Author's response to reviews

Title: Genetic influences on right ventricular systolic pressure (RVSP) in chronic obstructive pulmonary disease (COPD)

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BioMed Central Editorial Team 11 May 2012
BMC Pulmonary Medicine

Dear Editorial Team

Manuscript number MS: 1566558453628298:
Genetic influences on right ventricular systolic pressure (RVSP) in chronic obstructive pulmonary disease (COPD)

Thank you for considering our revision to the manuscript, as suggested by the Reviewers.

We sincerely thank the Reviewers for their comments about our manuscript, and made the following revisions:

Reviewer 1 – Dr Yildiz

No changes were suggested by this Reviewer.

Reviewer 2 – Dr Chappell

We thank the Reviewer for the helpful comments, and have addressed these below and in the revised manuscript.

Discretionary Revisions:

1. Background, 1st paragraph: It would be useful to clarify how many patients with COPD are affected by PH, rather than just saying “a significant number”.

Response: Thank you, we have now clarified the estimated prevalence, citing the
existing review (reference 1).

Original: Pulmonary hypertension (PH) is a serious complication of chronic obstructive pulmonary disease (COPD) and develops in a significant number of patients with COPD, increasing their morbidity and mortality [1].

Revision: Pulmonary hypertension (PH) is a serious complication of chronic obstructive pulmonary disease (COPD) and develops in 30% to 70% of patients with COPD, increasing their morbidity and mortality [1]. (Page 3)

2. Discussion, 4th paragraph: It would be useful to know further details about the conflicting study described in this section (reference 34). For example, a brief comment on the study size, ethnicity of subjects etc.

Response: Thank you for this suggestion, we have now provided further details about the study cited in reference 34 – in particular, study size, results and location of the study.

Original: This is in contrast to a previous study which found higher frequency of ACE DD genotypes in male smokers with COPD than in male smokers with normal lung function [34]. Additional studies are required to clarify this relationship.

Revision: This is in contrast to a previous study of Caucasian Mediterraneans which found a higher frequency of ACE DD genotypes in 74 male smokers with COPD than in 77 male smokers with normal lung function (odds ratio 2.2) [34]. (Page 10)

Major Compulsory Revisions:

1. My main concern with this manuscript is the significant number of statistical tests which have been carried out without any correction for multiple testing. I appreciate that the authors state they did not carry out these corrections due to the “exploratory nature” of the study (Discussion, final paragraph), but I feel that this is still something that should have been considered, even if by simply presenting both initial and corrected results.

Response: We agree with the Reviewer that the issue of multiple testing should be considered. We believe that this study is exploratory in examining all of these 7 SNPs together in one study, in a sample size of 278, which is relatively large compared with previous genetic association studies of RVSP in COPD patients. We have acknowledged that further replication in other cohorts is needed. We have added a statement saying ‘and the statistical significance of the results should be considered in light of this’.

Original: Given the exploratory nature of this study, we did not correct for multiple
comparisons. Even with this relatively large cohort of patients, replication in other cohorts is needed.

Revision: Given the exploratory nature of this study of 7 SNPs and RVSP in COPD, we did not correct for multiple comparisons, and the statistical significance of the results should be considered in light of this. Even with this relatively large cohort of patients, replication in other cohorts is needed. (Page 11)

The analyses of genotypes and RVSP were done with ANOVA for additive genetic model and t-tests for the dominant and recessive models. This was already described in the Results (Page 5):

Original: Associations of genotypes with RVSP or lung function were performed using ANOVA for additive genotype models (AA vs AB vs BB), and t-tests for dominant (AA vs AB + BB) and recessive (AA + AB vs BB) genotype models. (No change to text)

2. Table S3: Two comparisons are done for most of the polymorphisms (eg ACE II/ID vs DD as well as II vs ID/DD), but only one is done for the NOS3 VNTR (aa/ab vs bb). I would be grateful if the authors could confirm their reasons for this.

Response: The NOS3 VNTR 4aa genotypes were present in only 8 subjects, and this number was too small to meaningfully compare in the t-test analyses.

This explanation has now been added to the legend of Table S3.

3. Background, 2nd paragraph: “Polymorphisms exist in vasodilator (nitric oxide synthase)…” This sentence indeed identifies that polymorphisms exist in the genes listed (which are the ones analysed in the current study), although polymorphisms also exist in the other genes which are referred to in the studies listed in the previous sentence of this section. For example, Reference 5 contains the analysis of a SNP in IL-6 in COPD-related PH. How and why did the authors chose the genes and polymorphisms included in the current study? Further information needs to be included regarding this.

Response: We agree with the Reviewer that SNPs in other genes may also be candidates for effects on pulmonary hypertension in COPD. We selected the current candidate genes based on relevance, previous association with vascular disease and focused on those genes that were vasoactive mediators. At the Reviewer’s suggestion, to further explain this rationale, we have now added a sentence at the end of the Hypotheses paragraph:

Revision: We selected variants previously associated with vascular disease, in vasoactive mediators of biological importance in pulmonary hypertension. (Page 3)

4. Statistical methods, 2nd paragraph: It is good to see the authors consider power calculations in the manuscript. However, it would be useful to see this
expanded in the Results or Discussion section, especially given the negative results observed for many of the polymorphisms that have been studied. If the negative results were associated with the lowest power for example, this would need to be commented on.

