ORIGINAL RESEARCH

# Lrp1 Regulation of Pulmonary Function
## Follow-Up of Human GWAS in Mice

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## Abstract

Human genome-wide association studies (GWASs) have identified more than 270 loci associated with pulmonary function; however, follow-up studies to determine causal genes at these loci are few. SNPs in low-density lipoprotein receptor–related protein 1 (LRP1) are associated with human pulmonary function in GWASs. Using murine models, we investigated the effect of genetic disruption of the *Lrp1* gene in smooth muscle cells on pulmonary function in naive animals and after exposure to bacterial LPS or house dust mite extract. Disruption of *Lrp1* in smooth muscle cells leads to an increase in tissue resistance, elastance, and tissue elastance at baseline. Furthermore, disruption of *Lrp1* in smooth muscle increases airway responsiveness as measured by increased total lung resistance and airway resistance after methacholine. Immune cell counts in BAL fluid were increased in animals with *Lrp1* disruption. The difference in airway responsiveness by genotype observed in naive animals was not observed after LPS or house dust mite extract exposure. To further explore the mechanisms contributing to changes in pulmonary function, we identified several ligands dysregulated with *Lrp1* disruption in smooth muscle cells. These data suggest that dysregulation of LRP1 in smooth muscle cells affects baseline pulmonary function and airway responsiveness and helps establish *LRP1* as the causal gene at this GWAS locus.

**Keywords:** genome-wide association studies; lung function; low-density lipoprotein receptor–related protein 1; genetic variation; chronic obstructive pulmonary disease

Genome-wide association studies (GWASs) provide an unbiased, comprehensive approach to identifying genetic variants associated with disease relevant phenotypes. GWASs of pulmonary function have identified more than 270 genetic loci related to these traits (1, 2). Pulmonary function is a readily available and reliably measured index of the physiological state of the lungs that is used clinically to diagnose and assess the progression of chronic obstructive lung disease (COPD), asthma, and other lung conditions (3–5).

SNPs within the low-density lipoprotein receptor–related protein 1 (*LRP1*) gene associated with the pulmonary function trait of the ratio of forced expiratory volume in 1 second ($FEV_1$) to forced vital capacity (FVC) have been identified in GWASs (6). Although GWASs have been very successful in identifying genetic loci associated with many phenotypes and diseases, identifying the causal genes or variants has proved more elusive. Experimental animal studies can establish causality but are costly and time consuming and thus have rarely been applied to follow-up GWAS associations.

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## Clinical Relevance

Human genome-wide association studies (GWASs) have identified more than 270 loci associated with pulmonary function; however, follow-up studies to determine causal genes at these loci are few. SNPs in low-density lipoprotein receptor–related protein 1 (*LRP1*) are associated with the pulmonary function trait of the ratio of forced expiratory volume in 1 second to forced vital capacity. Although GWASs are very successful in identifying genetic loci associated with many phenotypes and diseases, identifying the causal genes or variants has proved more elusive. Experimental animal studies can establish causality but are costly and time consuming and thus have rarely been applied to follow-up GWAS associations. There are no studies following up the GWAS associations with pulmonary function for SNPs in *LRP1*. We provide updated GWAS results confirming the association between SNPs within *LRP1* and the ratio of forced expiratory volume in 1 second to forced vital capacity in a multiethnic population of more than 90,000 individuals. Using animal models, we found that mice without *Lrp1* in smooth muscle have altered baseline pulmonary function and exhibit increased airway responsiveness. These data recapitulate human findings from GWASs, lending evidence for a causal relationship between LRP1 and pulmonary function not possible to establish from association studies alone. Establishing causal genes underlying GWAS findings is an essential first step toward clinical translation.

There are no studies following up the GWAS associations with pulmonary function for SNPs in *LRP1*.

LRP1 is a ubiquitously expressed, versatile scavenger receptor first characterized as mediating the uptake of extracellular or membrane-associated molecules (7). Further research into the function of LRP1 has described additional functions of the protein, which some suggest may be tissue specific (8). Deletion of *Lrp1* in mice is embryonically lethal, outlining the importance of this receptor in embryogenesis and development (9). Within the immune system, this cell surface transmembrane scavenger receptor plays a significant role in regulation of cellular junctions and modulation of the inflammatory response (8). LRP1 in smooth muscle cells is known to mediate the endocytosis of numerous ligands that regulate the extracellular environment and promote survival of these cells (10, 11).

LRP1 has a wide range of functions in different tissues reflecting its diversity of interactions, activities, and expression. This receptor has been shown to interact with more than 100 proteins, including ~40 extracellular ligands including lipoproteins, extracellular matrix proteins, proteases, protease-inhibitor complexes, and growth factors (12). These extracellular interactions coupled with unique, potentially tissue-specific intracellular interactions help to modulate cell migration, survival, proliferation, and differentiation. Although LRP1 is ubiquitously expressed, this protein is most abundant in the brain, lung, and muscle (7, 13). Recent data support a key role for LRP1 in lung immunity (14) and lung remodeling (15). No study has outlined the role of LRP1 in the modulation of pulmonary function.

In this study, we report the development of a novel murine model to facilitate studies on the function of LRP1 in the lung. Using a smooth muscle–specific Cre recombinase, we disrupted *Lrp1* and assessed the impact of this disruption on pulmonary function. To identify the mechanisms contributing to the observed phenotypes, we used well-established pulmonary phenotyping techniques and proteomic assessment of BAL fluid to identify peptides altered by LRP1 disruption in the smooth muscle. This work in mice provides significant evidence that *LRP1* is causal for the GWAS signal for modulation of pulmonary function identified in humans.

## Methods

### Human GWASs of *LRP1* SNPs and Pulmonary Function Traits and Expression Quantitative Trait Analysis of Sentinel GWAS SNPs in *LRP1*

*See* data supplement.

### Murine Studies

$Lrp1^{flox}$ (B6; 129S7-$Lrp1^{tm2Her}$/J) mice were purchased from The Jackson Laboratory and maintained as Het x Het breeders to generate floxed mutant mice that were genotyped per the Jackson Laboratory protocol. These mice were bred to mice expressing Cre recombinase driven by the smooth muscle (SM) specific Tagln (Transgelin) promoter to generate experimental knockout (SM-$Cre^{+}$-$Lrp1^{flox/flox}$; represented as $Lrp1^{-/-}$) and control (SM-$Cre^{-}$-$Lrp1^{flox/flox}$; represented as $Lrp1^{+/+}$) animals. Given that SM-$Cre^{+}$ and SM-$Cre^{-}$ did not significantly differ in pulmonary function (Figure E1 in the data supplement), SM-$Cre^{-}$-$Lrp1^{flox/flox}$ animals were used as controls. Mice were fed NIH-31 rodent chow *ad libitum* and housed with alternating 12-hour light-dark cycles. All animal work described in this study was conducted according to National Institutes of Health guidelines and approved by the National Institute of Environmental Health Sciences Animal Care and Use Committee.

