Author's response to reviews

Title: c.620C>T mutation in GATA4 is associated with congenital heart disease in South India

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Author's response to reviews: see over
To
Dr. Sergi Castellvi-Bel
Editor
BMC Medical Genetics

Date: 20-11-2014

Re: Resubmission of revised manuscript entitled “c.620C>T mutation in GATA4 is associated with congenital heart disease in South India (MS: 1704347284141499)”.

Dear Dr. Sergi Castellvi-Bel:
Thanks for giving us an opportunity to revise and further improve our manuscript according to the reviewer’s suggestions. Here, we would like to resubmit our revised manuscript entitled “c.620C>T mutation in GATA4 is associated with congenital heart disease in South India (MS: 1704347284141499)” for publication in your esteemed journal “BMC Medical Genetics”.

According to the editor and reviewer’s suggestions, we have revised the whole manuscript. You will find below details of our point-by-point responses to the reviewer’s comments.

We think the manuscript has been greatly improved after revisions and we hope that you will now find it suitable for publication in your esteemed journal “BMC Medical Genetics”.

Sincerely yours,

Sanjay K Banerjee.

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An autonomous institute of Dept. of Biotechnology, Ministry of Science & Technology, Government of India
**Point-by-point responses to the reviewer’s comments**

Reviewer (Cecilia Vecoli) -1 Comments

**Major Compulsory Revisions**

Which is the novel promoter mutation reported in the title? Which its functional/clinical significance?

rs61277615 is the novel promoter mutation reported in the title. Since this mutation has already been reported in healthy individuals, we have now removed and changed the title as “c.620C>T mutation in GATA4 is associated with congenital heart disease in South India”. This SNP is significantly associated with ASD (p value=0.008514).

The conclusions show nothing new.

Although GATA4 mutations has been reported to be associated with CHD, but the SNP rs61277615 has not been reported to be associated with ASD in any of the world population hence this information is novel and important Indian prospective, as the population structure in India is unique. (Dhandapany PS et al, 2009, Reich et al, 2009)

The paper is not clear and the results are confused. In table 2, the allelic distributions of 5 polymorphisms among patients and controls have been reported. Why since in table 3, all the SNPs are reported?

In Table 2, we have reported all the SNPs observed in this study. However, in Table 3, we have listed only SNPs, which are significantly associated with CHD, with their frequency, Odd-ratio and p-Value.

Only 3 patients had SV and were not further analyzed. Why have they been sequenced? In table 2 the allelic distributions of polymorphisms among patients and controls has been reported splitting the patients according to three pathologies. Why? Do the authors think that these 3 diseases could have a different GATA4 genetic variants-dependent etiology? If yes, 33/32/32 patients for disease are a too small numbers. If no, the authors should perform a single analysis including all 100 patients (including the SV ones).
We agree with the reviewer that the sample size is small. Since most of the CHD patients are <5 years, collecting blood samples with the consent of parents is extremely difficult, although we tried to get additional samples. As desired by the reviewer, we have combined all 100 samples and performed the analysis. Interestingly, all the markers found to be associated in independent analysis were also showed association, when all the four diseases were combined. This data has been incorporated in the supplementary.

Tests of Hardy Weinberg equilibrium revealed that more variants violated the equilibrium at the p < 0.05 level. Thus these polymorphisms should be excluded from further analysis.

As desired, we have excluded the SNPs which were not Hardy Weinberg equilibrium.

Similarly, the five SNPs in the 3’UTR region did not satisfy the HW.

Three markers in 3’UTR i.e. rs904018, rs12825 and rs3203358 are in Hardy Weinberg equilibrium at the p≤0.01 level. This might be because of small sample size. Hence we are not used for further Insilico analysis. But two marker rs884662 and rs12458 are in Hardy Weinberg equilibrium. We further performed Insilico analysis of these two variations and observed that alter their miRNA binding.

Minor Compulsory Revisions:

The introduction should be more focused. Too many figures and Tables have been reported. i.e. Figures 2 and 3 do not help support or explain the text and needs either significant revision or to be removed.

We have rewritten the introduction. As Figure 2 explains two recessive mutation (rs61277615 and rs73203482) and its inheritance. We feel it is worthwhile to keep in the manuscript. Considering the reviewers suggestion, figure 3A has been moved to supplementary figures. Figure 3B (now figure 4) explains the binding of splicing factors (SRSF6) in both wild and mutant GATA4, hence we are retaining it.

At page 9, the analysis of parents’ samples appeared: why? How many parents did you have analyzed?
We found two recessive mutation (rs61277615 and rs73203482) in patients. We have collected parent’s samples for those patients who have either rs61277615 or rs73203482 or both the variations in GATA4 gene. Parent’s genetic analysis revealed that they are heterozygous. We have revised the method part and clearly indicated which parents samples were collected for genetic analysis.

