Genomewide Linkage Analysis Identifies Novel Genetic Loci for Lung Function in Mice

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Rationale: **Pulmonary function, including lung volumes and compliance, may be genetically determined, but few genetic polymorphisms have been identified that control these traits. We used an experimental approach and performed the first whole genome scan for pulmonary function in mice.** *Objectives and Methods:* **To identify novel chromosomal regions contributing to lung function, quantitative trait locus linkage analysis was applied in N_2 backcross and F_2 intercross mice derived from two inbred strains—C3H/HeJ and JF1/Msf—with extremely divergent phenotypes.** *Main results:* **Significant linkages to total lung capacity with LOD (logarithm of the odds) scores up to 6.0 were detected on chromosomes 15 and 17, to dead space volume and lung compliance on chromosomes 5 and 15 (LOD scores higher than 4.0), to lung compliance also on chromosome 19 (LOD score of 5.8), and to diffusing capacity on chromosomes 15 and 17 (LOD scores up to 5.0). The region of interest on chromosome 17 near *D17Mit133* contains a syntenic region to human chromosome 6q27, which was recently identified to be linked to lung function in humans. The identified intervals harbor valuable candidate genes, such as the *relaxin1* and *transforming growth factor β receptor 3* gene, which revealed missense polymorphisms between the parental strains.** *Conclusion:* **The study provides evidence for linkage of different measures of lung function on murine chromosomes 5, 15, 17, and 19 and suggests novel candidate genes that may also affect the expression of human pulmonary function.**

Keywords: genetics; linkage mapping; murine lung function; pulmonary diseases

Chronic lower respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by chronic inflammation with an accelerated decline in lung function. In the case of COPD, individuals with a higher initial level of lung function are less likely to develop clinically significant diseases than those with a lower initial level of lung function (1). Several familial studies and ethnic differences give evidence for the heritability of pulmonary function (2–8). Therefore, genetic factors influencing lung development (e.g., via growth factors) may, by defining the initial level of lung function, contribute to the clinical manifestation of chronic respiratory diseases. Genomewide scans have been conducted in humans for the traits forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) and the ratio FEV_1/FVC (1, 5, 9–14). These studies identified linkage to loci on 13 different chromosomes with replicated linkage reports on chromosomes 2, 5, and 6 (12, 14).

Experimental studies on the inheritance of respiratory function are not often available, although considerable interstrain differences in mice have been reported (15–18). In addition, morphometric studies revealed differences in the lung structure among mouse inbred strains (19–20). Studies of knockout and transgenic mice implicated a number of signaling molecules involved in lung development (e.g., overexpression of Pdgfb results in fibrosis and airspace enlargement) (21). Therefore, we used an experimental approach and performed the first whole genome scan for pulmonary function in mice using two strains with extremely divergent phenotypes (Figure 1): C3H/HeJ (C3; *Mus musculus domesticus*) and JF1/Msf (JF1; *Mus musculus molossinus*). Linkage analysis was performed in N_2 backcross and F_2 intercross progeny derived from C3 and JF1 for the phenotypic traits total lung capacity (TLC), dead space volume (V_D), lung compliance (C_L), and diffusing capacity (D_{CO}). Significant linkages with logarithm of the odds (LOD) scores up to 6.0 were detected on chromosomes 5, 15, 17, and 19. The region of interest on chromosome 17 contains a region syntenic to human 6q27 that is indicated to be important for lung function in humans (14). Within the regions of interest are several genes of unknown function, but a promising candidate is the *transforming growth factor β receptor 3* (22) gene, which is involved in embryonic lung development. Some of the results of this study have been previously reported in the form of abstracts (23–26).

METHODS

Animals

The C3 strain was obtained from The Jackson Laboratory (Bar Harbor, ME) and the JF1 mice were provided by the animal facility of the GSF (Munich, Germany). F_1 hybrids were produced by crossing either JF1 female with C3 male ($JF1C3F_1$) or reciprocal crossing of C3 female with JF1 male ($C3JF1F_1$) (Figure 2). The N_2 backcross was produced by crossing a $C3JF1F_1$ female back to a C3 male animal ($C3JF1C3N_2$). The F_2 generation resulted from matings of $C3JF1F_1$ animals ($C3JF1F_2$). From the parental strains and both F_1 generations, 16 males and 16 females were analyzed for lung function. Concerning the segregating generations, 150 female animals of F_2 and N_2, respectively, were phenotyped and genotyped. All animals were characterized at an age of 12–14 weeks as described below.

Phenotypic Characterization

Lung function measurements were performed in anesthetized, intubated mice by using a computer-controlled piston-type servo ventilator (17, 18) (*See also* http://aretha.jax.org/phenome for additional information). The ventilator provides for positive pressure ventilation and for defined respiratory maneuvers for lung function tests. Respiratory flow and volume signals were given from movements of the respirator piston. Concentrations of the respiratory and the test gases He and $C^{18}O$ were measured by a magnetic sector field mass spectrometer. A miniaturized pressure transducer enables the measurement of airway opening pressure (Pao). A second pressure transducer, located at the end of a thin-

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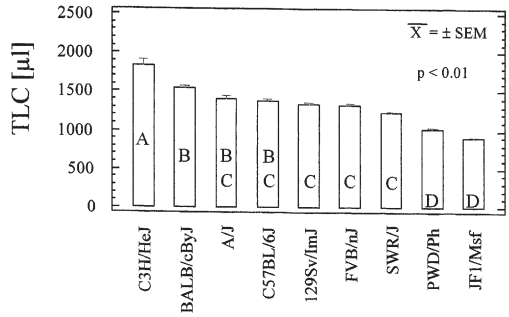

Figure 1. Comparison of lung size (total lung capacity, TLC) in inbred strains of mice (17). Sixteen male animals (age 12–14 weeks) from each of the indicated strains were analyzed for TLC. Measurements from individual animals of each strain were used to calculate a mean TLC value (*y* axis) and the standard error of the mean. Strains are arranged on the *x* axis by descending lung sizes. Strains with comparable lung sizes are in one group indicated by the same capital letter. Significant interstrain differences are marked by different capital letters.

walled, water-filled tube that is connected to an esophageal cannula, allows monitoring of the esophageal pressure (Poe). The volume signal, Pao, Poe, and gas concentration signals were continuously recorded on a multichannel recorder. Additionally, during the lung function measurements, all signals of interest were digitized and recorded on a PC for subsequent data analysis.

