

Author's response to reviews

Title: Polymorphisms of the Flavin Containing monooxygenase 3 (FMO3) gene do not predispose to essential hypertension in Caucasians.

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Author's response to reviews: see over

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Dear BioMed Central Editorial Team

This letter accompanies our revised version of “Polymorphisms of the Flavin Containing monooxygenase 3 (FMO3) gene do not predispose to essential hypertension in Caucasians.” Please find below our responses to the reviewers comments. Reviewers’ comments are in quotes and italics and our responses are in normal text (changes 1-10, numbers highlighted in bold) We have also changed some items (not suggested by reviewers) that we thought we could make clearer (changes 11-13).

List of changes that have been made to the manuscript

Reviewer: Brian James Morris

“Please present P values to 2 significant figures, not 3. The latter is superfluous.”

1.

P values have been changed throughout text and in tables 3 and 6 to 2 significant figures instead of 3

Reviewer: Elizabeth A Philips

“The experimental design and statistical analysis seem sound. The premise for the design of the experiments i.e. the link between FMO3 polymorphic variation and hypertension was not strong from the available evidence. The authors have indeed gone on to show a negative correlation between FMO3 variants and hypertension.”

2.

We have improved and clarified the description of the background to the hypothesis by replacing paragraph 3 of the background section from;

We have previously identified a number of individuals with trimethylaminuria who manifest idiopathic hypertension. A number of probands with TMAuria have independently reported adverse reactions to tyramine-containing foods and symptoms suggestive of disordered biogenic amine metabolism including encephalopathic hypertension. Probands with *FMO3* null mutations, whose effect is the loss of the functional *FMO3* enzyme, also have increased excretion of catecholamines, consistent with our *in vitro* observations that null *FMO3* alleles confer loss of *FMO3* mediated oxime formation from biogenic amines. In addition, Cashman et al have reported a proposed link in African-American males between hypertension and increased excretion of trimethylamine. This evidence suggests an involvement of *FMO3* in hypertension.

to;

We have previously noted that a number of patients with TMAuria have idiopathic hypertension. In addition, Cashman et al have reported a proposed link in African-American males between hypertension and increased excretion of trimethylamine. We have identified increased excretion of catecholamines in a proband with TMAuria who is homozygous for a deletion of the *FMO3* gene suggesting a possible association between abnormal catecholamine metabolism and variants of the *FMO3* gene. Endogenous substrates for *FMO3* include tyramine and phenylethylamine and tyramine has a known pressor effect. As high levels of circulating catecholamines contribute to hypertension, it is proposed that polymorphisms of *FMO3* gene could contribute to impaired catecholamine metabolism and hypertension.

*“Background: Change the last sentence of the paragraph on TMAuria to read. ‘We and others have ..’ and insert the reference of. Dolphin, C.T., Janmohamed, A., Smith, R.L., Shephard, E.A. and Phillips, I.R. (1997) A missense mutation, pro153leu (C>T), in the gene encoding flavin-containing monooxygenase 3, *FMO3*, underlies fish-odour syndrome. Nature Genet. 17, 491-494.”*

3.

The sentence in question above has been changed to read “We and others” and the suggested reference by Dolphin et al has been inserted.

Reviewer: Peter Wilhelmus de Leeuw

“The authors fail to provide evidence that would make the search for the role of the polymorphisms biologically defensible. What evidence is there that the polymorphisms under study would indeed lead to alterations in haemodynamic regulation”

As change 2 described above

“Previously, the authors found in their Quebec population evidence for an altered distribution of the polymorphisms among hypertensives. The discrepancy with the current

data, however, remains undiscussed. If the authors really thought that the polymorphisms could be biologically important, they should address this issue.”

4.

As the Quebec population data was never peer reviewed, in compliance with the reviewers comments we have decided to omit this reference

“On page 12, the investigators state that FOM3 has only a minor effect on catecholamines. Why then the current studies?”

As change 2 described above

“Table 3 does not read easily.”

5.

Table 3 has been redesigned in landscape format to make it clearer.

