Author's response to reviews

Title: Disease progression and genetic contribution in autoantibody negative type 1 diabetes Results from The Hvidore Study Group

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Author's response to reviews:

Cover Letter

Dear Editor in Chief

We hereby forward our answers to the reviewers. We have appreciated their thorough revision of the manuscript and address the questions and comments in the following pages point by point. Accordingly, changes are made in the manuscript.

Kind regards

Sven Pörksen

Answers to reviewers:

Reviewer 1:
Questions:
1. In the abstract and introduction, both aims of the present study are explicitly
pointed out, i.e. the comparison of pediatric type 1A and 1B diabetes and the genetic contribution to type 1 B diabetes. This nice study provides answers to both questions. The title of the manuscript does not fully reflect these major findings of this study. This is also the case for the last two sentences of the abstract that focus on the findings from one patient. Changes of the title and the conclusions (abstract) are therefore advised.

2. In the last paragraph of page 21 it is stated that “mutations in ABCC8 can cause idiopathic diabetes” in the 13 year-old patient. The in vitro data clearly support a functional role of this gene variant. On the other hand, considering the antibody data presented in the manuscript, there is little doubt that the 13 year-old indeed had autoimmune diabetes. It is therefore not essential to discuss to what extent diabetes in this patient has been caused by immune or non-immune mechanisms.

3. Effective sulfonylurea treatment requires intact beta cell function, as defined by C-peptide positivity. Treatment in the patient was performed 8 years after the initial diagnosis at a time when autoimmunity was far advanced and C-peptide was negative. It seems therefore not surprising that glibenclamide was ineffective at that time.

4. Figures 2B and 2C are derived from public database information and do not contain original data nor molecular modeling data. Inclusion of these figures is optional.

1. New title: “Disease progression and genetic contribution in autoantibody negative type 1 diabetes – Results from the Hvidøre Study Group.”

Deletion of the last part of the conclusion: “Mutations in ABCC8 may be a, so far, unidentified cause of AAB-negative childhood-onset diabetes. Superimposed type 1 diabetes in the present case may leave SU treatment ineffective.” (page 10)

2. Title of paragraph changed to: Mutation in ABCC8 (p 7, L 25)

3. Page 8, L 21 changed to: “We believe that the patient developed T1D in addition to the ABCC8-diabetes as he now is insulin-dependent, C-peptide- and IA-2A-negative but GADA-positive (14.9 U/ml, cut-off limit is 10 U/ml) and therefore had no beneficial effects of sulphonylurea treatment.”

4. We have chosen to skip these figures
Reviewer 2
Questions:

MAJOR COMPULSORY REVISIONS:

1. A major concern in regard to establishing the frequency of the “Ab-” phenotype in this cohort is lack of measurement of the ZnT8 autoantibody. Inclusion of ZnT8 autoantibody data would make this paper much stronger since it could well shift several of the Ab- pts into the Ab+ category. This is turn could affect some of the conclusions in regard to the sustained preservation of beta cell function in Abpatients. It could actually heighten the difference in regard to this outcome between Ab+ and Ab- groups, although it would make the latter group smaller. At any rate, it has to be done, since by now ZnT8 is now quite well established as a bona fide disease-relevant beta cell autoantibody. In fact, in table 1B, there is a blank column and a mysterious entry in the legend that suggests that this blank column had been created for ZnT8 Ab data. Are the authors choosing not to present this data for some reason? If so, this should be stated and discussed.

2. The study is described as a “multicenter … investigation with 18 participating centers from … countries in Europe and Japan”. However, there are no authors from the Japanese center(s). I raise this issue because the non-Caucasian ethnicities are not described. What ethnic groups comprised the 16% who were non-Caucasian? This is of relevance to understanding why ethnic frequencies were not different between the Ab+ and Ab- groups.

3. Somewhat related to my comment #2, it is surprising that there were no differences in HLA risk group frequencies between the Ab+ and Ab- groups. The authors should discuss the potential implications of this lack of difference. For example, could it be in part because of “occult” ZnT8 Ab+ patients in the Abgroup?

In other studies comparing patients with Ab+ to Ab- phenotypes of insulinopenic diabetes (e.g., in adults with A+B- compared to A-B- forms of ketosis-prone diabetes: Nalini et al, Diabetes Care 31(6):1195-1200, 2008) there are distinct differences in the frequencies of high-risk HLA class II alleles.

4. It is stated (p. 7) that 6 of the 22 Ab- patients had first-degree relatives with diabetes, and that apparently was the basis for investigating only these subjects for the two MODY genes. What were the phenotypes of diabetes in these relatives, and how was the information obtained so as to be reasonably sure that
such relatives were absent in those without a “family history of diabetes”. The authors should provide more details about the relatives’ phenotypes (at least some details of their ages of onset, potential for being type 1 or type 2, etc).

