**Author’s response to reviews**

**Title:** HNF1A gene p.I27L is associated with early-onset, maturity-onset diabetes of the younglike diabetes in Turkey

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**Author’s response to reviews:**

We express our special thanks to the reviewer for his/her careful reading of the paper, and for providing valuable comments and suggestions which have helped improved both the content and the presentation. We believe that the revision has been successful and the paper has been improved. The responses to reviewer’s comments are available in this document.

HNF1A gene p.I27L is associated with early-onset diabetes mellitus in Turkish population.

Main comments

Reviewer reports:

Monika Buraczynska (Reviewer 1):

The manuscript presents data from the study on the association of HNF1A gene polymorphism with early onset of diabetes mellitus in Turkish population. Since the literature on the subject is scarce the paper could be potentially interesting for those in the field. However, some issues should be addressed before acceptance.
1. The description of patient group is very unclear. Did all 565 subjects presented with clinical suspicion of MODY (see abstract)? If indeed all of them were suspected of having MODY, the age at diagnosis should be \( \leq 25 \) years. In the Patients and methods section the authors state that inclusion criterion was "age at onset above 35 years". And again, in Table 1 the mean age at diagnosis is 25 years. Besides, the mean age of patients is 28.17 years. So how come for inclusion in the study the disease onset was set at > 35 years? Please clarify this issue.

- ‘All 565 subjects presented with clinical suspicion of MODY.’ This sentence was corrected in abstract. ‘Mean age of control was 49.18±3.38 year’. This sentence is added to results. Only 3 diabetic patient’s age at diagnosis was 28, 31 year and 33 year. So, we excluded from study, the onset of diabetes age was 24.08±4.82 year (5-25 year). This sentence was rewritten in results. The diabetic patient’s mean age 28.177 was age at study time. So, this data is deleted in Table 1. Inclusion in the study the disease onset was below 25 year. This data is corrected in patients-methods.

2. All p values in Table 1 should be checked. For example, for creatinine (that the authors mistakenly call "creatine") p is \(< 0.001\) not 0.156 as they state.

- Creatinine was corrected. ‘Creatinine was 1.24±8.91 p=0.018’ was corrected in table 1. This was mistakenly written.

3. Table 3 - in the footnotes for this table the authors write "SNPs were expressed as allelic frequency (q) or prevalence of genotypes (%)”. However, there are no allelic frequencies or percentage of genotypes given in this table!

- ‘SNPs were expressed as allelic frequency (q) or prevalence of genotypes (%)’ sentence was deleted from Table 3.

4. The authors did not mention what was the power of the study. The sample size calculation has to be shown in the Methods section, along with the information which parameters were used to calculate power and sample size.

‘The power analysis was performed according to http://osse.bii.a-star.edu.sg/calculation2.php.

This study was recruited 749 subjects to have 68 % power with % 5 type 1 error level to detect a minimum clinically significant difference”. This sentence was included in statistical analyses.
5. The English language should be extensively revised. Grammar and spelling still need to be corrected.

- The English language was revised.

Monika Dmitrzak-Weglarz (Reviewer 2): The text still needs language correction. The lack of the use of correct articles, the lack of an appropriate form of singular or plural and typos are often repeated errors.

- English language was revised.

It is moving away from the use of MODY with the number; it is necessary to complete the correct names with gene mutations.

- ‘After genetic analysis, diabetic patients (n=46) had HNF1A, HNF1B, HNF4A, GCK gene mutations and diagnosed as, respectively, MODY3, MODY5, MODY1 and MODY3.’ was added to methods.

The scheme of including patients in the study is incomprehensible. Of the 567 patients with suspected MODY, 76 patients were excluded who had mutations in one of 4 genes, including HNF1A. Subsequently, variants in the HNF1A gene were again examined in the remaining patients and controls. Please complete the information on which variants were examined in the first part of the study.

- In the first part of the study, GCK, HNF1β and HNF4A single nucleotide polymorphisms were studied. This data was included in material part.

Over 30 genes contribute to an increased risk of developing type 2 diabetes. The most important risk factor is the TCF7L2 allele, which increases the risk of developing diabetes 1.5 times and has not been included in the study. It is necessary to provide a more detailed justification for the selection of polymorphisms studied only in one gene, without the others having a more significant impact.

- In the first part of the study, we found that more than 100 diabetic patients had HNF1A SNPs including I27L, S487N and A98V. Those diabetic patients did not have MODY mutations. We wonder whether these HNF1A SNPs would increase the risk of developing early-onset diabetes or not. So, healthy controls examined to have HNF1A SNPs. ‘The aim of this study was to obtain the effects of MODY associated single nucleotide polymorphisms on developing diabetes. This is the first study investigating the association between HNF1A
SNPs and having early-onset non-monogenic diabetes in Turkish population’ was added to introduction part.

Adoption of an arbitrary age of 45 as an early and late age cut-off point is not a right approach, mainly since the average age of onset of the subjects was 28.17 +/- 12.66. Determine the cut-off point individually for the study group by determining the median age of diagnosis +/- 2SD. It may turn out that there will be a completely different distribution of patients and the frequency of polymorphisms.

- Mean age of control was 49.18±3.38 year. The onset of diabetes age was 24.08±4.82 year (5-25 year). The diabetic patient’s mean age 28.177 was age at study time. The early or late-onset of diabetes was identified by using age 45 years, as described in previous studies, Holmkvist 2006 and Gamboa-Melendez 2012. Holmkvist and Gamboa-Melendez showed that late-onset diabetes would onset over 45 year. ‘If we select control subjects whose mean age diagnosis is +/- 2SD as almost below 28 year, these subjects will develop diabetes further life’. This sentence was included in material part.

At work, it should be supplemented how many patients meet the criteria for early and late onset of type 2 diabetes. The results should also be supplemented with a comparison between these two groups.

- ‘All control subjects (n=263) did not have diabetes. All diabetic patients (n=486) met the criteria for early onset of type 2 diabetes’. This sentence was added to material part.

When using the Bonferroni correction, it is not possible to consider a statistically significant value of p <0.05. Please complete the calculation.

- We analyze only two groups, so we did not perform ANOVA and bonferroni correction. ‘Comparisons of variables between three groups were analyzed by analysis of variance (ANOVA) with Bonferroni adjustment for multiple comparisons or Kruskal-Wallis test when appropriate.’ was deleted in statistical analyses. This sentence was mistakenly written.

After completing the results, the text, in particular, the conclusions should be modified.

In the conclusions, the authors should put information on the usefulness of the obtained results and the recommendation on the presence or absence of diagnostic/practical value of the studied polymorphisms.
- The conclusions and discussion were modified according to results.

Literature needs updating, no citation from 2017-2019.

- New citation was included the study.