Reviewer: Ingolf Cascorbi

“The hypothesis of the study is based on the observation, that subjects having deleterious FMO3 mutations occasionally have hypertension. However, the functional significance of the polymorphisms investigated is less pronounced. What are the putative effects on catecholamine levels in vivo?”

We haven't carried out any *in vivo* studies of catecholamine levels in subjects of varying genotypes. However we have improved and clarified the background to the tested hypothesis as per change 3.

“The information on a significant association among hypertensives in a Canadian population given in the introduction part should be omitted, since these results were not peer reviewed.”

As change 4 described above

“1. The AIB phase II study group may be considered as a population survey. However, information on the exact number of subjects considered as hypertensive is missing. Only less than 4% took antihypertensive medications, this is a much lower percentage than obtained in an average Caucasian population.”

6.

The low levels of hypertensive drug therapy in the AIB phase II study group is due to the requirement at phase I to be free of hypertension. We have changed the paragraph describing the AIB phase II study population in the methods section from:

The first population was recruited from the Allied Irish Bank Phase II (AIB Phase II) study that commenced in 1989 and consists of 387 bank employees (Table 1). Blood pressure was measured every 30 minutes for 24 hours using a validated monitor – the SpaceLabs 90207. The mean daytime and night time systolic (SBP) and diastolic (DBP) values were used for analysis in each case. Hypertension was defined as having daytime blood pressure of 135/85mm Hg or greater. This group was collected between 1998 and 2002 and was between the ages of 30-70 years. It was noted if individuals consumed more than 2 alcohol-containing drinks per day.

to:

The cohort of 815 current and retired bank employees and their spouses, were Caucasian, and free of diagnosed hypertension, when recruited to the Allied Irish Bank (AIB) Study. At the baseline (phase I) examination, conducted between 1989 and 1991, age, gender, smoking habit, alcohol consumption, salt intake, family history of cardiovascular events, past medical history, current drug treatments, height, weight and CBPM were recorded, and 24-hour ABPM was performed. Between 1996 and 2001, all phase I participants were invited to a follow-up (phase II) examination. After a mean interval of 8.4 years, 441 subjects responded, and underwent repeats of the phase I assessments. In addition, blood was drawn for biochemical measurements and for extraction of DNA. We excluded 54 participants because of technically unsatisfactory ABPM (n=9) or because DNA extraction was not successful (n=45). Thus the total number of participants included in the present analysis was 387, of which 224 were men and 163 were women. All subjects provided written informed consent to each phase of the study. The study protocols of the two phases were approved by the Beaumont Hospital research ethics committee

“2. The cardiovascular disease group was selected due to cardiac symptoms, therefore hypertension is only one risk factor contributing to the inclusion criteria. The entire study should be therefore better considered as association study of FMO3 polymorphism to coronary artery disease, not to hypertension itself. However, there are large studies in this area, enrolling patients, with coronar-angiographically verified coronary artery disease. Aside history of hypertension (which is a weak parameter) any analysis should consider the major risk factors such as diabetes, hypercholestroemia, bmi, age, gender and smoking in a logistic regression analysis. Moreover, nearly all CAD patients, subjects considered to be hypertensive or not, received four antihypertensive drugs in parallel.”

7.

We chose hypertension as our primary endpoint for the reasons discussed above, and do not believe it is helpful to change the primary endpoint at this stage. In response to the reviewer’s interest in seeing a test of the role of FMO3 in CAD, we have added an

analysis of CAD. We have added this description to the 4th paragraph of the statistical analysis section:

Logistic regression analysis was used to predict if any of the SNPs influence the risk of cardiovascular disease, including diabetes, BMI, hypercholesterolemia, smoking status, age and gender in the model.

and the findings to the 8th paragraph of the results section:

Likewise it was shown by logistic regression that none of the SNPs were significant predictors for cardiovascular disease when the CVD and the AIB Phase II study groups were compared (E158K p=0.55, V257M p=0.76, E308G p=0.63). In this model, the covariates of diabetes, hypercholesterolemia, smoking, age, sex and BMI were included, and only diabetes was found to be a significant predictor.