### Immunofluorescence

Pulmonary tissue was sampled from control and knockout animals and assessed for LRP1 expression using immunofluorescence. *See* data supplement.

### Pulmonary Function Assessment

Knockout and control animals between 9 and 12 weeks of age underwent pulmonary function analysis using the flexiVent Legacy system or flexiVent FX2 system (SCIREQ, Inc) as previously described (16). *See* data supplement.

### BALF Cell Analyses

After exsanguination, BAL fluid (BALF) samples were collected with Hanks' balanced salt solution (H6648; Sigma-Aldrich). BALF from each mouse was centrifuged to separate cells from supernatant. Cellular fractions were treated with ammonium–chloride–potassium buffer, centrifuged, resuspended in Hanks' balanced salt solution, and quantified with a TC20 Automated Cell Counter (Bio-Rad), and cytospins were prepared for cell differential analysis.

### BALF LRP1 Ligand Concentrations by ELISA or Bio-Plex

The concentrations of ligands of LRP1 were measured in the cell-free supernatant using Bio-Plex mouse assays and ELISAs. Matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) levels were assayed using the Bio-Plex mouse assay and Bio-Plex suspension array system (171

AM001M; Bio-Rad) according to the manufacturer's instruction. ELISA kits were used according to manufacturer's instruction to measure the levels of elastase (ab204730; Abcam), urokinase (ab198512; Abcam), and plasminogen activator inhibitor-1 (ab197752; Abcam) in the BALF.

### BALF Proteomics

After samples were digested overnight with trypsin, the digests were analyzed by liquid chromatography/mass spectrometry (LC/MS) on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific) interfaced with an M-Class nanoAcquity Ultra Performance LC (UPLC) system (Waters Corporation). Proteins from the LC/MS data were identified and quantified using Proteome Discoverer (ThermoFisher Scientific). Each sample was acquired in triplicate and the results searched against the RefSeq mouse protein database. *See* data supplement for details.

### Statistical Analysis

For assessment of genotype differences in pulmonary function parameters at baseline and after methacholine administration, we used a Student's *t* test. Values were reported as mean ± SEM and considered significant if the *P* value for difference by genotype was less than 0.05. *See* data supplement regarding proteomic analyses.

## Results

### Human GWASs

Using data from an updated large multiethnic GWAS meta-analysis (1), we confirmed earlier reported associations (6) from European ancestry individuals that SNPs within *LRP1* are associated with $FEV_1$/FVC (Figure 1A). Two common intronic SNPs in *LRP1* were identified at genome wide significance ($P < 5.00 \times 10^{-8}$): rs7968719 (minor allele frequency 0.49, $P = 4.34 \times 10^{-9}$) and rs11172113 (minor allele frequency 0.43, $P = 4.04 \times 10^{-8}$). Genome-wide significant associations were not seen for *LRP1* SNPs in relation to $FEV_1$ (Figure 1B, smallest *P* value $= 6.12 \times 10^{-4}$ for SNP rs61461775) or FVC (Figure 1C, smallest *P* value $= 6.9 \times 10^{-3}$ for rs34505337).

### Human eQTL Analysis

Among the significant *cis*-expression quantitative trait locus (eQTL) results in Genotype-Tissue Expression (GTEx; obtained from https://www.gtexportal.org/

