Author's response to reviews

Title: TCF7L2 and therapeutic response to sulfonylureas in patients with type 2 diabetes

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Author's response to reviews: see over
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Re: Revision of Manuscript

Dear Dr. Meyre,

thank you very much for your encouraging letter and for the opportunity to revise our manuscript entitled “TCF7L2 and therapeutic response to sulfonylureas in patients with type 2 diabetes” (Holstein et al.). We considered all comments raised by the Reviewers and revised our manuscript accordingly. We thank the Reviewers for their constructive criticism which, we feel, substantially improved the quality of our manuscript. All changes in the revised manuscript are indicated in red and underlined. Please also find attached our point-by-point response to the Reviewers’ concerns.

We sincerely hope that we satisfactorily addressed all issues and the paper could now be accepted for publication in BMC Medical Genetics.

With best regards,

sincerely,

Peter Kovacs

Comments to the Reviewers:

Reviewer: Jose Florez

1. The definition of sulfonylurea failure: the addition of any other antidiabetic medication (e.g. metformin, thiazolidinediones, exenatide, DPP-IV inhibitors), not just insulin, could also be indicative of sulfonylurea failure. Some information on whether other options were available to the physicians in this study would be helpful. Furthermore, the decision to escalate therapy
is also driven by physician inertia; given the availability of HbA1c as an index of treatment response, this biochemical measure alone would seem to be more objective and less prone to non-genetic environmental factors. Why not simply use HbA1c ≥7% as the definition of failure, regardless of how treatment was escalated?

- We absolutely agree that addition of any other antidiabetic drugs could be indicative of SU treatment failure and that attempt should be made to minimise non-genetic environmental factors. According to the Reviewer’s suggestions we now used HbA1c >7% as the definition of SU treatment failure and revised the manuscript adequately. As included in the revised manuscript (Subjects section), given the modified definition for SU treatment failure “Ninety-seven patients failed to respond to SU treatment according to our definition of A1C ≥7.0% after 6 months of treatment (76 patients treated with glimepiride, 19 with glibenclamide and 2 with gliquidon). The mean (±SD) daily dose of SU agents was comparable between subjects who failed to respond to SUs and the controls (5.0 ± 3.7 mg vs. 6.8 ± 3.7 mg, \( P = 0.13 \) for glibenclamide; 2.5 ± 1.6 mg vs. 2.5 ± 1.4 mg for glimepiride, \( P = 0.99 \)).”

In this part of the manuscript, we also included information on additional medication with metformin and patients who received insulin.

2. Confounding by severity of disease: the authors show that patients who required insulin in addition to a sulfonylurea were also younger at the onset of disease and had longer duration of type 2 diabetes. Therefore their genotype at TCF7L2 may have simply affected the manifestation of disease (in timing and/or severity), and not be specific to sulfonylurea response per se. This suspicion is further supported by the effect of adjustment for diabetes duration, which makes the \( P \) value under the additive model non-significant. A study where participants are matched for clinical characteristics at baseline (preferably in a prospective fashion) would be a more definitive way to answer this question. To bolster their claims, the authors should draw attention to the small change seen in the odds ratio after adjustment for diabetes duration, suggesting that the attenuation of effect is small even if the \( P \) value becomes non-significant.

- As mentioned in our response to Reviewer’s comment 1, we now used HbA1c >7% as the definition of SU treatment failure. In contrast to the previous data, modified definition of SU treatment failure resulted in several changes in presented data. Among others, age at onset of diabetes and diabetes duration did no longer differ between the groups of patients with SU treatment failure and the controls. Based on the changed dataset we revised our manuscript including the Results, Discussion and Tables adequately.

3. Lack of another treatment modality to support specificity: in contrast with the previous GoDARTS report, which compared the effect of genotype in people treated with metformin or a sulfonylurea, this study only examines sulfonylurea-treated patients. To demonstrate that the TCF7L2 effect is specific to the mechanism of action of sulfonylureas, it would have been nice to show that this genotype does not influence response to a non-insulin secretagogue. Can the authors repeat their analyses on the 72 metformin-treated individuals only?

- We thank the Reviewer for this suggestion. We now run the analyses on the 72 patients treated with metformin and found no effect of the genotype on response to this non-insulin secretagogue \([P=0.98; \text{OR } 1.01 (0.50-2.03)]\). The data further support the notion that TCF7L2 effect is specific to the mechanism of action of SUs. This has now been included in the Results and addressed briefly in the Discussion.
Results (page 6): “To investigate whether the rs7903146 effect is specific to the mechanism of action of SUs, we evaluated the genotype effects on response to a non-insulin secretagogue metformin. By analysing 72 metformin-treated individuals only, no effect of the genotype on SU treatment failure was found [P=0.98; OR 1.01 (0.50-2.03)].“

Discussion (page 8): “Even though limited by the small sample size (N=72), we also failed to observe any influence of TCF7L2 genotypes on the response to metformin, as a non-insulin secretagogue, thus further supporting the notion that TCF7L2 effect is specific to the mechanism of action of SUs.”

