Author's response to reviews

Title: Polymorphism of the FABP2 gene: a population frequency analysis and an association study with Cardiovascular Risk Markers in Argentina.

Authors:

Laura L C Gomez (lgomez@fcm.uncu.edu.ar)
Sebastian S M Real (sreal@unsl.edu.ar)
Marta M S Ojeda (msojeda@unsl.edu.ar)
Sergio S Gimenez (sergio.gimenez@osep.mendoza.gov.ar)
Luis L S Mayorga (lmayorga@fcm.uncu.edu.ar)
Maria M Roque (mroque@fcm.uncu.edu.ar)

Version: 3 Date: 17 April 2007

Author's response to reviews: see over
Author’s response

Title: Polymorphism of the FABP2 gene: a population frequency analysis and an association study with Cardiovascular Risk Markers in Argentina.

Authors:
Laura C Gomez
Sebastián Real
Marta Ojeda
Sergio Gimenez
Luis S Mayorga
María Roqué

Version 2. Date April 17th

Dear Editor:
We are pleased to send you a revised version of our manuscript which includes the revisions suggested by the reviewers. We consider the manuscript has been improved with their comments.

Looking forward hearing from you,

Maria Roqué, Ph.D.
Laboratorio de Biología Celular y Molecular
Facultad de Ciencias Medicas
Universidad Nacional de Cuyo
Mendoza, Argentina
Response 1:
The reviewer's observation is correct regarding the non-significant difference between Thr54 carriers and non-carriers for high cholesterol and BMI. The statistical non-significance of this difference is mentioned as first issue, in the Results and Conclusions of the Abstract (pages 1, 2): "No significant association was found with any of the tested markers in the context of our Argentine nutritional and cultural habits." Moreover, we found no association between the Thr54 allele and any of the five selected markers.", in the last paragraph of Results (page 5): “Our study reveals no significant association (p> 0.05) between the Thr54 allele and any of the analyzed parameters (Table 2). The ORs represent the magnitude of association between the genotype and disease; consequently ORs with a 95%CI including the value 1 are indicative of no difference between Thr54 carriers and non-carriers for that factor. These results are in accordance with other previous publications that found no major contribution of allele Thr54 on these phenotypic parameters”, last paragraph of the Discussion (page 6) : “Our results indicate that in the assessed population, the allele Thr54 does not influence the glycemia levels nor the blood pressure (odds ratio approaching 1). The Cholesterol levels, BMI and CVD index are not significantly influenced by the Thr54,” and Conclusions (page 6): “Moreover, we found no association between the Thr54 allele and any of the five selected markers. “ of the Main Text.

We considered that, despite the non-significance (for p=0.05) of the analysed associations, it was worth noting that three markers showed OR’s > 1, and that when these markers were analysed in combination, an increased number of Thr54 carriers was observed with high cholesterol and BMI (17/97 vs 10/105 in the non-carriers). We analyzed the differences between these numbers with a statistic tool (StatsDirect) and found non statistical significance until p=0.1. However this tendency is still not significant for p<0.1, we considered it of relevance to be mentioned, given that other markers did not show this subtle pattern.

Taking in consideration the reviewer’s comment, we introduced modifications in some sentences, to avoid any confusion regarding the statistical non-significance of our observation, emphasizing the limit of the study and mentioning this subtle difference in observed numbers:

“The observed tendency to increased total cholesterol and elevated BMI in Thr54 carriers, even though not significant (p<0.1), could be worth of further investigation to establish whether the Thr54 variant should be taken into consideration in cardiovascular prevention strategies” (page 2, in the Conclusions of the Abstract section).

“Despite the non-significant association results, we consider it worth noting that the ORs for Cholesterol (2.01), BMI (1.44) and CVD risk (1.25) are >1. When we analyzed the distribution of Thr54 carriers and non-carriers combining two of these three parameters, we observed an
increased number of polymorphism carriers with high cholesterol and high BMI (Figure 1). Notice in Figure 1a that the quadrant corresponding to high cholesterol and high BMI shows more carrier individuals (17/97) than non-carriers (10/105). This not significant difference (p<0.1) but subtle tendency was not observed in the rest of the parameter combinations that were analyzed (data not shown). These results reveal that even though the markers for Cholesterol and BMI do not show a significant association with the polymorphism when they are analyzed on their own, a subtle tendency is observed when they are analyzed in combination”.

(page 5, in the Results section)

Response 2:
We performed the study suggested by the reviewer. We analysed the five markers in male Thr54 carriers respect to female Thr54 carriers. We did not find any significant difference (for p=0.05) between gender for BMI, cholesterol, blood pressure, and glycemia. When we analysed the Risk index, the OR for male Thr54 carriers was 11.49 (significant) and for male non-carriers was 11.1 (significant), concluding that the male gender is a risk component on itself, independently of the genotype. This is the reason why we did not include the results in the manuscript.

If the reviewer considers this information useful, we find it reasonable to include it in the Result section.