Response: We thank the Reviewer for suggesting that we provide additional details about the power calculations. In fact, all genotypes except the NOS3 VNTR met the assumptions listed in the power estimates, and the NOS3 VNTR was the only positive association in the RVSP analysis. We have further clarified this in the Methods by providing more detail:

Original: For the t-test analyses of RVSP using dominant or recessive models, power calculations indicated that a total of 190 participants with RVSP measurements were required to detect a difference of 15% in mean RVSP in the cohort, with 80% power (based on mean RVSP of 44 mmHg, SD 13 mmHg, P value of 0.05, and ratio of genotype groups 1:2)

Revision: For the t-test analyses of RVSP using dominant or recessive models, power calculations indicated that a total of 190 participants with RVSP measurements were required to detect a difference of 15% in mean RVSP in the cohort, with 80% power (based on mean RVSP of 44 mmHg, SD 13 mmHg, P value of 0.05, and ratio of genotype groups of at least 1:2, which was the case for all variants studied, except the NOS3 VNTR which had a lower ratio) (Page 6)

5. Genotyping: It would be useful to have brief details of the quality control measures in place for the genotyping assays, particularly given the deviation from Hardy-Weinberg equilibrium which is mentioned later in the manuscript (Point 7).

Response: Yes we did repeat testing which confirmed the genotypes and we have now added a sentence at the end of the Genotyping paragraph.

Revision: To ensure reproducibility, 10% of the samples were chosen randomly and repeated for each polymorphism; these genotypes were confirmed by this repeated testing. (Page 5)

6. Results (Echocardiography), 1st paragraph: “Both these subgroups had similar characteristics to the whole cohort.” What about the significant difference seen for FEV1/VC ratio shown in Table 1 (p=0.026)?

Response: Thanks, we have now corrected this and included a brief statement about the lower FEV1/VC ratio:

Original: Demographic and disease characteristics were similar between patients with and without measurable RVSP.

Revision: Demographic and disease characteristics were similar between patients with and without measurable RVSP, except for a lower FEV1/VC ratio in those without measurable RVSP. (Page 7)

7. Results (Genotypes) and Results (Association of genotypes with RVSP)
measurements): The NOS3-Glu298Asp SNP showed minor deviation from Hardy-Weinberg equilibrium in the total sample (p=0.02), and the EDN1 SNP shows some deviation in the RVSP group (p=0.04) though there is no comment about possible causes for this. I would like to see the authors comment on these results, particularly to reassure readers that this is not due to genotyping error.

Response: We noted that there was minor deviation from Hardy-Weinberg equilibrium, and the reasons could include chance or differences in population sampling. As described above for Comment 5, we did repeat genotyping for all variants, and these were concordant, so we do not believe that genotyping error was present. We have now added a further statement about this in the Limitations section of the Discussion:

Revision: Two of the SNPs showed minor deviation in Hardy-Weinberg equilibrium, although these did not show positive associations. The reason for the minor deviation could include chance or differences in population sampling: we had performed repeat genotyping in 10% of samples and the results were concordant. (Page 11)

8. Tables S3-S5: If mean differences between groups are being presented, the 95% confidence interval for these differences should also be included.

Response: Thanks, the 95% confidence intervals have now been included in Tables S3 to S5.

Revision: Please see Tables S3 to S5.

9. Discussion, 2nd paragraph: The authors propose that the NOS3 VNTR polymorphism may have a functional effect, but should also consider and discuss the possibility of other polymorphisms which are in linkage disequilibrium with the VNTR.

Response: Thanks, we have now included this suggestion.

Original: The exact functional mechanisms of how the VNTR affects either nitric oxide or vascular remodelling, needs further elucidation.

Revision: The exact functional mechanisms of how the VNTR, or a nearby SNP which is in linkage disequilibrium with it, affects either nitric oxide or vascular remodelling, needs further elucidation. (Page 10)

10. Conclusions: The association between ACE genotype and lower FEV1 is only significant in one of the t-tests (p=0.09 ANOVA, p=0.028 t-test II/ID vs DD, p=0.68 t-test II vs ID/DD), so I wonder how robust this association is. This locus is not one which reached significance in recent GWAS for lung function – perhaps the authors should acknowledge this in the manuscript.

Response: We acknowledge that the ACE association was identified in one of the 3 genetic models tested. Whilst we recognise that ACE was not significant in the GWAS for lung function, these GWAS were generally population-based studies of lung function. Even in the COPD GWAS, the comparisons were COPD
vs control, rather than lung function impairment within COPD patients as a measure of disease severity. We have now added a further comment about the lack of association in the other genetic models tested.

Original: Patients with the ACE II or ID genotypes showed a significantly lower FEV1 % predicted, albeit a clinically small difference, than the ACE DD genotypes.

Revision: Patients with the ACE II or ID genotypes showed a statistically significantly lower FEV1 % predicted, albeit a clinically small difference, than the ACE DD genotypes. Analysis of the other genetic models for ACE genotypes did not show associations. (Page 10)

Once again, we thank BMC Pulmonary Medicine for considering this revised version.

Yours sincerely

Janet Shaw, on behalf of co-authors