Author's response to reviews

Title: Evaluation of Four Novel Genetic Variants Affecting Hemoglobin A1c levels in a Population-Based Type 2 Diabetes Cohort (The HUNT2 Study)

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Author's response to reviews: see over
To the Editor
BMC Medical Genetics

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Dear Editor,

We are very grateful for the many valuable comments to our manuscript and for the possibility of resubmission. We have revised the manuscript according to the editorial requests and the issues raised by the reviewers, and believe that it now has been greatly improved.

The manuscript has increased somewhat in length in order to respond to the questions and comments raised by the reviewers. We have also done some minor linguistic and grammatical changes to improve the readability.

The manuscript is not under consideration for publication elsewhere and has been prepared according to the journal’s instructions to authors. It has been seen and approved by all authors. All authors have made substantial contribution to conception, design, analysis and/or interpretation of the data.

We thank you once more for considering this manuscript as an article in BMC Medical Genetics and hope that it now will be acceptable for publication.

Yours sincerely,

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Response to the reviewers' comments and questions:

**Reviewer 1: Hélène Choquet**

**Reviewer's report:**

Review of the paper “Evaluation of Four Novel Genetic Variants Affecting Hemoglobin A1c levels in a Population-Based Type 2 Diabetes Cohort (The HUNT2 Study).”

In this report, J. Hertel and colleagues evaluated the effect of four SNPs (located near the BNC2, SORCS1, GSC and WDR72 loci) previously associated with HbA1c in a context of type 1 diabetes on glycemic control in type 2 diabetes (T2D). They tested association between the four polymorphisms and HbA1c and non-fasting glucose levels in 1,486 subjects with T2D issued from a Norwegian population-based cohort.

The paper is well written and the data clearly presented. However, the current study may be significantly improved.

**Minor points:**

Q1. It might be interesting to test others polymorphisms previously reported associated with HbA1C levels in diabetic patients in the HUNT2 Study, such as the -429T>C promoter polymorphism of the RAGE gene (Laki J et al., Mol immunol, 2007).

A1. We fully agree with the reviewer that it would be both interesting and highly relevant to test other genetic variants previously reported to be associated with HbA1c levels in both diabetic and non-diabetic patients in the population-based type 2 diabetes cohort of the HUNT2 study. However, when we initiated the current study we decided to only focus on the four novel variants reported by Paterson et al in the DCCT study. On the other hand, for the referees we would like to inform that we have now started a project where we will test several of the genetic variants reported by Soranzo N et al., Diabetes, 2010 and Pare G et al, Plos Genet, 2008 in two larger and more extensively characterized (in respect of glycemic control-related traits) Norwegian cohorts.

Q2. It would be relevant to test others common variants previously reported associated with HbA1C levels in non-diabetic populations. For example, the SNPs from these two papers (Soranzo N et al., Diabetes, 2010 and Pare G et al, Plos Genet, 2008) might be tested in the HUNT2 Study.

A2. See answer to question 1.

Q3. Did the authors have access to other glycemic control-related traits such as fasting glucose?

A3. Unfortunately, we did not have access to other glycemic control-related traits than HbA1c.
Q4. Results subsection: In the reviewer’s view, the sentence “However, the results for SORCS1 (rs1358030) demonstrated the largest effect sizes ... and direction consistent with decreased glucose level” must be discarded knowing that “none of the risk alleles reached statistical significance with either increased HbA1c measures or increased non-fasting serum glucose levels”.

A4. We agree that this sentence might have put too much emphasis on non-significant findings. However, considering our results in light of previous reported results and features for SORCS1 we cannot refute a possible link between SORCS1 and glycemic control in type 2 diabetes. Thus, we think it is appropriate to mention that the direction of effect was consistent with increased HbA1c and glucose levels as seen in the DCCT study. We have now changed the sentence to: “Although the observed effects was non-significant and of much smaller magnitude than previously reported in type 1 diabetes, the SORCS1 SNP showed a direction of effect consistent with increased HbA1c and glucose levels, with an observed effect of 0.11% (P=0.13) and 0.13 mmol/l (P=0.43) increase per risk allele for HbA1c and glucose, respectively (Table 3).”

Major points:

Q1. In this report, the authors suggest that “further studies in other populations are needed to elucidate whether these novel sequence variants affect glycemic control in type 2 diabetes”. In these days of meta-analysis and collaborations it would be sound to use data from genome-wide association studies to confirm the lack of effect of these four SNPs on HbA1c levels in T2D subjects in a large meta-analysis.

A1. We fully agree with the reviewer and believe that the best course of action in a second step is to evaluate the effect of these four SNPs using data from GWA studies or other large-scale association studies in a large meta-analysis. However, to date, the majority of GWA and genetic association studies conducted on glycemic control-related traits have focused on fasting plasma glucose levels and only a few of glycated haemoglobin (HbA1c) levels. Furthermore, most of these studies have been performed in non-diabetic participants. Thus, we believe that more studies need to be carried out on HbA1c in type 2 diabetic individuals before such an approach will be beneficial. Nevertheless, although such an approach would be sound for this paper, it is out of the frame for our project and further strategy regarding the evaluation of these SNPs.

