Human Molecular Genetics, 2003, Vol. 12, No. 10 **1199–1210**
DOI: 10.1093/hmg/ddg125

Genome-wide linkage analysis of bronchodilator responsiveness and post-bronchodilator spirometric phenotypes in chronic obstructive pulmonary disease

Lyle J. Palmer[1,3,*], Juan C. Celedón[1,3], Harold A. Chapman[4], Frank E. Speizer[1,3], Scott T. Weiss[1,3] and Edwin K. Silverman[1,2,3]

[1]Channing Laboratory and [2]Division of Pulmonary and Critical Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA, [3]Harvard Medical School, Boston, Massachusetts, USA and [4]Division of Pulmonary and Critical Care Medicine, University of California at San Francisco, San Francisco, California, USA

Received January 30, 2003; Revised and Accepted March 14, 2003

Chronic obstructive pulmonary disease (COPD) is a common, complex disease associated with significant and increasing morbidity and mortality. The cardinal feature of COPD is persistent airflow obstruction, measured by reductions in quantitative spirometric indices including forced expiratory volume at one second (FEV_1) and the ratio of FEV_1 to forced vital capacity (FEV_1/FVC). However, many patients have substantial improvement in spirometric measures with inhaled bronchodilator medications, and bronchodilator responsiveness (BDR) has been associated with disease severity and progression. To identify susceptibility loci for BDR phenotypes, we performed a 9 cM genome scan in 72 pedigrees ($n = 560$ members) ascertained through probands with severe, early-onset COPD. Multipoint variance component linkage analysis was performed for quantitative phenotypes including BDR measures and post-bronchodilator FEV_1 and FEV_1/FVC. Post-bronchodilator FEV_1 was linked to multiple regions, most significantly to markers on chromosome 8p (LOD = 3.30) and 1q (LOD = 2.24). Post-bronchodilator FEV_1/FVC was also linked to multiple regions, most significantly to markers on chromosome 2q (LOD = 4.42) and 1q (LOD = 2.52). When compared with pre-bronchodilator spirometric indices, the post-bronchodilator values demonstrated increased evidence of linkage in multiple genomic regions. In particular, the LOD score for the 8p linkage to FEV_1 roughly doubled from 1.58 to 3.30. Candidate regions on chromosomes 4p (LOD = 1.28), 4q (LOD = 1.56), and 3q (LOD = 1.50) gave the strongest evidence for linkage to BDR measures. Our results provide evidence for significant linkage to airflow obstruction susceptibility loci on chromosomes 2q and 8p, and further suggest that post-bronchodilator spirometric measures are optimal phenotypes for COPD genetic studies. This study has also identified several genomic regions that could contain loci regulating BDR in early-onset COPD families.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD; MIM 606963), the fourth leading cause of death in the United States, is strongly influenced by cigarette smoking (1). Chronic airflow obstruction, typically assessed by spirometry, is a cardinal feature of COPD; however, the development of chronic airflow obstruction is extremely variable among smokers (2). Spirometry involves measurements of forced exhalation after the subject has inhaled to total lung capacity. Individuals with airflow obstruction have reductions in the ratio of the forced expiratory volume at one second (FEV_1) to forced vital capacity (FVC); the severity of airflow obstruction is related to the level of FEV_1 in such individuals.

Although COPD is characterized by a component of irreversible airflow obstruction, many subjects with COPD

*To whom correspondence should be addressed at: Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA. Tel: +1 6175250872; Fax: +1 6175250958; Email: lyle.palmer@channing.harvard.edu

Human Molecular Genetics, Vol. 12, No. 10 © Oxford University Press 2003; all rights reserved

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

demonstrate substantial improvement in spirometric measures after receiving inhaled bronchodilator medications (3). In patients with COPD, symptomatic treatment with inhaled bronchodilators is the most common form of therapy (4–6), and the initial degree of reversibility of airflow obstruction following such therapy has been reported to be related both to annual decline in lung function (7,8) and to survival (9,10).

Severe alpha 1-antitrypsin (AAT) deficiency (MIM 107400) is a well-established but uncommon genetic determinant of COPD (11). General population and twin studies of spirometric measurements have suggested that factors other than AAT deficiency may influence genetic susceptibility to COPD (12–14). Studies in relatives of COPD patients have demonstrated familial aggregation of spirometric and bronchodilator responsiveness phenotypes (15–19).

To identify novel genetic determinants of COPD-related phenotypes, we enrolled and phenotyped pedigrees ascertained through single probands with severe, early-onset COPD who did not have severe AAT deficiency. Previously, we have demonstrated increased risk to current or ex-smoking first-degree relatives of early-onset COPD probands for airflow obstruction, chronic bronchitis and increased bronchodilator responsiveness (BDR) (19,20). Genome scan linkage analyses in the same data set identified several chromosomal regions with suggestive evidence for linkage to qualitative and quantitative COPD-related phenotypes, and one chromosomal region (on 2q) with significant evidence of linkage to pre-bronchodilator FEV_1/FVC levels (21,22). We now report the linkage analysis results of an autosomal genome scan of post-bronchodilator spirometry and bronchodilator responsiveness indices in our extended severe, early-onset COPD pedigrees.

RESULTS

Demographics and spirometry in early-onset COPD families

The characteristics of the study population are presented in Table 1. Very severe airflow obstruction in the early-onset COPD probands was noted, with mean post-bronchodilator FEV_1 values of 20.2% predicted (SD = 7.8) and mean post-bronchodilator FEV_1/FVC values of 36.4% predicted (SD = 12.3). The risk of developing COPD increases with age; therefore, it is not unexpected that parents had lower mean spirometric values than siblings, and that siblings had lower mean spirometric values than children.

Phenotypic modeling

Variance component analyses using SOLAR suggested that, after adjustment for all covariates, the narrow-sense heritability (h_N^2) of the pre-bronchodilator FEV_1 levels was 33.3% (SE = 6.3%), i.e. additive genetic effects (σ_A^2) contributed one-third of the total variance. The h_N^2 of the post-bronchodilator FEV_1 levels was 34.9% (SE = 5.9%). σ_A^2 was significantly greater than zero for both the pre- and post-bronchodilator FEV_1 ($P < 0.0000001$). The variance component modeling suggested that sex ($P < 0.0000001$), age ($P < 0.0000001$), height ($P < 0.0000001$), and linear ($P < 0.0000001$) and quadratic ($P < 0.000001$) terms for pack-years of smoking were highly significant for pre- and post-bronchodilator FEV_1. Race was a significant covariate for post-bronchodilator FEV_1 ($P < 0.01$), but not pre-bronchodilator FEV_1 ($P = 0.07$); post-bronchodilator FEV_1 levels were lower in the African-American subjects included in the study. FEV_1 levels were lower in older subjects and females and decreased with cumulative tobacco smoke exposure and shorter stature.

The h_N^2 of pre-bronchodilator FEV_1/FVC ratio was 29.7% (SE = 5.1%); the h_N^2 of the post-bronchodilator FEV_1/FVC ratio was 28.7% (SE = 5.0%). σ_A^2 was significantly greater than zero for both the pre- and post-bronchodilator FEV_1/FVC ratio ($P < 0.0000001$). Age ($P < 0.0000001$) and linear ($P < 0.0000001$) and quadratic ($P < 0.000001$) terms for pack-years of smoking were highly significant for both the pre- and post-bronchodilator FEV_1/FVC ratio. FEV_1/FVC levels were lower in older subjects and decreased with cumulative tobacco smoke exposure.