8.

We agree that >90% of subjects in the CAD population designated as non-hypertensive were taking blood pressure lowering drugs for the symptomatic relief or for CAD protection. We have added the following sentence to the results section, paragraph 2:

Blood pressure measurements are only presented to characterise, rather than compare, the groups, since medication is lowering blood pressure in most of the CAD group, but not in most of the AIB Phase II study group.

“3. In table 6 both study populations are mixed, however, since nearly all CAD patients received blood pressure affecting medications a comparisons of blood pressure does not appear to be possible.”

9.

The title for Table 6 contained an error. The results in this table are based on the analysis of the AIB Phase II study group only rather than both groups of subjects as stated. This error has now been corrected.

“Was the genotyping methodology verified by DNA sequencing?”

10.

The following sentence has been added to the last sentence of the FMO3 polymorphism genotyping methods section.

Confirmation of the validation of the mutation detection method was based on the use of known positive DNA controls (which had been sequenced) for the three polymorphisms provided by McGill University (Montreal).

11.

Last sentence in the abstract was changed from:

Conclusions: These results suggest that the variants in the *FMO3* gene do not predispose to essential hypertension in this population

To:

Conclusions: These results suggest that the variants in the *FMO3* gene do not predispose to essential hypertension in these two Irish populations.

12.

We feel that our description of the analysis being “stratified by gender” as slightly misleading, so we have changed from the words “stratified by gender” to “separate analysis was carried out for males and females” throughout the text as follows.

Statistical analysis, paragraph 5, changed from:

All of the above analyses were repeated stratifying according to gender.

To:

All of the above analyses were performed separately for males and females.

Results, paragraph 5, changed from:

When stratified by gender, there were still no statistically significant associations between any of the specific haplotypes and daytime SBP in the AIB Phase II study (males overall $p=0.912$, 3df and females overall $p=0.606$, 3df) or with hypertension status in the CAD patients (males overall $p=0.587$, 3df and females overall $p=0.816$, 3df).

To:

When separate analysis was done for males and females, there were still no statistically significant associations between any of the specific haplotypes and daytime SBP in the AIB Phase II study (males overall $p=0.91$, 3df and females overall $p=0.61$, 3df) or with hypertension status in the CAD patients (males overall $p=0.59$, 3df and females overall $p=0.82$, 3df).

Results paragraph 6, changed from:

Stratifying the analysis by gender, there was still no association between genotypic frequencies in the AIB Phase II study group (females E158K $p=0.919$, V257M $p=0.861$, E308G $p=0.131$, males E158K $p=0.371$, V257M $p=0.871$, E308G $p=0.384$).

To:

Analysing males and females separately, there was still no association between genotypic frequencies in the AIB Phase II study group (females E158K $p=0.92$, V257M $p=0.86$, E308G $p=0.13$, males E158K $p=0.37$, V257M $p=0.87$, E308G $p=0.38$).

Results paragraph 7, changed from:

When this analysis was stratified by gender, still no statistically significant differences are noted (females E158K p=0.785, V257M p=0.541, E308G p=0.290, males E158K p=0.709, V257M p=0.221, E308G p=0.661).

To:

When this analysis was performed separately for males and females, still no statistically significant differences are noted (females E158K p=0.79, V257M p=0.54, E308G p=0.29, males E158K p=0.71, V257M p=0.22, E308G p=0.66).

Discussion, paragraph 1 changed from:

For this reason the analysis was stratified by treating males and females as separate groups.

To:

For this reason the analysis performed separately for males and females.

Discussion paragraph 2 changed from:

Neither did the stratified analyses show any statistically significant associations.

To:

Neither did the separate analyses for males and females show any statistically significant associations.

13.

We have changed the name of the “CVD group” to the “CAD group” throughout the manuscript as we feel that this is a more accurate description of the study group.

We hope that the revised manuscript is clear and we look forward to hearing from you again soon.

Best regards
Ciara Dolan