Peer Review File

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Response to reviewer A

Response: Thank you for your comments on our work, which were valuable when we were revising our manuscript. We have carefully read all the comments and have addressed them in the revised manuscript. We have highlighted the changes with yellow in our manuscript MS. The point by point response is displayed below.

Comment 1: The title indicates association of SNP rs2043211 with positive rate of GADA, however there are not any statistical significant differences in allele and genotype distribution in T1DM group compared to control subjects. rs2043211 is only associated with percentage of GADA positive T1DM patients that carry risk TT genotype. The data did not provide robust evidence for association of rs2043211 with GADA since the p value is borderline and there is no correlation with the level of GADA. Moreover, the phrase "positive rate of GADA" is quite confusing.

Reply 1: Thanks for your suggestions. We have changed the title into "Polymorphism of the Inflammasome-related Gene CARD8 Is Associated with GADA Positivity in Patients with Type 1 Diabetes Mellitus". Indeed, no significant differences in the distributions of alleles or genotypes of rs10403848 and rs2043211 were observed between cases and controls. The polymorphism rs2043211 was associated with the rate of GADA positivity among T1DM patients but not correlated with the level of GADA. Also, we have changed the "positive rate of GADA" into "the rate of GADA positivity" to be clearer.

Changes in the text: We changed the title (see Page 1, line 1-3). We have modified our text as advised (see Page 2, line 15 and line 21).

Comment 2: Page 2 Line 21-21: The sentence is unclear. Do the Authors mean that T1DM accounts for 5-19% of autoimmune disorder?

Reply 2: Thanks for your remainder. To be clearer, we have changed this sentence into "The autoimmune diabetes accounts for 5-19% of diabetes".

Changes in the text: We have modified our text as advised (see Page 3, line 4).

Comment 3: Page 2 Line 28: The hereditary risk of T1DM is not reported as 80-85%. The concordance rate for genetically identical monozygotic twins of parents with type 1 diabetes is estimated to be less than 50%. The pathogenesis of T1DM is a consequence of complex process, with strong genetic component, however

epigenetics factors and environmental components contribute to T1DM development as well.

Reply 3: Thanks for your remainder. Our previous expression is misleading. To be clearer, we have modified this sentence into "Previous research, including GWASs (genome-wide association studies), linkage analysis, and candidate gene studies, has identified more than 60 susceptibility loci, explaining 80-85% of the heritability of T1DM". (ref. 5 and 6)

Changes in the text: We have modified our text as advised (see Page 3, line 10-12).

Comment 4: Page 3: The explanation of CARD8 role in pathogenesis of autoimmune disorder in not very clear and precise. Authors used too many generalities. Introduction section requires major revision with carefully interpretation of cited literature data.

Reply 4: Thanks for your suggestions. We have interpreted the cited literature concerning the CARD8 role in the pathogenesis of autoimmune disorder. We have added "*In vivo* study has indicated that CARD8 decreased the expression of IL-1β through interacting with wild-type NLRP3, and knockdown of CARD8 could lead to enhanced expression of IL-1β in human monocyte-derived macrophages". "A meta-analysis indicated that the polymorphism of CARD8 was significantly associated with decreased incidence of ileal CD (Crohn's disease) as well as stenotic or fistulizing CD, which suggests a protective effect in these CD types. In addition, the SNP of CARD8 was identified to be associated with increased risk of gout in the Chinese and European population. And in a Swedish population consisting of 492 patients and 793 population-based controls, the minor allele of rs2043211 was associated with a decreased risk of AS (ankylosing spondylitis).

Changes in the text: We have modified our text as advised. (see Page 4, line 10-12 and line 15-22).

Comment 5: Study Subjects: The Authors often use phrase "finally" in the context of study groups. Was there any tentatively T1DM or control group? Information related to inclusion, exclusion criteria and demographic characteristic should be descried in concise way in this section. Moreover, there is no information regarding control group and the enrollment criteria?

Reply 5: Thanks for your suggestions. We have deleted the phrase "finally". Information in related to inclusion and exclusion criteria and demographic characteristic has been added. "The two groups were matched in sex (male/female) (275/235 vs. 273/258, p=0.418). The inclusion criteria were as follows: (1) fulfilling the WHO diagnostic criteria for diabetes (1999); (2) acute onset; (3) insulin dependency within 6 months after diabetes diagnosis; (4) positive for at least one

islet-autoantibodies: GADA (glutamic acid decarboxylase antibody), IA-2A (protein tyrosine phosphatase antibody), and ZnT8A (zinc transporter 8 antibody). Participants with other autoimmune diseases or combined with malignant tumors were excluded." The enrollment criteria of control group have also been added. "The healthy controls must meet the following requirements: (1) FPG (fasting plasma glucose) < 5.6mmol/L; (2) 2h-PPG (postprandial plasma glucose) < 7.8mmol/L in 75g OGTT (oral glucose tolerance test). Besides, participants with autoimmune diseases, cancers, family history of diabetes were excluded." The demographic characteristic was presented as Table 2.
Changes in the text: We have added some data. (see Page 5, line 10-21 and Table 2 in Page 15).