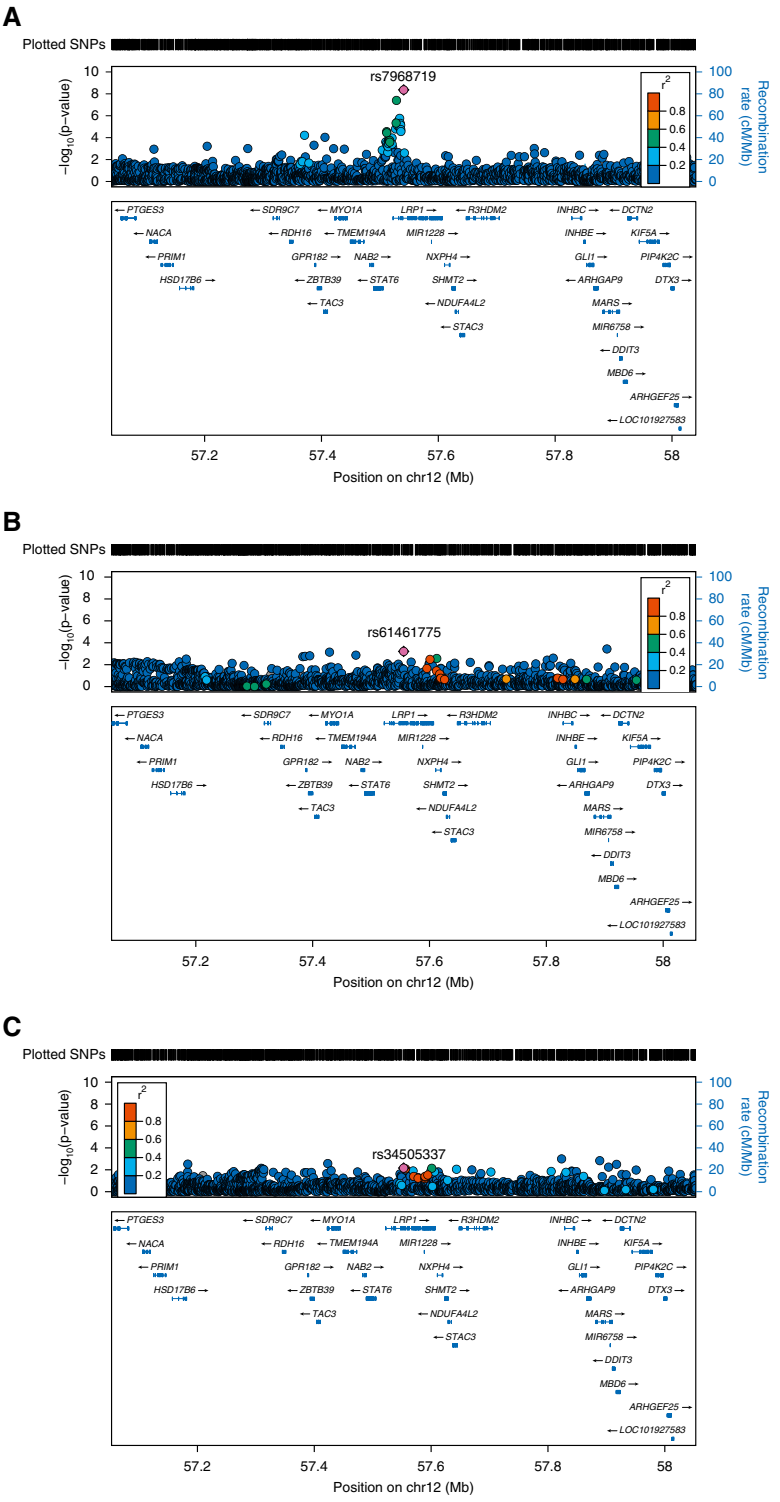


**Figure 1.** *LRP1* (low-density lipoprotein receptor–related protein 1) is associated with pulmonary function parameters in a multiethnic meta-analysis. Locus zoom plots for lead variants in or near *LRP1* identified in the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium multiethnic meta-analysis of 1,000 genomes variants and the following pulmonary function measures: (*A*) The ratio of forced expiratory volume in 1 second ($FEV_1$) to forced vital capacity (FVC), (*B*) $FEV_1$, and (*C*) FVC. Insertion/deletion variants are omitted. Linkage disequilibrium is based on hg19/1000 Genomes Mar 2012 EUR (http://locuszoom.sph.umich.edu/). Nearby genes not shown on plots include SLC26A10 and B4GALNT1.

home/ on January 3, 2019), rs7968719 and rs11172113, the genome-wide significant sentinel SNPs for *LRP1* in the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) multiethnic meta-analysis of $FEV_1/FVC$ (1), indicated significant cis-eQTLs in three tissues: skin and artery (aorta and tibial). Because GTEx includes only a few hundred (or fewer) samples for each tissue (e.g., 383 lung tissue samples and 369 whole blood samples), we also looked up *LRP1* variants in the following eQTL data sources: 5,311 blood samples in the Westra and colleagues study (17), and 2,116 blood samples in the BIOS (Biobank-based integrative omics study) Consortium (18). Both rs7968719 and rs11172113 indicated significant cis-eQTLs in blood in both studies.

### *Lrp1*$^{-/-}$ Mouse Characterization

Using specific antibodies and immunofluorescence, we confirmed a significant decrease in LRP1 protein expression in smooth muscle cells of *Lrp1*$^{-/-}$ mice relative to *Lrp1*$^{+/+}$ control mice (Figure 2). The changes in LRP1 expression were cell type specific as staining with markers for endothelial (CD31) and epithelial (EpCAM) cells did not yield significant differences in colocalization between *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice (Figure E3). Examination of hematoxylin and eosin–stained sections revealed no obvious differences in lung histology or architecture with genetic disruption of *Lrp1* (data not shown).

### Baseline Pulmonary Function Is Impacted by *Lrp1* Disruption

When analyzing the baseline pulmonary function in naive animals, we observed a significant increase in elastance, tissue resistance, and tissue elastance and a concomitant decrease in compliance in *Lrp1*$^{-/-}$ animals compared with *Lrp1*$^{+/+}$ controls (Figure 3A). We also observed a significant increase in airway responsiveness to methacholine as assessed by resistance, airway resistance, and tissue resistance (Figure 3B and Table E1). We observed no changes in the numbers of neutrophils, eosinophils, or total lymphocytes in BAL fluid between *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ animals, but did discern an increase in total cells and macrophages (Figure 3C).

