Title: T null and M null genotypes of the Glutathione S-transferase gene are risk factor for CAD independent of smoking

Authors:

Khaled K. Abu-Amero (kamero@kfshrc.edu.sa)
Olayan O. Al-Boudari (olayan@kfshrc.edu.sa)
Gamal G. Mohamed (gmohamed@kfshrc.edu.sa)
Nduna N. Dzimiri (dzimiri@kfshrc.edu.sa)

Version: 2 Date: 29 March 2006

Author’s response to reviews: see over
Article Ref # 1741364518955723 \[T^{null} \text{ and } M^{null}\text{ genotypes of the Glutathione S-transferase gene are risk factor for CAD independent of smoking}\]

Dear Sir/Madam

We have revised the manuscript putting into consideration all the reviewers’ comments.

Please find below our detailed responses to the reviewers’ comments.

We hoped we have responded adequately to the queries of the reviewer and that our manuscript is now suitable for publication in the BMC Medical Genetics journal.
A. Detailed responses to the queries of Reviewer Lülüfer Tamer

1. Results can be shortened in abstract section
   The results section in the abstract was shortened as requested by the reviewer.

2. In methods, exclude criteria can be given for control and patient (cancer, autoimmune disease etc.)
   The inclusion and exclusion criteria for both CAD and controls are now detailed in the methods section [see page 4-5].

3. In methods, to give methods of TG and cholesterol will be better (name of system or principal of manual method)
   The automated method used and the analyzer details are now detailed in the methods section [see page 5].

4. Demographic data of patients and controls can be given in a table.
   A new table was created which include the detailed demographic data for both CAD patients and controls [see Table 1, page 17].

5. In table 1, the percentiles of both CAD and control subjects can be indicated.
   The percentiles are mentioned now in Table 1 with the demographic data for CAD patients and controls.

6. The GSTT1 and M1 null genotypes in tables if they were thought as risk factors must be indicated. In table 4, the combination of genotype (M1 null) x smoking which has been thought as a risk factor, shown as protective factor. This might be raised in the statistical analysis period. There might be a mistake in the definition of risk when entering the categorical covariates.
   The GSTT1 and M1 null genotypes are now detailed clearly in tables 4 and 5.
   With regard to the second point, it is noteworthy that that there is an interaction between the effects of two exposures, if the effect of one varies dependent on the level of the other. For example, the protective effect of breastfeeding against infectious diseases in early infancy is more pronounced among infants living in
poor environmental conditions than among those living in areas with adequate water supply and sanitation facilities. An alternative term for interaction is “effect modification”. In this example, we can think of it as the quality of environmental conditions modifying the effect of breastfeeding. Interaction, effect modification and heterogeneity are different ways of describing exactly the same thing.

Odds ratio for (genotype X smoking, 0.469) is chosen specifically to test for interaction, since their estimated value is of no use by itself. An interaction here means that the smoking differences are not the same for both genotypes and, equivalently, that the genotype difference is not the same for smokers and non-smokers. If there is a significant interaction, as is in this case, then it will usually be best to report the effect of smoking separately for each level of the genotype as we did in table 6.

For more on information on interaction please see chapter 26 in ‘Statistical models in epidemiology, David Clayton and Michael Hills, 1993, Oxford science publications).
B. Detailed responses to the queries of Reviewer Maria Grazia Andreassi

The authors should provide more details on severity of CAD. Indeed, it would be interesting to evaluate the distribution of GST genotypes in relation to the disease severity.

1. In a way our, CAD group is already a selected group with severe CAD (with 70% narrowing of the coronary arteries). Hence this group cannot be further sub-classified. In order to make that clear we have now added the phrase “and can be defined as having severe disease” on page 4 of the revised manuscript.

2. Furthermore, analysis of the data based on the number of affected vessels (1, 2 or 3) showed no association with $T^{\text{null}}$ and $M^{\text{null}}$ allele distribution (results not shown).

In Tables 4 where authors show a significant interaction between the GSTM1 genotype with smoking, a protective OR = 0.469 is presented: which jointly predictive variables (described as interactive effect) was entered into the model? Please clarify.

It is noteworthy that there is an interaction between the effects of two exposures, if the effect of one varies dependent on the level of the other. For example, the protective effect of breastfeeding against infectious diseases in early infancy is more pronounced among infants living in poor environmental conditions than among those living in areas with adequate water supply and sanitation facilities. An alternative term for interaction is “effect modification”. In this example, we can think of it as the quality of environmental conditions modifying the effect of breastfeeding. Interaction, effect modification and heterogeneity are different ways of describing exactly the same thing. Odds ratio for (genotype X smoking, 0.469) is chosen specifically to test for interaction. Their estimated value is of no use by itself. An interaction here means that the smoking differences are not the same for both genotypes and, equivalently, that the genotype difference is not the same for smokers and non-smokers. If there is a significant interaction, as is in this case,
then it would be best to report the effect of smoking separately for each level of the genotype as we did in table 6.