Response 3:
The suggestion is very interesting. It is, however, impossible to perform in this study. The included subjects were recruited in Public Administration Offices by the Regional Cardiovascular Prevention (RCP) Program. The data for this study was obtained simultaneously with the risk markers collected by the RCP program. We are not able to re-contact those 202 volunteers. In a further study of our group, the amount of consumption of beef will be included as data to be collected.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Previous studies have suggested that the FABP2 is related to insulin resistance. If you have any data on insulin resistance, please describe this in the revision.
Response 2:
The data of the Chilean and Brazilian studies have been included in the Discussion section (page 5): “Other South American frequency analyses of Thr54 were reported by a Chilean group, who found a q=0.4 in 63 obese and not obese women [16], and a Brazilian study that reported a q=0.25 in 1042 diabetes type 2 individuals [17]. Even though both analyses are performed on specific study samples, i.e. obese and diabetic individuals, the Brazilian frequency in 1042 individuals is similar to that observed by our group in Argentina.”

Response 3:
The suggestion of the reviewer has been included in the Discussion section (page 5): “In the sample tested, the observed Thr54 frequency (q=0.277) is similar to that reported in eleven different European countries (0.276) [16], suggesting that the colonising European populations, mainly from Italy and Spain, introduced the same original allele frequency in this continent and that this frequency has remained conserved.”

Response 4:
The suggestion of the reviewer has been included in Methods, Subjects (page 3): “We recruited 202 volunteers (mean age 53 years) from various Public Administration Offices, including 86 men (aged between 35 and 75 years, mean age 51) and 116 women (aged between 45 and 75 years, mean age 56). The means in the sample were: cholesterol level 198,34 (190,52 for men and 204,27 for women), diastolic blood pressure: 79,38 (82,93 for men and 76,96 for women), systolic blood pressure: 127,22 (131,15 for men and 124,26 for women) and BMI: 28,45 (28,86 for men and 28,13 for woman).”
Reviewers: Viswanathan Mohan

Discretionary Revisions (which the author can choose to ignore)

The following are the concerns that need to be addressed:
1. The introduction needs to be shortened by 50%

Response 1:
The introduction has been significantly shortened.

2. The sample size is too small to come to any meaningful conclusions.

Response 2:
We have included in the Discussion section (page 6) the limitations of our study:
“This observation does, however, not prove association or causality. The sample size was small, and these observed tendencies require further investigation in order to reveal whether the influences become significant and whether it could be related to the meat and fat-rich diet prevailing in Argentina.”

The Thr54 population frequency analysis revealed a $q = 0.277$ with a 95% confidence interval of 0.234-0.323. We consider this result meaningful and relevant, as the first population frequency analysis in Argentina with statistical significance.

The genotype-phenotype association analysis was based on 202 data. Even though it is a small sample, we compared our work with other published data with a similar association aim, and found many publications with similar sample sizes, i.e:

- Brown MD et al. Metabolism. 2001;50(9):1102-5. Sample Size: 60

Even though many other studies have a bigger sample size, and however we know the limitations of our study, we consider that the 202 data used for this work contributes to results of statistical significance that could be useful for further studies in the field.
Response 3:
The suggestion of the reviewer has been included in Methods, subjects (page 3): “We recruited 202 volunteers (mean age 53 years) from various Public Administration Offices, including 86 men (aged between 35 and 75 years, mean age 51) and 116 women (aged between 45 and 75 years, mean age 56). The means in the sample were: cholesterol level 198,34 (190,52 for men and 204,27 for women), diastolic blood pressure: 79,38 (82,93 for men and 76,96 for women), systolic blood pressure: 127,22 (131,15 for men and 124,26 for women) and BMI: 28,45 (28,86 for men and 28,13 for woman).”

Response 4:
The title of table 1 has been substituted as suggested by the reviewer.

Response 5:
CDV has been replaced by CVD.

Response 6:
This suggestion is very interesting. It is, however, not possible to perform in our sample. The reason of this, is the low number of Thr54 homozygous that does not allow a statistical analysis to compare Ala/Thr54 heterozygous and Thr54 homozygous. We found many other studies that decided to constitute one same group with heterozygous and thr54 homozygous. We have mentioned this in Result section (page 4).

Response 7:
This suggestion is also very interesting. It exceeds however, the aim of this work. The included subjects were recruited in Public Administration Offices by the Regional Cardiovascular Prevention (RCP) Program. The data for this study was obtained simultaneously with the risk markers collected by the RCP program. We are not able to re-contact those 202 volunteers. In a further study of our group, the amount of secreted triglycerides could be included as data to be collected.
8. The authors need to review works carried out in other populations. 
Eg. Guettier et al, Clin Endocrinol Metab. 2005; 90: 1705-11 
Vimalesswaran et al, Metabolism. 2006; 55:1222-6

Response 8:
We have read the suggested publications, and included their conclusions in our Discussion (page 6) and in References (page 8): “Many earlier studies have reported associations of this polymorphism and insulin resistance, BMI, dyslipidemia, stroke, metabolic syndromes and hypertriglyceridemia [2,6,17,18,19,20,21].”


21. Vimalesswaran KS, Radha V, Mohan V. Thr54 allele carriers of the Ala54Thr variant of FABP2 gene have associations with metabolic syndrome and hypertriglyceridemia in urban South Indians. Metabolism 2006; 55:1222-1226