Inspiratory reserve capacity was defined as that volume slowly inspired over 10 seconds from a relaxed expiratory level (FRC) to a tracheal pressure of +25 cm H_2O. To account for differences in lung size between animals or mice strains, the duration of inspiration rather than the inspiratory flow rate was standardized during this and all other test maneuvers. Expiratory reserve volume was defined as that volume slowly expired over 10 seconds from FRC to a tracheal pressure of −10 cm H_2O. For the determination of FRC by the helium dilution technique, a rebreathing volume of 80% inspiratory reserve capacity labeled with 1% He in 21% O_2, balance N_2, was applied at a rate of 50/min for 15 cycles. The inspiratory and the mixed helium concentrations were determined by mass spectrometry and used for calculation of FRC. The quasi-static compliance of the respiratory system was determined from the linear portion of the pressure–volume curve obtained during a 6-second lasting exhalation from TLC to almost residual volume. Accordingly, the compliance of the lung was derived from the transpulmonary pressure–volume curve.

Series VD was obtained from single-breath washin measurements. A test gas containing breath (1% He in air) was applied over 3 seconds from relaxed expiratory level to TLC and back to slightly below FRC over 7 seconds without a breathhold. VD was determined according to the conventional Fowler method. Because of the experimental setup, VD includes the volume of the tracheal cannula (16 µl) and excludes the upper airways.

The single breathholding method as recommended by the European Respiratory Society and adapted to the present experimental conditions was used to determine the DCO (27). Inspiration of test gas (0.3% $C^{18}O$, 1% He, 21% O_2, balance N_2) started from the relaxed expiratory level to TLC over 3 seconds followed by a breathholding time of 3 seconds

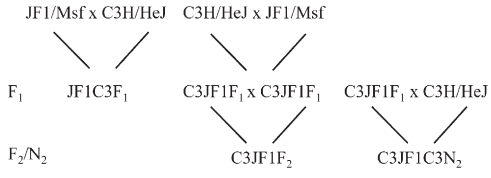

Figure 2. Breeding scheme to establish the F_1 (JF1C3F_1; C3JF1F_1), N_2 (C3JF1C3N_2), and F_2 (C3JF1F_2) generation from the parental strains C3H/HeJ (C3) and JF1/Msf (JF1).

and an expiration slightly below FRC. The alveolar volume was determined according to equation 9 of Cotes et al. (27); DCO was determined by use of equation 8 of Cotes and colleagues (27).

The sequence of the measurements was established in a strictly standardized protocol, which was equal for each of the animals. Measurements were performed in duplicate and the mean of both was used for further data analysis. Finally, the weight was taken from each animal.

Genotyping

Genomic DNA was isolated from tail clips according to the standard protocol of Sambrook and Russel (28). DNA samples were typed by polymerase chain reaction (PCR) with simple sequence length polymorphism markers purchased from MWG Biotech (Ebersberg, Germany) and Qiagen Operon (Hilden, Germany) according to a touchdown-PCR protocol (29). PCR products were analyzed on a MegaBACE 1000 DNA Analysis System (Amersham Biosciences, Freiburg, Germany). For an initial screen, 140 microsatellite markers were distributed at an average spacing of 10.5 cM across each chromosome. Subsequently, fine mapping was performed with 70 additional markers. The only new polymorphic marker *MM5BM1* was generated from contig nt_039308 and amplified with the PCR primers 5′-gCAACCACAgCA CACACTTC-3′ (forward) and 5′-CTCCTCCTCCACCAACAgAC-3′ (reverse).

Linkage Analysis

Map orders and distances from the Mouse Genome Informatics database (http://www.informatics.jax.org) were used and checked with the backcross and intercross genotype data separately using MAPMAKER/EXP, Version 3.0b (30). On chromosome 19, where discrepancies between physical (NCBI build32) and Mouse Genome Informatics genetic map exist, a marker order according to the physical map (NCBI build32) fit better and estimated distances were used. The algorithm of MAPMAKER/QTL assumes that the quantitative trait values follow a normal distribution across the population. Phenotype distributions of all traits were checked by the Kolmogorov-Smirnov test before linkage analysis. Interval mapping (31) under the option of free genetics was performed by MAPMAKER/QTL, Version 1.9, using the traits body weight (Bw), TLC, specific lung size (TLC/Bw), CL, specific lung compliance (CL/TLC), VD, and DCO. Specific values were calculated to rule out that the observed differences in absolute parameters are simply allometric differences from differences in body or lung size. LOD scores were calculated at 1-cM intervals throughout the genome. LOD scores of more than 1.9 and more than 3.3 were used as threshold levels for statistically suggestive and significant linkage, respectively, in the genomewide search of N_2 mice, and greater than 2.8 and greater than 4.3 for the F_2 mice (32).

Statistics

Regression analysis was used to establish a linear model that describes the relationship between the two variables including the correlation coefficient. The linear relationship was considered significant at $p < 0.01$. The correlation between two variables was assumed to be "weak" with r^2 values below 0.35. It was considered "intermediate" with r^2 values between 0.35 and 0.60, and considered "strong" with r^2 values higher than 0.60.

