Variable Susceptibility to Cigarette Smoke–Induced Emphysema in 34 Inbred Strains of Mice Implicates *Abi3bp* in Emphysema Susceptibility

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Abstract

Chronic obstructive pulmonary disease (COPD) is caused by a complex interaction of environmental exposures, most commonly cigarette smoke, and genetic factors. Chronic cigarette smoke exposure in the mouse is a commonly used animal model of COPD. We aimed to expand our knowledge about the variable susceptibility of inbred strains to this model and test for genetic variants associated with this trait. To that end, we sought to measure differential susceptibility to cigarette smoke–induced emphysema in the mouse, identify genetic loci associated with this quantitative trait, and find homologous human genes associated with COPD. Alveolar chord length (CL) in 34 inbred strains of mice was measured after 6 months of exposure to cigarette smoke. After testing for association, we connected a murine candidate locus to a published meta-analysis of moderate-to-severe COPD. We identified deleterious mutations in a candidate gene *in silico* and measured gene expression in extreme strains. A/J was the most susceptible strain in our survey (Δ CL 7.0 ± 2.2 μ m) and CBA/J was the least susceptible (Δ CL -0.3 ± 1.2 μ m). By integrating mouse and human genome-wide scans, we identified the candidate gene *Abi3bp*. CBA/J mice harbor predicted deleterious variants in *Abi3bp*, and expression of the gene differs significantly between CBA/J and A/J mice. This is the first report of susceptibility to

cigarette smoke–induced emphysema in 34 inbred strains of mice, and *Abi3bp* is identified as a potential contributor to this phenotype.

Keywords: emphysema; COPD; animal model; *Abi3bp*

Clinical Relevance

Cigarette smoke–induced emphysema in the mouse is a model of human emphysema that has contributed to our knowledge of the molecular mechanisms of chronic obstructive pulmonary disease. Although it was previously shown that inbred strains of mice have variable susceptibility to chronic cigarette smoke, this was only demonstrated in a limited number of strains. We show that mice of 34 inbred strains have a variable susceptibility to cigarette smoke–induced emphysema. We tested for genetic association with this trait and integrated the findings with the results of a meta-analysis of human chronic obstructive pulmonary disease genome-wide association studies. Using this approach, we identified the gene *Abi3bp* as a candidate gene contributing to emphysema susceptibility.

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Chronic obstructive pulmonary disease (COPD) is defined as a progressive and irreversible airflow limitation, and is a major cause of worldwide morbidity and mortality (1). The disease is caused by a complex interaction of environmental exposures, most commonly cigarette smoke, and genetic factors (2). One of the major pathological processes that contribute to this disease is pulmonary emphysema (destruction of the distal airspaces), which results in a loss of elastic recoil in the lung and air trapping (3).

Studying emphysema in humans involves several challenges, notably the difficulty of accurately quantifying the extent of emphysema in the lung and accounting for factors such as body size and degree of inflation, as well as heterogeneous environmental effects. An alternative is to use mouse models of pulmonary emphysema in which environmental exposure is tightly controlled and the extent of emphysema can be directly measured in the lung. Mice have been shown to develop emphysema after chronic cigarette smoke exposure, and this approach has been successfully employed to model human disease (4). Studies using this model have greatly contributed to our understanding of the mechanisms of COPD (5). However, although variable susceptibility to cigarette smoke-induced emphysema has been demonstrated in five inbred strains of mice, such variability in other commonly used inbred strains has not been previously reported, nor have the genetic contributions to that variability been studied (6).

In humans, genome-wide association studies (GWASs) have been conducted to identify susceptibility variants associated with COPD (2). Although the majority of these studies tested for association with phenotypes such as COPD affection and spirometric traits, several recent studies have identified susceptibility loci associated with radiologic measures of emphysema, as well as specific patterns of emphysema (7–9). These studies demonstrated that some loci associated with emphysema are shared with COPD more broadly, whereas others, such as an association near the gene *BICD1*, appear to be uniquely associated with emphysema (8). To date, no genome-wide assessments using cross-strain comparisons of mouse models of COPD have been conducted, although such studies have effectively identified candidate genes

in other lung diseases, including asthma and pulmonary hypertension (10, 11).

In this study, we confirm that mice have variable susceptibility to cigarette smoke-induced emphysema as measured by alveolar chord length (CL), and show that this trait is continuous when measured in 34 inbred strains. By testing for association of this quantitative trait across the genome and integrating our findings with a human genome-wide scan of COPD, we identify a candidate gene for future study.