5. It is reasonable as well as useful to suppose that an Ab- group of new onset type 1 diabetic children might be enriched in monogenic forms of the illness, but why restrict the MODY gene candidates to HNF1a and HNF4a? Why not GCK, PDX and others?

6. More information is needed regarding the cutoff values for GADA. What was the basis for the cutoff of 10 units/ml? Was it set at the 99th percentile of a control group (as for the IA-2 Ab measurement)? If so, what were key characteristics of the control group? Ethnicity of the control group can affect background titers and percentile cutoffs for GADA, and thus the frequency of GADA positivity in the study group.

7. Given the lack of information about the non-Caucasian ethnicities, the small numbers of non-Caucasian subjects, and point #6 above, it appears unjustified to conclude (page 9) that ethnicity has a minor role in Ab- T1D in childhood.

MINOR ESSENTIAL REVISIONS:
1. On page 7, the reader is referred to figure 1 to demonstrate that the Ab-patients had lower “blood glucose fluctuations” than the Ab+ group. The term “fluctuations” is misleading, as it suggests that data were obtained from something along the lines of continuous glucose monitoring. In fact, I believe figure 1C shows change over two time points after a glucose challenge – at least, I think so; this figure needs a clear legend.

2. It would be helpful if ethnicity data were provided in Table 1A.

3. On page 7, it is stated that Ab- patients had 0.65% lower HbA1c than Ab+ patients – after what duration of time post-diagnosis?

4. Spelling is inconsistent, e.g. “sulphonylureas” (p. 10) vs. “sulfonylureas”.

5. The word “apparent” is unnecessary at the bottom of page 3.

6. The construction “…magnitude of immunological markers..” (page 3) is awkward. Are the authors referring to the number of autoantibodies?

Answers:

Major:

1. We have, of cause, thought about including measurements of the ZNT8-AB. At the time of statistical analysis and preparation of the manuscript measurement of antibodies was not performed As the process in this multicentre-study, involving
a large magnitude of contributors, had come this far we gave priority to a fast publication of these, in our opinion, very important findings on AAB negative type 1 diabetes. We are aware of the fact, including the ZnT8-AB would strengthen that the outcome to some extend. On the other hand our study, in its unique longitudinal design, is able to confirm anticipation of a milder disease progression in patients that tested negative on ICA, GADA and IA-2A, which are commonly used in both scientific settings and especially in clinical situations and still only a few laboratories have access to the methodology. – Discussed P 9, L14

2. Our study was designed in a way to make it able to distinguish between the majority of patients, white Caucasians, and the rest of the population, categorized as “Not white Caucasian”. Focus of the study was not primarily the identification of ethnic differences. Only 4 of the participants came from Japan.

3. As presented by Mortensen et al. in Pediatric Diabetes our study showed no HLA based susceptibility on any of the investigated parameters (standard bicarbonate at onset, stimulated C-peptide etc.). It suggests that HLA genotypes are predisposing to the onset of the disease but the partial recovery of the injured beta-cell depends on other factors, including the cytokine attack, improvement of peripheral insulin sensitivity and a regenerating beta-cell mass.


4. Data on family history of diabetes was self-reported by questionnaire. A MODY form of diabetes was suspected in families with first degree relatives in three consecutive generations with onset before the age of 25yrs.

5. In those children with a family history of diabetes we decided to exclude the most common forms of MODY presenting in a way that resembles onset of type 1 diabetes and therefore could be confused with the latter. This comprises mutations in HNF1alfa and HNF4alfa.

6. Measurements of GADA were performed at the Dep. of Autoimmunology, Statens Serum Institut, Copenhagen, Denmark.


7. We agree with the reviewer and delete this paragraph (page 9)

Minor:
1. Changed to "blood glucose changes" (p7). New legend figure 1. C:" The blood glucose change (90 minute value minus fasting value) during meal-stimulation differed significantly between autoantibody-negative and autoantibody-positive patients 12 months after disease onset (P = 0.004)."

2. As ethnicity in our study does not show any significant contribution to outcome
we chose not to include these individual information. (The group of 22 patients remaining autoantibody-negative did not differ significantly from the autoantibody-positive group with respect to sex (P = 0.40), age (P = 0.49), ethnicity (P = 0.80), HLA risk groups (p=0.68) or INS-VNTR genotypes.)

3. The difference in HbA1c comprised the whole 12 months follow-up period (P 7, L 18) and was the statistical result of a multiple regression analysis in a repeated measurement model.

4. Corrected

5. Deleted

6. Sentence changed to: “The risk for developing T1D seems to increase with genetic susceptibility in combination with the presence of immunological markers of beta-cell autoimmunity.”