The h_N^2 of the BDRABS was 26.3% (SE = 9.2%); σ_A^2 was significantly greater than zero ($P = 0.0002$). Age ($P = 0.03$), height ($P = 0.05$) and race ($P = 0.002$) were significant covariates for BDRABS; responsiveness increased with increasing height and decreasing age, and was lower in African-American subjects. The h_N^2 of the BDRPRED was 24.2% (SE = 8.6%); σ_A^2 was significantly greater than zero ($P = 0.0002$). Race was the only significant covariate for BDRPRED ($P = 0.002$); responsiveness was lower in the African-American subjects. The h_N^2 of the BDRBASE was 10.1% (SE = 4.8%); σ_A^2 was significantly greater than zero ($P = 0.01$). Race ($P = 0.04$) and both linear ($P = 0.0005$) and quadratic ($P = 0.04$) terms for pack-years of smoking were significant for the BDRBASE. BDRBASE increased with cumulative tobacco smoke exposure and was lower in African-Americans.

Genome-wide multipoint variance component linkage analysis

Genome-wide multipoint variance component linkage results for post-bronchodilator FEV_1 and FEV_1/FVC are presented in Figures 1 and 2 and summarized in Table 2. The multipoint linkage results for pre-bronchodilator FEV_1 and FEV_1/FVC, which have been previously reported for the larger sample of subjects with pre-bronchodilator spirometry (21), are also shown at identical chromosomal positions for comparison purposes.

For post-bronchodilator FEV_1, multipoint LOD scores above 1.0 were found on seven chromosomes. Significant linkage (23) was found on chromosome 8p23 (LOD = 3.30 at ~2 cM from pter, proximal to marker ATA27A03) (Table 2, Fig. 1). Other suggestive linkages were found on chromosomes 1p21 (LOD = 2.24, 136 cM, flanked by markers GATA124C08 and GATA133A08), 8q24 (LOD = 2.01, 163 cM, flanked by markers GATA50D10 and UT721) and 19q13 (LOD = 1.94, 78 cM, at marker Mfd232). The major linkages to post-bronchodilator FEV_1 were reflected in the major linkages to pre-bronchodilator FEV_1, although the LOD scores were generally higher using post-bronchodilator FEV_1. The biggest difference between the pre- and post-bronchodilator FEV_1 linkages was for the chromosome 8p linkage; the LOD score was roughly doubled (to 3.30) using the post-bronchodilator FEV_1.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

Table 1. Characteristics of early-onset COPD genome scan families [Mean (SD)]

Variable	Probands ($n=69$)	Parents ($n=45$)	Siblings ($n=143$)	Children ($n=120$)	Other relatives ($n=153$)	Spouses ($n=30$)
Age (years)	47.6 (5.4)	72.5 (8.3)	46.8 (8.5)	24.7 (5.8)	54.0 (17.9)	51.8 (6.1)
Pack-years of smoking	39.1 (21.6)	38.6 (39.8)	22.4 (21.1)	4.3 (6.4)	22.2 (24.6)	37.4 (35.0)
Pre-bronchodilator FEV_1 (% predicted)	17.2 (6.2)	64.5 (25.5)	80.8 (22.9)	91.2 (11.7)	82.0 (19.2)	85.5 (23.1)
Post-bronchodilator FEV_1 (% predicted)	20.2 (7.8)	68.0 (25.1)	84.0 (22.5)	94.5 (11.4)	85.2 (18.1)	88.8 (21.7)
Pre-bronchodilator FEV_1/FVC (% predicted)	37.2 (11.4)	76.7 (18.1)	84.9 (15.3)	92.5 (7.8)	88.8 (11.8)	89.5 (13.3)
Post-bronchodilator FEV_1/FVC (% predicted)	36.4 (12.3)	76.8 (18.3)	87.5 (15.6)	95.5 (6.6)	91.1 (12.0)	91.3 (13.6)
BDRABS (ml)[a]	87.7 (73.8)	77.8 (112.8)	103.9 (131.2)	127.8 (155.0)	95.6 (129.5)	121.0 (157.9)
BDRPRED (%)[b]	3.0 (2.5)	3.5 (5.1)	3.2 (4.0)	3.3 (4.2)	3.2 (4.0)	3.4 (4.0)
BDRBASE (%)[c]	17.1 (13.4)	7.3 (10.7)	5.1 (7.7)	3.9 (5.3)	4.8 (7.3)	5.2 (8.0)

[a]BDRABS = (postbronchodilator FEV_1 − prebronchodilator FEV_1).

[b]$$\text{BDRPRED} = \left[\frac{(\text{postbronchodilator FEV}_1 - \text{prebronchodilator FEV}_1)}{\text{predicted prebronchodilator FEV}_1} * 100\right].$$

[c]$$\text{BDRBASE} = \left[\frac{(\text{postbronchodilator FEV}_1 - \text{prebronchodilator FEV}_1)}{\text{prebronchodilator FEV}_1} * 100\right].$$

For post-bronchodilator FEV_1/FVC, multipoint LOD scores above 1.0 were found on nine chromosomes; the highest LOD score was 4.42 on chromosome 2q36 (222 cM, flanked by markers GATA4G12 and GATA23D03; Table 2, Fig. 2). Other promising linkages were found on chromosomes 1p31 (LOD = 2.52, 118 cM, flanked by markers GATA6A05 and GATA124C08) and 17q21 (LOD = 2.44, 67 cM, at marker ATC6A06). Again, the linkages to the post-bronchodilator FEV_1/FVC were mirrored in the linkages to the pre-bronchodilator FEV_1/FVC. As for FEV_1, the LOD scores were generally lower for the pre-bronchodilator FEV_1/FVC. This difference was most noticeable for the chromosome 1p linkage, where the LOD score was increased by 0.63 for the post-bronchodilator FEV_1/FVC (Table 2).

Two chromosomal regions demonstrated some evidence for linkage to both FEV_1 and the FEV_1/FVC ratio. The broad chromosome 2q region (two LOD unit support interval between 210 and 245 cM from pter) that demonstrated strong evidence for linkage to post-bronchodilator FEV_1/FVC also had an LOD score of 1.13 for post-bronchodilator FEV_1. The chromosome 19q region also demonstrated some evidence for linkage to both post-bronchodilator FEV_1 and FEV_1/FVC. However, in general, there was little overlap between the linkages to FEV_1 and FEV_1/FVC.

Genome-wide multipoint variance component linkage results for the bronchodilator responsiveness phenotypes BDRABS, BDRPRED and BDRBASE are presented in Figure 3 and summarized in Table 3. Regions on six chromosomes showed some evidence of linkage to BDRABS. The most significant linkages to BDRABS were on chromosomes 4q31 (LOD = 1.56, 146 cM, at marker GATA107) and 3p12 (LOD = 1.50, 112 cM, at marker GATA128C02). The linkages to BDRPRED generally mirrored those to BDRABS, although with slightly lower LOD scores (Table 3). For the BDRBASE response phenotype, chromosomal regions on two chromosomes showed some evidence of linkage. The most significant linkage was to the chromosome 4p16 region (adjusted LOD = 1.28, 13 cM, at marker GATA22G05). The chromosome 3q25 (adjusted LOD = 1.07, 170 cM, at marker GATA8F01) region also showed some evidence for linkage.

While there was close overlap between the linkage results for the BDRABS and BDRPRED responsiveness phenotypes, there was little overlap between these measures of responsiveness and the linkage results for the BDRBASE phenotype. Similarly, there was no obvious overlap between linkages found for the spirometric indices (Table 2) and those found for the bronchodilator response phenotypes (Table 3).