Materials and Methods

Animal Experiments

All animal experiments were performed in accordance with the Institutional Animal Care and Use Committee of the University of Pittsburgh School of Medicine. Animals were housed in a pathogen-free barrier facility that maintains a 12-hour light/dark cycle in Plexiglas cages (one to four mice per cage) with free access to autoclaved water and irradiated pellet food. Animal health, weight, and overall behavior were monitored throughout the experiments.

Female mice of 34 inbred strains (129S1/SvImJ, A/J, AKR/J, BALB/cByJ, BALB/cJ, BPN/3J, BTBRT+tf/J, BUB/BnJ, C3H/HeJ, C57BL/10J, C57BL/6J, C57BLKS/J, C57BR/cdJ, C57L/J, CBA/J, CE/J, DBA/1J, DBA/2J, FVB/NJ, I/LnJ, KK/HlJ, LG/J, LP/J, MRL/MpJ, NOD/ShiLtJ, NON/ShiLtJ, NZO/HlLtJ, NZW/LacJ, P/J, PL/J, RIIS/J, SJL/J, SM/J, and SWR/J) were exposed to 6 months of either room air (NS, $n = 4$ –7/group) or cigarette smoke (SM, 4 cigarettes/d, 5 d/wk, $n = 3$ –7/group) beginning at 10 weeks of age, as previously described (12). Kentucky Reference Cigarettes (1R5F) were obtained from the Tobacco and Health Research Institute of the University of Kentucky (Lexington, KY). After smoke exposure, the mice were killed and tracheostomized, and the lungs were removed and inflated with 10% buffered formalin to a constant pressure of 25 cm water for 10 minutes. The lungs were fixed for 24 hours in formalin and then embedded in paraffin. Serial midsagittal sections were obtained and stained with modified Gill's stain. Using Scion Image software (version 4.0.2; Scion Corp., Frederick, MD), the mean alveolar CL was calculated on 10 randomly selected 200 $\times$ fields per slide. Airway and

vascular structures were masked from the analysis and the images were manually thresholded. The CL was determined in both horizontal and vertical planes, allowing for the calculation of alveolar airspace areas. Finally, the quantitative phenotype that was used in genetic association testing (SMCL; see further below) was determined by the following equation: $\log(\text{SM} - \text{NS})$. This log-transformed value was used to ensure phenotype normality. Measurement of body weights and tissue elastance is described in more detail in the online supplement.

Genetic Association Testing

Testing for association between SMCL and 4 million single nucleotide polymorphisms (SNPs) published by the National Institute of Environmental Health Sciences was performed using the genome-wide efficient mixed model algorithm (GEMMA), which partially corrects for the complex population structure of the inbred strains in this survey (13, 14). These SNPs were aligned to NCBI37, and all genomic locations reported in this study reflect that unless otherwise noted. To account for nonvariant sites and sites in complete linkage disequilibrium in all 34 strains, we employed simpleM to determine the effective number of tests, which we then used to calculate Bonferroni-corrected significance thresholds in this study (15, 16).

Identification of Genomic Regions Enriched for Nominal Associations

A candidate region was defined as all SNPs yielding nominal associations ($P < 10^{-3}$) that were within 1 Mb (Mb = 1 million base pairs) of another nominal SNP. The number of nominal associations in each region was recorded and 1 Mb was added in each direction to the location of the boundary SNPs to account for the significant linkage disequilibrium that can occur between inbred mouse strains (17).

Identification of Nominal Associations in Human GWAS Data

A region in the human genome that is homologous to the candidate region with the most significant association identified in the mouse strain survey was identified using mouse-human homology maps publicly available from the National Center for

Biotechnology Information (18). Human homologs to all genes in the candidate region were identified. The human homologous region was identified as continuous blocks of these genes and separating noncoding regions.

Searching for Nominal SNPs in a Meta-analysis of Human COPD

The human homologous region was searched for nominal associations ($P < 10^{-4}$) in a previously published meta-analysis comparing smokers with moderate-to-severe COPD and normal spirometry from the COPDGene, ECLIPSE, NETT/NAS, and Norway GenKOLS studies (19). Nominal associations were recorded and the closest genes to these associations were identified as genes of interest. Manhattan plots of searched regions were generated using LocusZoom (version 1.1; <http://locuszoom.sph.umich.edu/locuszoom/>) (20).

