-21R to screen? Did these SNPs represent Tag SNPs which captured all common variation in these gene regions? If so can the authors please state this and if not can the authors please perform Tag SNP screening of all common variation in these genes.

Response: For IL-21 gene, we selected rs907715, rs4833837, rs2221903 and rs2055979 covering a large region in the IL-21 gene which captured all common variation. For IL-21R gene of 48.4kb with 13 tag SNPs in Hap-CHB population, only rs3093301 and rs2285452 in different block are confirmed to associate with SLE as showed in Introduction, paragraph 3. That is why we just screened these two loci.

9. Results, 3.1 Association of the IL-21 gene polymorphisms with GD and HT, paragraph 1, can the authors need to also report HWE for their GD and HT cases as well as for their controls.

Response: HWE was used to assess whether the sample represent the ethnical population. But GD and HT patients were different from the normal population. We calculated the p values of HWE for GD and HT group, unfortunately both of them were more than 0.05, that’s why the results were not shown.

10. Results, 3.2 Association of the IL-21R gene polymorphisms with GD and HT, paragraph 1 currently reads ‘As shown in Table 2, the minor allele frequencies of rs3093301 and rs2285452 in IL-21R gene were 47.1% and 11.5%, respectively, in controls, 48.0% and 11.0% in GD patients and 42.3% and 7.9% in HT patients. None of the alleles exhibited any association with the genetic susceptibility to GD or HT (rs3093301: PGD =0.752, PHT=0.138; rs2285452: PGD=0.797 PHT=0.068).’ as the results are negative can this paragraph be altered to “As shown in Table 2, the rs3093301 and rs2285452 SNPs in IL-21R showed no association with GD or HT (P=0.797,0.068).”

Response: It was altered.

11. Results, 3.4 Gene-gene interaction analysis in HT, due to the small number of HT samples screened I am not sure that the authors would have had power to undertake such an analysis and think it would be better to remove this whole paragraph and any associated discussion of these results from the manuscript.

Response: It was removed.

12. Discussion, paragraph 3, can the authors please also comment on who your study supports or does not support the findings by Plagnol et al

Response: Jia’s et al found rs907715 of IL-21 gene being associated with GD in a Chinese population while Plagnol found only rs2069763 and did not find
association of rs2069762 and rs6822844 of IL-2 with GD, maybe there is ethnicity effect contributing to these results differences. Therefore, we cannot exclude that the SNPs of the IL-21 and IL-21R genes are only an indicator of a candidate gene contributing to AITD. Further studies such as identifying the causal SNP for this association, gene-environment interaction in GD and HT cohort studies required to clarify the effect of IL-21 and IL-21R in the development of AITD susceptibility.

13. Discussion, paragraph 3, The authors talk about the power of their datasets by saying they have 0.8. This value alone is meaningless. Can the authors please state at what OR this is and for what SNP minor allele frequency this is applicable. As such it would be helpful for the authors to assay what effect size their study could exclude for IL-21R to provide greater clarity for the reader.

Response: Discussion, paragraph 3, line 8. “Genetic power in our report was about 0.8 ” is replaced by ” The minor allele frequency is 0.2, which gives our study genetic power of about 0.8 with OR of homozygote 2.0, and of heterozygote 1.5.

14. Discussion, paragraph 4, the authors need to add a few lines saying that replication of these associations is required in larger independent datasets to verify these results.

Response: In Discussion, paragraph 4, line 5-6, the sentence “Replication of these associations between IL-21 and IL-21R gene and AITD is required in larger independent databases of different cohorts to verify” was added.

15. Throughout the manuscript, although the English is of a high standard within the manuscript can the authors please just double check the manuscript for consistency of abbreviation and typos

Response: The manuscript was double checked and revised by native English, speaker, highlighted in yellow.

Minor comments:
1. Introduction, paragraph 4, can ‘Vincent Plagnol [17]reported’ can it be changed to ‘Plagnol et al [17] reported’.

Response: It was altered.

2. Introduction, paragraph 4, the authors state ’Vincent Plagnol [17]reported that the SNPs in chromosome 4q27 were associated with the GD susceptibility.’ can ‘chromosome’ be changed to ‘chromosome’.

Response: It is changed.

3. Results, 3.1 Association of the IL-21 gene polymorphisms with GD and HT, paragraph 1 and 2, these two paragraphs need to be combined as one larger paragraph.
Response: they are combined

4. Discussion, paragraph 1, line 2, please change ‘IL-21 receptor’ to ‘IL-21R’ to make it consistent with the rest of the manuscript
Response: It was changed.

5. Discussion, paragraph 1, the authors state ‘The dysregulation of IL-21 and IL-21R plays a role in multiple immune-mediated diseases, including SLE, psoriasis, RA and other chronic inflammatory diseases. Like other autoimmune diseases, GD and HT are chronic conditions initiated by the loss of immunological tolerance to self-antigens. Researches have showed many immune-related genes may participate in the development of AITD.’ Can references be added to the end of each line and can ‘researches’ be changed to ‘researchers’
Response: In Discussion, paragraph 1,line 7 and 9, references were added and ‘researches’ was changed to ‘researchers’.

Review# 2: BOUGACHA-ELLEUCH Noura
1. The manuscript should be reread to correct many English mistakes, mainly in the introduction and discussion sections.
Response: The whole manuscript was reread by a native English speaker and mistakes were corrected.

2. Authors should add the different SNPs positions relative to the described genes (IL21 and IL21R genes).
Response: Materials and methods 2.2 genotyping, paragraph 4, line5-6, the following sentence were added. “Rs907715, rs4833837, and rs222190 are located on intron 2 of IL-21R and rs2055979 on intron 3. For IL-21R gene, rs3093301 is in intron 2 and rs2285452 in exon 10.”

3. In the results section, authors report a CI of 1.459-94.130 (Association of IL21 gene polymorphisms with GD and HT section). This range of CI is strange. How authors could explain it?
Response: The length of confidence interval (CI) is not only related to the sample size but also to the frequency of the haplotype. Smaller samples or less frequency of the haplotype farther away from 0.5 results in the longer confidence interval.

4. In the discussion section, authors should discuss the different modes of transmission (recessive ,dominant..).
Response: In genetics, a recessive gene is an allele that causes a phenotype (visible or detectable characteristic) that is only seen in a homozygous genotype (an organism that has two copies of the same allele) and never in a heterozygous genotype. While dominance is a relationship between alleles of
a gene, in which one allele masks the expression (phenotype) of another allele at the same locus. Our study didn’t find any synergistic effect of the risk genotypes and we were unable to conclude whether these two genes were either recessive or dominant over the other gene.

5. In table 3, the sum of many genotype frequencies is not 100. **Response:** For the genotype frequencies with three decimal places were calculated with half adjusting, the sum of some genotype frequencies was 99.9.

We sincerely appreciate the excellent comments by the reviewers and feel that the manuscript has been significantly improved as a result. Please let me know if you have any further questions or suggestions.

Thank you for your consideration.

Sincerely yours,

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