RESULTS

Lung Function Phenotype of Parental Strains and Offspring

Lung function tests were performed in 16 female and 16 male C3, JF1, and F_1 animals of both reciprocal crosses (C3JF1F_1; JF1C3F_1). From the segregating generations, 150 female N_2 backcross (C3JF1C3N_2) and 150 female F_2 intercross (C3JF1F_2) mice were phenotyped.

For TLC, C3 mice exhibited the largest and JF1 showed the smallest lungs (Figure 1). Concerning the lung function parameters CL, VD, and DCO, the two strains also demonstrated highly divergent phenotypes with higher values in C3 and lower values in JF1 (Table 1). To rule out that the observed differences in absolute parameters are simply allometric differences because

of differences in body or lung size, normalized values were calculated. By normalization, differences remained but were slightly reduced.

Animals of both reciprocal F_1 crosses mainly revealed intermediate phenotypes (Figure 3). Because the C3JF1F$_1$ animals demonstrated the more homogenous values for lung function parameters, this cross was chosen for setting up N_2 and F_2 generations (Figure 2). C3JF1F$_1$ animals tended to present smaller values for lung function parameters, especially for TLC, being closer to JF1. Thus, for greater efficiency in genotype analysis, C3 animals were established as the parental inbred strain for the backcross.

Phenotype and genotype analysis for lung function in N_2 and F_2 offspring was focused on female mice for practical reasons.

For the lung function parameters in 150 N_2 (Figure 4) and 150 F_2 (Figure 5), mice values were continuously distributed as determined by the Kolmogorov-Smirnov test supporting polygenic influences on lung function. One exception was found for the C_L in F_2 animals, which was not normally distributed (Figure 5). In this case, a $\log_{10}$ transformation was used for QTL analysis.

For all parameters, values of N_2 animals spanned the entire range of values found for the F_1 (C3JF1F1) and inbred backcross (C3) parents. Values of the F_2 animals were broadly distributed to encompass the range of values seen in the progenitor strains C3 and JF1. Concerning the parameter TLC, the phenotype of the F_2 animals was shifted more toward the JF1 strain (Figure 3).

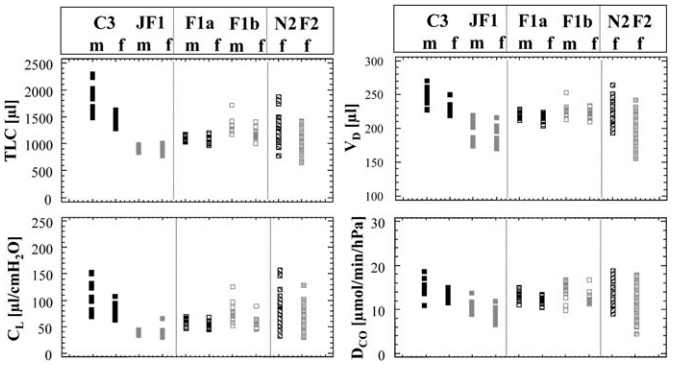

Figure 3. Scatterplots of TLC, dead space volume (V_D), lung compliance (C_L), and diffusing capacity (D_{CO}) from individual animals in parental, F_1, N_2, and F_2 mice show high values in C3 and low values in JF1 mice representing phenotypes for lungs at low and high risk, respectively, to develop clinically significant lung diseases. Intermediate phenotypes are found in F_1 animals. Values of N_2 and F_2 mice are broadly distributed to encompass the range of values seen in the parental animals. C3: C3H/HeJ; JF1: JF1/Msf; F1a: C3JF1F$_1$; F1b: JF1C3F$_1$; N2: C3JF1C3N$_2$; F2: C3JF1F$_2$.

Correlation Analyses

Correlation analysis was applied to determine the relationship between TLC and Bw. The correlation coefficients suggested a weak association of TLC and Bw in both N_2 ($r = 0.52$) and F_2 ($r = 0.49$) animals. This means that Bw accounted for approximately 25% of the variance in TLC in N_2 (Figure 6) and F_2 (Figure 7) mice.

To examine the influence of TLC on the different lung function parameters, correlation analysis was also applied (Figures 6 and 7). Results showed intermediate correlation of TLC and V_D ($r \leq 0.71$). For the parameters C_L and D_{CO}, strong correlations to TLC were observed ($r = 0.81$–0.93). Highest correlation was seen between TLC and D_{CO} in F_2 animals in which TLC explained approximately 86% of the variance in D_{CO}.

QTL Linkage Analysis in Backcross and Intercross

Linkage analysis was performed in 150 N_2 backcross and 150 F_2 intercross female mice employing, initially, 140 microsatellite markers with an average spacing of 10.5 cM across each chromosome.

