Author's response to reviews

Title: Association of genetic variants in the promoter region of genes encoding p22phox (CYBA) and glutamate cysteine ligase catalytic subunit (GCLC) and renal disease in patients with type 1 diabetes mellitus

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

Dear Professors,

We are resubmitting the manuscript entitled “Association of genetic variants in the promoter region of genes encoding p22phox (CYBA) and glutamate cysteine ligase catalytic subunit (GCLC) and renal disease in patients with type 1 diabetes mellitus”. We are grateful for the opportunity to revise the paper and for the comments that helped to improve this manuscript. All criticisms were considered and revised accordingly to the suggestions.

Thanking in advance, I remain truly yours.

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ANSWERS TO REVIEWER COMMENTS:

Reviewer #1

Minor points:

1. Generally in the manuscript, the official rs numbers should be appeared when it is possible. It is commonly accepted to use the official rs numbers when referring to the SNPs (e.g. GCLC -129 C#T = rs1788390 and GPX3 -65 T#C = rs8177412). This would not only simplify reading the manuscript but also differentiate between the known and the novel SNPs (such as CYBA -675 T#A that is not referenced).

As suggested, the official rs numbers were used.

2. The Authors might detail the abbreviations “MDRD”, “UAER” and “ACR”.

As suggested, the abbreviations were detailed.

3. The sentence “Fifteen percent of the SNPs evaluated by RFLP and by PCR ... were confirmed by direct sequencing and no misgenotyping was detected” might be reformulated. The Authors should give the genotype success rates for each SNP and if they double genotyped a subfraction of the sample, it may be worthwhile to provide a concordance rate for each SNP.

The phrase was reformulated in the revised version of the manuscript and the genotype success rates for each SNP were informed as well as the concordance rate after double genotyping.

4. It may be worthwhile to present briefly the clinical characteristics (sex ratio, age, age at diabetes, arterial hypertension, etc. ...) of the studied subjects according to the case-control status in a table.

A table was inserted in the revised version of the manuscript containing demographic, clinical and biochemical characteristics according to GFR status.

5. The Authors might precise which software they used to assess the statistical power.

The software used to calculate the statistical power was mentioned in Material and Methods (Cats Power Calculator).
6. In the Discussion subsection, the authors might name the number of the SNP in the sentence “No previous studies have associated this SNP ...”. Furthermore, the part of this sentence “but a study performed in a Swedish population of type 1 diabetes patients found that those with the CT genotype ...” might be reformulated to improve the understanding. **SNP rs number was inserted and the phrase was reformulated in the revised version of the manuscript.**

7. In Table 1, the authors should provide the exact accounts of genotypes distributions. **The numbers were inserted in Table 1.**

8. It would be relevant to advert the study of Hodgkinson AD and colleagues in the introduction or discussion part. (Hodgkinson AD, et al. Association of the p22phox component of NAD(P)H oxidase with susceptibility to diabetic nephropathy in patients with type 1 diabetes. Diabetes Care. 2003) **The study of Hodgkinson et al. was inserted in the Discussion section.**

9. The reference 18 does not appear in the References subsection and the reference 17 exists neither in the text nor in the References subsection. **The reference numbers were corrected in the revised version of the manuscript.**

10. Results presented in Table 3 should be more detailed in the Results subsection. **As suggested, the results presented in Table 3 were detailed in the Results section.**

11. The conclusion sentence “The functional SNPs ... require validation in additional cohorts” should be reformulated such as “Furthermore, replication studies for these functional variants will need to be carried out.” **The conclusion sentence was reformulated.**

Reviewer #2

**Major points:**

*Subject and methods*

1. Are controls age/gender matched?  
   Yes. This information was inserted in the revised version of the manuscript.

2. Are there different ethnic groups within patients?  
   **We defined ethnic background according to the subjects’ self determination. Ninety percent of the patients defined themselves as Caucasian, 7.3% as African descendants**
and 2.7% as Asian descendants. However previous genetic studies already demonstrated that due to the great admixture which characterizes the Brazilian population, color, as determined by physical evaluation, is a poor predictor of genomic African ancestry (Parra et al. PNAS 2003: 100: 177). For that reason, we preferred to omit the ethnic background.

3. The description of patients with (n=104) or without (n=196) overt DN is unclear as the total adds up to 300 not 401. Is that because overt DN is undetermined for some patients? Please clarify. The total number of patients considered for this analysis was smaller because patients with microalbuminuria were excluded – this information was inserted in the revised version of the manuscript.

4. Please replace rs1788390, located on chromosome 21 and not chromosome 6 (GCLC) by the correct dbSNP ID. The rs number was corrected (rs17883901)

* Results

5. What are the phenotypes of the few CYBA-A/A and GCLC-T/T patients? There are only three CYBA A/A patients and they are all GCLC C/C. None of them presents overt diabetic nephropathy or GFR < 60 mL/min/1.73 m².

