Author’s response to reviews

Title: The PTPN22 C1858T gene variant is associated with changes in residual beta-cell function in new-onset type 1 diabetes

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Version: 2 Date: 20 January 2011

Author's response to reviews: see over
**Dear Editor-in-Chief and Associate Editor**

Thank you very much for the possibility to submit a revised version of our manuscript. We have carefully revised our paper and addressed the reviewer's comments and are hereby giving a point-by-point response to the concerns. The changes in the manuscript are highlighted in yellow.

Sincerely yours,

Lotte Brøndum Nielsen

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**Reviewer's report**

**Title:** The PTPN22 C1858T gene variant is associated with changes in residual beta-cell function in new-onset type 1 diabetes

**Version:** 1  **Date:** 24 December 2010  
**Reviewer:** Raffaella Buzzetti

**Reviewer's report:**

In the present study the Authors investigated the association of the C1858T variant with residual beta-cell function, glycemic control, daily insulin requirements, diabetic ketoacidosis (DKA) and diabetes-related autoantibodies (IA-2A, GADA, ICA, ZnT8Ab) in children during the first year after diagnosis of type 1 diabetes.

Major compulsory revisions:
1) The prevalence of ZNT8 autoantibodies in type 1 diabetes patients should be reported.

Answer: 68 % of the patients showed positivity to either ZnT8RAb or ZnT8WAb 1 month after disease onset. This information is now described in the results-section (P6 L3-4).

2) In Table 1, the Authors should specify whether the clinical characteristics described were at clinical diagnosis.

Answer: The clinical characteristics described in Table 1 were at onset, this is now stated in the caption of Table 1 (P13). ‘At onset’ was following removed from Table 1 L 2 and 3.

3) In Table 1: low, moderate and high risk HLA genotypes should be specified.

Answer: DR 03/04 and DR 04/04 were considered as high HLA risk genotypes; DR 03/03 and DR 04/08 were considered to convey moderate risk, while all other HLA DR genotypes were classified as low risk genes. The specified HLA risk genotypes are included in the legend of Table 1 (P13).

4) In Research Design and Methods they reported that 84% of patients were white Caucasians. The Authors should specify the other populations of patients analyzed.

Answer: This observational study was conducted in 18 centres representing 15 countries in Europe and Japan. The patients participating in the study were asked if ‘the patient were
white Caucasian? Yes or No. Therefore we do not hold information about ethnicity of the remaining 16 %, only that 4 patients are Japanese corresponding to 2 %. For review purpose only we have included a Table giving an overview of the nationalities of the enrolled patients:

<table>
<thead>
<tr>
<th>Centre</th>
<th>Patients enrolled in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>31</td>
</tr>
<tr>
<td>France</td>
<td>8</td>
</tr>
<tr>
<td>Germany – Berlin</td>
<td>19</td>
</tr>
<tr>
<td>Germany – Giessen</td>
<td>7</td>
</tr>
<tr>
<td>Holland</td>
<td>4</td>
</tr>
<tr>
<td>Ireland</td>
<td>18</td>
</tr>
<tr>
<td>Italy – Chieti</td>
<td>11</td>
</tr>
<tr>
<td>Italy - Parma</td>
<td>15</td>
</tr>
<tr>
<td>Japan</td>
<td>4</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>12</td>
</tr>
<tr>
<td>Macedonia</td>
<td>23</td>
</tr>
<tr>
<td>Norway</td>
<td>11</td>
</tr>
<tr>
<td>Portugal</td>
<td>27</td>
</tr>
<tr>
<td>Spain</td>
<td>7</td>
</tr>
<tr>
<td>Sweden</td>
<td>14</td>
</tr>
<tr>
<td>Switzerland</td>
<td>17</td>
</tr>
<tr>
<td>UK - Glasgow</td>
<td>16</td>
</tr>
<tr>
<td>UK - Leicester</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>257</strong></td>
</tr>
</tbody>
</table>

5) The BMI values should be reported as SDS-BMI.

Answer: Unfortunately the international nature of this study precludes a detailed comparison with national standard growth charts there are up to date. Therefore we have refrained from giving the SDS-BMI score.

6) In Discussion section, they should reported a possible pathophysiological explanation of the association between T allele of C1858T and high levels of proinsulin.

Answer: The discussion was expanded with a possible explanation for the role of the PTPN22 T1858 allele related to severity of beta-cell destruction and to the autoimmune response (P7 L14-20).

