Author's response to reviews

Title: Leukotriene B4 receptor locus gene characterisation and association studies in asthma

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Author's response to reviews: see over
Response to reviewers

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Leukotriene B4 receptor locus gene characterisation and association studies in asthma
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BMC Medical Genetics

We thank the Editor and referees for their constructive comments and have addressed the issues raised in point by point manner and modified the manuscript accordingly.

Editorial Requirement:

1) The manuscript has been overseen by an English native speaker.

2) The comment for informed consent provided by the subjects was inserted into the methods section as required

- The following comment was inserted into the methods section, see page 9, line 12

“Informed consent was provided by the adult (or parent/guardian for child subjects).”
Reviewer’s report 1

Title: Leukotriene B4 receptor locus gene characterisation and association studies in asthma
Version: 1 Date: 6 August 2012
Reviewer: Maarten van den Berge

Reviewer's report:
In this manuscript, the authors have characterized the genes for LTB4 receptors. They show that LTB4R1 and LTB4R2 mRNA is ubiquitously expressed in the lung and have variation in 5’-untranslated regions and predicted promoter regions. In addition, the genotyped 6 SNPs selected for ability to tag linkage disequilibrium or inferred function. No association between SNP polymorphisms and presence or severity of asthma was found. The manuscript is well written. I have the following comments.

Major comments.
1. Page 8. Polymorphism screening The authors performed direct sequencing in (n=greater than or equal 35 ?? how many) ) individuals. Which material was used for sequencing? Blood or tissue? Were did these samples come from? Do the authors refer to the commercial RNA obtained from 3H biomedical? This should be more clearly described.

- The following addition regarding this point has been included in the text. See page 8, line 7.

“....using DNA extracted from whole blood of 35 individuals from the Nottingham Adult Asthma Cohort recruited on the basis of physician diagnosed asthma and no other respiratory illness with <10 pack-years smoking history. These subjects had severe asthma as defined by British Thoracic Society (BTS) step ≥ 3.”

2. Page 11 results 5 RACE data for LTB4R1 and LTB4R2 was generated for lung tissue. Similar to the point above. Were did the lung tissue come from?
The lung RACE ready cDNA was generated from total lung RNA purchased from Ambion. This has been made clear by the modification of the following sentence on page 7, line 19:

“RACE-ready lung cDNA was synthesised from 1µg total lung RNA obtained from Ambion (Huntingdon, UK) as described.”

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.
Reviewer’s report 2

Title: Leukotriene B4 receptor locus gene characterisation and association studies in asthma
Version: 1 Date: 24 August 2012
Reviewer: Kelan Tantisira

Reviewer's report:
In this manuscript, Tulah and colleagues seek to understand the role of LTB4 receptor genetic variation on asthma susceptibility and on lung function within asthma. Following a nice characterization of the receptor structure itself, they report genotyping of a subset of resequenced SNPs and lack of association of these variants with asthma and FEV1 in asthmatics.

Major Comments:
1. SNP Selection - Despite this methods section, it is unclear if all of the common variation within this region (understandably, rare variants were not included) is actually covered by the selected SNPs for two reasons:
a). While the authors refer to use of 1000 genomes data to evaluate dbSNP variants, it is unclear how these are incorporated into the genotyping selection, if at all. There are 100 annotated dbSNP variants, of which several have MAFs in excess of 5% but do not appear to be represented in the genotyped variants.

The timeline of this study predates the release of 1000 genomes data for the SNP selection component; therefore SNPs were actually selected based on our sequencing data in the severe asthma patients and available data in the HapMap database (Build 36). Both datasets were utilised in the Tagger program with similar SNPs selected.

We have inserted the following sentence: page 9, line 16.

“Sequencing data from the severe asthma subjects and available HapMap data (Build 36) were used to select the six SNPs for analysis. We acknowledge a limitation of the current study is the use of this available HapMap build that has now post SNP selection been superseded by 1000 genomes data for SNP selection”
b). Based on figure 3, the LD metric used to determine the selected SNPs appears to be D'. For association testing, R-square values have consistently been cited as a better metric to use.

We agree with the reviewer and as requested have modified Figure 3 to encompass R-square values.

The authors should address each of these issues in SNP inclusion criteria or state that there are limitations to their genotyping schema.

2. The authors list very little in the way of details in regard to the clinical cohorts, and do not detail any potential confounders or details of multivariable analyses within their methods or results sections. In addition to traditional covariates, information related to atopic status and/or controller medication administration would appear to be crucial in the interpretation of the association results data (especially the FEV1 and severity analyses). Have these been done?


However, to further define the population we have also included BTS step as an indicator of asthma severity/control and medication use between the cohorts. Atopic status based on skin prick test is provided.

- Our analyses with %predicted FEV₁ and BTS score 1 to 5 in the Adult Asthma Cohort was not corrected for any confounders. This was to enable comparision between the Adult Asthma Cohort analysis and the family based association analysis. This is because the family based association test (FBAT) does not involve correction for confounders and so involves uncorrected analysis.
We have inserted the following sentence in the methods sections, see page 10, line 14

“Analyses in the adult asthma cohort were not corrected for any potential confounders to enable comparison of the association analysis between family-based and adult cohorts.”

Minor Comments:

1. Given that only common (and not complete common) variation was the focus of the association analyses, it is premature to state the conclusions that "LTB4R polymorphisms are not susceptibility markers" (or severity/lung function markers).

2. The lung function analyses demonstrate z-scores and betas in the same direction for the family and population based analyses. What happens when the evidence from the cohorts is combined?

3. Given #2 above, since there is a consistent direction, but no significant association within a cohort, despite the power calculations provided, it should be stated that there may be a subtle effect that the current study is underpowered to detect.

The following sentence has been inserted into page 18, line 8
“Although there was no significant association, our data does show some evidence of a constant direction of effect, suggesting this study may be underpowered to detect a subtle effect.”

4. For the lung function analyses, it should also be noted by the authors that the FEV1 was relatively normal in all populations. Therefore, a ceiling effect might have helped to prevent the detection of a significant association.

- We acknowledge this and have included a sentence in the discussion, page 19.

“Similarly, our asthma subjects had relatively preserved lung function which may have impeded our ability to detect association with FEV1 (% Predicted).”

5. Given that spirometry was obtained, did the authors consider evaluating other measures of lung function (e.g. FEV1/FVC)? This would help to make the story more complete.

- We did not have the data to conduct an FEV1/FVC analysis in the family based analyses (no FVC data) and so did not complete this analysis as it could not be completed for both the family cohort and Adult Asthma Cohort.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
No competing interests.