Author's response to reviews

Title: The role of ALOX5AP, LTA4H and LTB4R polymorphisms in determining baseline lung function and COPD susceptibility in UK smokers

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

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The role of ALOX5AP, LTA4H and LTB4R polymorphisms in determining baseline lung function and COPD susceptibility in UK smokers
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BMC Medical Genetics

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

Reviewer’s report 1

Title: The role of ALOX5AP, LTA4H and LTB4R polymorphisms in determining baseline lung function and COPD susceptibility in UK smokers

Version: 2 Date: 22 August 2011
Reviewer: Ian Yang

Reviewer's report:
Polymorphisms in the leukotriene pathway have been implicated in asthma and its subtypes. However, the role of SNPs in this pathway is untested in COPD. This genetic association study addressed the potential involvement of important SNPs in this pathway in COPD susceptibility and lung function impairment. Excellent rationale for selecting these SNPs was provided in the Background and Discussion. The testing of these SNPs is novel, and the genotyping methods were robust. Rigorous correction for multiple comparisons was performed. Results were clearly presented.

Major Compulsory Revisions

1. Power – As the primary outcome showed no association, it would be very useful to have estimations of the power of this study, particularly given that the smoker control subject
numbers were relatively small. Also, the power of the lung function comparisons would be worthwhile estimating. This would give confidence about excluding type I and type II errors.

- The following addition regarding the power of this study has been included in the methods section: See page 8, line 15

“With respect to power (based on lowest and highest minor allele frequency), there was between 77-99% power to detect a 50ml difference in FEV$_1$ and between 58-99% power to detect a 5% difference in FEV$_1$/FVC ratio. Analyses of COPD susceptibility were relatively underpowered, with between 28-91% power with an odds ratio of 1.5 and 68-99% power with an odds ratio of 2.0. All analyses considered an error rate of 5%”.

2. Lung function as % predicted – The authors have used FEV1 as the main outcome measure (together with FEV1/FVC) in the lung function analysis, which is appropriate. Did they also have access to height measurements in order to calculate predicted values? Comparisons of genotypes with % predicted FEV1 would be worthwhile, if available.

- Our previous experience of genetic association studies of lung function phenotypes including FEV$_1$ in multiple populations has suggested that using the % predicted FEV$_1$ does not adequately correct for covariates age and gender (unpublished data). Therefore we have used the actual FEV$_1$ value in the current study and corrected in the linear regression model. This is an established approach e.g. see recent GWAS studies using actual FEV$_1$ (Repapi E, Sayers I, Wain LV,........ Elliot P, Strachan DP, Hall IP & Tobin MD. Nature Genetics Jan;42(1):36-44).
- Completing % predicted FEV$_1$ analyses for the current paper would add little.

3. Effect sizes – Were the positive differences in lung function between genotype groups considered clinically important?
While this study is essentially a negative report, the suggestive data for LTA4H rs1978331 equates to FEV\textsubscript{1} TT 1.468±0.039L, TC 1.599±0.034L and CC 1.594±0.057L. Therefore for the rs1978331 TT versus TC = 131ml difference, TT versus CC = 126ml difference

We have added an appropriate comment in the discussion regarding clinical relevance: See Page 16, line 12.

“While no association survived the Bonferroni correction, additive model analyses with rs1978331C (LTA4H, intron 11) showed a p=0.029 with an increase in FEV\textsubscript{1} and p=0.020 with FEV\textsubscript{1}/FVC ratio. The mean FEV\textsubscript{1} and FEV\textsubscript{1}/FVC values for the TC heterozygotes and CC homozygotes were similar, but the presence of the minor C-allele for these genotype groups gave higher trait values when compared to the TT homozygotes, suggesting a dosage effect does not occur. For rs1978331 TT versus TC genotype groups there was a 131ml difference in FEV\textsubscript{1} and for TT versus CC a 126ml difference in FEV\textsubscript{1} was observed. The level of FEV\textsubscript{1} at a given time depends on 1) the maximum lung function obtained during development, and 2) the rate of decline of lung function with age. Lung function reaches a maximum by age 25–35 years (Kohansal, R. et al. The natural history of chronic airflow obstruction revisited: an analysis of the framingham offspring cohort. Am. J. Respir. Crit. Care Med. 180, 3–10 (2009)). In smokers the rate of decline in FEV\textsubscript{1} is accelerated and has been calculated to be ~38.2ml/year in males and 23.9ml/year in females (Kohansal, R. et al.) therefore the differences observed between LTA4H rs1978331 genotypes can be considered clinically relevant and equate to >3 years decline in FEV\textsubscript{1}. These findings therefore provide tentative evidence suggesting that variants in LTA4H may determine clinically relevant lung function levels in smokers.”