Identification of Candidate Genes

All variants that resulted in a change in the amino acid sequence of the encoded protein (missense variants, stop-gained variants, and frameshift variants) predicted to be deleterious by the 'Sorting Tolerant From Intolerant' (SIFT) algorithm (SIFT < 0.05) were identified in Ensembl (release 84, dbSNP142) in each mouse homolog of the genes of interest in this study (21, 22). The genotype of each of these coding sequence variants in CBA/J and A/J mice was downloaded from Ensembl and the genotype of any variant that differed between these two extreme strains was subsequently downloaded for all available strains included in this study.

mRNA Expression

Lung tissue was homogenized in Trizol solution (Thermo Fisher, Grand Island, NY) and total RNA was isolated according to the manufacturer's instructions. RNA was quantified and used for reverse transcription with reverse transcriptase (Applied Biosystems, Grand Island, NY). Total cDNA was used for real-time PCR using primer and probe sets specific for the target genes (Applied Biosystems, Grand Island, NY). Relative fold change was calculated by comparing abundance

normalized to GAPDH between samples using the $\Delta\Delta CT$ method.

Statistics

Two-tailed t tests were used to compare CLs after cigarette smoking or room air. Error bars, unless otherwise noted, represent the standard error of the mean (SEM). Statistics were calculated with R (<http://cran.r-project.org>).

Results

Susceptibility to Cigarette Smoke-Induced Emphysema Varies Continuously in Inbred Mouse Strains

We measured the CL in 34 inbred mouse strains after 6 months of exposure to

cigarette smoke (SM) or room air (no smoke exposure, NS) (Table 1). There was significant variability in the response to SM compared with NS (Table 1; Figure 1). This trait was continuous across the 34 inbred strains, with CBA/J mice having essentially no response to cigarette smoke ($\Delta CL -0.3 \pm 1.2 \mu m$) and A/J mice being the most susceptible to cigarette smoke ($\Delta CL 7.0 \pm 2.2 \mu m$) (Figure 1; Table 1). The mean change in CL across all strains was $3.7 \pm 1.6 \mu m$, and mice of the 129S1/SvImJ strain most closely reflected this mean. Although the majority of the strains demonstrated a significant change in CL after cigarette smoke exposure, seven strains (BPN/3J, CBA/J, ILN/J, KK/HIJ, NOD/ShiLtJ, NZO/HILtJ, and RIIS/J) did not (Table 1). This trait

Table 1. Variable Susceptibility to Chronic Cigarette Smoke Exposure as Measured by Alveolar Chord Length Is Seen in 34 Inbred Strains of Mice

	NS			SM			P value*	Δ CL [#]
	n	Mean CL	SEM	n	Mean CL	SEM		
129S1/SvImJ	4	28.55	0.48	5	32.18	0.67	4.58E-02	3.63
A/J	5	33.78	0.74	6	40.77	2.11	1.83E-02	6.99
AKR/J	7	27.98	0.31	7	32.91	0.41	3.82E-04	4.93
BALB/cByJ	5	30.79	0.77	6	35.93	0.92	2.12E-04	5.14
BALB/cJ	5	30.16	0.79	6	32.64	1.15	4.10E-03	2.48
BPN/3J	4	27.49	1.00	5	30.62	1.07	6.13E-02	3.13
BTBRT+tf/J	5	31.00	0.83	6	35.59	0.57	9.79E-05	4.59
BUB/BnJ	4	30.79	0.91	5	33.01	0.73	3.28E-02	2.22
C3H/HeJ	5	40.49	0.41	6	46.76	0.48	1.95E-03	6.27
C57BL/10J	5	28.27	0.49	6	31.50	0.50	1.34E-03	3.23
C57BL/6J	5	29.67	0.79	6	36.02	0.90	8.21E-07	6.35
C57BLKS/J	5	34.05	0.32	6	38.57	0.85	1.27E-03	4.52
C57BR/cdJ	5	29.74	0.32	6	32.02	0.41	5.64E-03	2.27
C57L/J	5	32.29	0.56	6	37.60	0.25	2.35E-06	5.30
CBA/J	4	30.52	0.48	4	30.27	0.38	8.39E-01	-0.25
CE/J	5	30.67	0.80	6	32.94	0.60	4.09E-02	2.26
DBA/1J	5	28.75	1.04	6	31.92	0.53	5.23E-04	3.17
DBA/2J	5	28.92	0.59	6	33.31	0.44	5.96E-03	4.38
FVB/NJ	5	28.73	0.69	6	33.77	0.28	2.63E-03	5.04
I/LnJ	5	33.12	1.28	6	34.65	0.78	1.13E-01	1.53
KK/HIJ	4	31.19	1.17	3	34.10	0.90	1.22E-01	2.92
LG/J	5	33.92	1.24	5	36.64	0.68	2.25E-02	2.72
LP/J	5	30.72	0.39	6	35.03	0.33	1.52E-02	4.31
MRL/MpJ	5	31.19	0.36	4	34.59	0.89	3.91E-03	3.39
NOD/ShiLtJ	4	27.80	0.36	5	30.93	1.09	6.80E-02	3.13
NON/ShiLtJ	5	40.03	0.59	6	44.40	0.62	1.49E-02	4.37
NZO/HILtJ	5	32.88	0.57	6	36.31	0.55	5.97E-02	3.43
NZW/LacJ	5	30.79	0.68	6	32.57	1.58	9.09E-03	1.78
P/J	5	29.23	0.76	6	34.85	0.59	1.41E-02	5.62
PL/J	5	30.02	1.13	6	32.96	0.93	6.19E-04	2.93
RIIS/J	5	27.82	0.65	6	30.07	1.34	1.04E-01	2.25
SJL/J	5	29.50	0.31	6	34.50	0.51	6.58E-04	4.99
SM/J	5	32.11	0.55	4	34.67	0.60	1.27E-02	2.56
SWR/J	4	31.48	1.18	5	36.68	0.84	2.02E-03	5.20