QTL analysis showed evidence for loci affecting lung function on 9 of the 19 autosomes. In the N_2 backcross, two suggestive and seven significant linkages with LOD scores up to 5.0 were found. Linkage analysis further revealed six suggestive and six significant linkage results in F_2 animals obtaining LOD scores up to 6.0. For Bw, one suggestive linkage was located on chromosome 17 in N_2 animals. By use of 70 additional markers, significant linkage results were confirmed and refined on chromosomes 5, 15, 17, and 19 (Table 2). On chromosome 5, significant linkage results were obtained for V_D and specific lung compliance (C_L/TLC) in F_2 animals. Chromosome 15 showed significant linkage to TLC and C_L in both N_2 and F_2 mice. In addition, in N_2 animals, V_D, and in F_2 animals, specific lung size (TLC/Bw) and D_{CO}, were significantly linked to chromosome 15. On chromosome 17, significant linkage was obtained to TLC and D_{CO} in N_2 animals. Chromosome 19 revealed significant linkage results to C_L and C_L/TLC in N_2 mice (Figures 8 and 9). Regions of interest on

TABLE 1. BODY WEIGHT AND LUNG FUNCTION VALUES OF MALE AND FEMALE C3H/HEJ AND JF1/MSF MICE (N = 16; AGE = 12–14 wk)

Strains Sex	C3H/HeJ Male	C3H/HeJ Female	JF1/Msf Male	JF1/Msf Female
Bw (g)	29.0 ± 0.7	22.4 ± 0.6	22.7 ± 0.7	16.5 ± 0.4
Lung volumes				
TLC (µl)	1837 ± 79	1443 ± 29	904 ± 11	874 ± 17
TLC/Bw (µl/g)	63.5 ± 2.8	64.9 ± 2.1	40.2 ± 1.5	53.6 ± 1.7
V_D (µl)	245 ± 4	227 ± 2	201 ± 4	193 ± 3
V_D/TLC (%)	13.6 ± 0.5	15.8 ± 0.3	22.3 ± 0.3	22.1 ± 0.5
Compliance of the lung				
C_L (µl/cm H_2O)	100.3 ± 8.7	84.8 ± 3.6	37.8 ± 1.1	35.5 ± 2.2
C_L/TLC (µl/cm H_2O/ml TLC)	54.9 ± 2	58.5 ± 1.7	41.8 ± 1.3	40.9 ± 3
Pulmonary diffusing capacity				
D_{CO} (µmol/min/hPa)	14.1 ± 0.5	12.4 ± 0.3	9.9 ± 0.4	8.3 ± 0.4
D_{CO}/V_A (µmol/min/hPa/ml V_A)	8.7 ± 0.4	9.3 ± 0.2	11.8 ± 0.3	9.9 ± 0.4

Definition of abbreviations: Bw = body weight; C_L = static compliance of the lung; D_{CO} = diffusing capacity for carbon monoxide; TLC = total lung capacity; V_A = alveolar volume; V_D = dead space volume. (Data for C3H/HeJ mice reprinted from Reinhard *et al.* [17].)

Values are reported as mean ± SEM.

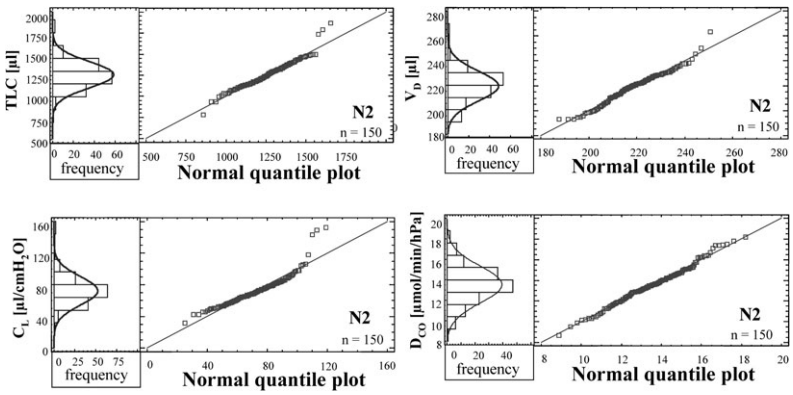

Figure 4. Histograms and normal-quantile plots show the distributions of values for TLC, VD, CL, and DCO of N_2 animals. The *diagonal* indicates the normal distribution. All parameters are normally distributed in N_2 animals.

chromosomes 5 and 17 are syntenic to regions that have been identified in humans to be significantly linked to FEV_1, FVC, or FEV_1/FVC (13, 14), whereas regions on chromosomes 15 and 19 represent novel loci.

Chromosome 5 revealed significant linkages to VD and CL/TLC in F_2 animals (Figures 8 and 9). VD was significantly linked to an interval spanning *D5Mit133* (32.0 cM) to *D5Mit290* (36.0 cM) with a peak LOD score of 4.4 at the location of *D5Mit255* (34.0 cM). Close to *D5Mit133* is where the *superoxide dismutase 3* gene (*Sod3*) is located (31 cM). *Sod3* was suggested as a candidate gene in the National Heart, Lung, and Blood Institute Family Heart Study (NHLBI FHS) and linked to the ratio of FEV_1/FVC (13). The region of interest significantly linked to CL/TLC encompassed *D5Mit20*, *D5Mit155*, and *D5Mit403*. The peak LOD score of 4.8 was obtained close to *D5Mit155* at 54.0 cM. Within this region, the *transforming growth factor* β *receptor 3* (*Tgfbr3*) gene is located. The expression of Tgfbr3 was shown to be essential for optimal transforming growth factor-β signaling during embryonic lung development (22). Sequencing of the *Tgfbr3* gene revealed three missense polymorphisms in exons 4, 9, and 12 between the two parental strains C3H/HeJ and JF1/Msf: His96Tyr, Ser391Pro, and Gly581Asp.