4. Incomplete analyses: it is not clear why genotype was not used as a predictor in the univariate regression analysis presented in Table 2 (although it is mentioned as an independent predictor in the Abstract and Discussion). A stepwise regression in this model would have helped sort out to what extent the effect of genotype is confounded by other variables.

- We originally omitted the genotype from Table 2, since genetic associations data were presented in detail in Table 3. According to the Reviewer’s comment, we now included the TCF7L2 genotype in Table 2. Following the Reviewer’s suggestion for using HbA1c>7% as a definition of SU treatment failure (comment 1), analyses with the revised dataset identified rs7903146 genotype as the only predictor of SU treatment failure and so, no stepwise regression was performed.

5. Unclear justification for the recessive model: multiple studies have demonstrated that this locus exerts its effects via an additive model. Thus there is no a priori justification to use a recessive model, unless it is being used simply to attain statistical significance.

- Yes, we agree with the Reviewer and are aware that based on previous studies, the TCF7L2 risk alleles exert their effects through an additive mode of inheritance. Having limited numbers of homozygous TT carriers, we used the recessive model to increase sample sizes of the genotype groups for statistical analyses. According to the Reviewer’s comment we now omitted the recessive model from the manuscript and only present the data in an additive model.

6. Low sample size: for genetic variants that confer a modest effect on risk, a much higher sample size is typically needed. The authors should provide power calculations describing the magnitude of the effect they are expected to detect, and how that compares with the previous publication.

- The Reviewer is perfectly right - one of the major limitations of our study is the low sample size and so, limited statistical power. Taking into account genotype frequencies and the sample size in our study we had a power of 80% (at α =0.05) to detect genetic risk (odds ratio) of 1.8 for treatment failure in additive mode of inheritance (using software Quanto version 1.2.2). In contrast, the GoDARTS study by Pearson et al (Diabetes, 2007) had 80% power to detect risk (OR) as low as 1.3. This has now been included in the Discussion of the revised manuscript (page 8).

7. Other endpoints: if genotype at rs7903146 makes patients more resistant to the action of sulfonylureas, one would expect that carriers of the risk genotype should be less likely to become hypoglycemic. This endpoint could provide supportive evidence, particularly in the context of a protocol originally designed to study hypoglycemia.
- Many thanks for this suggestion. We indeed looked at the relationship between the TCF7L2 genotype and severe hypoglycaemia events. Unfortunately, we could not observe any differences in the frequency of the diabetes risk allele between patients with and without hypoglycaemia (P=0.30). We addressed this issue in the Discussion (page8) as part of the study limitations:

“Given the TCF7L2 diabetes risk genotypes make patients more resistant to the action of sulfonylureas, one would expect that carriers of the risk genotype should be less likely to become hypoglycemic. However, in our study we could not observe any differences in the frequency of the diabetes risk allele between patients with and without hypoglycaemia (P=0.30).”

Reviewer: Marie Pigeyre

2 major revisions:

1) I think the pretreatment HbA1c should be added in the univariate regression logistic, because it is probably a predictor factor of sulfonylurea failure.

- We absolutely agree with the Reviewer in this point. However, HbA1c levels prior to the treatment are unfortunately not available for the present study and therefore, could not be considered for the analyses. It is noteworthy however, that pretreatment HbA1c has been considered in the GoDART study by Pearson et al. (Diabetes 2007) and inclusion of this variable as covariate strengthened the association between sulfonylurea response and genotype at rs7903146. This has now been included in the revised manuscript (page 7):

“Considering pretreatment HbA1c levels as covariate in logistic regression analyses even strengthened the association between sulfonylurea response and genotype at rs7903146 [15].”

2) It would have been interesting to study also the rs12255372 at TCF7L2, because it is also associated of the increased risk of T2D.

- Since TCF7L2 rs7903146 diabetes risk genotype has been reported as having the strongest association with T2D (Zeggini E, McCarthy MI, Diabetologia 2007 – cited in the revised manuscript) and since both SNPs, rs12255372 and rs7903146 are in nearly complete linkage disequilibrium (LD), only the rs7903146 was chosen for the present analyses. As a matter of fact, in a previous study in a German population (Körner et al, JCEM, 2007 – also cited in our manuscript), we genotyped both SNPs and based on high LD (r²=0.8 and D'=1) we found nearly identical association results for them. Therefore, to avoid any redundancy in further studies (including the present one) we have only genotyped one SNP as a representative variant for the LD group.

3) This study replicates the previous findings of the GoDARTs study. So, even if there are some methodological differences, the new knowledge brought by these data remains limited.
Indeed, our study could confirm previous findings by Pearson et al (Diabetes, 2007). Nevertheless, as we also stated in the Introduction (page 3), ‘‘Since a causal phenotype-genotype relationship can not be established with one initial report, replication studies are the backbone to the genetic epidemiology of complex diseases [16], and play a crucial role in pharmacogenomics as well.’’ Therefore, we strongly believe that the manuscript might be of interest to those working on genetics of complex diseases, particularly those dealing with diabetes.