Definition of abbreviations: CL, chord length; NS, no smoke exposure; SM, exposure to 6 months of cigarette smoke.

* P values are the result of two-tailed t tests between SM and NS groups by strain.

#Difference between mean SM CL and mean NS CL.

Figure 1. Inbred strains of mice have variable susceptibility to cigarette smoke-induced emphysema. Shown is the absolute difference in chord length between mice exposed to cigarette smoke and those exposed to room air, with SEM. The solid horizontal line represents the mean difference in all strains and the dashed horizontal lines represent the mean of all strains $\pm$ SD.

did not always directly correlate with changes in body weight (Table E2 in the online supplement) or with other measures of cigarette smoke damage, such as tissue elastance (Table E3).

The Genetic Contribution to Cigarette Smoke-Induced Emphysema Is Complex in the Mouse

We tested for association of SMCL with known genotypes of 34 inbred strains of

mice using GEMMA (13). No association with a single variant reached genome-wide ($P < 6.3 \times 10^{-7}$) or genome-wide suggestive ($P < 1.3 \times 10^{-5}$) significance after correction for the 79,492 effective tests tested in this study. We determined the effective number of tests using simpleM for the specific strains in our panel (Figure 2) (15). The most significant association we identified was with rs46114044 located at chr16:54194946 ($P = 7.21 \times 10^{-5}$; Table E1). The top 50 most significant associations can be seen in Table E1. To account for the significant linkage disequilibrium in the mouse, we defined a 4.3 Mb region surrounding this association (see MATERIALS AND METHODS) at chr16:54194946–58510508 as our candidate region to integrate with human GWAS results.

Identification of Candidate Associations from a Human GWAS

We mapped the candidate region from the mouse to its homologous region at 3q (chr3:97,806,017–102,198,685) in the human genome using a gene-based approach. We then searched the results of a previously published GWAS meta-analysis testing for genetic association with moderate-to-severe COPD in these human genomic regions, and identified any nominal associations yielding $P < 1 \times 10^{-4}$ (19). Only one association met this threshold, located at 3q12.2. The most significantly associated variant at this locus was rs62280585 ($P = 7.72 \times 10^{-5}$), which is located between the genes *IMPG2* and *ABI3BP* (Figure 3).

Abi3bp Contains Nonsynonymous Mutations and Is Differentially Expressed Between Extreme Strains

We attempted to identify coding nonsynonymous SNPs (cnSNPs) in the murine homologs of *ABI3BP* and *IMPG2* to identify potentially causal variants. We compared the most susceptible strain, A/J, with the most resistant strain, CBA/J, and determined whether either strain held the alternate allele of a cnSNP predicted to be deleterious by SIFT. Two nonsynonymous mutations in *Abi3bp*, rs253717433 and rs45694439, are predicted to be deleterious, and the alternate allele of each of these variants occurs in CBA/J mice but not in A/J mice (Figure 4A). Of note, one of these variants, rs253717433, occurs in exon 5 of

Figure 2. Manhattan plot of associations with the log-transformed difference in chord length between mice exposed to cigarette smoke and mice exposed to room air. Each dot represents a single-nucleotide polymorphism (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.

