Author's response to reviews

Title: Association of the eNOS E298D polymorphism and the risk of myocardial infarction in the Greek population

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Author's response to reviews: see over
RESPONSE TO COMMENTS

REVIEWER #1 (Anke Tonjes):

**Major issues**

**Comment 1:**

The authors did not include a power calculation. The statistical power should be calculated based on the sample size of the presented study, MAF, expected effect size and mode of inheritance. The provided reference is not appropriate.

**Answer:**

The relevant analysis of the current study was performed on QUANTO, using recessive model. Setting MAF at 0.3, expected OR from 1.5 to 2.5 (univariate OR for the relationship between TT/GG+GT and the risk for MI) and power at 80%, we found the results shown below (QUANTO output). Thus minimum OR, given the sample size of our study, was 2.00, which is in accordance with our findings. The previously provided reference in the statistical section has been deleted.

<table>
<thead>
<tr>
<th>RG</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5000</td>
<td>646</td>
</tr>
<tr>
<td>1.6000</td>
<td>471</td>
</tr>
<tr>
<td>1.7000</td>
<td>363</td>
</tr>
<tr>
<td>1.8000</td>
<td>291</td>
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<tr>
<td>1.9000</td>
<td>241</td>
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<tr>
<td>2.0000</td>
<td>204</td>
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<td>175</td>
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<tr>
<td>2.2000</td>
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<tr>
<td>2.3000</td>
<td>136</td>
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<tr>
<td>2.4000</td>
<td>122</td>
</tr>
<tr>
<td>2.5000</td>
<td>110</td>
</tr>
</tbody>
</table>

N is the number of cases required for the desired power
The required number of controls is 1xN
Comment 2:
The authors should include the meta-analysis now mentioned in the response to the comments in the discussion in the manuscript (also regarding effects sizes, association with other phenotypes, possible interactions etc).

Answer:
The meta-analysis is now mentioned in the discussion part of the manuscript.

Comment 3:
In the methods section it is stated that TT was compared to GT+GG but different models are given in the tables. The authors should please consider the classical additive/recessive/dominant models as requested and provide the reason for preferring the recessive mode of inheritance for the reader (e.g. based on the results of the meta-analysis of Casas et al.).

Answer:
In statistical methods, it is actually stated that: “To study the univariate association of MI with the eNOS genotype we modeled the data through logistic regression analysis (crude odds ratios). Subsequently, a multivariate analysis was performed to estimate the risk of MI for TT versus GT+GG after adjustment for a series of possible risk factors and confounders (adjusted odds ratios)”. Thus, univariate estimates for all possible combinations are provided (Table 3) firstly, and subsequently we focused our research on TT versus GT+GG (Table 4). So there is no gap between text and tables. Concerning your comment “consider the classical additive/recessive/dominant models”, please find those models in Table 3.

Given that the model TT vs GG+GT revealed the higher OR, we decided to investigate further its relationship with the risk of MI. This was not possible to be done for further combinations eg. GG vs TT+GT or GT vs TT+GG given: (a) the limited extent of a publication, (b) the risk of bias when performing a variety of multiple comparisons without a scientific foundation behind. Thus our opinion is to focus the analysis on TT vs GG+GT, which is in accordance with the meta-analysis of Casas et al.
Comment 4:
The provided study limitations do not appropriately reflect study specific aspects such as sample size, distribution of confounding risk factors etc.

Answer:
In respect with sample size, a relevant phrase has been inserted in discussion. At the onset of the study, a literature search was conducted in order to identify possible confounding factors. According to the findings, the most common confounders were actually all included in this study.

Comment 5:
The effect sizes are rather large in comparison to the meta-analysis of Casas et al and also in comparison to the effect sizes of other risk variants for complex phenotypes. Furthermore, the OR increases with ascending number of covariates (table 4). Based on the small sample size (15 subjects with TT genotype in the control group and 27 in the case group) the chance of biased effect size due to low case number per strata is quite high. The authors should debate this scenario and add it in the discussion. Furthermore, even the recessive model seems to fit best in the meta-analysis the additive model should still be considered if sample size is small.

Answer:
We identified a significant association between homozygous carriers of the T allele and the occurrence of MI and this association was also found in the meta-analysis of Casas et al. Our effect sizes are indeed larger than the respective effect size of the meta-analysis. Nevertheless there are three studies included in the meta-analysis (Hingorani et al, Shimasaki et al, Colombo et al), which found even higher odds ratios than our study. Moreover Casas et al incorporated in their article studies with sample size smaller than ours. Given the rare frequencies of some genotypes in the general population, this kind of studies cannot achieve high number of participants. A relevant phrase has been inserted in discussion section.

Minor issues
Comment 6:
Table legends are incomplete. Table1: on which test is the P-value based on?, Table 2: legend is missing, which test was used?, Table 3: legend is missing, please provide additive mode of inheritance as well.
Answer:

Relevant footnotes are inserted in Tables 1, 2 and 3 and more detailed description of statistical tests is provided in statistical section. Furthermore, we incorporated in Table 3 the relevant estimates for the additive model.

Comment 7:
The authors now performed a logistic regression adjusted for other known risk factors of MI. Why were age and BMI not used as quantitative covariates?

Answer:

BMI was used in all analyses as a continuous variable. Thus in Table 4, BMI was actually inserted as a continuous variable but we opted to define the increment at 2 kg/m$^2$ for better interpretation of results. Concerning age, the selection of the cut-off point of 60 years was based on the mean estimate of the age of the total sample size of the study. Mean as well as median age of the 422 study participants was almost 60 years. In addition, we have run alternative models with insertion of age as a continuous variable, but the results remained almost unchanged. Given that the binary format is easier to be interpreted by a non-familiar with statistics reader we preferred to present age as a dichotomous variable.

REVIEWER #2 (Stavroula Kanoni):

Discretionary Revisions

Comment 1:
The authors should justify their selection of the cut-off point of 60 years of age, for the use of age as a nominal variable in their analyses (Tables 1 and 4).

Answer:

The selection of the cut-off point of 60 years was based on the mean estimate of the age of the total sample size of the study. Mean as well as median age of the 422 study participants was almost 60 years. In addition, we have run alternative models with insertion of age as a continuous variable, but the results remained almost unchanged. Given that the binary format is easier to be interpreted by a non-familiar with statistics reader we preferred to present age as a dichotomous variable.