Author's response to reviews

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

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Editorial Office
BMC Medical Genetics

Dear Editors:

On behalf of my co-authors, I would like to resubmit manuscript 1973263057918672 entitled “ITGB5 and AGFG1 Variants are Associated with Severity of Airway Responsiveness” for publication as an original article. We thank the reviewers who commented on the original draft for their thoughtful and helpful suggestions, which we have incorporated in the current version as detailed in the “Response to Reviewers” below. Because of the changes we made to the manuscript, we believe that the paper is much improved compared to the original version.

Please let me know whether we can provide further information or you have any questions regarding this submission. Thank you for your consideration.

Sincerely,

Blanca E. Himes, Ph.D.
Response to Reviewers’ Comments

REVIEWER 1

CRITIQUE 1: The major limitation is the heterogeneity by age, asthma status and smoking history among the discovery and replication samples. While the discovery population includes asthmatic children, replication samples were not children. Although the DAG study was conducted among adult asthmatics, the LHS study included adult smokers and excluded subjects who were not using asthma medications regularly. As the authors pointed out, these heterogeneity may have influenced the replication finding. Suggest that the author conduct a sensitivity study among non-smoking DAG subjects to find out whether they could replicate the findings.

RESPONSE 1: We agree that it is unfortunate that our cohorts were not more homogeneous, and/or of a sample size large enough to be able to discern differences based on cohort-specific phenotypes. We did adjust for smoking status in DAG to reduce any confounding that may have resulted from this variable and preferred this approach over a division into smaller sub-groups due to the even greater reduction in statistical power that would result from doing so. Nonetheless, as suggested above, we tried the association among non-smokers only (n=364), and we were unable to replicate results.

CRITIQUE 2: Given the lung-function distribution, it appears that some of the LHS study subjects may have met the criteria for COPD. Also, some LHS subjects required a very high-dose of methacholine to elicit AHR, which is beyond the distribution of LnPC20 is the discovery sample. It will be useful to conduct another sensitivity analysis by restricting the replication in LHS subjects who did not have COPD and had similar distribution of LnPC20 as that of the discovery sample.

RESPONSE 2: Yes, all LHS subjects had COPD. Sorry this was not made clear in the original version; we have included a better description of this cohort. Because these subjects were recruited to have COPD and not asthma, we are unable to obtain an LnPC20 distribution that resembles that of our asthma cohorts.

CRITIQUE 3: Include asthma and COPD status in Table 1 (since it appears that some asthmatics and COPD cases might be in the LHS study).

RESPONSE 3: A sentence that better describes the cohorts has been included in the Table 1 header.

REVIEWER 2

MAJOR
CRITIQUE 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?

RESPONSE 1: The four regions are reported in Table 2. The two that SNPs that were not in the genes are rs4861175 and rs296282. It is precisely because the SNPs within the two genes are in a region of LD that we focused on single SNPs for each gene that had the lowest p-value (i.e. strongest association). Figure 2 does provide the association results for all SNPs in the regions, and the two selected SNPs are highlighted because they had the lowest local p-values.

CRITIQUE 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?

RESPONSE 2: We agree that not having significant p-values after correcting for multiple-comparisons testing weakens the results, but we reported the eQTL data anyways because they may still represent a true association that we are statistically underpowered to detect. Yes, as stated in the Methods subsection “Genome-wide Gene Expression Data,” we focused on the 419 arrays (47,053 gene probes) from whole blood samples of 419 white CAMP subjects utilized in the current study. We have included a new figure (Figure 4) that includes the expression of an AGFG1 probe by genotype of SNP rs6731443. Because the AGFG1 SNPs in Table 2 are in LD with one another, the figures for the other SNPs are quite similar. We checked whether the level of AGFG1 mRNA detected correlated with LnPC20 levels in CAMP, and they did not. We have included an explicit statement about this in the manuscript.

CRITIQUE 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.

RESPONSE 3: We agree that LHS is not an ideal replication population. The LHS subjects did not have asthma at the time of enrollment, but a history of asthma was possible. To clarify that the association with AHR may be independent of the asthma phenotype, we have modified the title to be “ITGB5 and AGFG1 Variants are Associated with Severity of Airway Responsiveness.”

MINOR
CRITIQUE 1: Were AGFG1 and ITGB5 in HWE? It is not clear why the authors have used a threshold of 1E-3 for HWE.

RESPONSE 1: For GWAS, a HWE threshold of 1E-6 is the most common. Our threshold of 1E-3 is more conservative than this, and we screened our genotyped data based on this threshold to err on the side of excluding more SNPs that may not have been properly genotyped.

CRITIQUE 2: 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.

RESPONSE 2: The BMI across studies differs as expected by age. The pediatric cohorts (CAMP and CARE) have lower BMI, while the adult cohorts have similar BMI with mean ~25. The only population which included participants taking corticosteroids was DAG, and we did adjust for this in analyses. CAMP, CARE, ACRN, and LHS subjects were not taking asthma medications at the time of the AHR test as described in the Methods section.

DISCRETIONARY
CRITIQUE 1: 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 lnPC20, 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.

RESPONSE 1: We agree with this assessment. We have tried to perform GWAS that are age-specific, and most of the results are similar to those using the three populations at once. This is likely due to the uneven distribution of subjects and may not reflect the underlying differences in AHR that are age-dependent. When we obtain GWAS data for other asthma trials that include AHR measures, we would like to pursue further studies in more homogeneous populations.

CRITIQUE 2: 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.

RESPONSE 2: The DAG investigators have spent much time trying to ensure that the results of histamine vs. methacholine tests are similar, and they have found that this is the case. However, it is certainly true that DAG is different in many other ways from the other populations, and so, we also look forward to obtaining data for other cohorts that may help clarify the differences in populations.