DISCUSSION

In order to identify genetic factors influencing susceptibility to COPD, we have studied extended pedigrees of individuals ascertained through severe, early-onset COPD probands without severe AAT deficiency. Our 9 cM genome-wide linkage scan is the first reported investigation of human linkage to bronchodilator responsiveness phenotypes; some evidence of linkage to several chromosomal areas has been found. A striking finding of this study is that post-bronchodilator spirometry appears to provide more powerful phenotypes for genetic studies of COPD than conventional pre-bronchodilator spirometry.

The importance of familial determinants in the regulation of lung function as measured by spirometry is well established. Consistent with our results, pedigree-based studies of unselected, asthmatic and COPD families have shown significant evidence for familial aggregation of spirometric indices (24–29), suggesting that ~20–60% of total phenotypic variance may be accounted for by familial factors. The heritability of the post-bronchodilator spirometric indices was similar to the pre-bronchodilator spirometric indices. The heritability of bronchodilator responsiveness measures has not

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

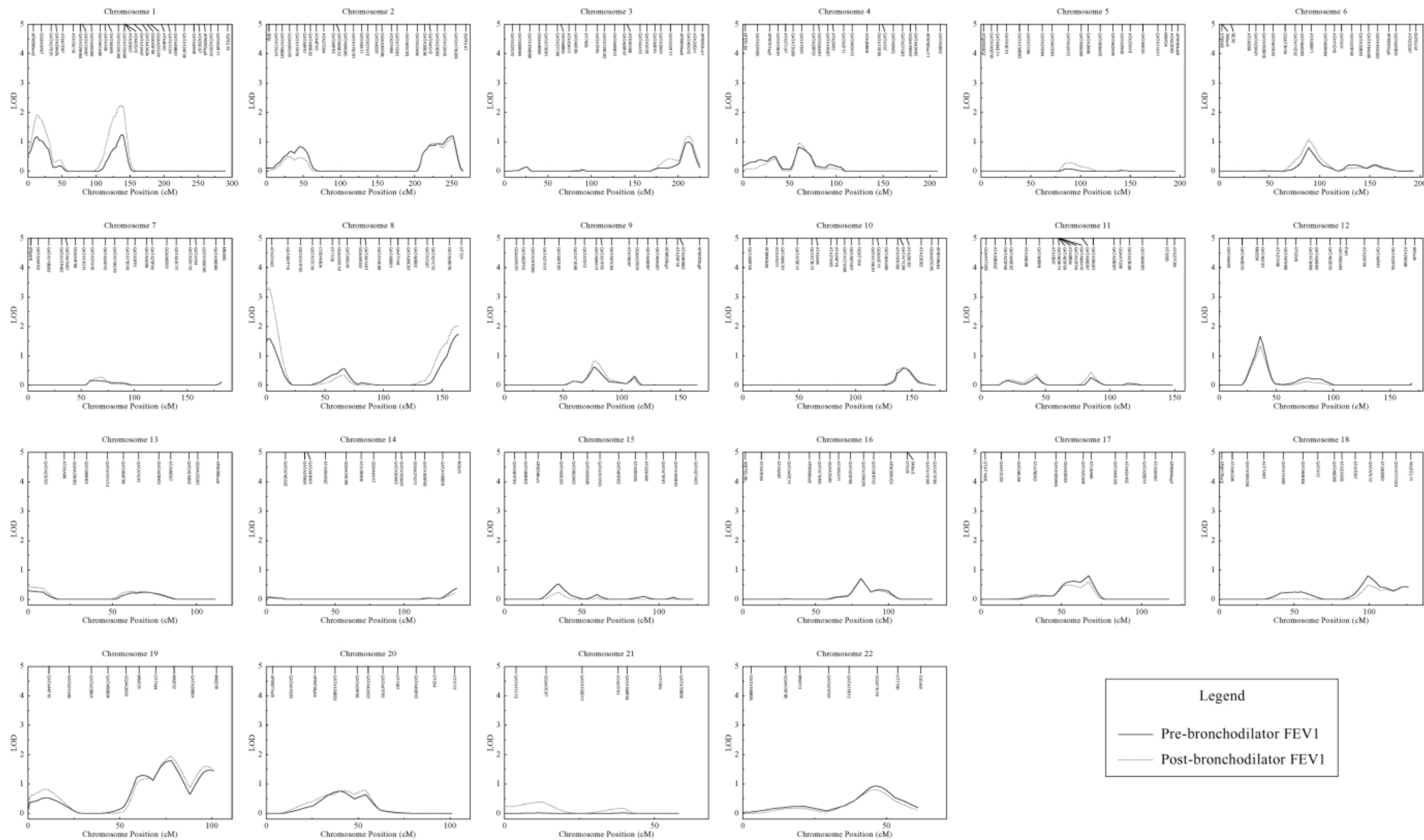

Figure 1. Multipoint variance component linkage analysis of all 22 autosomes in early-onset COPD pedigrees. Linkage analysis results are presented for post-bronchodilator FEV_1, with adjustment for relevant covariates. Linkage results for pre-bronchodilator FEV_1 are also presented for comparison purposes. The *x*-axis represents genetic distance in cM along each of the 22 autosomes, the *y*-axis represents the multipoint variance component LOD score. Markers are arrayed in map order along the top of each plot.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