### Pulmonary Function in *Lrp1*$^{-/-}$ Animals after LPS Exposure

After exposure to LPS, there was a significant decrease in baseline compliance

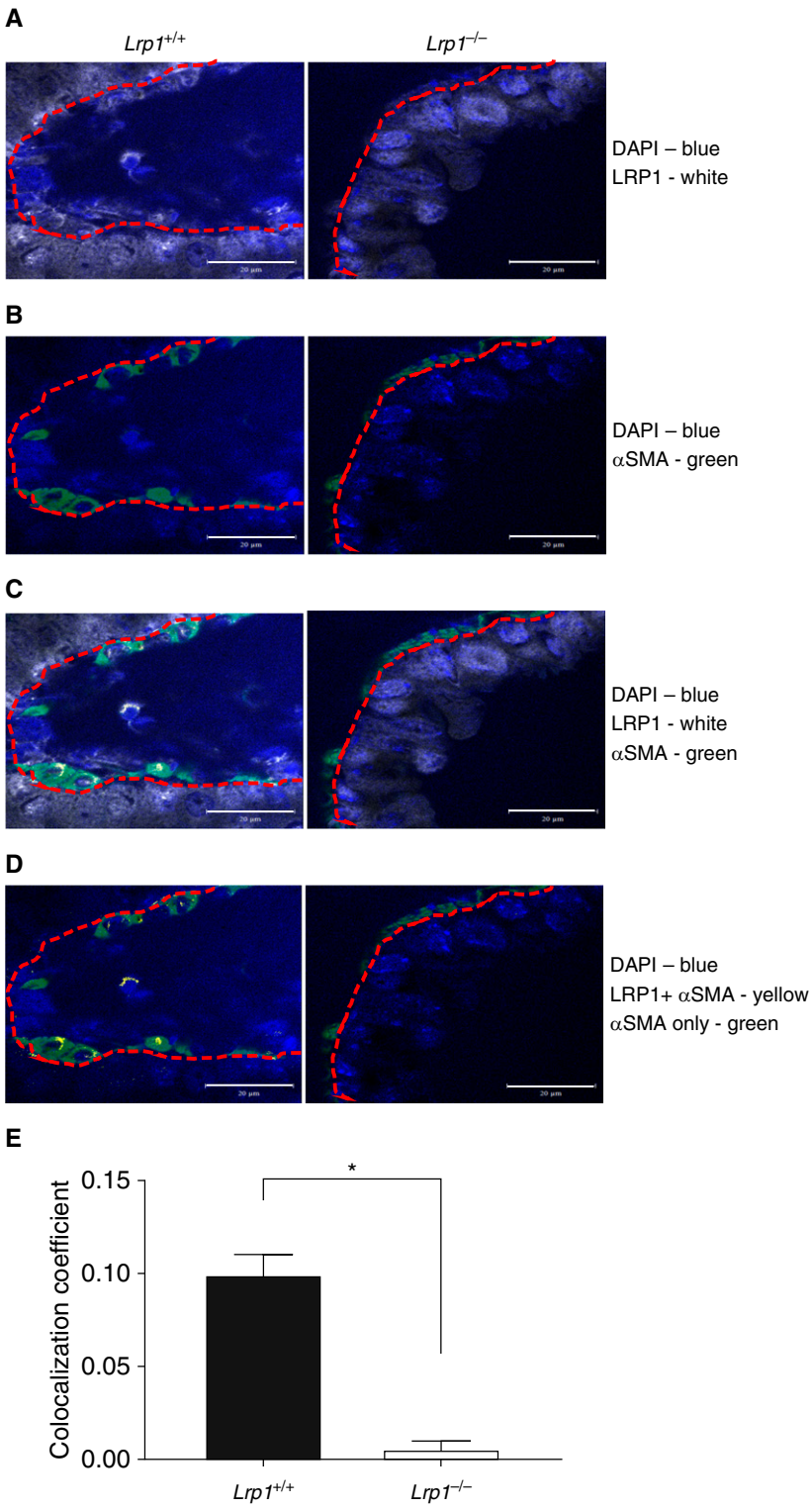


**Figure 2.** Representative immunofluorescent images of LRP1 colocalization with αSMA (α-smooth muscle actin) in *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ animals. (*A*) DAPI (blue) and anti-LRP1 (Cy5). (*B*) DAPI (blue) and anti-αSMA (Alexa488). (*C*) Merge of DAPI (blue), anti-αSMA (Alexa488), and anti-LRP1 (Cy5). (*D*) Areas of colocalization of anti-LRP1 and anti-αSMA with false color (yellow) and anti-LRP1 (Cy5) channel removed. Red dotted line is traced on the basement membrane separating smooth muscle from bronchial epithelium. Scale bars, 20 μm. (*E*) Quantification of colocalization signal in wild-type and *Lrp1*$^{-/-}$ animals. ($n = 3$/genotype, $^*P < 0.05$).

and significant increases in baseline elastance and tissue elastance in *Lrp1*$^{-/-}$ mice compared with *Lrp1*$^{+/+}$ controls (Figure 4A). Moreover, after exposure to LPS, both *Lrp1*$^{-/-}$ and *Lrp1*$^{+/+}$ animals exhibited significantly increased airway responsiveness to methacholine compared with saline (vehicle)-treated mice, with LPS-exposed *Lrp1*$^{-/-}$ animals responding similarly to their *Lrp1*$^{+/+}$ counterparts (Figure 4B and Table E2). Thus, LPS exposure abolished genotype differences in airway responsiveness to methacholine. Furthermore, although there was an increase in the number of total cells and neutrophils BAL fluid in LPS-exposed *Lrp1*$^{-/-}$ and *Lrp1*$^{+/+}$ animals compared with saline-exposed animals, there were no significant differences by genotype (Figure 4C). These data suggest that the innate immune response is unaffected by LRP1 deficiency.

### Pulmonary Function in *Lrp1*$^{-/-}$ Animals after Exposure to House Dust Mite Extract

After house dust mite extract (HDME) exposure, baseline pulmonary function did not differ significantly between *Lrp1*$^{-/-}$ mice and *Lrp1*$^{+/+}$ controls (Figure 5A). Moreover, after exposure to HDME, both *Lrp1*$^{-/-}$ and *Lrp1*$^{+/+}$ animals had significantly increased airway responsiveness compared with their respective vehicle-exposed controls, without significant differences by genotype (Figure 5B and Table E3). Eosinophils and total cells were increased in the BAL fluid of HDME-exposed *Lrp1*$^{-/-}$ and *Lrp1*$^{+/+}$ animals compared with their respective vehicle-exposed controls but there were no differences by genotype after exposure to HDME (Figure 5C). This suggests that the inflammation induced by HDME is similar in *Lrp1*$^{-/-}$ and *Lrp1*$^{+/+}$ animals.

### LRP1 Ligands Are Dysregulated in BAL Fluid of *Lrp1*$^{-/-}$ Animals

Given the observed differences in pulmonary function in naive *Lrp1*$^{-/-}$ mice relative to *Lrp1*$^{+/+}$ controls and the absence of differences in immune cell response to LPS and HDME, we evaluated whether genetic disruption of *Lrp1* in smooth muscle cells alters the levels of LRP1 ligands in the BAL fluid. We initially focused on LRP1 ligands previously shown to be associated with pulmonary disease including matrix metalloproteinase 9