Minor revisions:
1) In the Figures 1A and 1B, the Authors should add the p values.
Answer: The \( p \) values have been added on Figures 1A, 1B and 2

2) In Discussion section they reported: “The discrepancies in significant/non-significant findings on C-peptide levels between the two studies mentioned above may relate to the use of fasting contra stimulated C-peptide”. The number of references of the two mentioned studies should be reported.

Answer: The sentence has been rewritten and a reference number referring to reference (6) has been added in the text (P7 last two lines).

Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.
Reviewer's report
Title: The PTPN22 C1858T gene variant is associated with changes in residual beta-cell function in new-onset type 1 diabetes
Version: 1 Date: 2 January 2011
Reviewer: Takuya Awata
Reviewer's report:
In this concise manuscript, the authors have verified the possible association of the established susceptibility PTPN22 nsSNP (C1858T) with residual beta-cell function in patients with recent-onset type 1 diabetes. The aim of the manuscript is clear, the sample size is moderate, the results are intriguing but debatable. However, I recommend several revisions as follows.

Major Compulsory Revisions:
1. I feel that the 3rd paragraph of the discussion is not logically uncertain. Please rewrite it more evidently. As mentioned by the authors, the higher proinsulin levels at 6 or 12 months despite significant reductions of C-peptide may indicate that the proinsulin levels were fairly modified by IA's that might be mainly produced against exogenous insulin. Although the authors mentioned that "…, however, not affect the association between proinsulin and the PTPN22 variant in the statistical analysis", it appears that the higher proinsulin levels were related to the higher IA levels at 12 months but not at 6 months. Can the authors demonstrate the results of the statistical analysis as a graph or table?

Answer: Yes, we agree the 3rd paragraph of the discussion should be written in a more precise way and we have rewritten the paragraph accordingly (P7 L9-14). Proinsulin and IA were significantly related at all time points investigated (1, 6 and 12 months after disease onset), this association did not affect the relation between the PTPN22 variant and the proinsulin level. The relationship between IA and proinsulin is described in Table 2 (P13). The statistical analysis testing for this relationship is described in the statistical analysis-section (P5 L3-5).

Minor Essential Revisions:
2. More details of the total proinsulin assay should be described.

Answer: A sandwich ELISA assay using two monoclonal antibodies was used to determine the proinsulin level. The assay detects total proinsulin as well as the four metabolites: split(32-33), des(31-329, split(65-66) and des(64-65)-proinsulin. The detection limit is 0.3 pmol/l and the interassay coefficients of variation are <8.7%. This assay has no cross reactivity with insulin, C-peptide, IGF-I and IGF-II. This information has been added in the methods-section (P4 L11-14).

3. What about the mixed-meal? Was it constant among patients?

Answer: In order to estimate the residual beta-cell function (C-peptide and proinsulin) a Boost™-test (6 ml/kg (max: 360 ml, Mead Johnson, Evansville, IN, USA; 237 ml = 8 FL OZ contains 33 g carbohydrate, 15 g protein and 6 g fat, a total of 240 kcal)) was carried out at 1,
6 and 12 months ((± 1 week) after diagnosis in all 257 children with newly diagnosed type 1 diabetes. Blood was drawn 90 minutes after ingestion of the Boost™ drink. Serum samples were labelled and frozen at −20 °C until shipment on dry ice.

4. In Figure 1 and Figure 2, it may be better to change the labels of x-axis (month) to "0, 6, and 12"; and the kinds of lines (solid and broken) should be unified for PTPN22 genotypes,

Answer: Thank you for drawing our attention to the usage of different types of lines for the PTPN22 genotypes, it has been changed accordingly. The interval of the x-axis has been changed to ‘1, 6 and 12’, which are the time points for the measurements, in Figures 1A, 1B and 2.

5. How about the effects of HLA and INS genotypes on the levels of proinsulin and IA?

Answer: We did not find an effect of the different HLA risk genotypes on the proinsulin or the IA levels. We did find a difference between the INS -23HphI/VNTR genotypes on the IA levels at 1 month after onset, where the Class III (TT) genotype carriers have significantly lower IA levels compared to the other genotype groups (Nielsen LB et al 2006 Diabetologia (3)). We did not find a difference between this variant and the proinsulin during disease progression.

**Level of interest:** An article of importance in its field  
**Quality of written English:** Acceptable  
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.  
**Declaration of competing interests:**  
I declare that I have no competing interests.