The Long and Winding Road from GWAS to Obstructive Lung Disease: Is There a Role for LRP1?

The importance of quantifying lung function to characterize human physiology has been recognized since the second century when the Greek physician Claudius Galenus first measured respiratory volume by having a child breathe into a bladder (1). Modern pulmonary function testing (PFT), however, did not come into existence until 1846 when the English physician Hutchinson invented the water spirometer to measure the vital capacity of the lung (1, 2). Today, spirometry remains an essential component of PFTs that assesses respiratory health, diagnoses pulmonary disease, monitors disease severity, and predicts mortality (3). Genome-wide association studies (GWAS) have further advanced our understanding through the identification of at least 279 genetic loci that are associated with lung function (4). Despite the advances made by GWAS, a challenge facing the field is the establishment of causal relationships between these genetic loci and PFT measurements.

In this issue of the *Journal*, Nichols and colleagues (pp. 368–378) address the goal of establishing causality between a candidate genetic locus that regulates lung function and spirometry (5). The authors focused on the *LRP1* gene based upon a GWAS meta-analysis study of European subjects that identified a significant association between a reduction in the forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio and a single-nucleotide polymorphism (SNP) that mapped to a *LRP1* intron (6). The *LRP1* gene encodes LRP1 (low-density lipoprotein receptor–related protein 1), which is a member of the low-density lipoprotein receptor family that is highly expressed in the lung as well as the liver and brain (7). LRP1 functions as an endocytic receptor that may interact with over 100 different ligands, including multiprotein complexes, which clears proteins from the extracellular space and internalizes cargo for lysosomal degradation (8–10). LRP1 may also modify cell signaling, either indirectly by internalizing ligands and membrane proteins that participate in cell signaling pathways or directly through interactions with coreceptors, as well as adapter and scaffolding proteins (8–10). Furthermore, LRP1 can undergo regulated intramembrane proteolysis to release its intracytoplasmic domain, which may regulate cell signaling, and shed its extracellular domain to function as a soluble receptor (9, 10). Several functions of LRP1 in the lung have been identified. LRP1 has been shown to negatively regulate the antigen uptake and presentation functions of $CD11b^+$ dendritic cells in the lung, which attenuated allergic sensitization, type 2 immune responses, and eosinophilic airway inflammation in a murine model of house dust mite (HDM)-induced airways disease (11). Additionally, knockdown of LRP1 in human lung fibroblasts increased the expression of ACTA2 to promote cellular differentiation into myofibroblasts with a contractile phenotype that can increase tissue fibrogenesis (12).

Here, Nichols and colleagues first analyzed a multi-ethic GWAS meta-analysis to confirm that two intronic SNPs in the *LRP1* gene were associated with FEV_1/FVC (3). Next, the authors created a cell-specific knockout mouse in which the *Lrp1* gene was deleted in smooth muscle cells driven by the smooth muscle actin promoter ($Lrp1^{-/-}$ mice), which allowed investigations to examine the role of LRP1 in modulating pulmonary function. They show that $Lrp1^{-/-}$ mice have baseline abnormalities in pulmonary function with decreased compliance as well as increased elastance, tissue resistance, and tissue elastance. Furthermore, $Lrp1^{-/-}$ mice showed significantly increased airway responsiveness to methacholine challenge. The authors next assessed pulmonary function in $Lrp1^{-/-}$ mice following exposures to LPS and HDM. $Lrp1^{-/-}$ mice again displayed decreased baseline compliance that was associated with increased elastance and tissue elastance following acute LPS exposure but not following sensitization and challenge with HDM. Interestingly, $Lrp1^{-/-}$ mice did not display genotype differences regarding airway hyperresponsiveness to methacholine following exposure to either LPS or HDM. Thus, *Lrp1* appears to primarily regulate basal lung function in mice. Collectively, these results support a causal association between SNPs in the *LRP1* gene with pulmonary function in mice and humans.

How does LRP1 modify smooth muscle function to cause basal abnormalities in lung physiology? Having shown a causal relationship between the *LRP1* gene and pulmonary function, the authors hypothesized that $Lrp1^{-/-}$ mice might have dysregulated expression of LRP1 ligands in BAL fluid (BALF). They used mass spectroscopy to identify that BALF levels of 160 proteins differed between $Lrp1^{-/-}$ and $Lrp1^{+/+}$ mice, of which 7 were proteins known to interact with LRP1. LRP1 functions as a receptor that clears serpin-enzyme complexes from the plasma, and SERPINA1 was the most upregulated protein in this proteomic analysis of BALF from $Lrp1^{-/-}$ mice, whereas the LRP1 ligand APOE (apolipoprotein E) was the most downregulated protein (8, 9). Modified BALF levels of cytoskeletal proteins were also identified by mass spectroscopy, whereas increases in urokinase were detected by ELISA. As discussed by the authors, a limitation of their study is that it does not establish a molecular mechanism by which LRP1 modifies pulmonary physiology. Thus, additional studies will be needed to test the authors' hypothesis that dysregulated expression of LRP1 ligands contribute to physiological lung function abnormalities. Furthermore, the significance of the proteomic findings in BALF identified by mass spectroscopy will require

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confirmation by experiments utilizing additional quantitative methods.

It is intriguing that APOE was identified in the proteomic analysis as the most highly downregulated protein in BALF from *Lrp1*$^{-/-}$ mice, which suggests a possible role for a APOE as a candidate LRP1 ligand that modifies smooth muscle function. APOE was the first identified LRP1 ligand, and several studies have suggested a link between APOE and lung function in both mice and humans (9). For example, *Apoe*$^{-/-}$ mice have impaired developmental alveologenesis that is associated with increased airflow resistance at age 3 months (13). Both *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice that had been sensitized and challenged with HDM have a phenotype of increased airway reactivity to methacholine (14). Furthermore, an analysis of participants in the Long Life Family Study found that the FEV_1/FVC ratio was reduced among carriers of the APOE ε4 allele, who were primarily women older than 70 years without lung disease (15).

Thus, the study by Nichols and colleagues provides valuable future directions to further define the role of LRP1 in regulating smooth muscle function in the lung. How do the intronic *LRP1* SNPs regulate the expression of LRP1 at the mRNA and protein levels? Are the intronic *LRP1* SNPs associated with lung disease? What are the relevant LRP1 ligands that modulate airway smooth muscle function? Does LRP1 modify cell signaling or contractility in lung smooth muscle cells? The answers to these questions have the potential to further expand the role of LRP1 in regulating lung physiology in health and disease. ■

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Xianglan Yao, M.D., Ph.D.
Stewart J. Levine, M.D.
Laboratory of Asthma and Lung Inflammation
National Heart, Lung, and Blood Institute, National Institutes of Health
Bethesda, Maryland

ORCID IDs: 0000-0003-0668-0353 (X.Y.); 0000-0001-9313-9746 (S.J.L.).

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