All lung function parameters (i.e., TLC, VD, CL, and DCO) were significantly linked to chromosome 15 (Figures 8 and 9). Both crosses showed significant evidence of linkage to TLC in a comparable region flanked by *D15Mit85* (16.4 cM) and *D15Mit105* (47.9 cM). In N_2, peak LOD scores of 4.7 and 4.9 mapped to the markers *D15Mit58* (18.2 cM) and *D15Mit152* (20.2 cM), respectively. A peak LOD score of 6.0 was seen in F_2 animals for the marker *D15Mit206* at 17.2 cM. In F_2 offspring, another linkage with significant LOD score mapped within the same region on chromosome 15 for TLC/Bw. The peak LOD score of 6.0 was observed for *D15Mit268* at 20.0 cM. For CL, again in both crosses, chromosome 15 revealed significant linkage results between *D15Mit85* and *D15Mit105*, giving further evidence of several genetic loci on this particular chromosome influencing lung function in mice. Highest LOD scores, on average 4.0 and 5.0, were seen in N_2 and F_2 animals, respectively, with the markers *D15Mit152* (20.02 cM) and *D15Mit105* (47.9 cM). Similarly, high LOD scores were seen for *D15Mit92* in N_2 and *D15Mit168* in F_2 females. The trait VD was also linked to chromosome 15 in N_2 animals. The peak LOD score (4.1) was detected between *D15Mit146* and *D15Mit105* and, thus, was again located within the region of interest already established for TLC and CL. This was also true for the linkage of the trait DCO with the peak LOD score of 4.8 close to *D15Mit85* in F_2 animals. This region of interest on chromosome 15 harbors for example the *tricho rhino phalangeal syndrome type 1* (*Trps1*) gene. Mice bearing a deletion of the GATA domain of *Trps1* die of neonatal respiratory failure (33). Although observations supporting a nonpulmonary basis for the respiratory failure are reported, we sequenced the *Trps1* gene because GATA proteins have unique functions in development, especially GATA6 in lung branching morphogenesis (34). However, no polymorphisms were detected between the two parental mouse strains.

On chromosome 17, significant evidence for linkage to TLC and DCO was found in N_2 progeny (Figures 8 and 9). For TLC, three regions of significant linkage were identified (*D17Mit156-D17Mit234*; *D17Mit180-D17Mit20*; *D17Mit218-D17Mit206*). The highest LOD score of approximately 5.0 was obtained in the region spanning the tested markers *D17Mit180* (29.4 cM), *D17Mit237, D17Mit70,* and *D17Mit20* (34.3 cM). The region containing *D17Mit218* and *D17Mit206* is syntenic to a region on human chromosome 18 (near D18S843) that was found to be linked to FEV_1 and FVC in the participants of the NHLBI FHS (13). Within this region, no obvious candidates are located. The trait

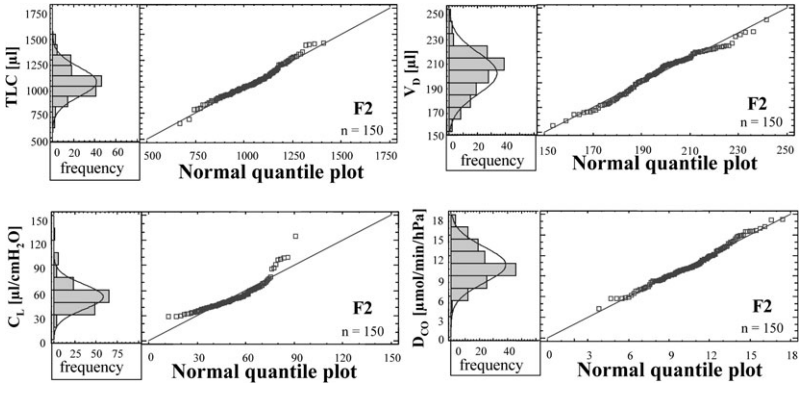

Figure 5. Histograms and normal-quantile plots show the distributions of values for TLC, VD, CL, and DCO of F_2 animals. The *diagonal* indicates the normal distribution. All parameters are normally distributed in F_2 animals except CL. In this latter case, a $\log_{10}$ transformation was used for QTL analysis.

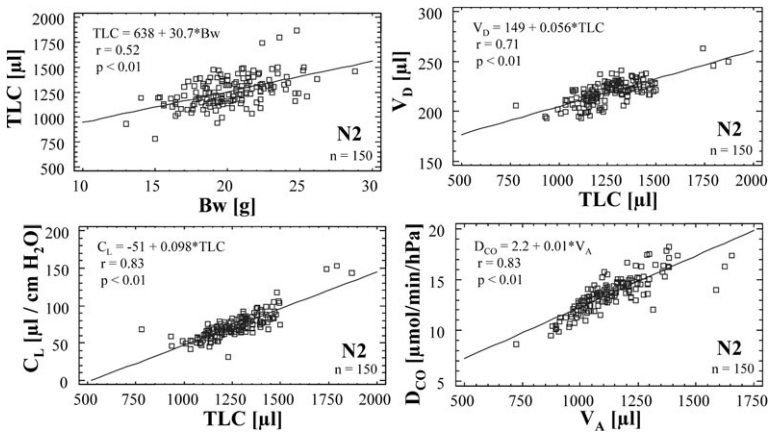

Figure 6. Correlation analyses that describe lung size (TLC) as a function of body weight (Bw) result in a weak association of these two variables in N_2 mice. Correlation analyses between different lung function parameters and lung size showed intermediate association between V_D and lung size, and strong correlations between C_L as well as D_{CO} and lung size.

D_{CO} showed linkage to an interval delimited by loci *D17Mit156* (7.1 cM) and *D17Mit234* (20.7 cM) and contains a syntenic region to human chromosome *6q27*, which was recently identified to be significantly linked to lung function in humans (14). Close to the peak LOD score of 5.0 at *D17Mit234*, the *retinoid x receptor* β (*Rxrb*) gene is located, which is involved in the regulation of lung development. Thus, *Rxrb* was sequenced, but no missense polymorphisms could be identified between the two parental strains.