Q2. Subgroup analyses have been performed (subjects with HbA1c > or < 7.0%).
Given the sample size (N=1,486 subjects), the reviewer suggests not to apply such subgroup analyses for statistical power issues. Analyses in not adequately powered subsamples strongly increase the risk of false positive associations.

A2. We were aware of the power issue when applying subgroup analyses and the related increased risk for achieving false positive associations. We have decided to follow the suggestion from the reviewer not to apply such subgroup analyses. Thus, all text
throughout the manuscript involved in describing the subgroup analyses has been removed.

**Reviewer 2: Anne Marie Minahan**

**Reviewer's report:**

Hertel JK et al.,
*Evaluation of four novel genetic variants affecting haemoglobin A1c levels in a population-based type 2 Diabetes Cohort (The HUNT2 Study)*

**In the current manuscript the authors describe a study where they examine the impact of SNPs in four gene loci on glycemic control in type 2 diabetes. The gene loci were chosen as they have recently been highlighted as potentially mediating glycemic control in type 1 diabetics. The manuscript is generally well written, with results clearly described and the discussion reflective of the results.**

**Q1. However, the results are overstated in places, with too much emphasis placed on non-significant findings, which is somewhat misleading. For example in the results section in the main body of the text and in the abstract, you talk about the size effects for the SORCS1 SNP on HbA1c (P=0.13) and glucose (P=0.43). These results are clearly not significant. The text needs to be modified throughout to reduce the emphasis on non-significant findings.**

**A1. The above-mentioned sentence has been modulated and toned down, and the results and text describing the subgroup analyses (subjects with HbA1c > or < 7.0%) have been removed from the manuscript. We now believe that text reflects the results in a more accurate way. See also the answer to question 4 for reviewer 1.**

**Q2. It would be useful to include in the introduction/discussion a description of the known function of BNC2, SORCS1, GSC and WDR72. If these are unknown then that should be stated.**

**A2. We agree and have now included some more detailed information of the function and other relevant data for the BNC2, SORCS1, GSC and WDR72 loci in the discussion section.**

**Q3. Can you justify why no correction of your P-value was conducted for multiple testing, as would be the accepted procedure?**

**A3. It is correct that we did not correct for multiple comparisons. We wanted to perform a pure replication of the four SNPs previously reported associated with glycemic traits (i.e. same phenotypes and identical SNPs), thus it could be argued that the a priori hypothesis was clearly established beforehand for each of the four SNPS and we therefore presented the results as is. We appreciate that correction for multiple testing...**
should be considered and we therefore have clarified that the results should be interpreted with this in mind.

Minor comments

Q1. Not clear what is meant by ‘repeated hemoglobin’ in the second line of the abstract.
A1. By repeated hemoglobin we meant repeated hemoglobin A1c (HbA1c) measures, and this has now been clarified in the abstract.

Q2. Abstract: Last line of the ‘results’ section is vague and unclear what is meant.
A2. We have removed this sentence.

Q3. Abstract and main text: last line of conclusion. Are you suggesting that all four SNPs should be studied in other type 2 diabetes populations? Your analysis does not provide any evident for an affect of the BCN2 or GSC SNP.
A3. Based on previous reported findings and features for the SORCS1 locus, the results reported by Paterson et al and our results, we are inclined to believe that the previous reported association between the SORCS1 SNP and glycemic control is better supported, whereas those at the other loci have less statistical support. Thus, we agree with the reviewer that investigation of the SORCS1 SNP in other type 2 diabetes populations is certainly more attractive than testing those at the three other loci. We have changed the last line of the conclusion to: “Hence, further studies in other populations are needed to elucidate whether these novel sequence variants, especially rs1358030 near the SORCS1 locus, affect glycemic control in type 2 diabetes.”

Q4. Methods: Although the study population characteristics have been described in other papers, it would be useful if some basic information could be included here. For example, age range, BMI range and how diabetes was defined.
A4. We have now added information regarding age range, BMI range, HbA1c range and how diabetes was defined in the Method section.

Q5. Method, HUNT2 subjects and ethics: not clear what the term ‘non-premeditated dropout rate from an extensive population based study’ means. This needs to be clarified in the text.
A5. We are sorry for this confusing sentence, and have now changed this part of the text to: “The study population has recently been described [22-24]. In short, the participants were ≥20 years of age (range 21-97) and represent the total diabetes population drawn from an extensive population-based study (the HUNT2 Study).”

Q6. MODY: put in acronym in full
A6. The acronym is now in full.
Q7. Results: Unclear what is meant by ‘The results did not change notably in view of other genetic models’ (not shown).

A7. Among the four SNPs tested, there was no strong evidence for non-additive effects of the minor allele. We have now written as follows: “The results did not change notably in view of dominant or recessive genetic models (not shown).”

Q8. You state in your discussion, that strength of your study is that ‘your genotyping strategy is strong’. Can you clarify in the text what is meant by this? Also the meaning does not emerge from the final sentence of the discussion.

A8. By a strong genotyping strategy, we meant that the technique and platform applied for genotyping in the current study is a robust and reliable genotyping strategy (as seen by the 100% concordance rate). However, we have now removed this sentence and leave for the reader to determine if the quality results presented in the Methods section is good.