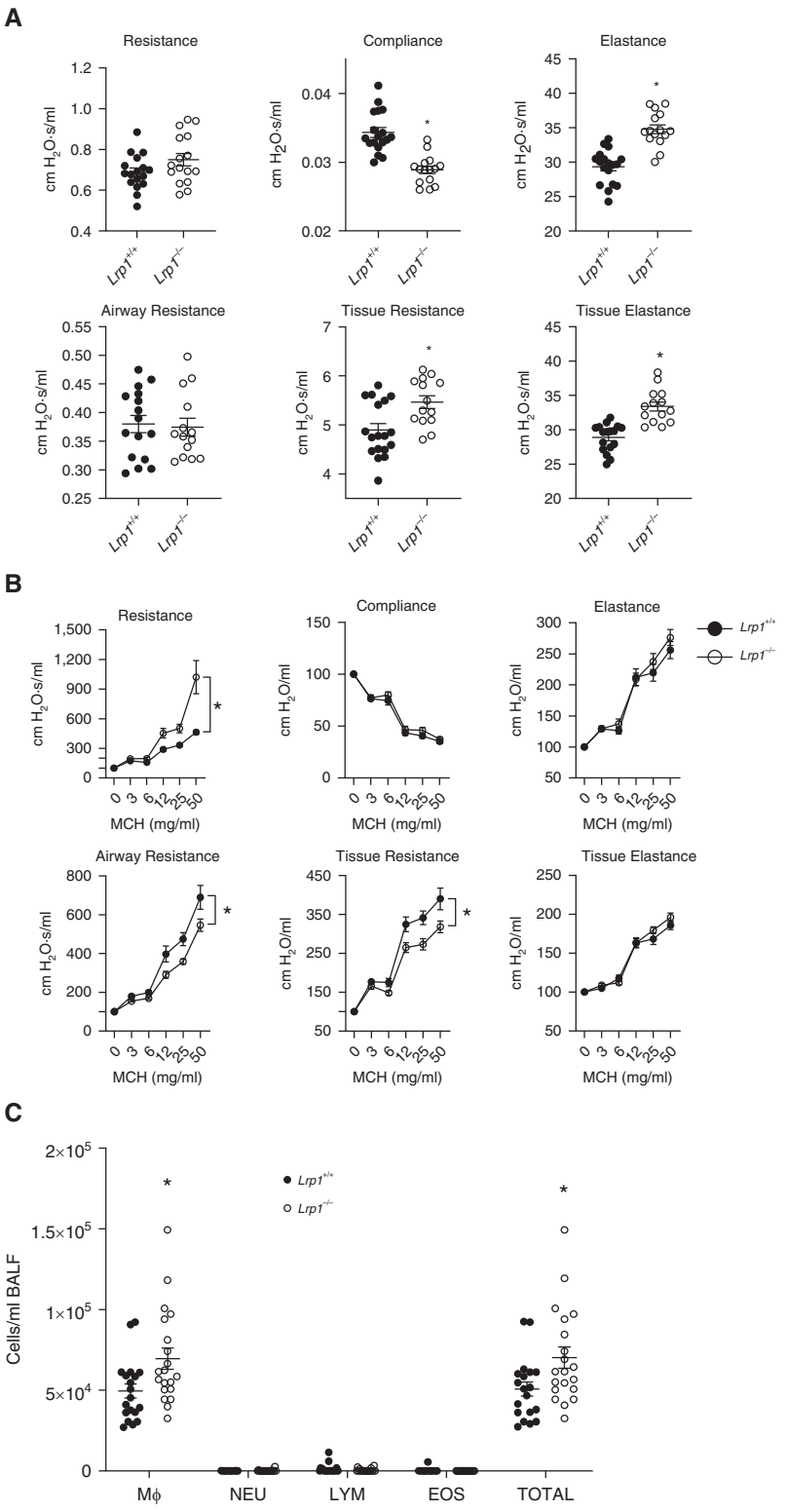


**Figure 3.** Smooth-muscle *Lrp1*$^{-/-}$ mice exhibit altered baseline pulmonary function, airway responsiveness, and BAL fluid (BALF) cell counts. (*A*) Baseline pulmonary function in *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice; Means and SEMs are plotted ($n > 18$/genotype). (*B*) Airway responsiveness to methacholine (MCH) in *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice; means and SEs are plotted ($n > 18$/genotype). (*C*) Cell differential counts between *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice for macrophages (Mϕ), neutrophils (NEU), lymphocytes (LYM), and eosinophils (EOS). *$P < 0.05$.

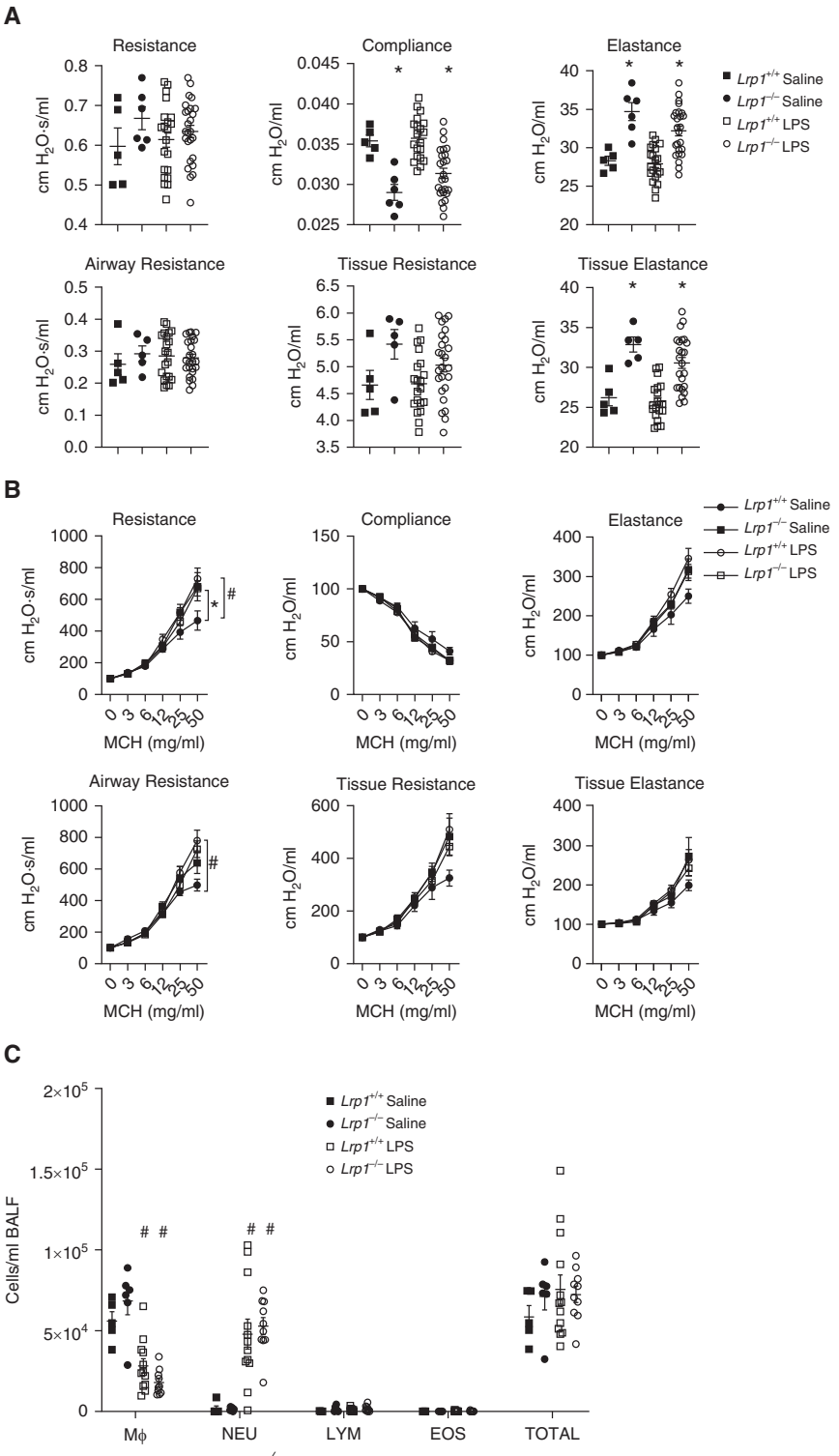


**Figure 4.** Smooth-muscle *Lrp1*$^{-/-}$ mice exhibit altered pulmonary function but not airway responsiveness or BALF cell counts after exposure to bacterial LPS. (*A*) Baseline pulmonary function in wild-type and *Lrp1*$^{-/-}$ mice 4 hours after exposure to bacterial LPS or saline. (*B*) Assessment of airway responsiveness to MCH in *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice after exposure to bacterial LPS or saline. (*C*) Cell differential counts between *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice for Mϕ, NEU, LYM, and EOS after exposure to bacterial LPS or saline. $N > 5$/genotype for saline exposure and $N > 20$/genotype for LPS exposure. $^{*}P < 0.05$ for comparison between genotypes with the same exposure. $^{\#}P < 0.05$ for comparison between saline and LPS exposure of the same genotype.