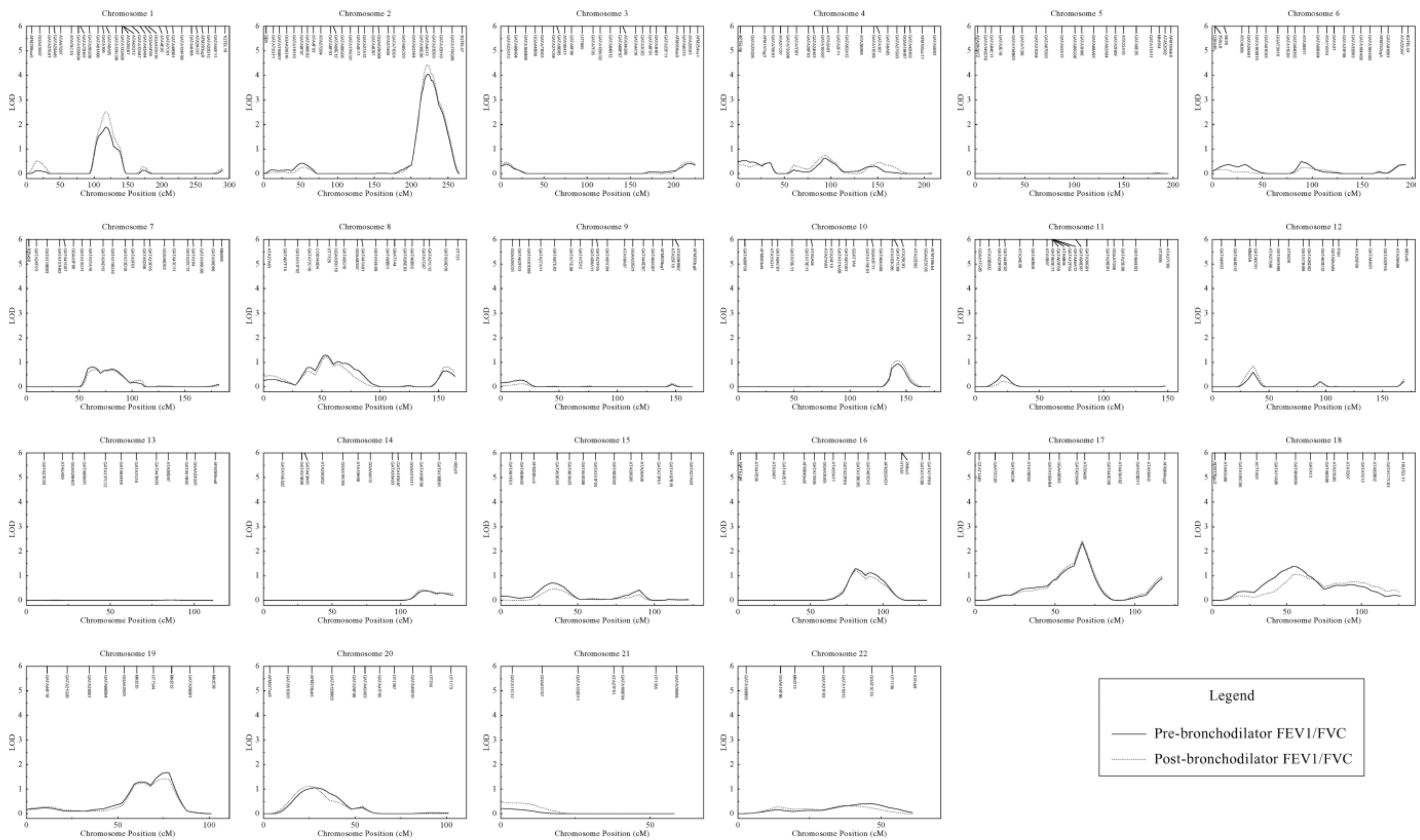

Figure 2. Multipoint variance component linkage analysis of all 22 autosomes in early-onset COPD pedigrees. Linkage analysis results are presented for post-bronchodilator FEV_1/FVC, with adjustment for relevant covariates. Linkage results for pre-bronchodilator FEV_1/FVC are also presented for comparison purposes. The *x*-axis represents genetic distance in cM along each of the 22 autosomes, the *y*-axis represents the multipoint variance component LOD score. Markers are arrayed in map order along the top of each plot.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

Table 2. Multipoint genome-wide linkage analysis of spirometry with SOLAR: chromosomal regions with LOD scores above 1.0. Pre-bronchodilator linkage results shown for comparison purposes

Chromosome	Phenotype							
	Post-bronchodilator FEV_1[a]			Pre-bronchodilator FEV_1[b]	Post-bronchodilator FEV_1/FVC[c]			Pre-bronchodilator FEV_1/FVC[c]
	Maximum LOD score	cM	*P*-value[d]	Maximum LOD score[e]	Maximum LOD score	cM	*P*-value[d]	Maximum LOD score[e]
1	1.91	14	0.005	1.17	2.52	118	0.001	1.89
1	2.24	136	0.002	1.24	—	—	—	—
2	1.13	252	0.02	1.19	4.42	222	0.00003	4.05
3	1.19	212	0.02	1.00	—	—	—	—
6	1.08	89	0.03	0.80	—	—	—	—
8	3.30	2	0.0002	1.58	1.22	54	0.02	1.29
8	2.01	163	0.004	1.73	—	—	—	—
10	—	—	—	—	1.07	142	0.02	0.93
12	1.33	36	0.02	1.66	—	—	—	—
16	—	—	—	—	1.22	81	0.02	1.30
17	—	—	—	—	2.44	67	0.001	2.35
18	—	—	—	—	1.05	56	0.02	1.39
19	1.94	78	0.004	1.80	1.42	75	0.01	1.67
20	—	—	—	—	1.12	25	0.02	1.05

[a]Model adjusted for age, sex, height, pack-years, $(\text{pack-years})^2$, race.
[b]Model adjusted for age, sex, height, pack-years, and $(\text{pack-years})^2$.
[c]Model adjusted for age, pack-years, $(\text{pack-years})^2$.
[d]*P*-values generated empirically by simulation (rounded to nearest decimal place).
[e]Maximum pre-bronchodilator LOD score within ± 2 cM of post-bronchodilator position.

previously been reported, and it is of interest that our results show strong genetic effects underlying these traits.

Smoking is clearly the critical environmental exposure for COPD (30,31), but precisely why some smokers develop COPD and others do not is unclear. It has been proposed that the development of COPD requires both environmental exposure and heightened sensitivity to the effects of these exposures (32). BDR is an important component of the COPD phenotypic spectrum. Previous studies have found that BDR measures were significantly correlated with baseline lung function, although the evidence has been somewhat controversial (3,10,33). Along with airway hyperresponsiveness to inhaled bronchoconstrictors, reversibility of airflow obstruction in response to administration of a bronchodilating agent is probably one of the key factors underlying disease progression and outcome in COPD (7). Increased BDR may be associated with increased host susceptibility to COPD or with earlier age-of-onset in susceptible subjects who smoke. Mechanistically, airway smooth muscle tone may be increased in COPD (34), and may explain the reversible component of airflow obstruction in COPD. Regardless of the mechanism, increased BDR has been shown to be associated with subsequent decline in pulmonary function (3,8,35,36). Therefore, genetic determinants of BDR probably represent important determinants of COPD.

There have been no previous linkage studies of BDR phenotypes. The linkages to BDR phenotypes in our study were modest and did not reach genome-wide significance (23). The most significant areas of linkage were on chromosomes 3p12 (BDRABS; LOD = 1.50), 4p16 (BDRBASE; adjusted LOD = 1.28), and 4q31 (BDRABS; LOD = 1.56). These modest linkages may reflect the inherent noisiness of the BDR phenotypes, which exhibit substantial day-to-day variability (coefficients of variation ranging from 57 to 61%) and poor reproducibility even in controlled research settings (37,38). Although these BDR phenotypes were increased in the current or ex-smoking first-degree relatives of our early-onset COPD probands (19), they may be less than optimal for genetic linkage studies which are critically dependent upon accurate phenotype definition within each individual.

The analysis of post-bronchodilator spirometry gave similar results to pre-bronchodilator spirometry, although linkages were generally more significant for the post-bronchodilator measures. Not surprisingly, the pre-bronchodilator results were very similar to a previously published whole genome scan for pre-bronchodilator spirometry (21) in a slightly larger sample of early-onset COPD families that included all of the subjects in the current analysis. These results, discussed in detail elsewhere (21), suggested significant linkage to an airflow obstruction susceptibility locus on chromosome 2q, which may reflect one or more genes influencing the development of airflow obstruction or dysanapsis. The one exception to the general pattern of modest improvement to linkage evidence for post- versus pre-bronchodilator spirometry was the linkage to chromosome 8p23, which roughly doubled and became significant at a genomewide level (LOD = 3.30) (23) when the post-bronchodilator FEV_1 was used. Of interest, the chromosome 8p linkage signal is located near a cluster of defensin genes (39)—cytotoxic proteins produced by neutrophils that may be involved in host defense and could modulate lung inflammation in COPD (40).