Statistical analysis is a title of Methods. It is not used for labeling a result section. All the references have to be reported as request by the Journal style i.e. ref34 It is enough to indicate the use of Plink software in the Statistical analysis sections (not in the mutational analysis too).

Thanks for the suggestion, we are replacing “statistical analysis” with “Genetic studies”. We have also changed all reference as per Journal style.

Reviewer-2 Comments

Major Compulsory Revisions

In the manuscript is interesting biological concept of trying to elucidate the etiology of CHD with genetic/environmental interactions. However, the study presents some limitations. The title and abstract not accurately reflect the manuscript as a whole, in particular, the findings. The authors could provide more details about the novel mutation and additional functional analysis could be provided.

rs61277615 is the novel promoter mutation reported in the title. Since this mutation has already been reported in healthy individuals, we have now removed and changed the title as “c.620C>T mutation in GATA4 is associated with congenital heart disease in South India”. This SNP is significantly associated with ASD (p value=0.008514).

The manuscript involves excellent work and interesting observations, but should be revised to improve readability. It is difficult to follow the logical flow of the manuscript. After reading through your manuscript I feel that the quality of written English needs to be improved before the manuscript can be considered further. I advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language.
Thanks for the valuable suggestion, accordingly we have taken care of English language and made required changes.

Reviewer-3 Comments

The title of the paper appears misleading, because the authors are not assessing the association of a novel mutation with CHD in South India population, rather they are trying to assess the association of a pool of mutations with CHD in an ethnic group living in southern India.

As suggested the title has been changed as “c.620C>T mutation in GATA4 is associated with congenital heart disease in South India”

The aim of the authors is the association of some GATA4 mutations to Congenital Heart Disease (CHD) in an ethnic group, those people which speaking the Dravidian language (Dravidians) and living in southern India. However, the selection of the study population is not clear. The assumption that the authors make to justify the setting of their discussion, namely the high rate of Consanguineous marriages in the Dravidians, do not directly imply the high frequency of three mutations in these people (the cited supplementary figure 2 unfortunately does not provide this information either). In addition, the articles cited to endorse a low frequency of mutations in patients with CHD of other populations, were not carefully studied. For example, the authors report that Peng and colleagues [11] found a frequency of 1,48% (2/135) of mutations in VSD Chinese patients, whereas Peng and colleagues report 1 mutation in 82 Chinese VSD (1,22%) and 1 mutation in 12 Chinese TOF (8,33%). Similarly, all the CHD Germans reported in [12] are 205, while the Germans VSD are only 6, so that the percentage of mutations is 16.7% (1/6) and not 0.49% (1/205), as reported by the authors of the article under review. According to these published data, the reference samples for analysis on individual CHD are not significant.

During sample collection, we took information of patient’s geographical location from their parents. All patient samples were collected from specialized hospital and they were belongs to same ethnic group (Dravidian). Due to consanguinity there is possibility increasing of recessive allele frequency. As Infants are the most affected age group of CHD, we were unable to collect extended pedigree. We have only collected patient and parent’s
samples of some families. We have checked our citation about low frequency of mutations in patients with CHD of other populations. We have revised the manuscript according to your suggestion. We did all corrections as “Peng and colleagues reported 1 mutation in 82 Chinese VSD (1.22%) and 1 mutation in 12 Chinese TOF (8.33%)”. According to published data with Germans VSD we have corrected the frequency as 16.7% (1/6) in our revised manuscript. Due to low sample size the analysis of individual CHD are not significant. But we did our analysis with 100 samples together and found same marker are associated with CHD.

In general, I think the authors have submitted a paper that still needed to be well thought out and harmonized. Although they found 6 mutations not in Hardy-Weinberg equilibrium, they reported these mutations in many tables and figures. The mutations were also considered in the 3’UTR in silico analysis. Moreover, they reported that for SV patients they haven’t found any mutations (this finding is not strange if we consider that the reference population was only of 3 subjects). Nevertheless, SV patients were reported in tables and figures. Information and data provided by the authors (including the supplementary material) are redundant and often misleading.

We agree with the reviewer that the sample size is small. Since most of the CHD patients are <5 years, collecting blood samples with the consent of parents is extremely difficult, although we tried to get additional samples. Three markers in 3’UTR i.e. rs904018, rs12825 and rs3203358 are in Hardy Weinberg equilibrium at the p≤0.01 level. This might be because of small sample size. Hence we are not used for further Insilico analysis. But two marker rs884662 and rs12458 are in Hardy Weinberg equilibrium. We further performed Insilico analysis of these two variations and observed that alter their miRNA binding.