(MMP9) (19), MMP2 (20), elastase (21), urokinase (22), and plasminogen activator inhibitor-1 (PAI-1) (23). We found that the concentration of urokinase was significantly increased in the BALF of *Lrp1*$^{-/-}$ animals compared with *Lrp1*$^{+/+}$ controls. No significant differences in the concentrations of the other ligands were found between the genotypes (Figure 6).

### BALF Proteomes Are Dysregulated in *Lrp1*$^{-/-}$ Animals

Because LRP1 has been reported to interact with more than 40 different proteins (24), we used a proteomic approach to further investigate differences in BALF proteins between *Lrp1*$^{-/-}$ and *Lrp1*$^{+/+}$ animals using label-free mass spectrometry. A total of 1,850 proteins were identified after quality control and data processing. Of the proteins identified, 160 proteins were significantly dysregulated (adjusted $P$ value $< 0.05$) in the BALF of *Lrp1*$^{-/-}$ compared with *Lrp1*$^{+/+}$mice. Of the proteins identified, 21 are known to associate with LRP1 and 7 of these associated proteins were significantly dysregulated in *Lrp1*$^{-/-}$ animals (Table 1). Some proteins that were significantly upregulated include members of the serpin family (e.g., SERPINA1D, 21-fold increase). Apolipoprotein E (APOE) was significantly (28-fold) decreased in the BALF of *Lrp1*$^{-/-}$ animals relative to *Lrp1*$^{+/+}$ controls. Some proteins altered by disruption of *Lrp1* in smooth muscle are cytoskeletal proteins, for example, tubulin and microtubule-associated protein, RP/EB family, member 1. The presence of cytoskeletal proteins in the BALF has been reported in previous studies of mice under control conditions (25–29).

## Discussion

In this study we used mouse models to follow up human GWAS–identified SNPs in *LRP1* associated with pulmonary function to ascertain whether *LRP1* is indeed the causal gene at this locus. We also provide updated GWAS results confirming the association between *LRP1* SNPs and $FEV_1$/FVC in a multiethnic population of more than 90,000 individuals (1). Furthermore, we investigate whether the sentinel *LRP1* SNPs are expression quantitative trait loci that influence gene expression in humans. Using a murine model of *Lrp1* disruption in smooth muscle

cells we investigated the role of LRP1 in modulating pulmonary function and observed a unique pulmonary phenotype with *Lrp1* knockout in smooth muscle showing a change in both baseline pulmonary function parameters and airway hyperresponsiveness compared with animals with intact *Lrp1*. We further investigated mechanisms that contribute to the unique pulmonary phenotype observed. Owing to the previously identified role of LRP1 in the modulation of the inflammatory response, we began by investigating the role of inflammation in the modulation of the inflammatory response in the lungs of $Lrp1^{-/-}$ animals and concluded that removal of LRP1 in the smooth muscle does not overtly impact the inflammatory response. Given our evidence that the inflammatory response modestly affects and is not responsible for the pulmonary phenotype, we investigated the dysregulation of ligands of LRP1 within the BALF and found that expression of some proteins known to be associated with pulmonary function and asthma are altered. Taken together, these data help to confirm that LRP1 is the gene at the locus identified in GWASs responsible for modulating pulmonary function in humans and establish that the pulmonary phenotype observed in animal models may in part be due to dysregulation of LRP1 ligands.

A previous GWAS meta-analysis had established the association of SNPs in *LRP1* with $FEV_1$/FVC in individuals of European descent (6). In the current study, we confirm this association in a much larger, multiethnic population. Of note, a recent large GWAS from the UK Biobank has implicated one of these two SNPs (rs11172113) in the additional spirometric trait of peak expiratory flow (2). However, simple association in GWASs does not establish *LRP1* as causal at this locus. GWAS significant sentinel *LRP1* variants identified for $FEV_1$/FVC were significant cis-eQTLS in GTEx in skin (sun-exposed lower leg) and two arterial tissues (aorta and tibial artery) and in blood in two larger data sets. Interestingly, the two sentinel SNPs for lung function have also been associated in GWASs with aortic aneurysm (30) and arterial pulse pressure (31). Although we did not identify significant eQTLs in the lung, a heterogeneous tissue in GTEx samples (32), this result confirms that the sentinel *LRP1* GWAS SNPs

