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Reviewer A

Summary:

The manuscript titled "β -cell Function After 10 Years of Autoimmune Type 1 Diabetes: Prevalence, Possible Determinants, and Implications for Metabolism" by Cheng and Yin et al is a rigorous study looking at the preservation of beta-cell function in the Chinese population. This study appears to add to the existing literature but as it stands is limited by a clear description of the multivariate analyses that were done.

Major Comments:

Comment 1: Would consider including high risk HLA genotypes from other ethnicities as well.

Reply 1: Thank you very much for your suggestion. In this study, we investigated the relationship between susceptible HLA haplotypes and the long-term β-cell function preservation in Chinese T1D patients. The HLA haplotypes included were DR3, DR4 and DR9, which were demonstrated by former studies to confer T1D susceptibility in Chinese population (Notably, DR3 and DR4 were also high-risk HLA haplotypes in Caucasians, while DR9 was a Chinese or East Asian specific T1D risk haplotype).

We didn't include high risk HLA haplotypes from other ethnicities because former studies revealed that a particular haplotype or genotype may manifest a distinctive impact on T1D susceptibility in different populations. Data from our group[1] and Yang et al[2] demonstrated that the T1D susceptible HLA haplotypes for Chinese differed greatly from those in Caucasians. Therefore, inclusion of the high-risk HLA haplotypes or genotypes from other ethnicities here would probably make no more sense and make the results difficult to interpret. So, in this study, we only explored the relationship between ethnic-specific HLA haplotypes and β-cell function preservation in Chinese T1D patients.

According to your suggestion, we further investigated the association between the high -risk HLA genotypes and β-cell function preservation. The DR3/DR3, DR3/DR9 and DR9/DR9 genotypes were rare in Caucasian, but they were frequent in Chinese, and were all Chinese-specific T1D risk genotypes. While the DR3/DR4 genotypes were the major

risk factor in Caucasians but only manifested a susceptible trend for T1D risk in Chinese. We investigated the associations between β-cell function preservation and the above HLA genotypes respectively, but no significant results were found. We presented the results of Chinese-specific HLA genotypes (DR3/DR3, DR3/DR9 and DR9/DR9) in the revised manuscript.

Changes in the text: We further investigated the association between the suspectable HLA genotypes and β-cell function preservation (see Page 9, Line175-176, and Page 15, Line 304-311).

Comment 2: Line 174-176- ANOVA should be used for parametric test, Wilcoxon Rank Sum and Kruskal Wallis are the appropriate nonparametric tests.

Reply 2: Thanks a lot for your correction. We are sorry that this is a clerical error. In fact, the normally distributed data was compared by ANOVA analysis, and non-normally distributed data was analyzed by Wilcoxon Rank Sum and Spearman correlation tests.

Changes in the text: We modified the 'Statistical analysis' part. And now the text reads as 'normally distributed data was compared by ANOVA analysis, and non-normally distributed data was analyzed by Wilcoxon Rank Sum and Spearman correlation tests' (see Page 11, Line225-227).

Comments 3: Line 208-212: Make it clear that the numbers are given first for FCP and then for PCP.

Reply 3: Thank you for your comment. We modified the expression in this part so as to make the text more understandable.

Changes in the text: We modified the expression in the first paragraph of the 'Prevalence of β-cell function' part, and the text now reads as 'we found that the FCP levels of 67.9% (74/109), 29.4% (32/109) and 2.7% (3/109) of patients fell into the undetectable, minimal, and preserved range, respectively. And the PCP levels of 66.0% (72/109), 25.7% (28/109) and 8.3% (9/109) of patients were undetectable, minimal, or preserved, respectively' (see Page 13, Line 260-270).

Comments 4: - Section starting at Line 234: are these univariate, multivariate analyses or both? This should be made clear.

Reply 4: Thanks for your comments. In this part, we used multivariate analyses to investigate the associations between variations (including sex, BMI, age of onset, disease duration, DKA occurrence at disease onset, HLA-genotypes and autoantibody status) and β-cell function preservation. Data of this part were presented in the modified Table 2. In the RCS analyses, univariate analyses and multivariate analyses (adjusting for sex, BMI, disease duration and antibody status) were also both used. These processes were described in more detail in the 'Statistical analysis' part and the 'Possible determinants of residual β-cell function' part of the results.

