## PEER REVIEW HISTORY



## ARTICLE DETAILS

| | |
|---|---|
| **TITLE (PROVISIONAL)** | The relationship between islet autoantibody status and the clinical characteristics of children and adults with incident type 1 diabetes in a UK cohort |
| **AUTHORS** | Bravis, Vassiliki; Kaur, Akaal; Walkey, Helen; Godsland, Ian; Misra, Shivani; Bingley, Polly; Williams, Alistair; Dunger, David; Dayan, Colin; Peakman, Mark; Oliver, Nick; Johnston, Desmond |

## VERSION 1 – REVIEW

| | |
|---|---|
| **REVIEWER** | Richard David Leslie<br>Blizard Institute,<br>4 Newark Street<br>London E1 2AT<br>UK |
| **REVIEW RETURNED** | 08-Dec-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | My previous comments have been addressed. They might make a small change to the comment relating to c-peptide. Since they will have sera collected in theory they should also be able to estimate a random C-peptide on each case.<br><br>The following references could bring the paper up to date<br>Also are comments on some references which over 20 years old and have been supplanted by other papers from the same groups.<br>Metabolic risk profiles in diabetes stratified according to age at onset,<br>islet autoimmunity and fasting C-peptide.<br>Wod M, Yderstræde KB, Halekoh U, Beck-Nielsen H, Højlund K.<br>Diabetes Res Clin Pract. 2017 Dec;134:62-71.<br><br>Adult-onset autoimmune diabetes: current knowledge and implications<br>for management.<br>Buzzetti R, Zampetti S, Maddaloni E.<br>Nat Rev Endocrinol. 2017 Nov;13(11):674-686.<br><br>Frequency and phenotype of type 1 diabetes in the first six decades of<br>life: a cross-sectional, genetically stratified survival analysis from UK Biobank.<br>Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT.<br>Lancet Diabetes Endocrinol. 2017 Nov 30. pii: S2213-8587(17)30362- 5. |

| | |
|---|---|
| | Association of Type 1 Diabetes vs Type 2 Diabetes Diagnosed During<br>Childhood and Adolescence With Complications During Teenage Years<br>and Young Adulthood.<br>Dabelea D, Stafford JM, Mayer-Davis EJ, D'Agostino R Jr, Dolan L, Imperatore G, Linder B, Lawrence JM, Marcovina SM, Mottl AK, Black<br>MH, Pop-Busui R, Saydah S, Hamman RF, Pihoker C; SEARCH for Diabetes in Youth Research Group.<br>JAMA. 2017 Feb 28;317(8):825-835.<br><br>The following references should be reviewed for more recent references from the same groups:<br>Ref 12<br>Ref 13<br>Ref 15<br>Ref 9 should replace Ref 17<br>Ref 18 is very similar and more recent than Ref 4<br>Ref 32 can be supplanted by paper in JAMA as above<br>Ref 34 and Ref 13 could be replaced by many recent papers from the same group |

| | |
|---|---|
| **REVIEWER** | Steve Bain<br>Diabetes Research Unit Wales<br>Institute of Life Science<br>Swansea University |
| **REVIEW RETURNED** | 13-Dec-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | A well-written manuscript.<br><br>I think BMI of children should also be reported since z-score will not resonate with most BMJ Open readers.<br><br>I also think the authors miss out on a major learning opportunity, namely that so many of these patients were overweight or obese. This is pretty much the major new finding of this study and it should be used to emphasise to general practitioners that not all new cases of type 1 diabetes are thin. It presumably reflects the general increase in weight of the younger population. |

| | |
|---|---|
| **REVIEWER** | Beverley Shields<br>University of Exeter, UK |
| **REVIEW RETURNED** | 14-Dec-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | This paper describes the characteristics of patients close to diagnosis with Type 1 diabetes, with particular focus on differences between patients with positive or negative antibodies, differences between White and non-White ethnic groups and differences between children and adults. The methods are generally described well and the study has been conducted to a high standard, but there are almost too many results presented in the paper so the main message of the paper gets a bit lost. I think there could be areas where it is made clearer. |

