Author's response to reviews

Title: Association between -T786C NOS3 polymorphism and resistant hypertension: A prospective cohort study

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Author's response to reviews: see over
Dear Editor of BMC Cardiovascular Disorders,

We have addressed the constructive and helpful comments of your reviewers below and modified the paper accordingly. We believe that with your help the manuscript is much improved and hope that you find it suitable for publication on this occasion.

Yours sincerely,

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Reviewers' comments:

Reviewer #1: The article submitted by Cruz-Gonzalez and colleagues are well written, simplifying the study understanding. The aim was to investigate if the promoter (-786T>C) and exon 7 (G894T / Glu298Asp) polymorphisms of the eNOS gene are associated with resistant. The question posed by authors was well defined, however a improved literature review is extremely necessary, including, for example, recent articles evaluating eNOS in resistant hypertension (Sandrim et al, 2006), functional effects of eNOS variants (Joshi MS, 2007), etc. Moreover, it is essential to add in results section the clinical patient characteristics. About the results found by authors, if it be consider a Bonferroni correction (P=0.05/2=0.025) the difference found by authors would be not significant. At least, this may be including in discussion. How the power calculation was done? Finally, the authors genotyped only hypertensives subjects, and information about normotensives frequencies (healthy subjects) are lacking. The authors should include this group in the study and/or put in discussion some information about the eNOS polymorphism in Spanish healthy population.

Response:

- We have improved literature review according to the reviewer comment.
- We have included a new table with the patient characteristics.
- We agree that using the Bonferroni correction the difference would be not significant, this limitation has been included in the manuscript. However there is controversy regarding the use of this correction and some authors claim that “Bonferroni adjustments are, at best, unnecessary and, at worst, deleterious to sound statistical inference” (Perneger T. What's wrong with Bonferroni adjustments? BMJ 1998; 316: 1236-1238), and in the multivariate regression analysis we showed a significant difference comparing C allele homozygotes (-786CC) and carriers of ≥ 1 T allele (-786TT, -786TC); RR 2.09 (95% CI: 1.03-4.24) p 0.004.
- We have included the data of a healthy control group as the reviewer suggested.
- Sample size and statistical power were estimated according to the preliminary results obtained in this study as there was a lack of previous studies on this topic. Sample size was estimated for a 95% confidence intervals and 80% statistical power.

Reviewer#2
1. The purpose of the study was to determine a possible association between the -786T>C and G894T (Glu298Asp) polymorphisms of the NOS3 gene and resistant hypertension. The
question posed by the authors well defined in the main text of the MS, but not in the Abstract section. My first suggestion is to define the aim in the abstract section.

*We have added the aim to the abstract section according to the reviewer’s comment*

2. Methods are appropriate and well described.

3. Data are sound.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? I would suggest adding the cumulative number in Tables 1 and 2 in rows and columns (for example: Table 1, GG genotype 18 + 87 = 105 – add number 105 in column, etc.)

*We have added the cumulative numbers as the reviewer suggested*

5. Are the discussion and conclusions well balanced and adequately supported by the data? Discussion is well balanced and adequately supported by the data. My suggestion is to add the power of the study and comment the power of the study with regard to the number of cases with resistant hypertension

*We have added the power of the study and its calculation in the statistical analysis section*

6. Are limitations of the work clearly stated? The limitation of the study might be the number of cases with resistant hypertension. My suggestion is to calculate the power of the study and comment the power of the study with regard to the number of cases with resistant hypertension.

*We have included the power of the study in the manuscript*

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? To my knowledge used the authors crucial published genetic MS with regard to eNOS3. There is, however, one report about the Glu298Asp SNP of the eNOS synthase gene that was associated with drug-resistant hypertension from another EU county (Chech republic) that could be mentioned (Jáchymová M, Horký K, Bultas J, Kozich V, Jindra A, Peleska J, Martásek P, Biochem Biophys Res Commun, 2001).

*This study was referenced in the introduction section but we have added a comment in the discussion section*

**Reviewer#3**

**Major Compulsory Revisions**

1. In this study, a clinical definition of resistant hypertension is used which involves excluding those subjects with: a.) secondary causes of hypertension; b.) white coat hypertension; c.) inadequate dosing of antihypertensive medications; and d.) non-adherence to treatment.
Detailed information regarding how subjects were included or excluded in the study, for at least each of these factors, must be delineated.

*This point is well taken but we don’t have that specific data*

Protocols and validation of measures such as analytic control and adherence tests should be included. What types of diets were subjects ingesting during the period of observation? Were dietary influences controlled during the period of blood pressure assessment? Pertaining to the assessment of target organ damage, were funduscopy and echocardiography measures validated?