Figure 3. Manhattan plot of a human genomic region on chromosome 3 (chr3) syntenic to the mouse candidate region. A region at 3q12 in the human genome, homologous to a candidate region on chromosome 16 in the mouse genome, contains a single nominal association with COPD. The Manhattan plot shows the location on chromosome 3 and gene annotations on the x-axis and strength of association on the y-axis. Linkage disequilibrium with the most significant SNP estimated from the CEU population (Utah residents with Northern and Western European ancestry) genotyped in the 1,000 Genomes Project is illustrated by different-colored SNPs. The plot was created with LocusZoom (version 1.1).

Abi3bp, which is shared among all known transcripts of the protein. The only other strain in this study harboring this allele is SM/J, which showed a small but significant response to cigarette smoke (ΔL_m $2.6 \pm 0.7 \mu m$; Table 1). The alternate allele of rs45694439 is harbored by several susceptible strains, but this mutation only occurs in one of the four known transcripts of this gene (Figure 4A; Table E1). Neither of the extreme strains harbors the alternate allele of any of the nonsynonymous mutations in *Impg2* with a predicted deleterious effect (Figure 4B).

Finally, we looked at the RNA expression of *Abi3bp* in the two extreme strains used in this study to identify potential functional effects. There was a significant difference in the levels of expression of this gene, with an ~ 100 -fold greater level of expression in A/J mice, which are susceptible to cigarette

smoke-induced emphysema than in CBA/J mice, which are resistant (Figure 4C).

Discussion

In this study, we measured alveolar CL, a surrogate for airspace size and an important measure of emphysema, after chronic cigarette smoke exposure in 34 inbred strains of mice. This study adds to our understanding of the variable susceptibility to cigarette smoke-induced emphysema in mice, which was originally described in five inbred strains (NZWLac/J, C57BL6/J, A/J, SJ/L, and AKR/J) (6). By measuring this trait in 29 additional strains, we found that these mice exhibit a continuous variation in the response to chronic cigarette smoke exposure, ranging from showing essentially no response (CBA/J) to being extremely susceptible (A/J). Importantly, we identified several common inbred strains, in addition

to the previously reported NZWLac/J, that are extremely resistant to cigarette smoke and show no significant change in CL after chronic smoke exposure. At the same time, we found that commonly used strains were comparatively more susceptible to cigarette smoke-induced emphysema than was previously reported; for example, C57BL/6J mice were the second most susceptible strain in our study.

As this was the first time that cigarette smoke-induced emphysema was measured in a large panel of inbred mice, it offered the opportunity to test for associations between the trait and previously genotyped variations among these strains. This inbred-strain-association approach has been used to detect association with a number of traits in mouse models of human disease and physiology (23, 24). However, due to the high interrelatedness of inbred strains, its statistical power has sometimes been limited for complex traits without

Figure 4. *In silico* and molecular evidence for *Abi3bp*. Nonsynonymous variants predicted to be deleterious by the ‘Sorting Tolerant from Intolerant’ (SIFT) algorithm in (A) *Abi3bp* and (B) *Impg2* on mouse chromosome 3. A/J mice harbor the reference allele for all labeled variants, and CBA/J mice harbor the alternate allele of those labeled in red. (C) Relative gene expression of *Abi3bp* in A/J and CBA/J mice exposed to cigarette smoke (SM, $n = 5$ /strain) or room air (NS, $n = 5$ /strain) compared with CBA/J NS. RNA was isolated from whole lungs after 6 months of exposure to room air (NS) or cigarette smoke (SM). P values were determined by two-tailed t test of between-group comparisons ($***P < 0.001$).

supporting genetic data from an alternate approach, such as linkage analysis (14, 25). Although other approaches are available that may have greater power to achieve this goal, such as the Hybrid Mouse Diversity Panel, they also require phenotyping in many more strains of mice (26). Since the cigarette smoke-induced model of emphysema in mice is both time and labor intensive, we conducted a classic inbred-strain study hoping to improve our ability to detect true positives by combining our data with human GWAS results, an approach previously used in asthma (10).

