Genetic Loci Influencing Lung Function

A Genomewide Scan in the Framingham Study

OSCAR JOOST, JEMMA B. WILK, L. ADRIENNE CUPPLES, MICHAEL HARMON, AMANDA M. SHEARMAN, CLINTON T. BALDWIN, GEORGE T. O'CONNOR, RICHARD H. MYERS, and DANIEL J. GOTTLIEB

Boston University School of Medicine and Boston University School of Public Health; Research Service, Boston VAMC, Boston; and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts

Prior studies have found cross-sectional lung function to be highly heritable. In the present study, we used a 10-cM genome-wide scan of 1,578 members of 330 families participating in the Framingham Study to test for linkage of genetic markers to level of lung function as determined by spirometry during middle age. At this age, lung function measures may reflect the effects of genes influencing lung growth and development, as well as of those influencing decline in lung function during adulthood. We performed spirometry on 345 members of the Original Cohort and 1,233 members of the Offspring Cohort of the Framingham Study. The effects of age, height, body mass index, and smoking status on spirometric measures were adjusted through linear regression models created separately for men and women in each cohort. Standardized residuals for FEV_1, FVC, and the ratio of FEV_1 to FVC were obtained from these models. The residual spirometric measures were analyzed for linkage to the genome scan markers through the use of variance component models in the Sequential Oligogenic Linkage Analysis Routines software program. The loci most strongly influencing FEV_1 and FVC colocalized on chromosomes 4, 6, and 21. FEV_1 was most influenced by the locus on chromosome 6 (logarithm of the odds favoring genetic linkage [LOD] = 2.4), whereas chromosome 21 contained markers with the strongest linkage to FVC (LOD = 2.6).

Keywords: lung function; spirometry; chronic obstructive pulmonary disease; linkage (genetics)

Chronic obstructive pulmonary disease (COPD) affects approximately 10% of the United States population over the age of 55 (1) and ranks as the fourth leading cause of death, responsible for more than 100,000 deaths per year (2). Although COPD may not become manifest until the seventh decade of life, mildly reduced pulmonary function in early middle age, as measured spirometrically, predicts the subsequent development of COPD (3, 4). Moreover, reduced pulmonary function predicts both cardiovascular and all-cause mortality (5–8). Cigarette smoking is the most important environmental factor influencing pulmonary function (9). Environmental factors such as occupational exposures, air pollution, childhood respiratory illness, diet, and exposure to respirable allergens may also contribute to impaired pulmonary function (10, 11).

Although environmental factors clearly influence the level of pulmonary function, there is also evidence for an important contribution of genetic factors. A number of studies have found that after adjustment for age, cigarette smoking, and body habitus, there is a strong correlation in level of pulmonary function among biologically related individuals (12–18). These studies suggest that up to 50% of the residual variance in level of pulmonary function may be explained by genetic factors. Segregation analyses of pulmonary function have yielded conflicting results about the presence of a major Mendelian gene for pulmonary function. Rybicki and colleagues found evidence of a major gene effect on FEV_1 in families ascertained through a proband with COPD, but not in a smaller sample of randomly ascertained families (12). A segregation analysis of the Framingham Study cohort found no evidence of a major gene effect on FEV_1; rather, the results were consistent with a polygenic mode of inheritance and/or with shared environmental factors (13). In the Humboldt Family Study, a segregation analysis of FVC yielded evidence for a major gene, but environmental factors also remained significant (19). A recent segregation analysis, done with data from the Family Heart Study cohort, found evidence for a dominant major gene influencing FEV_1, but no compelling genetic model for FVC (15).

The Framingham Study, a longitudinal cohort study begun in 1948, provides a unique population in which to study the genetics of pulmonary function. Many siblings and spouse pairs were enrolled in the Original Cohort of the study, and recruitment of their offspring in 1971 produced numerous extended pedigrees for analysis. Spirometry has been performed during adulthood in each generation of these pedigrees. Additionally, a 10-cM genome scan has been performed on 330 of the largest extended families in the Framingham Study. These data provided us with the opportunity to explore linkage to the spirometric measures FEV_1, FVC, and the ratio of FEV_1 to FVC (FEV_1/RVC ratio). This is the first reported genome scan exploring genetic linkage to lung function.