*The diet of the patients was controlled by the physician at the Hypertension Unit. The funduscopy and the echocardiogram were performed by a single-operator blinded to the patient’s condition.*

2. A table of all baseline characteristics including statistical comparison between the resistant hypertensive and control groups should be included. This table should include several relevant factors such as body mass index or other suitable anthropometric values and lipid values. Likewise a table of antihypertensive medication use between groups would be useful. Results of the 24-hour ambulatory blood pressure assessment were not included. Details of the multivariate analysis should be included. Were multiple models investigated? The issue and handling of multiple comparisons should be explicitly specified in the methods section. It would appear that no correction of p-values for multiple comparisons was applied in this study.

*A table of the baseline characteristics has been included in the manuscript. Details of the multivariate analysis have been described in the statistical analysis section.*

*No correction of p-values for multiple comparisons have been applied and this has been included in the limitations section. Although we agree that using the Bonferroni correction the difference would be not significant, there is controversy regarding the use of this correction and some authors claim that “Bonferroni adjustments are, at best, unnecessary and, at worst, deleterious to sound statistical inference” (Perneger T. What’s wrong with Bonferroni adjustments? BMJ 1998; 316: 1236-1238). Furthermore, in the multivariate regression analysis we showed a significant difference comparing C allele homozygotes (-786CC) and carriers of ≥ 1 T allele (-786TT, -786TC); RR 2.09 (95% CI: 1.03-4.24) p 0.004.*

3. Acknowledgment of limitations should be explicitly included. This cohort has possibly been used other studies (J Hum Hypertens. 2009 Mar 12 - epub ahead of print) which would also
have an effect on the multiple comparisons issue as noted above. This issue should be addressed.

*We have included that comment in the limitations section*

Minor Essential Revisions

1. Grammatical errors and awkward sentences were noted in several places. For example, on page 2 (abstract), … “therapy could be determined at [the] endothelial level.” On page 4, “Once excluded the secondary causes of hypertension and those patients who did not adhere lifestyle measures…”.

*We have corrected the grammatical errors.*

Discretionary Revisions

1. To provide additional perspective, the discussion would benefit from a brief synopsis of the studies of these particular polymorphisms and other nitric oxide synthase variants with respect to hypertension including references to positive, negative, and indeterminate studies.

*We have included a new sentence with new references (including a recent metaanalysis) in the discussion.*

2. Was there consideration of a haplotype analysis in any fashion?

*The haplotype analysis was performed (data not shown) but we felt that it did not improve the global message of the manuscript.*

**REVIEWER#4**

Major Compulsory Revisions

The selection criteria are clear, however no indications are given about the various therapeutic regimens that patients received nor, at least, about the first choice drug that was used, an information that might be important in order to correlate the genetic background with the responsiveness to therapy. The studied subpopulations comprise respectively 48 resistant hypertensive patients and 232 responsive patients. (Of note, table 1 includes 254 controlled hypertension subjects. Could you explain this discrepancy?).

*The first choice drug was left to the discretion of the individual physician*

*We have modified the discrepancy in table 1 (it was a typographical error)*

The author studied two well known polymorphisms and both ID SNPs code and information on the allelic distribution in a Caucasian control population should be included.

*We have added the data of a control group.*

*We have included ID SNPs*
It should be worth to enlarge the group of resistant hypertension patients in order to enhance the statistical power of the study, or at least to provide information about the statistical power of the population studied.

*We have provided the statistical power of this study.*

From a strictly statistic point of view, the authors used chi square and Fisher’s exact test to compare the subgroups of patients, however, a correction for multiple tests should also be included, since more than one polymorphism has been studied. It should be relevant to combine the two polymorphisms and to reconstruct the haplotype, if the linkage disequilibrium pattern allows it, as the two polymorphisms are quite near one to the other.

*No correction of p-values for multiple comparisons have been applied and this has been included in the limitations section. Although we agree that using the Bonferroni correction the difference would be not significant, there is controversy regarding the use of this correction and some authors claim that “Bonferroni adjustments are, at best, unnecessary and, at worst, deleterious to sound statistical inference” (Perneger T. What’s wrong with Bonferroni adjustments? BMJ 1998; 316: 1236-1238). Furthermore, in the multivariate regression analysis we showed a significant difference comparing C allele homozygotes (-786CC) and carriers of ≥ 1 T allele (-786TT, -786TC); RR 2.09 (95% CI: 1.03-4.24) p 0.004.*

However, the most important point is that the authors need to analyse at least a second population of hypertensive patients in order to confirm their findings.

*We agree that these results should be confirmed in a second population*

Minor Compulsory Revisions

It should be worth to analyse also the allelic distribution of other polymorphic variants that are known to influence the development of hypertension (such as, for example ACE I/D genotype) and other new genes emerging from WGAS (Whole Genome Association Studies).

*This is a very interesting comment, we will take it into account in future projects*

Should the influence of the polymorphism of NOS3 be confirmed, the authors need to emphasize the possible clinical implications, such as modifications in management and/or personalization of the approach to patients.

*We consider that these results should be confirmed. We have emphasized the possible clinical implications in the discussion.*

Although the paper does not seem to add much information on the comprehension of the causes of resistant hypertension and it provides only a weak cause-effect relationship between the disease and one polymorphism, it could be reconsidered for publication after major revision.