Reviewer's report

Title: ITGB5 and AGFG1 Variants are Associated with Severity of Airway Responsiveness

Version: 2 Date: 21 May 2013

Reviewer: Lisa Cameron

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The Authors have made revisions and added a further figure. Overall the manuscript is improved but there are still some remaining questions.

Minor Essential Reviews

1. Methods for cis-eQTL analysis are not clear. Does Table 3 show allelic or genotype association? While the methods section indicates genotypes of a SNP were investigated and show rs6731443 is significant in Table 3, it is not clear if this is significant in Figure 4 and whether the effect was dominant or recessive - is the analysis of 0 vs 1+2 significant, ie is there an additive/dominant allelic effect?. A better description of how the eQTL analysis was performed is necessary. As a minor point, I believe the section of the title for Table 3'(ie, nominal p-value >0.05)' should read: p<0.05. For example, a schematic such as that found in Hao PLOS Genetics 2012 would be useful.

2. Figure 2 is very dense, indicating all the genetic variability within this region and the p-values. The two selected SNPs were chosen due to having the lowest p-values, rather than using any functional predictions. Where within the AGFG1 gene is rs6731443 located? For the rs673144 panel, the SNP within the conserved transcription factor binding site (denoted as *), which also shows high LD and p-value, may be an important functional SNP. Is association with mRNA for AGFG1 significant for this SNP? Furthermore, there is a one blue circle that shows low LD, but high p-value, which may represent an independent risk. Is this SNPs significantly associated with mRNA? Since the SNP unique identifiers are not provided in Figure 2 (albeit there are too many), it is not possible to cross reference these data with Table 3. This information should be added to the Supplementary data if at all possible, as it would be helpful for future investigations of functional SNPs.

3. Since the replication for the AGFG1 SNP was observed with the LHS study, this may imply the gene is involved generally in AHR, independent of asthma. While this is discussed/summarized in the Discussion, most of the paper has been written with the focus on identifying and replicating genetic variation underlying AHR in asthma. READ: last line of first para of Background “Our goal was to measure the association of genetic variants with AHR severity in asthmatics via a GWAS” AND last para: “After attempting to replicate primary findings in two independent populations ....” This implies they are asthma
populations, of which one is not. As such, the paper was somewhat confusing. This could be addressed more carefully.

Discretionary

4. As the number of samples in this study is large, the gold standard for validating mRNA expression analysis (qRT-PCR) would be challenging. There is newer high throughput technology Nanostring that could be applied. Validation of AGFG1 mRNA expression by another method is important and the Authors should discuss the lack of these data and its potential implications.

**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:**

I declare I have no competing interests