Author’s response to reviews

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

Authors:

Blanca E. Himes (rebeh@channing.harvard.edu)
Weiliang Qiu (stwxg@channing.harvard.edu)
Barbara Klanderman (rebjk@channing.harvard.edu)
John Ziniti (rejpz@channing.harvard.edu)
Jody Senter-Sylvia (rejms@channing.harvard.edu)
Stanley J. Szefler (szeflers@njhealth.org)
Robert F. Lemanske Jr (rfl@medicine.wisc.edu)
Robert S. Zeiger (Robert.S.Zeiger@kp.org)
Robert C. Strunk (strunk@kids.wustl.edu)
Fernando D. Martinez (fernando@resp-sci.arizona.edu)
Homer Boushey (homer.boushey@uscf.edu)
Vernon M. Chinchilli (vchinchi@phs.psu.edu)
Elliot Israel (EISRAEL@partners.org)
David Mauger (dmauger@hes.hmc.psu.edu)
Gerard H. Koppelman (reghk@channing.harvard.edu)
Maartje AE Nieuwenhuis (m.a.e.nieuwenhuis@umcg.nl)
Dirkje S. Postma (d.s.postma@umcg.nl)
Judith M. Vonk (j.m.vonk@umcg.nl)
Nicholas Rafaels (nrafaels1@jhmi.edu)
Nadia N. Hansel (nhansel1@jhmi.edu)
Kathleen Barnes (kbarnes@jhmi.edu)
Benjamin Raby (rebar@channing.harvard.edu)
Kelan G. Tantisira (rekgt@channing.harvard.edu)
Scott T. Weiss (scott.weiss@channing.harvard.edu)

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Author’s response to reviews: see over
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Editorial Office
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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 reviewer who commented on the revised draft. We have incorporated her suggestions 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

MINOR

CRITIQUE 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’(i.e., 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.

RESPONSE 1: We believe that the methods for the cis-eQTL analysis are clear, and we performed standard genotype analysis for which the corresponding results are conveyed in Figure 4. The methods description and P-values in Table 3 are accurate. We did not perform dominant/recessive tests. The trend in Figure 4 has the significance level noted in Table 3 by the methods described. Perhaps the reviewer expected a more dramatic effect to be noticeable in Figure 4 “by eye”, and not seeing one that aligns with expectations makes the statistical results seem less intuitive.

CRITIQUE 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.

RESPONSE 2: We agree that Figure 2 does not provide enough information for the reader to identify what SNPs are represented. We have included Supplementary Table 1 that contains results for all imputed SNPs in or within 50kb of the top two genes that are plotted in Figure 2 (i.e. AGFG1 and ITGB5). We have included information on the location of the two top SNPs (intronic) in the results section. We listed eQTL data for all of the SNPs in Table 3, which had the lowest P-values in our GWAS. While other SNPs in these genes may be stronger eQTLs, they would not be associated with our outcome of interest, and hence, we have excluded this information from the manuscript purposefully. Because functional information for SNPs can be easily gathered via tools such as Haploreg, we leave it to the reader to make further inquiries regarding SNP function as the data used to produce functional annotation is constantly being updated.

CRITIQUE 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.
RESPONSE 3: We have modified the Background and Conclusion sections to be clearer about the origin of each cohort.

CRITIQUE 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.

RESPONSE 4: We have included mention of the functional validation necessary to confirm the eQTL findings in the Discussion section.