6. Did the authors look at the effect of the combination of CYBA+GCLC risk alleles in patients? Since the T/T genotype of the CYBA SNP is very frequent, 62 out of 70 (88.5%) of the patients carrying at least one risk allele (T) of the GCLC SNP also carry the risk allele of the CYBA SNP in homozygous (patients carrying only one T allele were not considered because the protective effect of the A allele of CYBA SNP was considered to be dominant). Thus, the percentage of patients with GFR < 60 mL/min in the group carrying GCLC C/T or T/T concomitantly with CYBA T/T (45.5%) was similar to the percentage of patients with GFR < 60 mL/min observed when only GCLC genotypes were considered (47.1% - Table 3) and higher than the frequency of GFR< 60 mL/min in carriers of risk alleles in either CYBA or GCLC SNPs (31.6%).

*Discussion

7. Did the authors look at the effect of the CYBA-T allele on hypertension in this cohort? We did not find any association between hypertension and the CYBA-T allele. Our explanation for this finding is that in type 1 diabetes patients, hypertension is normally a consequence of incipient or overt diabetic nephropathy and not essential hypertension, which was associated with the CYBA-T allele in the Spanish study by Moreno et al.
8. Were other SNPs tested in this cohort of patients, for example polymorphisms found by meta-analyses e.g. Mooyaart et al. Diabetologia 2011?
No. We studied only the three SNPs which have never been evaluated in diabetic nephropathy.

9. Reference [18] is not listed under “References” and there is no reference [17].
The reference numbers were corrected.

Minor Essential Revisions

*Abstract

10. Abstract/Background: “NOX/NADPH oxidase generates...” should be replaced by “NOX/NADPH oxidases generate...”
The phrase was reformulated as suggested.

11. Abstract/Background: “encoded by CYBA gene” should be replaced by “encoded by the CYBA gene”
The phrase was reformulated as suggested.

12. Abstract/Background: SNP IDs (rs numbers) should be indicated.
SNP ID numbers were indicated as suggested.

13. Abstract/Methods: “Patients were sorted according” should be replaced by “Patients were sorted into two groups according”. Define the number of patients in each group.
The phrase was reformulated as suggested.

14. MDRD needs to be written out in full when first mentioned, and also in “Subjects and methods”.
MDRD abbreviation was detailed as suggested.

15. Abstract/Results: “in the group carrying the TT genotype” should be replaced by “in the group carrying the T/T genotype”. Please check for consistency across the whole manuscript.
All genotypes were written in the same way across the manuscript.

16. Abstract/Conclusion: The space should be removed between -> and A.
As suggested, the space was removed.
*Background
17. “encoded by CYBA gene” should be replaced by “encoded by the CYBA gene” “coded by the GCLC gene” should be replaced by “encoded by the GCLC gene”

The phrases were reformulated as suggested.

*Subject and methods
18. “The study was carried out in compliance to the Institutions’ Ethics Committee and to Declaration of Helsinki” should be replaced by “The study was carried out in compliance with the Institutional Ethics Committee and the Declaration of Helsinki”

The phrase was reformulated as suggested.

19. “to each subject” should be replaced by “by each participant”

The phrase was reformulated as suggested.

20. Please add an alternative description for variants, e.g. in relation to a genomic reference sequence, particularly for the unregistered SNP.

An alternative description (location) was provided for all SNPs.


The primers and probe sequences were provided in the revised version of the manuscript.

*Results
22. Indicate that “data is not shown” for GPX3 results.

The information was added in the revised version of the paper.

23. Please refer to “CYBA” in this sentence: “The frequency of overt DN was significantly lower in the group of patients carrying genotypes TA+AA…”

The phrase was reformulated as suggested.

24. “Years-old” should be replaced by “Years old”

Replacement was performed as suggested.

*Discussion
25. “We evaluate a different functional…” , this sentence should be reformulated to clarify that it is the proportion of cases with low GFR that is lower in TA or AA cases, not different rates <60

The phrase was reformulated as suggested.

26. “the allele T” => “the T allele”
Replacement was performed as suggested.

27. “it is plausible to suppose that” => “it is plausible that”
Replacement was performed as suggested.

28. Specify that it is the T allele that was “previously associated to myocardial infarction”.
The phrase was reformulated as suggested

29. “effect of this SNP at the age of onset” => “effect of this SNP on the age of onset”.
The phrase was reformulated as suggested.

30. “this study widen” => “this study widens”.
The phrase was corrected.

*Table 1
31. TT should be replaced by T/T
Replacement was performed as suggested.

32. What is the significance of “***” after 73.7% for GPX3-TT in control subjects? Add a footnote if necessary.
** was a mistyping which was corrected in the revised version of the manuscript.

33. There is an extra space before C/C for GPX3 genotypes.
The extra space was removed.