Although the genetic evidence provided by human GWAS results offers improved resolution and additional confidence in the findings of an inbred-strain GWAS, murine genome-wide scans may also inform our interpretation of the results of future human COPD GWASs. Significant and replicated variants in human studies explain only a small portion of the heritability of COPD and other complex diseases (27, 28). Although some of this “missing” heritability is likely to be

explained by rare and structural variation, it has been demonstrated that for some traits, a significant portion can be explained by a common variation failing to reach genome-wide significance due to lack of power or phenotypic heterogeneity (29). Genetic evidence from homologous loci in both mice and humans can thus be helpful for identifying associations that fail to meet significance thresholds but may still represent susceptibility loci.

By integrating our mouse and human genome-wide scans in this way, we identified *Abi3bp* as a candidate gene in emphysema in a relatively large (4.3 Mb) region that we originally identified in our murine inbred-strain association study. ABI family member 3 binding protein (ABI3BP, also known as TARSH and eratin) is an extracellular matrix-binding protein that was first identified in a yeast two-hybrid screen as binding to c-Abl, and appears to be predominantly expressed in the lung (30, 31). Of note, a recent study combining genetic association testing and expression quantitative trait loci studies in asthma

identified *ABI3BP* as a candidate gene (32). Several studies have characterized *ABI3BP* as a putative tumor suppressor. Lung tumors exhibit marked reductions in expression and differential splicing of the gene product compared with healthy tissue (33, 34). Knockdown of *Abi3bp* by short hairpin RNA in mouse embryonic fibroblasts was shown to result in genomic instability, with a greater percentage of cells exhibiting multicentrosomes as well as increased colony formation compared with controls (35). Conversely, overexpression of *ABI3BP* reduced cellular proliferation in thyroid tumor cell lines via a p21-dependent senescence pathway (36). The gene appears to play a role in cellular proliferation in nontumor settings as well: mesenchymal and cardiac progenitor cells lacking *ABI3BP* demonstrate significant increases in proliferation and differentiation (37, 38). Additionally, a genome-wide copy-number variation study identified a significant association of *ABI3BP* with the chronic osteochondropathy Kashin-Beck disease

(39). That study further demonstrated increased gene expression in the cartilage of patients, raising the possibility that growth restrictions in chondrocytes lead to the disrepair and loss of function observed in these patients (39). Taken together, these data suggest a role for ABI3BP in regulating cellular proliferation across a wide spectrum of tissues and disease states. In our study, we showed that CBA/J mice, which are highly resistant to cigarette smoke-induced emphysema, carry predicted nonsynonymous deleterious mutations in *Abi3bp* and have significantly lower expression of the *Abi3bp* transcript. It is intriguing to consider that a functional decrease of this gene, which controls the growth and differentiation of stem and tumor cells, may also protect against cigarette smoke-induced emphysema, potentially by promoting the growth and survival of the lung epithelium. Future studies using *Abi3bp* knockout mice will be necessary to delineate this relationship more clearly.

This study has limitations. Although we measured the response to cigarette smoke-induced emphysema in 34 inbred strains, we were still unable to detect any regions that met strict multiple testing correction thresholds. As mentioned above, this is due partially to the genetic complexity of the trait and partially to the power of inbred-strain surveys; a larger survey might be able to overcome this limitation. Since we did not identify any regions with genome-wide significance, we chose to use only the most significant association to identify candidate genes. Although this was useful for identifying *Abi3bp*, it remains possible that other nominal associations could be of interest with additional supporting evidence. In addition, although the use of a mouse model of human disease offers a unique opportunity to compare regions of association between organisms, it also is limited by the similarity of the model to the disease. We chose to use the alveolar CL, which has been used as a correlate for emphysema in many translational studies, but we recognize that this phenotype does not always directly mimic emphysema with airflow obstruction in humans. This is underlined by the fact that not all strains that developed significant changes in CL had similar changes in tissue elastance, another trait that has been used to measure response to cigarette smoke in the mouse (Table E3).

We have demonstrated that susceptibility to cigarette smoke-induced emphysema is a variable trait in 34 inbred strains of mice. Although we were not able to identify any single variants associated with this phenotype at a genome-wide level, we integrated our mouse genome-wide scan with the results of a human GWAS and identified a novel candidate gene, *Abi3bp*. As the absence of *Abi3bp* has been shown to increase cellular growth in both tumor and stem cells, its potential role in pulmonary emphysema is intriguing and requires more investigation. ■

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