Changes in the text: The analytical processes were described in more detail in the 'Statistical analysis' part (see Page 11, Line 228-238) and the 'Possible determinants of β-cell function' part of the results (see Page14, Line 289-299). The results of the multivariate analyses were showed in Table 2.

Comments 5: - Analysis of association with HLA type- was this adjusted for age of onset, number Ab positivity (rather than just particular Ab positivity) or other parameters associated with residual beta cell function?

Reply 5: Thanks a lot for your question. We did adjust for the possible co-influence parameters when analyzed the association between HLA type and β-cell function preservation. But we found no statistically significant differences whether the parameters were adjusted or not. We described this issue in more detail in the revised article.

Changes in the text: The analytic process was described in more detail in the third paragraph of the 'Possible determinants of β-cell function' part (see Page 15, Line 304-311).

Comments 6: - Reference LADA as a disease entity in your discussion of adult onset T1D

Reply 6: Thanks for your constructive suggestion. We further discussed the relationship between LADA and adult-onset classical T1D in the 'Discussion' part.

Changes in the text: The relationship between LADA and adult-onset classical T1D were further discussed in the 9th paragraph of the 'Discussion' part (see Page 20, Line 422-434).

Comments 7: - Figure 2: Unclear what the difference in Figure 2A and Figure 2B without

reading the text. This should be labelled on the figures.

Reply 7: Thanks for your suggestion. The original Figure 2 has been modified as Figure 3 in the revised manuscript. Figure 3A and Figure 3B were derived from the univariate and multivariate analyses, respectively. We reperformed the RCS analysis during this revision and included the antibody status in the multivariate analyses this time. The differences of Figure 3A and Figure 3B were described in detail in the Figure legend.

Changes in the text: Description of Figure 3A and 3B were made in more detail in the figure legend (see Page 28, Line 596-604).

Comments 8: - Figure 2 and 3 present the same message therefore making them redundant.

Reply 8: Thanks a lot for your comment. The order of the original Figure 2 and Figure 3 was reversed during this revision. The modified Figure 2 and Figure 3 both presented the associations between residual β-cell function and age of onset, but they described this issue from different angles. Figure 2 of the revised manuscript simply showed the linear association between age of onset and FCP levels, which represented a quantitative correlation between the two parameters. While Figure 3 presented the odds ratio for β-cell function preservation of distinct age of onset. This graph qualitatively indicated that the possibility for β-cell function preservation increased with age of onset, and there was no specific cutoff point of age of onset which determined whether the β-cell function would be preserved or not, since the odds ratio for β-cell function preservation increased linearly with age of onset. Therefore, we think there are some differences in the messages presented by Figure 2 and Figure 3, which we described further in the text during this revision.

Changes in the text: The massages presented by Figure 2 and Figure 3 were described in more depth in the second paragraph of the 'Possible determinants of residual β-cell function' part (see Page 14, Line 293-299).

Comments 9: - Could combine Table 1 and 2 so that there are 4 columns: overall, depleted, residual and p value.

Reply 9: Thanks a lot for your suggestion. We combined Table 1 and 2 so as to make the article clearer and more concise.

Changes in the text: The Table 1 of the revised manuscript was a merger of the original

Table 1 and Table 2 (see Table 1 of the revised manuscript).

Comments 10: - Table 3: Is this a multivariate analysis? If univariate, you have already included much of this data in Table 2 and this table would therefore not provide more detail.
Reply 10: Thanks a lot for your comments. The data of the original Table 3 were mainly derived from univariate analyses. During this revision, we reperformed the multivariate analyses adjusting for parameters including sex, disease during, age of onset, BMI and autoantibody status. This result was shown in Table 2 of the revised manuscript.
Changes in the text: Table 3 of the original article was deleted, and the result from the multivariate analyses was shown in Table 2 of the revised manuscript (see the revised Table 2).

Minor Comments

Comments 11: - The entire manuscript needs to be reviewed for clarity, proper diction and English grammar.
Reply 11: Thank you for your advice. We asked a professional language polishing agency to help revise the English expression of this article.
Changes in the text: The entire article was re-edited by a professional language polishing agency. And the language certificate was provided along with the revised manuscript.

