Author's response to reviews

Title: DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets

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Author's response to reviews: see over
The BMC Medical Genetics Editorial Team

Dear Prof Hengmi Cui,

We would like to thank the reviewers and the Editor for helpful comments on our manuscript 154276788847199 ‘DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets’ by Elin Hall, Tasnim Dayeh, Clare L Kirkpatrick, Claes B Wollheim, Marloes Dekker Niertt and Charlotte Ling. Following the suggestions of the reviewers we have extensively modified and amended the manuscript to clarify and address all the issues raised. This includes additional analyses as well as clarifications and changes in the text, tables and the figures. The additions and changes to the manuscript are marked with red and underlined text. We have also had assistance of a fluent English speaking colleague to correct the language of our manuscript.

These comprehensive modifications have improved our manuscript considerably and we feel that the revised manuscript now merits publication in BMC Medical Genetics.

Enclosed please find our point-by-point answers to the reviewers’ comments together with information of the incorporated changes, the revised Manuscript, Tables, Figures and Supplementary Information.

We now hope that our study will be suitable for publication in BMC Medical Genetics and we are looking forward to your decision.

Sincerely Yours,

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Response to reviewer’s report.

Title of the ms: DNA methylation of the glucagon-like peptide 1 receptor (GLP1R) in human pancreatic islets

Response to Prof Xin Gao’s review report.

Major compulsory revision

1. Conduct western blot analysis to examine the protein expression of the gene GLP1R and MeCP2
Answer: We agree with the reviewer that it would be interesting to analyze protein levels of the GLP1R and MeCP2 in the human pancreatic islets. Unfortunately, protein is not available from the pancreatic islets of these patients included in this study. However, Shu et al (ref 14) have previously shown that the protein level of GLP1R is decreased in pancreatic islets of human donors with type 2 diabetes. We have added this information to the discussion on page 12 of the revised ms. Additionally, we analyzed gene expression of MECP2 in pancreatic islets of patients with type 2 diabetes and non-diabetic donors but could not detect any differences in expression of MeCP2 between islets from non-diabetic donors and those with type 2 diabetes. These data have been included on page 10 and Figure 4A of the revised ms.

2. Correlation between the degree of DNA methylation of CpG site +199/205 of the GLP1R gene in pancreatic islets and A) GLP1R mRNA expression, B) BMI and C) HbA1c levels in all individuals of the studied cohort.
Answer: Based on this review comment, we have now included in the manuscript that there are no significant correlations between DNA methylation of this CpG unit and expression, BMI or HbA1c on page 9-10 of the revised ms.

3. In the discussion section of the manuscript, the present study suggests that hyperglycemia and/or obesity may affect DNA methylation of GLP1R, rather than DNA methylation of GLP1R influencing hyperglycemia. This discussion should be expanded.
Answer: We appreciate this comment and we have expanded this part of the discussion on page 12 of the revised ms.

Discretionary Revisions (which are recommendations for improvement but which the author can ignore)

1. Measure the levels of S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy) in the pancreatic islets and plasma levels of homocysteine in T2DM and non-diabetic donors. This may indicate if the levels of methyl group donors affect the DNA methylation of GLP1R.
Answer: This is an interesting suggestion. The pancreatic islets are supplied from the Scandinavian transplantation unit, which is not at liberty to supply us with plasma of these human donors. It is hence impossible to analyze plasma levels of homocysteine. Furthermore, the number of pancreatic islets from the human donors is limited and we are therefore unable to analyze AdoMet and AdoHcy within the islets. However, a previous study has shown that type 2 diabetes patients have decreased S-adenosylmethionine levels in the erythrocytes compared with control subjects. We have added this information to the discussion on page 12-13 of the revised ms.
2. Measure DNA methylase levels and activity or the mRNA and protein levels of selected DNA methylases in the islets of the two groups. This may indicate the mechanism of DNA methylation of GLP1R.

Answer: Based on this valid comment, we have analyzed the mRNA expression levels of three DNA methyltransferases, DNMT1, DNMT3A and DNMT3B, in pancreatic islets of human donors with or without type 2 diabetes. These data have been added to page 10 and Fig 4 of the revised ms.

Minor Essential Revisions (such as missing labels on figures, or wrong use of a term, which the authors can be trusted to correct)

Fig 1B/3 figure legends should add * p<0.05. **??
Answer: We apologize for this omission and have tried to add missing labels, use the correct terms and we have changed the figure legends of Fig 1B and 3.

Response to Prof Puspa Pandey’s review report.

Major compulsory revisions

1. The introduction part of the manuscript is too short and does not explain the rationale and significance of the study. The authors can elaborate it so as to give the readers a good idea about the manuscript.

Answer: Based on this valid comment, we have expanded the introduction and we have tried to explain the rationale and significance of the study more clearly.

2. In Figure 1B, DNA methylation pattern in T2D and control is very low. The authors also have not mentioned how many samples (donors) were used in the study.

Answer: We agree with the reviewer that the difference in DNA methylation between T2D and control islets for CpG unit +199/+205 is quite small. However, it still significant (p=0.022). In the abstract, result section and the discussion we mention that this difference is small. The number of donors used in this study (55 non-diabetic donors and 10 donors with type 2 diabetes) is included in the abstract, the introduction, the method section, the result section and in Table 1.

3. Does the 376 CpG site only negatively correlated with the GLP1R expression? The authors did not mention the correlation with other CpG sites that they stated in Fig 1A.

Answer: We did not find any significant negative correlations between DNA methylation and GLP1R expression, or any significant positive correlations with DNA methylation and BMI or HbA1c for the other studied CpG sites. We have clarified this in the results on page 9-10 of the revised ms.

4. The authors did not provide information regarding DNA methylation patterns in other CpG sites besides -376 in alpha and beta cells. It would be nice if they can check the methylation patterns in other sites as well.

Answer: Based on this comment, we have added DNA methylation data of all studied CpG sites in Supplementary Table 3 and we mention these results on page 10 of the revised ms.

5. Results, Discussion and Conclusions sections are too short to explain all the things.

Answer: We have expanded all these sections in order to explain the results more thoroughly.

6. Too little information is provided in this manuscript. In fact, all the 3 figures can be combined into one. Very limited experiments are done to prove their hypothesis.

Answer: We have expanded the revised ms and added new data e.g. gene expression of DNMT1, DNMT3A, DNMT3B and MECP2 in Figure 4 as well as Supplementary Table 3. Our study includes one of the largest existing cohorts of human pancreatic islets and to our knowledge only
four studies have previously examined the impact of T2D on DNA methylation in human pancreatic islets (see references 8, 11, 13, 24). However, no previous study has analyzed DNA methylation of the GLP1R, which is an important candidate gene for T2D. We therefore believe the results of this study are important to the readers of BMC Medical Genetics. Furthermore, several of our results are presented only in text of the result section and in tables instead of figures.

Minor Essential Revisions:
1. The names of manufacturers should be uniform throughout the text. For instance, in materials and methods section, only Invitrogen and Roche is written however elsewhere name, city and country is also given.

Answer: We appreciate this comment and we have added city and country when missing.