Chromosome 19 of N_2 mice revealed the strongest evidence for linkage to C_L and C_L/TLC (Figure 8). Linkage to C_L was obtained at the location of *D19Mit40* (26.1 cM) with a LOD score of 5.8. The C_L/TLC showed linkage to the same region on chromosome 19 with a similarly high LOD score of 6.0 at *D19Mit40*. This region contains the *relaxin1* (*Rlx1*) gene. Relaxin-deficient mice develop an age-related progression of lung fibrosis associated with significant changes in lung function (35). Sequencing of the *Rlx1* gene revealed four missense polymorphisms in exon 2 between the two parental strains C3H/HeJ and JF1/Msf: Thr86Met, Leu98Pro, Leu109Phe, and Val173Ileu.

Summarizing the results of the whole genome scan for lung function parameters, significant linkages to lung size were detected on chromosomes 15 and 17; to dead space volume on chromosomes 5 and 15; to lung compliance on chromosomes 5, 15, and 19; and to diffusing capacity on chromosomes 15 and 17 (Table 2).

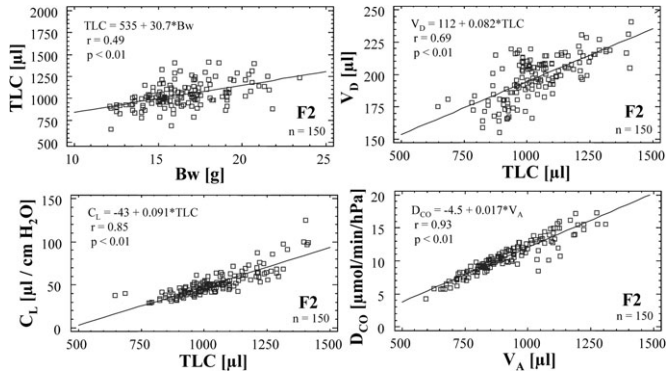

Figure 7. Correlation analyses that describe lung size (TLC) as a function of Bw result in a weak association of these two variables in F_2 mice. Correlation analyses between different lung function parameters and lung size showed intermediate association between V_D and lung size, and strong correlations between C_L as well as D_{CO} and lung size.

DISCUSSION

The individual susceptibility to several diseases and developmental abnormalities that affect lung function (i.e., asthma, COPD, and respiratory distress syndrome) is genetically based, but the genetic factors influencing lung function are still scarcely known.

In our study, lung function parameters failed to segregate as a simple mendelian trait, which points to complex genetic influences. Significant linkages to lung function parameters were detected on chromosomes 5, 15, 17, and 19. Linkage results in N_2 and F_2 animals partly overlapped, as on chromosome 15, but also differed for these two crosses. The differences may arise from the fact that analyzing a complex trait through a backcross is more efficient because one of only two different genotypes at each locus can occur. Thus, more QTLs were detectable in N_2 animals. On the other hand, analyzing the F_2 intercross additionally discovers recessive effects, which may explain linkage results on chromosome 5 that were only obtained in F_2 mice (36, 37).

Although different studies investigated genetic determinants of baseline breathing patterns (16, 38–45), lung mechanics (46), and genetic susceptibility to acute lung injury in mice (47–48), we report here the first QTL linkage analysis for lung function in mice. Data on the inheritance of lung function were collected in humans for the traits FEV_1, FVC, and the ratio of FEV_1/FVC (1, 5, 9–14). A genome-wide scan exploring genetic linkage to lung function in participants of the Framingham Study detected the strongest evidence of linkage to FEV_1 on chromosome 6 and for FVC on chromosome 21 (1). Subsequent fine mapping revealed further evidence for a gene on the q-terminus of chromosome 6 that influences FEV_1, FVC, and FEV_1/FVC ratio (14). This human region is syntenic to a region of mouse chromosome 17 that we found to be significantly linked to the traits D_{CO} and TLC. Because the corresponding parameters of our study and the studies performed in humans are TLC and FVC that both describe lung size, this finding further supports the presence of a gene close to *D17Mit133* that may influence lung size. As a candidate gene, the *secreted modular calcium-binding protein 2* (*SMOC2*) was proposed because it contains a protease inhibitor domain and protease inhibitors were found to be linked to pulmonary disease, in particular to COPD (14). In our study, we considered the *Rxrb* gene as a candidate on chromosome 17 because it is located closer to the peak than is the *Smoc2*, has been shown to be expressed in both fetal and adult human lungs (49), and is involved in alveologenesis (50–51). However, sequencing of the *Rxrb* gene revealed no missense polymorphisms between the two parental strains. This region also harbors genes that are involved in the pathobiology of asthma. Linkage