METHODS

Subjects

The origin and family structure of the Framingham Study cohorts have been previously described (13). The Study participants are white, and 91% are of western European descent. For the present analysis, we considered the largest 330 families in the Framingham Study, with 2,885 participants (1,213 in the Original Cohort and 1,672 in the Offspring Cohort), including 1,702 subjects genotyped in a genome scan. Among the 2,885 Framingham Study participants, 2,417 had both spirometry and risk factor data; among these, 1,578 had genotype data and were included in the linkage analysis. These 1,578 members of 330 families included 1,545 sibling pairs, 933 parent–offspring pairs, 468 avuncular pairs, 742 cousin pairs, and 87 spouse pairs. Clinical data and blood samples from these individuals were obtained with informed consent as approved by the Boston University School of Medicine Institutional Review Board for Human Research.

Spirometry and Risk Factors

Spirometric methods utilized in the Framingham Study have been previously described (7, 13, 20). Spirometric measurements obtained at Original Cohort Cycle 5 (conducted in 1958 and 1959), Cycle 6 (1960 and 1961), or Cycle 13 (1974 and 1975) examinations or at Offspring Cohort Cycle 3 (1984 to 1987) or Cycle 5 (1992 to 1995) exami-

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Correspondence and requests for reprints should be addressed to Daniel J. Gottlieb, M.D., The Pulmonary Center, R304, BUSM, 715 Albany Street, Boston, MA 02118. E-mail: dgottlieb@lung.bumc.bu.edu

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nations were used in our analysis. Predicted values for these measures were obtained by linear regression of lung function on age, age-squared, height, body mass index (kg/m^2), pack-years of cigarette smoking, and dummy variables for current and former smoking. To optimize the reliability of the predictive models, we included in our models all participants who did not have missing data for lung function and all independent variables, even if they were not in the 330 families of the genome scan. This group comprised 1,558 male and 2,281 female members of the Original Cohort and 1,911 male and 2,084 female members of the Offspring Cohort.

For each of the three measures of lung function (FEV_1, FVC, and FEV_1/FVC ratio), we constructed separate models for men and for women within each of the two cohorts. Residual lung function was defined as the difference between measured lung function and the lung function predicted by the regression model. Among subjects with acceptable spirometry at any of the examination cycles named earlier, 65% of those in the Original Cohort and 63% of those in the Offspring Cohort had spirometry measured at two of the selected examination cycles. In these subjects, the mean value of spirometry at the two time points was used in deriving the residual level of lung function, as were the mean age, height, weight, and pack-years of smoking. Standardized residual values of FEV_1, FVC, and the FEV_1/FVC ratio were calculated for each subject by dividing residual lung function by the SD for residual lung function within each sex- and cohort-specific group. Spirometric methods and equipment varied over the 38 year encompassed by this study (7, 13), and secular trends in height and weight were observed, with members of the Offspring Cohort being taller and heavier than members of the Original Cohort. The use of the standardized residuals as the phenotypic variables in the linkage analysis provided adjustment for sex effects and for both biologic and technical cohort effects on level of lung function.

Smoking behavior was ascertained at each Framingham Study clinical examination, allowing a determination of current smoking status (current, former, or never) and of lifetime smoking history (pack-years).

Genotyping

Genomic DNA was extracted from peripheral lymphocytes with a Blood and Cell Culture DNA Maxi kit (Qiagen, Inc., Valencia, CA). A genome-wide scan was done by the Mammalian Genotyping Service (Marshfield, WI). Screening Set version 8 of the Marshfield Center for Medical Genetics, comprising 399 microsatellite markers, covers the genome at an average density of 10 cM and has an average heterozygosity of 0.77 (21). The screening set and genotyping protocols are available at the website of the Center for Medical Genetics, Marshfield Medical Research Foundation. Map distances were taken from Screening Set version 9 and the Marshfield "build your own map" facility (available at: http://www.marshmed.org/genetics/).

Statistical Analysis

Variance component linkage analysis was done with the Sequential Oligogenic Linkage Analysis Routines (SOLAR) software system (22). This approach makes use of all the information present in pedigrees of any size or complexity. Although variance component models require relatively few assumptions about the mode of inheritance, they assume that the genetic effect is additive. The SOLAR system uses likelihood ratio tests to evaluate linkage by comparing a model that permits a particular locus (possible quantitative trait locus) to account for additive genetic variance, with a residual polygenic component, with a second, purely polygenic model. Multipoint analyses are based on an extension of a regression approach that, for each centimorgan within the genome, yields a weighted average of the identity-by-descent probabilities over the nearby two-point probabilities as proposed by Fulker and colleagues (23). Allele frequencies were calculated with data from the study participants.