Comment 12: - Line 87-88- what is meant by 'specific types of diabetes'?
Reply 12: Thanks a lot for your comments. We are sorry for the lack of accuracy in this expression. The correct expression here should be 'other types of diabetes caused by specific reasons', which comes from the WHO 1999 diabetes classification criteria[3].
Changes in the text: The text now reads as 'other types of diabetes caused by specific reasons' (see Page 6, Line119-122).

Comments 13: - Line 102: by urea (UA) do you mean uric acid? and creatinine (CREA) is usually abbreviated Cr.
Reply 13: Thank you for your comments. We are sorry for the mistakes. The expression

here should be ‘uric acid (UA)’. And ‘CREA’s were changed into ‘Cr’s throughout the whole text.

Changes in the text: We have corrected ‘urea (UA)’ into ‘uric acid (UA)’ (see Page7, Line 136), and ‘CREA’ s were modified as ‘Cr’s (see Page 7, Line136; Page 14, Line 287 and Table 1).

Comments 14: - Line 117: FCP and PCP need to be defined.

Reply 14: Thanks for your comments. We added the definitions of FCP and PCP in the ‘β-cell function assessment’ part.

Changes in the text: Definitions of FCP and PCP were made in the ‘β-cell function assessment’ part (see Page 7, Line 151).

Comments 15- Section starting on Line 140: add topic sentence—unclear why you are defining hypoglycemia.

Reply 15: Thank you for your comments. We have added the topic sentence here. Since hypoglycemia was the most common acute complication of T1D, we investigated its prevalence and severity in our subjects. The definition of hypoglycemia and its severity help the readers understand the glycemia control status of our subjects.

Changes in the text: We have added the topic sentence in the ‘Determination of Acute and Chronic Complication Status’ part (see Page 9, Line 187).

Comments 16- Line 190: specific that what is in the parentheses is the range.

Reply 16: Thanks for your comments. For non-normally distributed data, we used median (quartile range: 25th, 75th) to describe its distribution, which has been accounted in the ‘Statistical analysis’ part. According to your advice, we used median (quartile arrange: 25th, 75th) and range to describe age and age of onset of our subjects, and we've labeled the quartiles that appeared in parentheses throughout the text.

Changes in the text: The age and age of onset of our subjects were described by median (quartile range: 25th, 75th) and range (see Page 12, Line243-246). The quartiles appeared in the parentheses were labeled throughout the text (see Page 3, Line 66; Page 12, Line 243-246; Page 13, Line 278 and Table 1).

Comments 17- Line 196-204-Don't start sentences with numbers.

Reply 17: Thanks for your comments. We have revised the language expression of the whole text and corrected this mistake.

Changes in the text: We have corrected this mistake throughout the whole text.

Comments 18- Line 223: are you referencing the "mean HbA1c" here?

Reply 18: Thank you for your question. Yes, the values here presented the mean HbA1c levels of subjects in distinct groups. To avoid ambiguity, we converted the unit of HbA1c from '%' to 'mmol/mmol' and used mean±SD to describe its average level.

Changes in the text: The levels of HbA1c were described by mean±SD with the unit of 'mmol/mol' (see Page 4, Line67; Page 12, Line 247; Page 13, Line 279; Page 19, Line 408 and Table 1).

Comments 19-Line 233: within univariate analysis

Reply 19: Thanks a lot for your comment. But we are sorry that we didn't quite understand the exact meaning of this comment. Are you suggesting that we explain the analytic process of the variables here (just like the major comment 4)? Or would you please elaborate your question in more detail? We feel very grateful for this.

Changes in the text: We have not yet made modifications according to this comment.

Reviewer B

This is a well-done study that will help readers understand the characteristics of T1D in China and how it differs from what is seen in Caucasians, which has been studied in more detail.

Comment 1-One weakness is that the writing style is clumsy. Thus, the paper should be edited by someone experienced with scientific writing in English.

Reply 1: Thanks for your suggestion. We have asked a professional language polishing agency to revise the English expression of the article.

Changes in the text: The entire text was re-edited by a professional language polishing agency. And the language certificate was provided along with the revised manuscript.

Comments 2 -Line 85: It is surprising that antibodies against insulin were not included because that has become such a standard.