| | |
|---|---|
| | Major comments:<br>1) The aim of the manuscript (as stated at the end of the background) is to characterise people in relation to their autoantibody status, but this gets a bit lost due to all the other results added in. The three tables show three clear comparisons, and then there are two figures – one relating to predictors of antibody positivity/diabetes presentation, and the other relating to the association between age at diagnosis and antibody positivity. However, in the results the descriptions jump about between these tables and figures and it makes it quite hard to follow.<br>Could the results or figures be altered to be more in line with one another and be structured so as to relate to specific research questions? E.g. The results could be structured to first present the descriptives of the overall cohort, then describe the individual characteristics associated with diabetes presentation, and then the key results comparing antibody positive and negative patients (by individual characteristics first, then diabetes presentation) and finally which predictors are independent in a multivariable model. This would allow you to see the key results as to which factors are associated with antibody positivity at diagnosis, but also compare with whether they're the same factors as those predicting the symptoms and then the relationship between the symptoms and antibody status.<br>2) Are there any additions that could be made to the background to further explain why identifying the correlates of antibody positivity might be of interest? Is the idea to provide clinicians with more of an idea of when to test? Or is it purely for research interest in picking out those who might have a different phenotype?<br><br>More general comments on the statistical analysis:<br>1) Figure 1 –the association between age and antibody positivity looks non-significant as the point crosses the line. The units aren't presented but I'm presuming this is effect size per year? Could age be presented as a standardised variable (so the effect size is per SD increase rather than 1 year increase). It would make it easier to see and it might make the confidence intervals clearer.<br>2) For regression analysis, the variables themselves don't need to be normally distributed (it's the residuals that need to be normal) so transformation might not have been necessary – it's more important that there is good model fit and that the assumptions are met (e.g. association between predictor and either outcome (linear regression) or log odds of outcome (logistic regression) is linear; for linear regression, that the residuals are normally distributed and have constant variance). Were the appropriate checks of model fit carried out? If the model fit is reasonable without transformations, this would simplify analysis and interpretation of results.<br>3) Odds ratios in figure 1 should be presented on a log scale (e.g. halving of risk in one direction is equivalent to doubling the risk in the other direction, so equal distance should be shown for 0.5 from 1, and 2 from 1) |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1

Reviewer Name
Richard David Leslie

Institution and Country
Blizard Institute,
4 Newark Street
London E1 2AT
UK

Please state any competing interests or state 'None declared':
None though I am part of the T1DUK consortium which uses ADDRESS2 to ascertain patients.

Please leave your comments for the authors below:
My previous comments have been addressed.
They might make a small change to the comment relating to c-peptide. Since they will have sera collected in theory they should also be able to estimate a random C-peptide on each case.

Response: We are now measuring C-peptide in certain patients in samples taken specifically for this purpose. Unfortunately the samples which are obtained routinely are not taken and stored in a way suitable for C-peptide analysis.

The following references could bring the paper up to date Also are comments on some references which over 20 years old and have been supplanted by other papers from the same groups:

Metabolic risk profiles in diabetes stratified according to age at onset, islet autoimmunity and fasting C-peptide.
Wod M, Yderstræde KB, Halekoh U, Beck-Nielsen H, Højlund K.
Diabetes Res Clin Pract. 2017 Dec;134:62-71.

Adult-onset autoimmune diabetes: current knowledge and implications for management.
Buzzetti R, Zampetti S, Maddaloni E.
Nat Rev Endocrinol. 2017 Nov;13(11):674-686.

Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank.
Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT.
Lancet Diabetes Endocrinol. 2017 Nov 30. pii: S2213-8587(17)30362- 5.

Association of Type 1 Diabetes vs Type 2 Diabetes Diagnosed During Childhood and Adolescence With Complications During Teenage Years and Young Adulthood.
Dabelea D, Stafford JM, Mayer-Davis EJ, D'Agostino R Jr, Dolan L, Imperatore G, Linder B, Lawrence JM, Marcovina SM, Mottl AK, Black MH, Pop-Busui R, Saydah S, Hamman RF, Pihoker C; SEARCH for Diabetes in Youth Research Group.
JAMA. 2017 Feb 28;317(8):825-835.