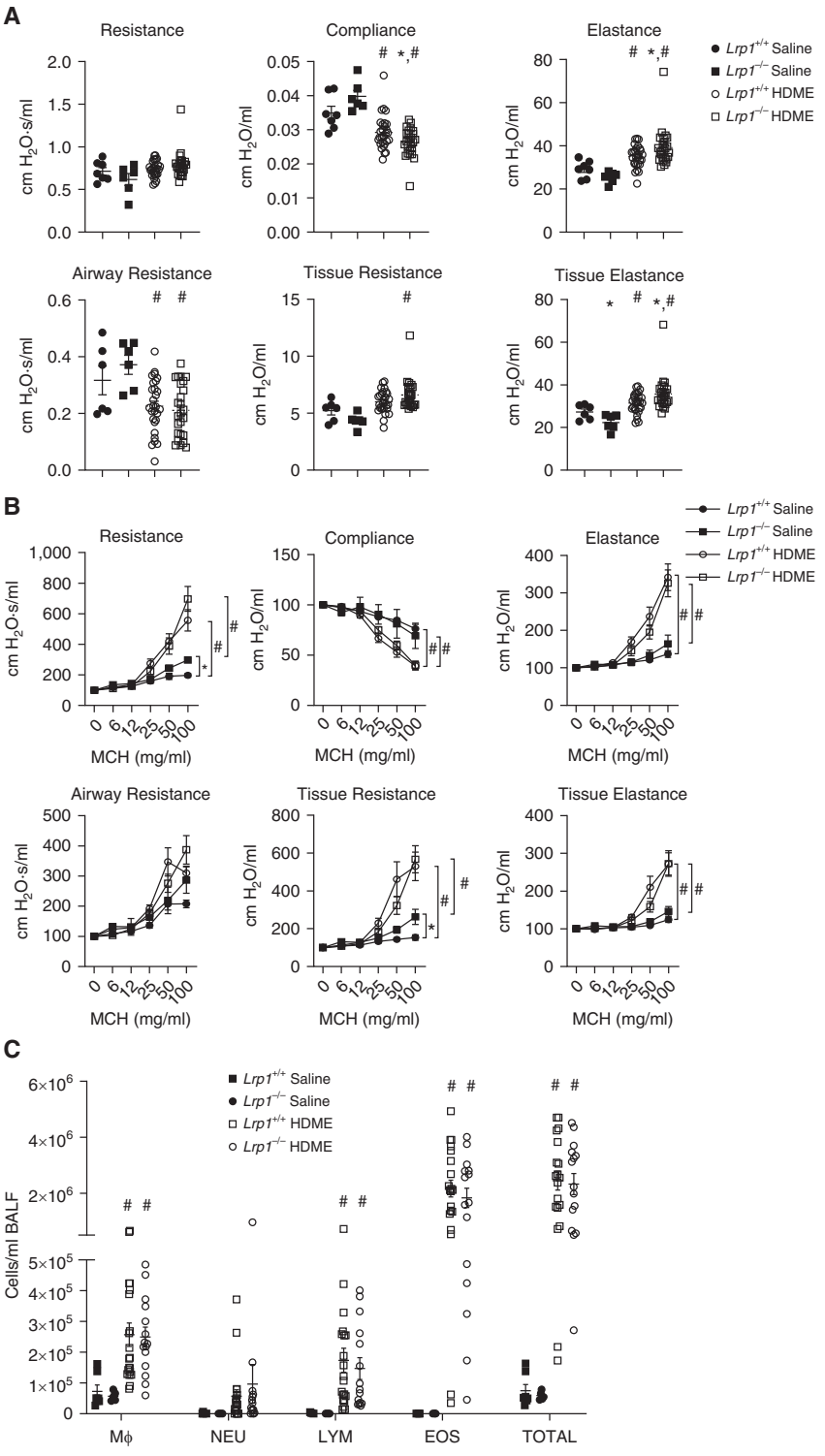


**Figure 5.** Smooth-muscle $Lrp1^{-/-}$ mice exhibit few differences in pulmonary function, airway responsiveness, or BALF cell counts after house dust mite extract (HDME) exposure. (*A*) Baseline pulmonary function in $Lrp1^{+/+}$ and $Lrp1^{-/-}$ mice after two sensitization exposures and three challenge exposures with HDME over 17 days. (*B*) Assessment of airway responsiveness to MCH in $Lrp1^{+/+}$ and $Lrp1^{-/-}$ mice. (*C*) Cell differential counts between $Lrp1^{+/+}$ and $Lrp1^{-/-}$ mice for Mϕ, NEU, LYM, and EOS. $N > 6$/genotype for saline exposure and $N > 24$/genotype for HDME exposure; $^{*}P < 0.05$ for comparison between genotypes with the same exposure. $^{\#}P < 0.05$ for comparison between saline and HDME exposure of the same genotype.

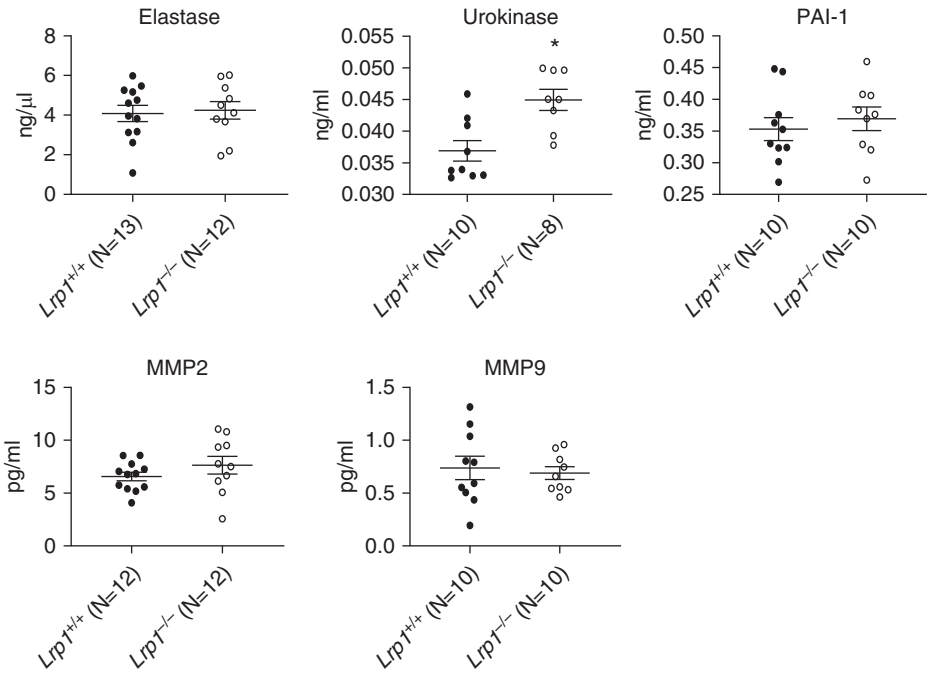


**Figure 6.** Smooth-muscle *Lrp1*$^{-/-}$ mice and LRP1-ligand concentrations in BALF. BALF was collected from *Lrp1*$^{+/+}$ and *Lrp1*$^{-/-}$ mice and assessed via ELISA for concentrations of known LRP1 ligands: elastase, urokinase, PAI-1, MMP2, and MMP9. $N \geq 8$/genotype. *$P < 0.05$. MMP2 = matrix metalloproteinase 2; MMP9 = matrix metalloproteinase 9; PAI-1 = plasminogen activator inhibitor-1.

correlate with gene expression. However correlational analysis cannot establish a causal locus. Therefore, we used murine models to conduct follow-up analyses, to better establish the causal relationship between LRP1 and pulmonary function parameters.

Experimental follow-ups of GWAS findings are not a common undertaking as they are expensive and labor intensive and there are many loci identified in GWASs for a given trait. Previous experimental studies by us and others have followed up a handful of GWAS loci for pulmonary function traits and helped to clarify the causal gene at the locus and potential mechanisms for the GWAS associations (16, 33–35). Thus, this manuscript adds to this small body of work that highlights the value of experimental studies to better understand GWAS findings. Human GWASs of pulmonary function traits were done in population studies where it is not possible to administer different exposures. The baseline differences identified in this study by removal of LRP1 in smooth muscle cells in an animal model mimic the human phenotype. Because interventions are not part of the human population-based GWASs, we could build on the human data by exposing mice to methacholine and the environmentally relevant exposure models of LPS and HDME.