Minor Essential Revisions

Discussion, paragraph 5 – change ‘neutrophillic’ to neutrophilic

- This has been corrected.
Discretionary Revisions

Correlation with GWAS – Did these SNPs show any signals in the recent COPD or lung function GWAS?

We have added text; See Page 17, line 12

“To our knowledge these SNPs did not show association with lung function and/or COPD in recent GWAS studies. We have also completed a comprehensive look up of genes previously associated with lung function including LTA4H and ALOX5AP in 20,288 individuals from the general population (the SpiroMeta consortium) and did not identify these genes as containing major determinants of lung function in this large general population cohort (Obeidat M., A comprehensive evaluation of potential lung function associated genes in the SpiroMeta general population sample. PLoS One. 2011;6(5):e19382. Epub 2011 May 20).”
Reviewer's report 2

Title: The role of ALOX5AP, LTA4H and LTB4R polymorphisms in determining baseline lung function and COPD susceptibility in UK smokers

Version: 2 Date: 24 August 2011
Reviewer: Gregory Hawkins

Reviewer's report:
This manuscript describes a genetic association analysis investigating the role of genes in the leukotriene pathway and potential effects on baseline lung function in smokers and COPD. Polymorphisms from the genes ALOX5AP, LTA4H and LTB4R were investigated due to their role in asthma. The hypothesis is that because these genes have been associated with asthma, there could be an equivalent genetic association between these genes and COPD. The study was performed in a large cohort (N=992). For logistic regression analysis, the subjects were stratified into smoking controls and subjects with COPD. After multiple correction adjustment, a single polymorphism in LTA4H was modestly associated with FEV1 and ratio. None of the genes were associated with COPD susceptibility or severity. Overall this is a simple, well designed study with a defined hypothesis. While the results are mostly negative, this study does effectively describe what genetic role these three genes may have in COPD.

Minor essential revisions

1. The authors repeatedly discuss baseline FEV1, however most readers will assume that this value isn’t adjusted for gender, height, etc… Have the authors considered using % predicted FEV1 as a better measure for presentation? It could the authors also present % predicted FVC data?

   - See comments to Reviewer 1.

2. Did the authors consider other genes in this pathway such as the receptors?

   - The aim of this study was to determine whether SNPs spanning those genes involved in the production (specifically ALOX5AP and LTA4H) and activity (specifically
LTB4R1 and LTB4R2) of LTB4 influenced baseline lung function and COPD susceptibility/severity in smokers. The hypothesis being that LTB4 production and activity is dysregulated in COPD and contributes to disease mechanisms (see manuscript introduction). We did not consider genes involved in cysteinyl leukotrienes due this reason e.g. LTC4S. We did not evaluate SNPs within ALOX5 which have the potential to influence LTB4 production, which is acknowledged as a limitation of the study. See Discussion, Page 17, line 6.

Comments from the Editor

Consent: Please state in the Methods section whether written informed consent for participation in the study was obtained from participants or, where participants are children, a parent or guardian.

Ethics: Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm). A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

We have added a comment in the Methods section Page 7, line 15:

“Ethical approval was obtained from the relevant ethics committees (Nottingham, Sheffield, Manchester, Leicester and Oxford) and informed consent from all subjects was obtained”.

Tables: Please ensure that the order in which your tables are cited is the same as the order in which they are provided. Every table must be cited in the text, using Arabic numerals. Please do not use ranges when listing tables. Tables must not be subdivided, or contain tables within tables. Please note that we are unable to display vertical lines or text within tables, no display merged cells: please re-layout your table without these elements. Tables should be formatted using the Table tool in your word processor. Please ensure the table title is above the table and the legend is below the table. For more information, see the instructions for authors on the journal website.

We have reformatted tables to conform to these guidelines.