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Reply to: Quantitative Histology Seriously Flawed by Lack of Lung Volume Measurement

From the Authors:

We appreciate the interest of Drs. Mitzner and Ochs in our study (1), and their comments based on their experience with methods for measuring lung structural changes in emphysema (2). We also recognize that there has been a discussion in the literature about the most accurate methods for assessing lung structure, and that the recommendations based on this discussion include assessment of fixed lung volume to account for volume-based variation in chord length (CL) (3). As noted by the letter writers, we did not measure fixed lung volume in the strain survey described in our paper. Nonetheless, we believe that there is disease-based relevance to our findings.

The largest barrier to the interpretation of CL in the absence of fixed lung volume, as described by Mitzner, is that the CL “will not be able to separate the effects on tissue destruction from those on tissue distension” (4). Both of these parameters represent pathological changes that occur in emphysema, with increased compliance occurring secondary to extracellular matrix loss and parenchymal destruction. So, in mice exposed to cigarette smoke compared with appropriate controls, whether due to distension or destruction, changes in CL clearly represent a response to cigarette

Figure 1. Manhattan plot of associations with percentage change in chord length. Each dot represents a SNP, with its genomic location represented on the x-axis and the strength of association on the y-axis. The top threshold (red) represents genome-wide significance after Bonferroni correction of the effective number of tests, the middle threshold (blue) represents suggestive significance, and the bottom threshold (green) is drawn at $P = 10^{-3}$ to demonstrate nominally associated SNPs that were used to identify candidate regions.

smoke, which we found to be variable between inbred strains (1). Assuming that volume and surface area are only likely to increase in response to this exposure, the letter writers are concerned that in strains with significant changes in CL, the extent of tissue destruction was inflated, particularly given that changes in lung volume may also be strain dependent (5). However, if some strains demonstrate increased volumes without tissue destruction and others show increased tissue destruction without increased volumes in response to cigarette smoke, there are evidently disease-related genetic factors regulating these differences that we can search for in an association study. Although it would have been ideal to perform separate studies looking at these factors as independent parameters of emphysema, using an unadjusted CL primarily affects our association study by increasing the phenotypic heterogeneity.

The statistical impact of phenotypic heterogeneity on association studies has been extensively studied in the human genetics literature because of the indirect and sometimes inaccurate clinical measures we rely on to define disease (6, 7). In studies of other complex diseases, it has been shown that improving the definition of a phenotype improves the power to detect associated variants but rarely results in a failure to detect variants identified with a less well-defined phenotype (8). In chronic obstructive pulmonary disease, we have seen that many variants associated with spirometric measures of disease, which are the result of multiple pathological processes, are also associated with radiological measures of emphysema, but that there are variants associated with emphysema that can only be detected by looking at these more refined radiological measures (9). As a simple demonstration of this effect, using the methods described in our paper, genetic association with the alternate but still relevant phenotype “percent change in CL” is also nominally associated with the region on murine chromosome 16 containing *Abi3bp* (Figure 1), despite the alterations in the susceptibility scale mentioned by the letter writers (1).

We recognize the important qualification presented by Mitzner and Ochs regarding the interpretation of our strain survey. However, we maintain that *Abi3bp* is a strong candidate worthy of further study. The phenotypic heterogeneity introduced by using CL without fixed lung volumes may have reduced our study’s power but is unlikely to reduce the relevance of our findings. Results of the strain survey were used to identify a large candidate region, but identification of the candidate gene within this region was based on associations from a meta-analysis of human chronic obstructive pulmonary disease. In addition, the gene is biologically plausible, and we presented *in silico* and molecular evidence to support its potential involvement in the observed phenotype. Only future studies of *Abi3bp* will confirm what mechanistic role, if any, this gene plays in the development of emphysema in humans.

However, as discussed in an editorial published with our work, its identification using this initial phenotype suggests the need for future genetic studies in mice using finer techniques and alternative resources such as the Collaborative Cross strain panel, as they are likely to successfully identify additional candidates, particularly when combined with supporting evidence from human disease (10). ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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