LRP1 has been demonstrated to have a wide variety of functions, but it is best known as a scavenger receptor. Furthermore, there is previous evidence for cell specificity of some of the functions of LRP1, prompting the need to investigate the impact of LRP1 in different cell types on pulmonary function. LRP1 has previously been shown to play a key role in vascular

**Table 1.** Proteins within the BAL Fluid Identified by Mass Spectrometry and Previously Associated with Low-Density Lipoprotein Receptor–related Protein 1

| Symbol | Protein Name | Adjusted *P* Value | Fold Change |
|---|---|---|---|
| APOE | Apolipoprotein E | 0.014 | −28.860 |
| CALR | Calreticulin | 0.064 | 23.348 |
| GRIN2A | Glutamate ionotropic receptor NMDA type subunit 2A | 0.641 | 1.404 |
| GLUL | Glutamate-ammonia ligase | 0.015 | −4.563 |
| HSP90AA1 | Heat shock protein HSP 90-α | 0.018 | −20.937 |
| HSP90B1 | Endoplasmin | 0.909 | −1.226 |
| HSPA5 | Heat shock protein family A member 5 | 0.010 | 9.401 |
| HPX | Hemopexin | 0.194 | 3.510 |
| IGFBP3 | Insulin-like growth factor–binding protein 3 | 0.386 | 1.694 |
| LPL | Lipoprotein lipase | 0.343 | −4.020 |
| MAPRE1 | Microtubule-associated protein RP/EB family member 1 | 0.131 | −2.652 |
| ROCK2 | Rho-associated coiled-coil–containing protein kinase 2 | 0.978 | 1.023 |
| SEPT7 | Septin-7 | 0.250 | −4.114 |
| SERPINA1D | Alpha-1-antitrypsin 1-4 | 0.003 | 21.471 |
| SERPINC1 | Serpin C1 | 0.333 | 2.226 |
| SERPIND1 | Serpin D1 | 0.070 | 3.295 |
| SERPING1 | Serpin G1 | 0.075 | 3.234 |
| TUBB4A | Tubulin β-4A chain | 0.130 | −4.047 |
| TUBB4B | Tubulin β-4B chain | 0.016 | −13.986 |
| YWHAG | 14-3-3 protein γ | 0.075 | 4.068 |
| VCL | Vinculin | 0.042 | 5.099 |

smooth muscle function (36, 37). In addition to documenting that *Lrp1* disruption in smooth muscle alters pulmonary function in the mouse and establishing this locus as causal for the human association, we attempted to identify mechanistic insight for this observation. *Lrp1* knockout induces rampant systemic inflammation suggesting that one canonical mechanism of LRP1 is to inhibit inflammatory reactions (38, 39). Thus, we began investigating this mechanism by treating $Lrp1^{-/-}$ animals with environmentally relevant exposures that are known to induce inflammatory reactions—LPS and HDME. After identifying that control and knockout animals responded similarly to these environmental exposures, we considered the other main function of LRP1: regulating the levels of its ligands within the extracellular space. Because LRP1 has more than 40 identified ligands, we used a combination of proteomics and well-established assays to gather information on the concentrations of these ligands within the bronchoalveolar fluid. Using these, we identified several ligands dysregulated by LRP1 removal that may contribute to the identified pulmonary phenotype. Although this study used proteomic approaches to identify some candidate proteins altered in the BALF of $Lrp1^{-/-}$ mice that could be involved in the pulmonary function phenotype, it does not establish a precise molecular mechanism. Future experiments to confirm the proteomic findings with another method and then to link dysregulation of these proteins to altered pulmonary function would be needed to establish whether any of the dysregulated ligands are responsible for the observed phenotype.

Deeper characterization of the cells in the BALF, as well as *in vitro* studies to understand how Lrp1 deletion in smooth muscle cells affects behavior of cytoskeletal proteins, would be of interest. Of direct relevance to this issue, a recent study examined the impact of *Lrp1* deletion in smooth muscle in the mouse and also found various cytoskeletal proteins to be altered (40). In both their direct examination of vascular smooth muscle and ours of BALF, several cytoskeletal proteins were altered in the same direction with *Lrp1* deletion in smooth muscle. At nominal $P < 0.05$ in both studies, ACTA2 and CLIC4 were decreased and levels of the following proteins were increased: ACTN4, MYH11, MYLK, and VCL. Both studies highlight the importance of LRP1 in the regulation of cytoskeletal proteins.

This is the first study to directly analyze the impact of a scavenger receptor on pulmonary function and to further investigate the mechanisms contributing to this phenotype. In the current study, urokinase and APOE were two ligands that were dysregulated in $Lrp1^{-/-}$ animals. Both of these ligands have been previously identified to be associated with pulmonary function and airway responsiveness (41, 42). In humans, urokinase is known to be increased in the BALF of humans with asthma (43), suggesting that in our study the increase in BALF urokinase levels may contribute to the increase in airway responsiveness. Decreased APOE levels in other animal models has been shown to increase susceptibility to cardiovascular and pulmonary injury (44, 45), especially after pulmonary environmental and occupational exposures (46–48). Furthermore, one study investigating the relationship between APOE and urokinase showed that decreased levels of urokinase lead to increased levels of ApoE, suggesting urokinase plays a role in regulating ApoE levels (49). The differential impact of ligands could be exacerbated with disruption of LRP1 in different cell types. Not only could this be due to the differential functions of LRP1 in different cell types, but further different ligands may differentially impact these different cell types.

In summary, following up human GWAS findings for *LRP1* and pulmonary function, we found that $Lrp1^{-/-}$ mice have altered baseline pulmonary function (decreased compliance, increased elastance, tissue resistance, and tissue elastance) and exhibit increased airway responsiveness. These data recapitulate human findings from GWASs, lending evidence for a causal relationship between *LRP1* and pulmonary function that was is not possible to establish from such association studies alone. We did not find that LRP1 deficiency leads to altered responses to either bacterial LPS or HDME. We found evidence that supported that ligand disruption contributes to the pulmonary phenotype observed in our animal model of *Lrp1* disruption. In conclusion, we present here strong evidence for a causal relationship between LRP1 and pulmonary function in mice that supports human GWAS findings. ■

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

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