Response: All of these references are now in the text. We have retained some of the older references where this was felt to be appropriate.

The following references should be reviewed for more recent references from the same groups:
Ref 12
Ref 13
Ref 15

Ref 9 should replace Ref 17
Ref 18 is very similar and more recent than Ref 4 Ref 32 can be supplanted by paper in JAMA as above Ref 34 and Ref 13 could be replaced by many recent papers from the same group

Response: Reference 12 – we could not find a more recent reference which was pertinent from this group but have added Dzidzonu et al, 2016, as a more recent paper from another group.
Reference 13 – we have now added a more recent reference from the SEARCH group – Black et al, 2013. We have left the original Dabelea et at, 2011 paper as it addressed well the specific point in the text.
Reference 15 is the recent BMJ Open description of the protocol for ADDRESS.
Reference 8 (not 9) is the NICE guideline on diagnosis and management of type 1 diabetes to which the reviewer refers. It has a detailed review of islet autoantibodies and we have repeated reference to it to reflect its being an excellent review. We have left reference 17 in the text as it refers to the method used for measurement of antibodies to ZnT8. We thank the reviewer for these suggestions.
Reference 16 (not 18) is similar to reference 4. We have left this in the paper however as it also refers to the method used in our paper.
Reference 32 is a recent (2016) study of high rates of ketoacidosis in Italy and we have left it in our paper for this specific purpose. The JAMA (2017) paper (see above) is now in the publication list.
Reference 34 has now been supplemented by Choleau et al, 2013.
Reference 13 has been supplemented by Dzidzomu et al, 2016.

Reviewer: 2

Reviewer Name
Steve Bain

Institution and Country
Diabetes Research Unit Wales
Institute of Life Science
Swansea University

Please state any competing interests or state ‘None declared’:
None

Please leave your comments for the authors below A well-written manuscript.

I think BMI of children should also be reported since z-score will not resonate with most BMJ Open readers.

Response: In our revised manuscript, rather than give unmediated BMI values for children, we have given the BMI z-score percentile equivalents. These now appear in either the Results text or as footnotes to the tables. We felt that giving percentiles was appropriate since children’s BMI values are only interpretable in terms of their z-scores or percentile equivalents in relation to age- and sex-matched reference data (for example, the overall median BMI in the children was 18.1kg/m2, which in an adult would imply appreciable underweight but in a male or female child of the median age 11.1 years implies a weight somewhat above average).

I also think the authors miss out on a major learning opportunity, namely that so many of these patients were overweight or obese. This is pretty much the major new finding of this study and it should be used to emphasise to general practitioners that not all new cases of type 1 diabetes are thin. It presumably reflects the general increase in weight of the younger population.

Response: The fact that 35% of the patients were overweight or obese is now emphasised in the text and has been put in the Abstract. The message has been reinforced by putting in the text data from the patients who had body weight measurements recorded shortly (<28 days) following diagnosis.

Reviewer: 3

Reviewer Name
Beverley Shields

Institution and Country
University of Exeter, UK

Please state any competing interests or state 'None declared':
None declared

Please leave your comments for the authors below This paper describes the characteristics of patients close to diagnosis with Type 1 diabetes, with particular focus on differences between patients with positive or negative antibodies, differences between White and non-White ethnic groups and differences between children and adults. The methods are generally described well and the study has been conducted to a high standard, but there are almost too many results presented in the paper so the main message of the paper gets a bit lost. I think there could be areas where it is made clearer.

Major comments:
1) The aim of the manuscript (as stated at the end of the background) is to characterise people in relation to their autoantibody status, but this gets a bit lost due to all the other results added in. The three tables show three clear comparisons, and then there are two figures – one relating to predictors of antibody positivity/diabetes presentation, and the other relating to the association between age at diagnosis and antibody positivity. However, in the results the descriptions jump about between these tables and figures and it makes it quite hard to follow.
Could the results or figures be altered to be more in line with one another and be structured so as to relate to specific research questions? E.g. The results could be structured to first present the descriptives of the overall cohort, then describe the individual characteristics associated with diabetes presentation, and then the key results comparing antibody positive and negative patients (by individual characteristics first, then diabetes presentation) and finally which predictors are independent in a multivariable model. This would allow you to see the key results as to which factors are associated with antibody positivity at diagnosis, but also compare with whether they're the same factors as those predicting the symptoms and then the relationship between the symptoms and antibody status.