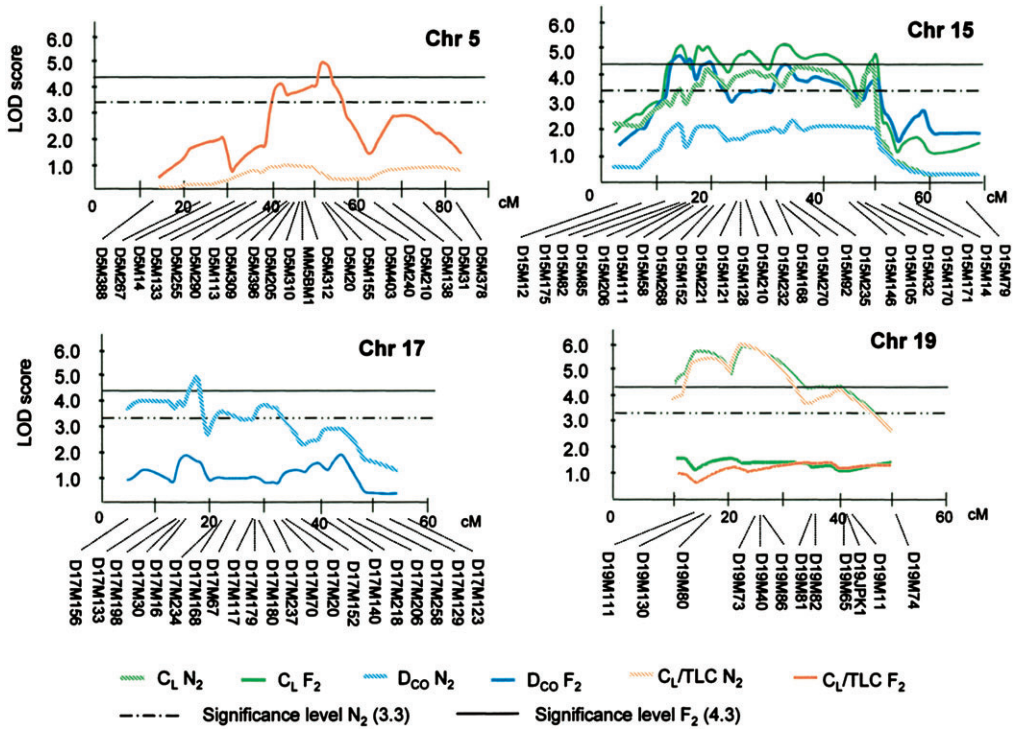

Figure 8. Lung function parameters C_L, C_L/TLC, and D_{CO}. LOD score curves for interval mapping on chromosomes 5, 15, 17, and 19 are shown for N_2 and F_2 mice. Map distance is presented in centimorgans. The positions of informative markers genotyped are shown below each plot. The *dashed horizontal bar* indicates the threshold (3.3) for genomewide significance for an N_2 backcross. The *solid horizontal bar* indicates the threshold (4.3) for genomewide significance for an F_2 intercross.

was seen near the major histocompatibility complex and the tumor necrosis factor gene clusters. Linkage analysis of an asthma-related phenotype in A/J and C57BL/6J mice implicated three loci significantly linked to airway hyperresponsiveness on chromosomes 2 (*Bhr1*), 15 (*Bhr2*), and 17 (*Bhr3*) (52). *Bhr2* and *Bhr3* overlap with the linkages we identified for several lung function parameters. Because the pulmonary response to a stimulating substance is related to the deposited dose of the substance, lung function parameters such as airway and lung size are very likely to influence airway hyperresponsiveness (53). Thus, genetic loci contributing to lung function parameters could also influence asthma-related phenotypes such as airway hyperresponsiveness. Two other regions identified in our study significantly linked to lung size on chromosome 17 spanning *D17Mit180* to *D17Mit20* (LOD score = 5.1) and *D17Mit218* to *D17Mit206* (LOD score = 3.9) do not contain obvious candidate genes, but do contain

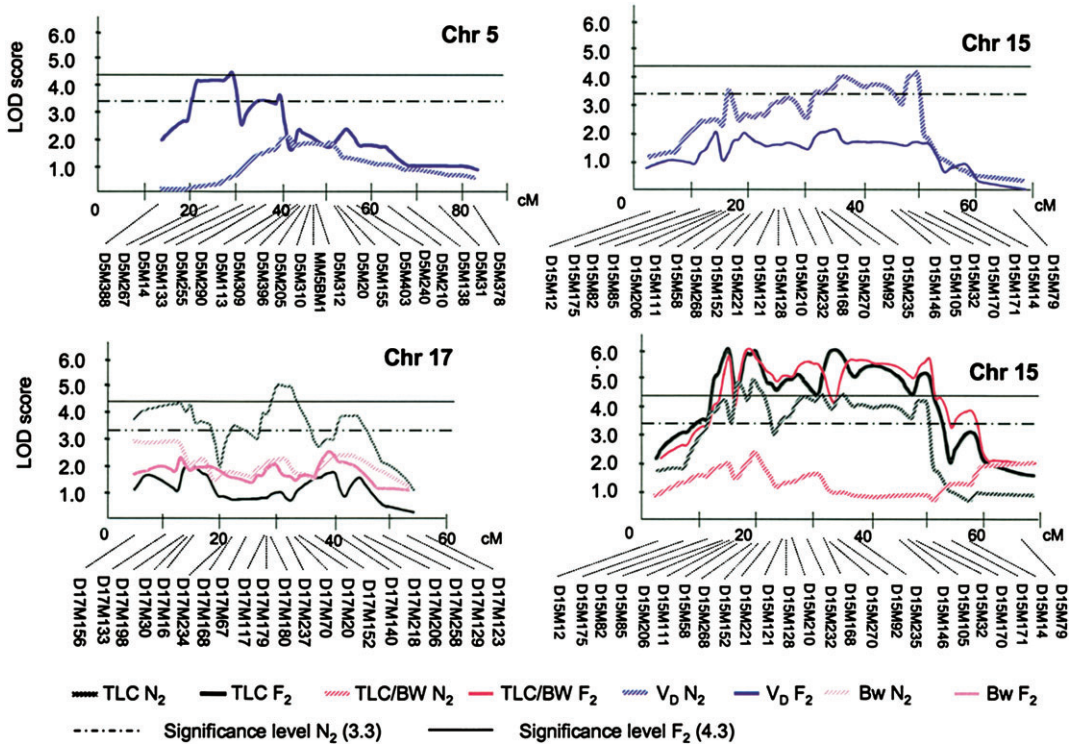

Figure 9. Lung function parameters TLC, TLC/Bw, and V_D, and Bw. LOD score curves for interval mapping on chromosomes 5, 15, and 17 are shown for N_2 and F_2 mice. Map distance is presented in centimorgans. The positions of informative markers genotyped are shown below each plot. The *dashed horizontal bar* indicates the threshold (3.3) for genomewide significance for an N_2 backcross. The *solid horizontal bar* indicates the threshold (4.3) for genomewide significance for an F_2 intercross.