For the pedigrees used in this study, we had approximately 80% power with the variance component method to detect a logarithm of the odds favoring genetic linkage (LOD) score of 2.0 or higher for a quantitative trait locus (QTL) that accounts for approximately 20% of the variation in the phenotype. This estimate of power is based on simulations done with these pedigrees that provide the expected LOD score for a QTL with specified heritability.

RESULTS

Characteristics of subjects included in the linkage analysis are presented in Table 1. Fewer men in the Offspring Cohort than in the Original Cohort, and more women in the Offspring Cohort than in the Original Cohort, reported ever smoking cigarettes, whereas the percentage who reported having quit smoking was greater in the Offspring Cohort than in the Original Cohort regardless of sex, reflecting the temporal trends in smoking habits among adults in the United States during the period under study. Lung volumes were greater in subjects in the Offspring than in the Original Cohort, reflecting the larger stature of the Offspring Cohort.

The SOLAR program provides an estimate of heritability as the percentage of variation in the trait attributable to genetic factors. SOLAR produced estimates of the heritability of FEV_1 of 35%, of FVC of 49%, and of the FEV_1/FVC ratio of 26%.

The highest multipoint variance component LOD score obtained for FEV_1 was 2.4, at the q terminus of chromosome 6 (Figure 1). This locus accounted for 24% of the variance in the FEV_1 trait, as estimated by the SOLAR system. The LOD score for FVC at this location was 1.1 and that for the FEV_1/FVC ratio was 1.4. No other LOD scores above 2.0 were found for FEV_1 in this scan. The next highest score, of 1.6, was localized at 76 cM from the p terminus on chromosome 4, and the third highest score, of 1.2, was localized at the p terminus of chromosome 21, both of which regions that also showed evidence of linkage to FVC (Table 2). Other LOD scores above 1.0 for FEV_1 were found on chromosomes 3 and 10.

The highest multipoint variance component LOD score for FVC was 2.6, for markers near the p terminus of chromosome 21. SOLAR results estimated that this locus accounted for 26% of the trait variance. The maximum LOD score for FEV_1 in this area was 1.2, and that for the FEV_1/FVC ratio was less than 0.5. No other LOD scores above 2.0 were found for FVC in this scan.

Chromosome 4 yielded an LOD score of 1.2 for FVC at 76 cM from the p terminus, a location that was coincident with the LOD score of 1.6 for FEV_1. The only other LOD scores above 1.0 for FVC were at the q terminus of chromosome 6 (LOD = 1.1), where the maximum LOD score for FEV_1 was located, and on chromosome 18 (LOD = 1.2).

An LOD score of 1.4 at the q terminus of chromosome 6 was the highest score for the FEV_1/FVC ratio, accounting for 19% of the variance in this trait. The only other genome locations with LOD scores above 1.0 for the FEV_1/FVC ratio were on chromosomes 5 (LOD = 1.1 at 139 cM), 10 (LOD = 1.1 at 113 cM), and 19 (LOD = 1.3 at 78 cM).

TABLE 1. CHARACTERISTICS OF 1,578 SUBJECTS INCLUDED IN THE GENOME SCAN*

	Men		Women	
	Original Cohort	Offspring Cohort	Original Cohort	Offspring Cohort
No. of subjects	133	609	212	624
Age, yr	54.0 (5.12)	48.4 (10.1)	55.2 (5.94)	49.1 (9.83)
BMI, kg/m^2	26.9 (2.92)	27.8 (3.87)	26.2 (4.53)	26.2 (5.52)
FEV_1, L	3.26 (0.62)	3.65 (0.76)	2.39 (0.46)	2.61 (0.55)
FVC, L	4.11 (0.71)	4.79 (0.88)	2.86 (0.47)	3.38 (0.62)
FEV_1/FVC ratio	0.77 (0.07)	0.76 (0.07)	0.80 (0.07)	0.77 (0.07)
Former smoker, %	18.8	39.4	9.3	34.4
Current smoker, %	58.4	27.9	36.1	29.1
Smoking history, pack-years	22.7 (17.5)	17.2 (22.3)	7.68 (13.0)	11.4 (16.2)

Definition of abbreviation: BMI = body mass index.

* All values are mean (SD), except for current and former smoking, which are percentages.