Response: We agree with referee 3 and have substantially re-structured the Results accordingly along the lines suggested by the referee. We have also reduced the amount of data shown, including removing two tables (one on children vs adults and one on ethnicity), in order for the paper to focus on the importance of autoantibody positivity/negativity.

2) Are there any additions that could be made to the background to further explain why identifying the correlates of antibody positivity might be of interest? Is the idea to provide clinicians with more of an idea of when to test? Or is it purely for research interest in picking out those who might have a different phenotype?

Response: We were interested in the heterogeneity in patients at first presentation and have focused on the role of autoantibody status as a determinant. We now conclude the Introduction to our revised

manuscript with the following sentence: “We aimed to characterise these people with reference to their heterogeneity, focusing on the associations of autoantibody status with variation in presentation characteristics”.

More general comments on the statistical analysis:

1) Figure 1 –the association between age and antibody positivity looks non-significant as the point crosses the line. The units aren’t presented but I’m presuming this is effect size per year? Could age be presented as a standardised variable (so the effect size is per SD increase rather than 1 year increase). It would make it easier to see and it might make the confidence intervals clearer.

Response: We thank the reviewer for this helpful suggestion. Odds ratios, coefficients and 95% confidence intervals for age in Figure 1 now derive from standardised data.

2) For regression analysis, the variables themselves don’t need to be normally distributed (it’s the residuals that need to be normal) so transformation might not have been necessary – it’s more important that there is good model fit and that the assumptions are met (e.g. association between predictor and either outcome (linear regression) or log odds of outcome (logistic regression) is linear; for linear regression, that the residuals are normally distributed and have constant variance). Were the appropriate checks of model fit carried out? If the model fit is reasonable without transformations, this would simplify analysis and interpretation of results.

Response: Regrettably, there was an error in our original ‘Methods-Statistical Analysis’ section, which has, understandably, caused some confusion for which we apologise. The original sentence in question read: “For regression analyses, non-normally distributed continuous variables were transformed to normalize their distributions.” This should have read: “For regression analyses, continuous variables were transformed as appropriate to improve residual distribution normality, reduce heteroskedasticity and improve model fit.” Of the two continuous variables we considered (symptom duration and age) only symptom duration was subject to any transformation. So, for example, in univariable analysis of age as a predictor of symptom duration, use of the square root-transformed rather than the untransformed variable increased the t statistic for symptom duration from 6.7 to 10.9 and reduced the chi square for the Breusch-Pagan test for heteroskedasticity from 596 to 133. Statistically significant heteroskedasticity still remained after transformation but, in a plot of residuals against fitted symptom duration, this was virtually invisible - the correlation coefficients between the squared residuals and the fitted values being 0.02 for untransformed and 0.05 for transformed symptom duration. However, we have now established that transformation of symptom duration made no difference whatsoever to our conclusions regarding associations between symptom duration and individual characteristics. In univariable and multivariable analyses age was the principle predictor at $p<0.001$ regardless of transformation, followed by female at $p<0.001$ in univariable and multivariable analysis with square root transformed symptom duration and at $p=0.02$ and $p<0.001$ in univariable and multivariable analysis, respectively, with untransformed symptom duration. For simplicity, we have, therefore, followed the reviewer’s suggestion and used the untransformed variable in our revised Figure 1. Accordingly, variable transformation is no longer mentioned in our revised manuscript With regard to our logistic regression models for the binary categorical variables ketoacidosis, osmotic symptoms, weight gain and fatigue, plots of log odds against the single continuous explanatory variable, age, were consistently linear.

3) Odds ratios in figure 1 should be presented on a log scale (e.g. halving of risk in one direction is equivalent to doubling the risk in the other direction, so equal distance should be shown for 0.5 from 1, and 2 from 1)

Response: Odds ratio plots in Figure 1 are now presented on a log scale.