Our study population was highly selected for COPD and is clearly different from study populations ascertained on the basis of asthma or atopy. However, it is of some interest to consider the potential overlaps between our linkage findings and those derived from studies of asthma genetics. Multiple

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

Figure 3. Multipoint variance component linkage analysis of all 22 autosomes in early-onset COPD pedigrees. Linkage analysis results are presented for bronchodilator responsiveness measured as an absolute change in FEV_1 (BDRABS), a relative change from baseline FEV_1 as a percentage of baseline FEV_1 (BDRBASE), and a relative change from baseline FEV_1 as a percentage of predicted FEV_1 (BDRPRED); in each case adjustment for relevant covariates was included. The *x*-axis represents genetic distance in cM along each of the 22 autosomes, the *y*-axis represents the multipoint variance component LOD score. Markers are arrayed in map order along top of each plot.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

Table 3. Multipoint genome-wide linkage analysis of bronchodilator responsiveness with SOLAR: chromosomal regions with LOD scores above 1.0

Chromosome	Phenotype								
	BDRABS (ml)[a]			BDRPRED (%)[b]			BDRBASE (%)[c]		
	Maximum LOD score	cM	*P*-value[d]	Maximum LOD score	cM	*P*-value[d]	Maximum LOD score[e]	cM	*P*-value[d]
2	1.18	159	0.02	—	—	—	—	—	—
3	1.50	112	0.01	1.36	112	0.01			
3	—	—	—	—	—	—	1.07	170	0.01
4	1.56	146	0.01	1.55	146	0.01			
4	—	—	—	—	—	—	1.28	13	0.01
10	1.22	110	0.01	1.15	109	0.02	—	—	—
16	1.04	0	0.02	1.03	0	0.02	—	—	—
22	1.33	2	0.01	—	—	—	—	—	—

[a]Model adjusted for age, height, pack-years, (pack-years)^2, and race.
[b]Model adjusted for race.
[c]Model adjusted for race, pack-years, (pack-years)^2.
[d]*P*-values generated empirically by simulation (rounded to nearest decimal place).
[e]LOD scores empirically corrected for kurtosis ($\times$0.815).

genomic regions have been linked to asthma-related phenotypes in genome scan linkage studies. BDR is also a characteristic feature of asthma in children and adults, and inhaled bronchodilator agents (β_2-agonists) are the most widely prescribed drugs in the initial treatment of patients with asthma (41). Evidence for linkage of asthma-related phenotypes to the three most promising regions for BDR in this study, chromosomes 3p, 4p and 4q, have been reported previously in some asthma genetics studies (42–45). Similarly, the chromosome 8p region that demonstrated a novel linkage to post-bronchodilator FEV_1 is located in the same general region as a linkage signal to asthma diagnosis in the Hutterite population (42) and to specific IgE reactivity to house dust mite in a British population (46). It remains unclear whether asthma and COPD share genetic determinants, and elucidation of the specific genetic determinants of each condition will be required to resolve this controversy definitively. However, our results are consistent with at least some shared genetic determinants for asthma and COPD.

Phenotype definition is a critical issue for genetic investigations of complex human diseases such as COPD (13). Our study has important implications for phenotype definition in future genetic studies of COPD. Spirometric indices assessed after administration of an inhaled bronchodilator were associated with increased evidence of linkage compared to pre-bronchodilator spirometry, suggesting that these measures may be optimal for genetic studies of COPD. Airflow obstruction in COPD typically has a reversible and an irreversible component; post-bronchodilator spirometric values may allow more focused assessment of the irreversible component. Thus, post-bronchodilator spirometric values may provide less heterogeneous phenotypes for the detection of COPD genetic effects.

The best method of calculating BDR for epidemiological studies remains unclear, and several measures are in common use (37,47–49). The most commonly used BDR measure clinically is the BDRBASE (47). The BDRBASE measure exhibited only marginal heritability (10.1%) and was markedly kurtotic; BDRBASE has been previously shown to be most dependent upon pre-bronchodilator FEV_1 and to have a higher coefficient of variation in COPD patients than the BDRABS or BDRPRED, which had similar coefficients of variation (37). The linkages to BDRBASE were markedly different than those to BDRABS, suggesting that BDRBASE may reflect a different dimension of BDR than the other measures. Our study has found that the BDRPRED linkage results mirrored those to BDRABS, although the BDRPRED results were slightly less significant (Table 3). Although it is unclear which measure will ultimately prove more useful for genetic analysis, these results suggest that the BDRABS and BDRPRED measures, once appropriately adjusted for important covariates, may be more useful for genetic studies of COPD than the BDRBASE measure.

This study has several important limitations. The generalizability of linkage results in our severe, early-onset COPD (primarily Caucasian) families to COPD at later ages or to other ethnic groups is undetermined. We have included the primary environmental determinant of COPD, cigarette smoking, as a covariate in our analyses, but we have not formally tested for genotype-by-environment interactions. Some subjects were on chronic bronchodilator medication, and they may have thus had decreased acute BDR. We used a spacer device in our BDR testing protocol for bronchodilator administration in order to reduce variability caused by errors in coordinating inspiration with actuation and in aiming a metered-dose inhaler; however, uniformity of actual dose received cannot be guaranteed. These factors could have contributed to the modest linkage results for BDR measures. Finally, we have not adjusted for the multiple phenotypes analyzed, because these bronchodilator responsiveness phenotypes are all significantly correlated with each other.

There are likely to be multiple genetic determinants of COPD, and these linkage analyses have identified multiple regions of interest across the genome. The analyses of post-bronchodilator spirometric phenotypes have identified a novel region of significant linkage (8p) in addition to the linkages previously identified by analysis of pre-bronchodilator spirometric phenotypes (21). The analysis of BDR measures has

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

identified several regions of potential linkage on which to focus fine mapping efforts. Evaluation of the regions of linkage within our study with additional STR markers will be required, and replication of our findings in other samples will be necessary. If novel genetic determinants of COPD can be identified, important new insights into COPD pathophysiology, and ultimately treatment, could result.

SUBJECTS AND METHODS

Families

The recruitment and phenotypic assessment of severe early-onset COPD pedigrees have been described previously (20,50). Ascertainment criteria for probands with severe early-onset COPD included: $FEV_1 \leq 40\%$ predicted, age ≤ 52 years, and absence of severe AAT deficiency (e.g. PI Z, PI null–null) (21,22). All available first-degree relatives, older second-degree relatives (half-sibs, aunts, uncles and grandparents), and 49 additional relatives of the ascertained COPD probands were invited to participate (21,22). Seventy-two pedigrees with mean size of 8.1 individuals (range 2–18) were included in the original genome scan linkage analysis.

A total of 607 individuals were initially typed for a whole genome scan. Twenty-two subjects were removed due to pedigree inconsistencies (see below), and a further 25 subjects (including three probands) did not have both pre- and post-bronchodilator spirometric data. The genome scan linkage analyses were thus based on the 560 members of 72 pedigrees for whom complete data on both pre- and post-bronchodilator spirometry, and therefore bronchodilator responsiveness phenotypes, were available. The study population was predominantly European-American, but included 10 African-American subjects.

Participants gave written informed consent and completed a protocol that included a questionnaire, spirometry before and after inhaled bronchodilator and phlebotomy. The study protocol was approved by the Human Research Committees of Partners Health Care (Brigham and Women's Hospital and Massachusetts General Hospital) and the Brockton/West Roxbury VA Hospital.

Questionnaire

Each participant completed a modified version of the 1978 ATS-DLD Epidemiology Questionnaire as previously described (20,51).

