Reviewer's report

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

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Reviewer: Lisa Cameron

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Summary
This study is a GWAS analysis of ~2 million imputed single nucleotide polymorphisms (SNPs) and their association with airway hyper-responsiveness (AHR), measured by the natural log of the dose of methacholine causing 20% drop in FEV1 (PC20). The primary GWAS was performed on 994 white subjects from 3 drug clinical trials (CAMP, CARE, ACRN). Significant association of SNPs in 2 genes, ITGB5 and AGFG1, were identified with p-values between 10^-6 and 10^-7. Associated AGFG1 SNPs were replicated in the LHS study, but not the DAG study. No ITGB5 SNPs were replicated in either population. The authors also performed microarray expression analysis on whole blood samples from white CAMP subjects (n=419) and assessed whether “genotypes of a SNP within 50kb from both ends of a gene were associated with the expression level of the gene after adjustment for age and gender”. From this analysis, the authors report that AGFG1 SNPs represent an eQTL for AHR. While this is an interesting and progressive approach to studying the genetics of asthma and related phenotypes, there are questions regarding study design and analysis.

Major Compulsory Revisions

1. The results section indicates that significant associations were found in ‘4 regions’. Were there other associated snps not reported here? If so, they should also be shown to strengthen the paper. Table 2 shows that the frequency of a number of the SNPs is very similar, yet Figure 2 and the abstract/results highlight one SNP for each gene. An LD plot of the multiple SNPs/gene should be provided to assess whether they represent the same or independent risk. For instance, rs6731443 and rs13382948 appear to be in LD, how did the authors decide to report rs6731443 as the risk SNP?

2. The eQTL data are interesting, but not significant so it is not clear how meaningful they are. Have the authors examined associations between expression levels and SNPs in just the CAMP population, since these are the same subjects? More information on the eQTL data is needed to help further the understanding of the underlying mechanisms. What genotype is associated and does the level of mRNA increase or decrease with increasing PC20? If the AGFG1 SNPs represent an eQTL, then the level of AGFG1 mRNA should be associated with lnPC20? Have the authors examined this? If so, why not?
3. Whether using the Lung Health Study as a replication population is appropriate is also a question. The LHS is focused on smokers, while the other studies had none to few smokers and excludes individuals on regular use of asthma medications. What is the % of asthma in that population? Since this is the only population where any SNP was replicated, it is unclear whether it is independent of asthma, though the title of the manuscript says ‘in asthma subjects’. This should be addressed.

Minor Essential Revisions
4. Were AGFG1 and ITGB5 in HWE? It is not clear why the authors have used a threshold of 1E-3 for HWE.

5. Looking at the 5 populations (Table 1) it appears that BMI is fairly different and therefore analyses should include adjustment for this. Whether adjustment for corticosteroids/medications was performed/needed for all populations was also not clear.

Discretionary Revisions
7. Like most GWAS there are very few hits. The SNPs reported here do not show significance at the genome wide level (they are <10-8), although the AGFG1 SNPs seem promising since they are replicated in the LHS population. A potential reason may be that the primary GWAS was done by merging 3 drug studies (CAMP, CARE and ACRN). However, these populations are considerably different. ACRN adult subjects and has fewer females, different times of wash-out and most importantly has very different InPC20, the primary end point. The CAMP and CARE studies are in children and are more similar. Population heterogeneity may have contributed to loss of power to detect other associations. The authors could examine the GWAS data using CAMP and CARE as the primary dataset and using ACRN as a replication population.

8. Lack of replication in the Dutch Asthma Genetics study (DAG) is also difficult to interpret since they examined airway hyper-responsiveness differently, using either histamine or methacholine and were using steroids. Could the data from those having a PC20 test with methacholine be analyzed? As such, is not clear whether this population is the best choice for replication, particularly for the ITGB5 SNPs, since the gene is likely involved in inflammatory responses known to be influenced by smoking and steroids.

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:

no