Reply 2: Thanks a lot for your comment. Antibodies against insulin were important for the progression of T1D. But in our study, all the subjects have been diagnosed with T1D for at least 10 years, and the exogenous insulin treatment they accepted would induce anti-insulin antibodies. The current methods of test could not distinguish the antibodies induced by endogenous or exogenous insulin. Therefore, detecting antibodies against insulin after exogenous insulin treatment would probable make no more sense. Studies demonstrated that a combination of GADA, IA2-A and ZnT8A would well define the autoimmune status of T1D[4-6]. According to your suggestion, we will test the antibodies against insulin in patients who have not been treated by exogenous insulin or were within 7 days of insulin treatment when conditions permit in future studies.

Changes in the text: We further discussed this issue in the 'Discussion' part (see Page 21, Line 449-451).

Comments 3-Line 107: Presumably the MMTT was 2 hour rather than 4 hour.

Reply 3: Thanks a lot for your comment. The 4-hour-MMTT dose better reflect the insulin secretion function of β-cells than a 2 hour one. While due to the limited conditions in the outpatient and the compliance of the patients, we failed to conduct a 4-hour MMTT in this study. We conducted the 2-hour-MMTT in this study also because we saw from some former studies that a 2-hour-MMTT could also to a large extent reflect the β-cell responsiveness to hyperglycemia stimulation [7, 8]. According to your suggestion, we will use the standard 4-hour-MMTT in the future studies to better reflect the β-cell function. This issue was further discussed in the 'Discussion' part.

Changes in the text: This issue was further discussed in the conclusion part (see Page21, Line 445-449).

Comments 4-Line 116: Please define FCP and PCP.

Reply 4: Thank you for your comment. We added the definition of FCP and PCP in the 'β-cell function assessment' part.

Changes in the text: We added the definitions of FCP and PCP in the 'β-cell function assessment' part (see Page 7, Line 151).

Comments 5-Line 165: The testing for neuropathy does not seem very rigorous.

Reply 5: Thanks a lot for your comment. We are sorry that the description of diabetic neuropathy here is not rigorous since the electrophysiological examinations were not described. We have modified the description of diabetic neuropathy diagnosis criteria in the 'Determination of Acute and Chronic Complication Status' part.

Changes in the text: The description of the diagnostic criteria for diabetic neuropathy now reads as 'A history of diabetes, and neuropathy at or after the diagnosis of diabetes were necessary for the diagnosis of DPN. It can be diagnosed if any one of the following examinations is abnormal: abnormal ankle reflex (or abnormal knee reflex); abnormal acupuncture pain sensation; abnormal vibration sensation or abnormal pressure sensation. If the diagnosis cannot be confirmed via the above symptoms, an electrophysiological examination should be performed to assess the nerve conduction (NC). The presence of an abnormality of NC and a symptom or symptoms, or a sign or signs of neuropathy confirms DPN. Neuropathy caused by other defined causes should be excluded.' in the 'Determination of Acute and Chronic Complication Status' part (see Page10, Line 208-217).

Comments 6-Line 289: Having positive tests for antibodies does not rule out the presence of T2D.

Reply 6: Thanks a lot for your constructive comment. According to the WHO 1999 criteria[3], both the diagnosis of T1D and T2D were etiological based. The positive test for antibodies indicated that autoimmune factors may participate in the disease progression. And the T2D phenotype patients with positive antibody testing were defined as LADA or LADY[9, 10], which were thought to be the slow-progressing subtypes of autoimmune T1D. Therefore, based on the above diagnosis criteria, we held the point that the positive tests for antibodies would rule out the presence of T2D.

Your comments made us think deeper on the expression of this part. It seemed that ruling out the presence of T2D should not be the significance of antibody positive testing in our

study. Since, T1D was diagnosed according to the WHO 1999 criteria, which emphasized the insulin secretion deficiency due to β-cell destruction. Therefore, T2D was well excluded during this step by the absolute deficiency of endogenous insulin and the dependence of exogenous insulin at disease onset. The significance of autoantibody positive testing in our study would lie in that it homogenized the etiology of the enrolled T1D patients. Therefore, the presence of the idiopathic T1D (T1BD) was well excluded, which allowed this study to better align with the further exploration of immune interventions. This issue was further discussed in the 'Discussion' part.

Changes in the text: The issue was further discussed in the 'conclusion' part (see Page 20, Line 434 and Page 21, Line 435-439).

Comments 7-Line 362: Same point as for line 289.

Reply 7: Same as reply 6.

Changes in the text: Same as reply 6.

References

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