Pulmonary function tests

Pulmonary function testing in these pedigrees has been reported previously (20,50). Spirometry was performed with a Survey Tach Spirometer (Warren E. Collins, Braintree, MA, USA), in accordance with American Thoracic Society guidelines (52). Although the absolute volume measurements were the primary spirometric phenotypes analyzed, pulmonary function test results also were expressed as percent of predicted using predicted equations from Crapo *et al.* (53) for adult Caucasian participants. For Caucasian participants under age 18, predicted values for FEV_1 were determined from Hsu *et al.* (54), and predicted values for FEV_1/FVC were determined from Knudson *et al.* (55). For African-American participants, predicted spirometric values were determined from Hankinson *et al.* (56). For four subjects with both pre- and post-bronchodilator spirometry, previously obtained pulmonary function test results were used; two subjects had undergone lung volume reduction surgery and two other subjects were located at great geographic distance. Height was measured in stocking feet at the time of spirometric testing.

Bronchodilator responsiveness testing

Subjects were asked to avoid bronchodilator medication use for at least 4 h prior to spirometry, unless respiratory symptoms required bronchodilator treatment. After the initial spirometry was performed, subjects were given 180 μg (two puffs) of albuterol through a metered dose inhaler with a spacer device. Approximately 15 min after albuterol administration, spirometry was repeated.

Genotyping and data management

The NHLBI Mammalian Genotyping Service performed genome scan genotyping in 607 individuals, using DNA that was extracted from blood samples with Puregene Kits (Gentra Systems) (21,22). We analyzed 377 autosomal short tandem repeat markers with an average spacing of 9.1 cM derived from Weber Marker Set 10. Marker and gene locations were determined using the Human Genome Working Draft (June 2002 freeze) and SNPper. The Kosambi mapping function was used for all multipoint analyses. The estimated genotype completion rate was 98.3%, and the estimated genotyping error rate was 0.75%.

To assess for Mendelian inconsistencies in our pedigree data, the RELCHECK program was used to determine pedigree relationships based on the genome scan marker data (57) as previously reported (21,22). For the remaining 560 genotyped individuals, inconsistencies at individual markers were identified using the PEDCHECK program and resolved (58). Marker allele frequencies were estimated by maximum likelihood estimation using the SOLAR program (59).

Linkage analysis

The quantitative phenotypes included in the linkage analysis were bronchodilator responsiveness and post-bronchodilator FEV_1 and FEV_1/FVC levels. Bronchodilator responsiveness to inhaled β-agonist was measured in three ways: (i) a measure of absolute change in unadjusted FEV_1 ('BDRABS'), (postbronchodilator FEV_1 − prebronchodilator FEV_1) (ml); (ii) a relative measure of change in percent predicted FEV_1 ('BDRPRED'),

$$\left[\frac{(\text{postbronchodilator } FEV_1 - \text{prebronchodilator } FEV_1)}{\text{predicted prebronchodilator } FEV_1} * 100\right] (\%)$$

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

and (iii) a relative measure of change in unadjusted FEV_1 ('BDRBASE'),

$$\left[\frac{(\text{postbronchodilator } FEV_1 - \text{prebronchodilator } FEV_1)}{\text{prebronchodilator } FEV_1} * 100\right](\%)$$

Covariate adjustments were performed within the linkage analysis, as described below, and modeling included the investigation of polynomial terms for covariates.

Variance component analyses assume multivariate normality. Variance components estimates are consistent regardless of the underlying distribution; however, second-order moments (standard errors) may be biased by non-normality (60). Kurtosis is the primary determinant of the effect of non-normality on the likelihood ratio test statistic in a variance component linkage analysis (61). Blangero *et al.* (61) indicate that trait distributions with $\kappa < 2$ can reasonably be analyzed under an assumption of multivariate normality. The kurtoses of the outcomes analyzed were all <2, with the exception of the BDRBASE ($\kappa = 5.39$). We estimated empirical *P*-values via simulation (see below) for all traits analyzed in order to derive correct test statistics for our linkage analyses. We further estimated a robust LOD score for BDRBASE by simulating the empirical distribution of the LOD scores under the assumption of multivariate normality (61) (the correction constant for the original BDRBASE LOD scores was 0.815).

Two-point and multipoint linkage analysis of the genome scan data were performed using a variance component method as implemented in the SOLAR program (version 1.7.4 running under Linux Mandrake version 8.0) (59). Using SOLAR, observed phenotypic variance was partitioned into genetic and non-genetic components by maximum likelihood methods. Additive models, without dominance variance, were used in these analyses. Each model assumed that the distribution of the response phenotype in a pedigree was multivariate normal, with a mean that depended upon a particular set of explanatory covariates. Genotypes were imputed for untyped individuals, conditional on all other marker data and pedigree structure, and marker-specific identity-by-descent (IBD) matrices among all relative pairs were estimated independently for all autosomal markers. Multipoint IBD matrices were then generated at 1 cM resolution. Expected genetic covariances among relatives in an extended family were specified as a function of the fixed effects of covariates, residual error and random effects reflecting polygenic factors and an unobserved quantitative trait locus (QTL) linked to an observed marker locus.

Covariates modeled in the variance component analyses included: age, age^2, sex, age $*$ sex, race, height, $height^2$, pack-years, and $pack\text{-}years^2$. Covariates that were significant at $P < 0.05$ in the polygenic modeling were retained in the linkage analysis model.

The statistical associations between covariates entered as fixed effects and the response variable were assessed by removal of terms from the mean model and calculation of a likelihood ratio χ^2 test statistic. The same approach was used as an approximate guide to the 'significance' of a departure of the value of a variance component from its null value (zero).

The null hypothesis of no linkage at a specific chromosomal location was tested by comparison of a polygenic model to a model with variance components for both a QTL and polygenic factors. Twice the difference in $\log_e$ likelihood of these two models gives a test statistic that is asymptotically distributed as a $\frac{1}{2}:\frac{1}{2}$ mixture of a χ^2_1 and a point mass at zero (62). The difference between these two $\log_{10}$ likelihood values corresponds to an LOD score for linkage.

The single ascertainment scheme was corrected for in the SOLAR program by conditioning the pedigree likelihood on the probability of the single proband's phenotype for each outcome (63).

Simulations were performed using SOLAR to assess the statistical significance of the linkage results. Genotypes for a fully informative unlinked marker were created, and the evidence for linkage was assessed in 100 000 replicates. The number of times that a LOD score exceeded a specified threshold provides an empirical *P*-value for that LOD score threshold.

ACKNOWLEDGEMENTS

We appreciate assistance from a variety of physicians in recruiting subjects for this study. We would like to thank Drs Jeffrey Drazen and Steven Shapiro for helpful discussions. We gratefully acknowledge the alpha 1-antitrypsin testing of early-onset COPD probands by Dr Edward Campbell. We are grateful for the high quality genotyping performed by the NHLBI Mammalian Genotyping Service. We are especially thankful for the enthusiastic support for this study from the members of the early-onset COPD families. This study was supported by NIH Grant HL 61575 (E.K.S.) and Program in Genomic Applications Grant U01 HL 66795 (S.T.W.) to the Channing Laboratory, Brigham and Women's Hospital; a gift from the Overholt Thoracic Foundation to the Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital; and HL 67204 (H.A.C.) to the University of California, San Francisco.

ELECTRONIC DATABASE INFORMATION

URLs for OMIM and accession databases: NHLBI Mammalian Genotyping Service/Marshfield Clinic, http://research.marshfieldclinic.org/genetics; Human Genome Working Draft (Golden Path), http://genome.cse.ucsc.edu; SNPper, http://pga.bwh.harvard.edu/; Online Mendelian Inheritance in Man (OMIM), www.ncbi.nlm.nih.gov/omim.