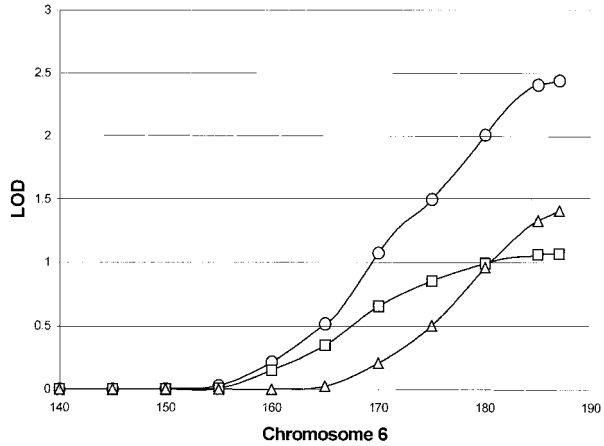

Figure 1. Multipoint LOD scores, plotted against genetic distance along chromosome 6, for FEV_1 (*circles*), FVC (*squares*), and the FEV_1/FVC ratio (*triangles*) in the genome-wide scan of 332 families.

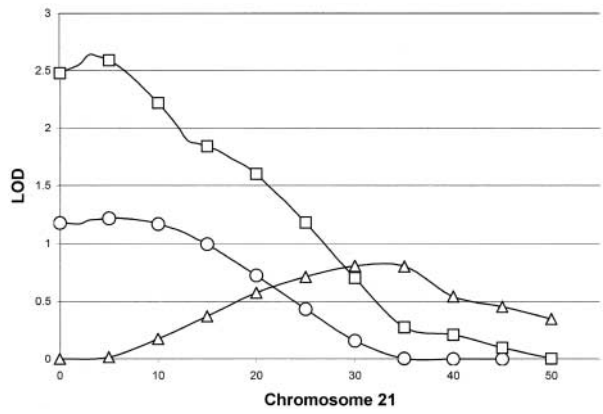

Figure 2. Multipoint LOD scores, plotted against genetic distance along chromosome 21, for FEV_1 (*circles*), FVC (*squares*), and the FEV_1/FVC ratio (*triangles*) in the genome-wide scan of 332 families.

DISCUSSION

This is the first published report of a genome scan for pulmonary function, which was conducted in 330 families participating in the Framingham Study. This study supports the conclusion of earlier studies that a significant genetic determination exists for lung function, with observed heritabilities of 35% and 49% for FEV_1 and FVC, respectively, and a heritability of 26% for the FEV_1/FVC ratio. The study also suggests that this genetic variation can be partitioned among distinct genomic loci, with evidence for multiple gene influences on different measures of lung function. Linkage analyses identified one genetic locus with a multipoint LOD score of 2.4 for FEV_1 and a multipoint LOD score of 2.6 for FVC, with no other loci having an LOD score above 2.0. The highest LOD score for the FEV_1/FVC ratio was smaller than that for either FEV_1 or FVC, at 1.4, and may be the result of the complexity of the individual underlying distributions influencing FEV_1 and FVC, or of the lower observed heritability of this measure.

The loci most strongly influencing FEV_1 and FVC colocalized on chromosomes 4, 6, and 21, although the patterns of linkage to the various spirometric measures differed. FEV_1 appeared to be more strongly influenced by the locus on chromosome 6 than was FVC, with some evidence for linkage at this locus to the FEV_1/FVC ratio as well. This pattern might be expected if a gene at this locus had a greater effect on airflow resistance than on lung volume. In contrast, the locus with the greatest influence on FVC, on chromosome 21, had a much weaker influence on FEV_1 and showed no evidence of linkage to the FEV_1/FVC ratio. This might be observed if a gene at this locus influenced lung volume independent of airflow resistance, although most known influences on lung volume have similar effects on both FEV_1 and FVC.

TABLE 2. GENOMIC LOCI LINKED TO LUNG FUNCTION WITH SCORES > 1.0 FOR THE LOGARITHM OF THE ODDS FAVORING GENETIC LINKAGE

		LOD Score for Linkage to Each Lung Function Measure		
Chromosome	Marker Location (cM from p Terminus)	FEV_1	FVC	FEV_1/FVC Ratio
3	45	1.1	–	–
4	76	1.6	1.2	–
5	139	–	–	1.1
6	q Terminus	2.4	1.1	1.4
10	124,113	1.2	–	1.1
18	14	–	1.2	–
19	78	–	–	1.3
21	p Terminus	1.2	2.6	–

Definition of abbreviation: LOD = logarithm of the odds favoring genetic linkage.