TABLE 2. OVERVIEW OF LINKAGES TO DIFFERENT LUNG FUNCTION PARAMETERS IN N_2 AND F_2 MICE

Chromosome	Trait	Peak LOD Score N_2	Peak LOD Score F_2
5	V_D	2.3	**4.4**
5	Specific lung compliance (TLC/Bw)	1.5	**4.8**
15	Lung size (TLC)	**4.9**	**6.0**
15	TLC/Bw	2.6	**6.0**
15	C_L	**4.3**	**5.0**
15	V_D	**4.1**	2.5
15	D_{CO}	1.3	**4.8**
17	Lung size (TLC)	**5.1**	2.0
17	D_{CO}	**5.0**	1.8
19	C_L	**5.8**	1.4
19	Specific lung compliance (C_L/Bw)	**6.0**	1.7

Definition of abbreviations: Bw = body weight; CL = lung compliance; DCO = diffusing capacity; TLC = total lung capacity (i.e., lung size); TLC/Bw = specific lung size; VD = dead space volume.

Numbers in bold indicate significant linkage (i.e., N_2: > 3.3; F_2 > 4.3).

several genes of yet unknown function. The latter interval contains a region syntenic to human chromosome 18 that was found to be linked to FEV_1 and FVC in the participants of the NHLBI FHS (13) and could thus be of particular interest.

Body weight was considered independently as a phenotypic trait in the linkage analysis because it accounts for approximately 25% of the variance in TLC. Suggestive linkage to Bw was observed in N_2 mice on chromosome 17. On the same chromosome, significant linkage to TLC was observed, but not to TLC/Bw. The LOD score curves for TLC and Bw show a similar shape (Figure 9), but much higher LOD scores for TLC compared with Bw (5.1 versus 2.9). These findings may be valued as a hint that the linkage to TLC on chromosome 17 is driven partly by linkage to Bw.

The highest number of significant linkages to lung function parameters was obtained on mouse chromosome 15. On chromosome 15, the *platelet derived growth factor* β (*Pdgfb*) gene is located close to a peak linked to *D15Mit105* for TLC, CL, and VD. The *Pdgfb* gene is considered a good candidate because transgenic mice overexpressing the *Pdgfb* gene from the lung-specific surfactant protein C promoter develop enlarged airspaces, inflammation, and fibrosis, indicating strong effects on lung growth and development (21). The broad region of interest on chromosome 15 also contains many genes of yet unknown function or genes that have not been described in a functional context with lung function, lung growth, or lung development. Thus, as yet unknown genes on chromosome 15 are likely to contribute to differences in lung function.

The region of interest linked to VD on chromosome 5 contains a syntenic region to human chromosome 4 that was identified to be linked to the ratio of FEV_1/FVC in the participants of the NHLBI FHS. Gene *Sod3* was given as a candidate (13). *Sod3* is the main antioxidant enzyme in the lung and is suggested as a candidate gene for COPD. A polymorphism in this gene was found in approximately 2% of a random population (54). From the candidates inferred from the results of our study, *Sod3* is the only one that has been studied in humans. A candidate gene in the peak region linked to CL/TLC on chromosome 5 is *Tgfbr3*, which directly affects transforming growth factor-β signaling during embryonic lung development (22). Supporting evidence for the relevance of the *Tgfbr3* gene is given by the detected missense polymorphisms between the two parental strains. These polymorphisms result in changes in the amino acid sequence, which may lead to alterations of the protein structure because of the specific properties of the exchanged amino acids. In particular, the change of Gly581Asp could be of functional importance because it is located in a highly conserved domain of the protein (*zona pellucida* domain) that also contains the putative binding site (55).

Significant linkages of CL and CL/TLC were detected for both parameters in the same region on chromosome 19 in F_2 mice. Close to the peak localizes an interesting candidate gene, the *Rlx1* gene. Relaxin-deficient mice ($RLX^{-/-}$) develop a progressive age-related phenotype of pulmonary fibrosis accompanied by altered lung function (35). The functional significance of the identified missense polymorphisms in the *Rlx1* gene between the two parental strains C3 and JF1 in our study is under closer scrutiny. Relaxin consists of two disulfide-linked chains (A and B). It is synthesized in a precursor form, which undergoes sequential proteolytic digestion of a signal peptide and of a connecting peptide between the two chains (56). One of our polymorphisms is located in the A chain, which seems to be important to determine the specific binding of relaxin to its own receptor and not to the receptors for the other hormones of the same family (57–59). The other three polymorphisms are found within the connecting peptide and, thus, could affect the proper folding as well as endoproteolytic cleavage. Particularly in the case of the changes of Thr→Met and Leu→Pro, alterations of the protein structure are expected because of the specific properties of the exchanged amino acids.

In conclusion, our study provides evidence that lung function is controlled by a number of loci on chromosomes 5, 15, 17, and 19, indicating complex genetic influences. Several interesting candidate genes have been observed within the regions of interest, some of them associated with lung development (e.g., *Tgfbr3*, *Pdgfb*). These genes with the respective polymorphisms will be subjected to further analyses. One approach will be the comparative sequencing of the attractive candidates in the other seven inbred strains previously phenotyped. The allelic states of validated candidates should be in concordance with the level of the corresponding lung function parameter. Differential gene expression studies are ongoing to determine the functional relevance of pathways involving these specific genes in the inheritance of lung function. To refine the positions of the identified loci creating consomic strains might be useful.

Conflict of Interest Statement: C.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; B.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; H.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; T.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; G.E. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; F.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; P.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.H.d.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; H.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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