REFERENCES

1. Davis, R.M. and Novotny, T.E. (1989) Changes in risk factors: the epidemiology of cigarette smoking and its impact on chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.*, **140**, S82–S84.
2. Silverman, E.K. and Speizer, F.E. (1996) Risk factors for the development of chronic obstructive pulmonary disease. *Med. Clin. N. Am.*, **80**, 501–522.
3. Kanner, R.E. (1984) The relationship between airways responsiveness and chronic airflow limitation. *Chest*, **86**, 54–57.
4. ATS (1987) Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am. Rev. Respir. Dis.*, **136**, 225–244.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

5. Siafakas, N.M., Vermeire, P., Pride, N.B., Paoletti, P., Gibson, J., Howard, P., Yernault, J.C., Decramer, M., Higenbottam, T., Postma, D.S. and Rees, J. (1995) Optimal assessment and management of chronic obstructive pulmonary disease (COPD). *Eur. Respir. J.*, **8**, 1398–1420.
6. Gross, N.J. (1986) COPD: a disease of reversible airflow obstruction. *Am. Rev. Respir. Dis.*, **133**, 725–726.
7. Barter, C.E. and Campbell, A.H. (1976) Relationship of constitutional factors and cigarette smoking to decrease in 1-second forced expiratory volume. *Am. Rev. Respir. Dis.*, **113**, 305–314.
8. Vollmer, W.M., Johnson, L.R. and Buist, A.S. (1985) Relationship of response to a bronchodilator and decline in forced expiratory volume in one second in population studies. *Am. Rev. Respir. Dis.*, **132**, 1186–1193.
9. Postma, D.S., Gimeno, F., van der Weele, L.T. and Sluiter, H.J. (1985) Assessment of ventilatory variables in survival prediction of patients with chronic airflow obstruction: the importance of reversibility. *Eur. J. Resp. Dis.*, **67**, 360–368.
10. Postma, D., deVries, K., Koeter, G. and Sluiter, H. (1986) Independent influence of reversibility of air-flow obstruction and nonspecific hyper-reactivity on the long-term course of lung function in chronic air-flow obstruction. *Am. Rev. Respir. Dis.*, **134**, 276–280.
11. Janus, E.D., Phillips, N.T. and Carrell, R.W. (1985) Smoking, lung function, and alpha 1-antitrypsin deficiency. *Lancet*, **1**, 152–154.
12. Sandford, A., Weir, T. and Pare, P. (1997) Genetic risk factors for chronic obstructive pulmonary disease. *Eur. Respir. J.*, **10**, 1380–1391.
13. Lomas, D.A. and Silverman, E.K. (2001) The genetics of chronic obstructive pulmonary disease. *Respir. Res.*, **2**, 20–26.
14. Redline, S., Tishler, P.V., Rosner, B., Lewitter, F.I., Vandenburgh, M., Weiss, S.T. and Speizer, F.E. (1989) Genotypic and phenotypic similarities in pulmonary function among family members of adult monozygotic and dizygotic twins. *Am. J. Epidemiol.*, **129**, 827–836.
15. Kueppers, F., Miller, R.D., Gordon, H., Hepper, N.G. and Offord, K. (1977) Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am. J. Med.*, **63**, 336–342.
16. Cohen, B.H. (1980) Chronic obstructive pulmonary disease: a challenge in genetic epidemiology. *Am. J. Epidemiol.*, **112**, 274–288.
17. Cohen, B.H., Ball, W.C., Jr., Brashears, S., Diamond, E.L., Kreiss, P., Levy, D.A., Menkes, H.A., Permutt, S. and Tockman, M.S. (1977) Risk factors in chronic obstructive pulmonary disease (COPD). *Am. J. Epidemiol.*, **105**, 223–232.
18. Beaty, T.H., Liang, K.Y., Seerey, S. and Cohen, B.H. (1987) Robust inference for variance components models in families ascertained through probands. II. Analysis of spirometric measures. *Genet. Epidemiol.*, **4**, 211–221.
19. Celedon, J.C., Speizer, F.E., Drazen, J.M., Weiss, S.T., Campbell, E.J., Carey, V.J., Reilly, J.J., Ginns, L. and Silverman, E.K. (1999) Bronchodilator responsiveness and serum total IgE levels in families of probands with severe early-onset COPD. *Eur. Respir. J.*, **14**, 1009–1014.
20. Silverman, E.K., Chapman, H.A., Drazen, J.M., Weiss, S.T., Rosner, B., Campbell, E.J., O'Donnell, W.J., Reilly, J.J., Ginns, L., Mentzer, S., Wain, J. and Speizer, F.E. (1998) Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease: risk to relatives for airflow obstruction and chronic bronchitis. *Am. J. Respir. Crit. Care Med.*, **157**, 1770–1778.
21. Silverman, E.K., Palmer, L.J., Mosley, J.D., Barth, M., Senter, J.M., Brown, A., Drazen, J.M., Kwiatkowski, D.J., Chapman, H.A., Campbell, E.J. *et al.* (2002) Genomewide linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic obstructive pulmonary disease. *Am. J. Hum. Genet.*, **70**, 1229–1239.
22. Silverman, E.K., Mosley, J.D., Palmer, L.J., Barth, M., Senter, J.M., Brown, A., Drazen, J.M., Kwiatkowski, D.J., Chapman, H.A., Campbell, E.J. *et al.* (2002) Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. *Hum. Mol. Genet.*, **11**, 623–632.
23. Lander, E. and Kruglyak, L. (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet.*, **11**, 241–247.
24. Higgins, M. and Keller, J. (1975) Familial occurrence of chronic respiratory disease and familial resemblance in ventilatory capacity. *J. Chron. Dis.*, **28**, 239–251.
25. Tager, I., Rosner, B., Tishler, P., Speizer, F. and Kass, E. (1976) Household aggregation of pulmonary function and chronic bronchitis. *Am. Rev. Respir. Dis.*, **114**, 485–492.
26. Lebowitz, M., Knudson, R. and Burrows, B. (1984) Familial aggregation of pulmonary function measurements. *Am. Rev. Respir. Dis.*, **129**, 8–11.
27. Rybicki, B., Beaty, T. and Cohen, B. (1990) Major genetic mechanisms in pulmonary function. *J. Clin. Epidemiol.*, **43**, 667–675.
28. Cotch, M., Beaty, T. and Cohen, B. (1990) Path analysis of familial resemblance of pulmonary function and cigarette smoking. *Am. Rev. Respir. Dis.*, **142**, 1337–1343.
29. Palmer, L.J., Knuiman, M.W., Divitini, M.L., Burton, P.R., James, A.L., Bartholomew, H.C., Ryan, G. and Musk, A.W. (2001) Familial aggregation and heritability of adult lung function: results from the Busselton Health Study. *Eur. Respir. J.*, **17**, 696–702.
30. US Department of Health and Human Services (1990) The health benefits of smoking cessation. US Goverment Printing Office, Washington, DC.
31. American Thoracic Society (1995) Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.*, **152**, S77–121.
32. Speizer, F.E. and Tager, I.B. (1979) Epidemiology of chronic mucus hypersecretion and obstructive airways disease. *Epidemiol. Rev.*, **1**, 124–142.
33. Ramsdale, E.H., Roberts, R.S., Morris, M.M. and Hargreave, F.E. (1985) Differences in responsiveness to hyperventilation and methacholine in asthma and chronic bronchitis. *Thorax*, **40**, 422–426.
34. Hughes, J.A., Tobin, M.J., Bellamy, D. and Hutchison, D.C. (1982) Effects of ipratropium bromide and fenoterol aerosols in pulmonary emphysema. *Thorax*, **37**, 667–670.
35. Campbell, A.H., Barter, C.E., O'Connell, J.M. and Huggins, R. (1985) Factors affecting the decline of ventilatory function in chronic bronchitis. *Thorax*, **40**, 741–748.
36. Taylor, R.G., Joyce, H., Gross, E., Holland, F. and Pride, N.B. (1985) Bronchial reactivity to inhaled histamine and annual rate of decline in FEV1 in male smokers and ex-smokers. *Thorax*, **40**, 9–16.
37. Dompeling, E., van Schayck, C.P., Molema, J., Akkermans, R., Folgering, H., van Grunsven, P.M. and van Weel, C. (1992) A comparison of six different ways of expressing the bronchodilating response in asthma and COPD; reproducibility and dependence of prebronchodilator FEV1. *Eur. Respir. J.*, **5**, 975–981.
38. Guyatt, G.H., Townsend, M., Nogradi, S., Pugsley, S.O., Keller, J.L. and Newhouse, M.T. (1988) Acute response to bronchodilator. An imperfect guide for bronchodilator therapy in chronic airflow limitation. *Arch. Intern. Med.*, **148**, 1949–1952.
39. Lehrer, R.I. and Ganz, T. (2002) Defensins of vertebrate animals. *Curr. Opin. Immunol.*, **14**, 96–102.
40. Matsushita, I., Hasegawa, K., Nakata, K., Yasuda, K., Tokunaga, K. and Keicho, N. (2002) Genetic variants of human beta-defensin-1 and chronic obstructive pulmonary disease. *Biochem. Biophys. Res. Commun.*, **291**, 17–22.
41. Palmer, L.J., Silverman, E.S., Weiss, S.T. and Drazen, J.M. (2002) Pharmacogenetics of asthma. *Am. J. Respir. Crit. Care Med.*, **165**, 861–866.
42. Ober, C., Tsalenko, A., Parry, R. and Cox, N.J. (2000) A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. *Am. J. Hum. Genet.*, **67**, 1154–1162.
43. Yokouchi, Y., Nukaga, Y., Shibasaki, M., Noguchi, E., Kimura, K., Ito, S., Nishihara, M., Yamakawa-Kobayashi, K., Takeda, K., Imoto, N. *et al.* (2000) Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31–q33 near the interleukin 12 B locus by a genome-wide search in Japanese families. *Genomics*, **66**, 152–160.
44. Ober, C., Cox, N.J., Abney, M., Di Rienzo, A., Lander, E.S., Changyaleket, B., Gidley, H., Kurtz, B., Lee, J., Nance, M. *et al.* (1998) Genome-wide search for asthma susceptibility loci in a founder population. The Collaborative Study on the Genetics of Asthma. *Hum. Mol. Genet.*, **7**, 1393–1398.
45. Dizier, M.H., Besse-Schmittler, C., Guilloud-Bataille, M., Annesi-Maesano, I., Boussaha, M., Bousquet, J., Charpin, D., Degioanni, A., Gormand, F., Grimfeld, A. *et al.* (2000) Genome screen for asthma and related phenotypes in the French EGEA Study. *Am. J. Respir. Crit. Care. Med.*, **162**, 1812–1818.
46. Kurz, T., Strauch, K., Heinzmann, A., Braun, S., Jung, M., Ruschendorf, F., Moffatt, M.F., Cookson, W.O., Inacio, F., Ruffilli, A. *et al.* (2000) A European study on the genetics of mite sensitization. *J. Allergy Clin. Immunol.*, **106**, 925–932.
47. Dales, R.E., Spitzer, W.O., Tousignant, P., Schechter, M. and Suissa, S. (1988) Clinical interpretation of airway response to a bronchodilator. Epidemiologic considerations. *Am. Rev. Respir. Dis.*, **138**, 317–320.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022