Factors that influence the rate of decline in lung function during adulthood, such as cigarette smoking, are important to the development of COPD. Several genes are implicated as risk factors for COPD. In each case, the mechanism for the association is presumed to be an increased risk of lung destruction leading to an excess decline in lung function. The Z allele of the gene on chromosome 14 for the antitrypsin protein, α_1-protease inhibitor, is associated with early onset of emphysema when homozygous (24); heterozygosity for the Z allele results in an increased risk for COPD in case–control studies (25). This allele is rare, however, being observed in only 2% to 3% of whites and in even fewer non-whites. Another variant, located in the 3′ direction from the gene, is more common among COPD patients, but is found only in about 5% of the general population in the United Kingdom (26, 27). A closely linked and structurally similar gene, for α_1-antichymotrypsin, has variants reported only among COPD patients, although they are present in only several percent of these patients (28). A polymorphism in the third exon of the gene for microsomal epoxide hydrolase, located on chromosome 1, results in slower activity of this enzyme. This variation was four times more common among COPD patients (24%) than among controls (29). That these genes failed to show linkage to lung function in our study suggests that although they may be important causes of COPD, they account for little of the total population variance in lung function in a community-dwelling population selected independently of the presence of lung disease.

Although genes that influence the rate of decline in lung function are likely to be important causes of COPD, a low level of lung function at the onset of adulthood might also be an important determinant of COPD risk. For any given rate of decline in lung function, individuals with a higher initial level of lung function are less likely than those with a lower initial level to develop clinically significant disease. Indeed, the cross-sectional level of lung function is a strong predictor of the subsequent development of COPD (3, 30, 31) and of both disability and death from COPD (32, 33). There is considerable variation in age-, height-, and sex-adjusted cross-sectional lung function among never-smokers without apparent respiratory disease. Individuals at the upper end of the normal range

have lung function values that are 50% greater than those at the lower end of the normal range (34). Because studies demonstrating heritability of cross-sectional lung function have typically studied families in which lung function in offspring was measured during childhood (14, 15, 17, 19), it is likely that much of the heritable variation in lung function is due to genes that influence lung growth and development. Although the present study evaluated the lung function of two generations of families during middle age, the relatively low heritability of longitudinal change in lung function in this population (35) and its generally good respiratory health make it likely that the observed linkages point to the locations of genes influencing lung growth and development rather than the rate of decline in lung function.

Certain limitations of the present study should be discussed. One potential limitation relates to possible imprecision in spirometric data collected approximately 20 yr earlier in the Original Cohort than in the Offspring Cohort, before the publication of American Thoracic Society guidelines for standardization of spirometry; however, our use of cohort- and sex-specific regressions and standardization of the residual lung function measures minimized the impact of cohort effects related to spirometric technique, as well as of true biologic cohort effects. Although we adjusted for height in the predictive models used to calculate lung function residuals, it is possible that the loci indicated in our analysis influence lung function through their effect on stature. Linkage analyses of height and other body measures in this population are being pursued by other investigators, and we will explore any concordance with the lung function loci reported here.

The family structure of the Framingham Study and the availability of both phenotype and genotype information from two generations studied during adulthood provide a valuable resource for study of the genetic determinants of lung function. The present study had 80% power, at an LOD score of 2.0, to detect only those genetic loci accounting for at least 20% of the variation in lung function. It is possible that many genes, such as the gene encoding α_1-protease inhibitor, have variants that confer a risk of COPD but occur at a sufficiently low frequency in the general population that they could not be detected in this study. Moreover, many such genetic variants may lead to abnormal lung function only in the presence of environmental exposures, such as to tobacco smoke. The power to identify such gene–environment interactions is necessarily even smaller. It is possible that a cohort with greater genetic homogeneity and a higher prevalence of smoking would be better suited to the detection of genes that confer risk of COPD in smokers. These considerations notwithstanding, the present study does point to several genetic loci that may have a large influence on lung function, most likely through influences on lung growth and development. Identification of genes at these loci that influence lung function is clinically relevant, since maximal lung size attained during growth and development may be an important determinant of the risk of clinically significant COPD in persons with an excess adult decline in lung function.

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