Comment 6: Page Line 8: What chemiluminescence and liquid chromatography kits were used to measure C-peptide and glycated hemoglobin? Moreover, the Authors did not indicate manufacturers of antibody assays.
Reply 6: Thanks for your remainder. We have added the information in related to chemiluminescence and liquid chromatography kits. The antibodies, GADA, IA-2A, and ZnT8, are autoantibody, and the detection methods have been described on our previous paper (reference 21 and 22).
Changes in the text: We have added some data. (see Page 6, line 1-2 and line 3).

Comment 7: Page 5 Line 2: What MassArray system was used in current study?
Reply 7: Thanks for your remainder. We have added information about MassArray system.
Changes in the text: We have added some data. (see Page 6, line 18).

Comment 8: Page 5 Line 5: HWE should be estimated in control and T1DM group.
Reply 8: Thanks for your remainder. We have added information about the HWE estimation in the T1DM group in Table 3.
Changes in the text: We have added some data. (see Page 16).

Comment 9: Page 5 Line 6-7: Major estimation of genotype distribution and allele frequencies was based on model with reference allele/genotype.
Reply 9: Thanks for your remainder. We have estimated both the genotype distribution and allele frequencies (Table 5 and Table 6).
Changes in the text: We have added some data. (see Page 18, Page 19 and Page 20).

Comment 10: Page 5 Line 18-19: Assuming that in control group only healthy subjects were included, is not surprising that fasting blood glucose level and

postprandial blood glucose level was significantly higher in T1DM group compared to controls.
Reply 10: Thanks for your remainder. We have modified our text as advised.
Changes in the text: Please see Page 7, line 16-19

Comment 11: Table 4: The table is unclear. In dominant, recessive, additive and over-dominant model specific combination of genotypes are compared between study and control group. In the table 4 there is no information about compared genotypes and the frequencies of the specific combination of genotypes.
Reply 11: Thanks for your remainder. We have added information about the comparison of genotypes and the frequencies of different combination of genotypes (Table 5 and Table 6).
Changes in the text: We have added some data. (see Page 18, 19 and 20).

Comment 12: Table 5, 6: Tables notes below the table body do not provide enough information concerning data including in the table. All abbreviations must be explained. Are the data presented as mean ± standard deviations? What do values in the brackets mean?
Reply 12: Thanks for your remainder. We have added the information in the table notes.
Changes in the text: We have added some data. (see Page 21 and Page 22).

Comment 13: Page 7 line 15: "The association studies between rs2043211 and autoimmune auto-inflammatory diseases are relatively more than polymorphism rs10403848. However, the results obtained are inconsistent sometimes." These sentences are unclear.
Reply 13: Thanks for your remainder. We have modified this sentence into "Association studies between rs2043211 and autoimmune and autoinflammatory diseases are relatively more common than such studies of rs10403848. However, the results obtained are sometimes inconsistent."
Changes in the text: We have modified our text as advised. (see Page 9, line 21-22 and Page 10, line 1).

Comment 14: Page 7 line 18: What does "stop allele" mean?
Reply 14: To be clearer, we have changed "stop allele" into "the allele containing a stop codon".
Changes in the text: We have modified our text as advised. (see Page 10, line 3).

Comment 15: Page 8 Line 8: Patients with TT genotype of rs2043211 had higher

positive rate of GADA, however TT genotype is not correlated with level of GADA. Patients carrying TT genotype present the lowest GADA level compared to heterozygous and wild homozygous. Can Authors explain it?
Reply 15: Thanks for your suggestion. We couldn't explain the potential mechanisms behind the controversial phenomenon after literature review. According to your suggestion, in the section of Discussion, we have proposed the question. "Patients with TT genotype of rs2043211 had higher rate of GADA positivity, however TT genotype is not correlated with level of GADA. Patients carrying TT genotype present the lowest GADA level compared to heterozygous and wild homozygous. Therefore, further investigation should focus on explaining the contradictory outcomes and revealing the exact function of the polymorphisms."
Changes in the text: We have modified our text as advised. (see Page 11, line 7-12).

Comment 16: Page 8 Line 9-10: "This result is consistent with previous finding that the production and distribution of islets antibodies exhibit heritable risk to some extent". Might Author explain it clearer?
Reply 16: Thanks for your remainder. We have added some interpretation. "It has been indicated that the HLA DR4 was positively associated IA-2A, and HLA DQ2 was negatively associated with IA-2A."
Changes in the text: We have modified our text as advised. (see Page 10, line 19-20).

Comment 17: Style and grammar of English should be corrected
Reply 17: Thanks for your suggestion. Our manuscript has been polished by AJE (American journal experts).

Response to reviewer B
Response: Thanks.