48. Eliasson, O. and Degraff, A.C. Jr. (1985) The use of criteria for reversibility and obstruction to define patient groups for bronchodilator trials. Influence of clinical diagnosis, spirometric, and anthropometric variables. *Am. Rev. Respir. Dis.*, **132**, 858–864.
49. Brand, P.L., Quanjer, P.H., Postma, D.S., Kerstjens, H.A., Koeter, G.H., Dekhuijzen, P.N. and Sluiter, H.J. (1992) Interpretation of bronchodilator response in patients with obstructive airways disease. The Dutch Chronic Non-Specific Lung Disease (CNSLD) Study Group. *Thorax*, **47**, 429–436.
50. Silverman, E.K., Weiss, S.T., Drazen, J.M., Chapman, H.A., Carey, V., Campbell, E.J., Denish, P., Silverman, R.A., Celedon, J.C., Reilly, J.J., Ginns, L.C. and Speizer, F.E. (2000) Gender-related differences in severe, early-onset chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.*, **162**, 2152–2158.
51. Ferris, B.G. (1978) Epidemiology standardization project. *Am. Rev. Respir. Dis.*, **118** (suppl.), 1–120.
52. ATS (1995) Standardization of spirometry: 1994 Update. American Thoracic Society. *Am. J. Respir. Crit. Care Med.*, **152**, 1107–1136.
53. Crapo, R.O., Morris, A.H. and Gardner, R.M. (1981) Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am. Rev. Respir. Dis.*, **123**, 659–664.
54. Hsu, K.H.K., Jenkins, D.E., Hsi, B.P., Bourhofer, E., Thompson, V., Tanakawa, N. and Hsieh, G.S.J. (1979) Ventilatory functions of normal children and young adults—Mexican-American, white, and black. I. Spirometry. *J. Pediatr.*, **95**, 14–23.
55. Knudson, R.J., Lebowitz, M.D., Holberg, C.J. and Burrows, B. (1983) Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am. Rev. Respir. Dis.*, **127**, 725–734.
56. Hankinson, J.L., Odencrantz, J.R. and Fedan, K.B. (1999) Spirometric reference values from a sample of the general, U.S. population. *Am. J. Respir. Crit. Care Med.*, **159**, 179–187.
57. Broman, K.W., Murray, J.C., Sheffield, V.C., White, R.L. and Weber, J.L. (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. *Am. J. Hum. Genet.*, **63**, 861–869.
58. O'Connell, J.R. and Weeks, D.E. (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am. J. Hum. Genet.*, **63**, 259–266.
59. Almasy, L. and Blangero, J. (1998) Multipoint quantitative-trait linkage analysis in general pedigrees. *Am. J. Hum. Genet.*, **62**, 1198–1211.
60. Beaty, T.H., Self, S.G., Liang, K.Y., Connolly, M.A., Chase, G.A. and Kwiterovich, P.O. (1985) Use of robust variance components models to analyse triglyceride data in families. *Ann. Hum. Genet.*, **49** (Pt 4), 315–328.
61. Blangero, J., Williams, J.T. and Almasy, L. (2001) Variance components methods for detecting complex trait loci. In Rao, D.C. and Province, M.A. (eds), *Genetic Dissection of Complex Traits,* Academic Press, New York, pp. 151–181.
62. Self, S.G. and Liang, K.-Y. (1987) Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *J. Am. Stat. Assoc.*, **82**, 605–610.
63. Lange, K., Weeks, D. and Boehnke, M. (1988) Programs for pedigree analysis: MENDEL, FISHER and dGENE. *Genet. Epidemiol.*, **5**, 471–472.

Downloaded from https://academic.oup.com/hmg/article/12/10/1199/641766 by guest on 21 August 2022