ASTHMA

Genome-wide linkage analysis of pulmonary function in families of children with asthma in Costa Rica

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Background: Although asthma is highly prevalent among certain Hispanic subgroups, genetic determinants of asthma and asthma-related traits have not been conclusively identified in Hispanic populations. A study was undertaken to identify genomic regions containing susceptibility loci for pulmonary function and bronchodilator responsiveness (BDR) in Costa Ricans.

Methods: Eight extended pedigrees were ascertained through schoolchildren with asthma in the Central Valley of Costa Rica. Short tandem repeat (STR) markers were genotyped throughout the genome at an average spacing of 8.2 cM. Multipoint variance component linkage analyses of forced expiratory volume in 1 second (FEV_1) and FEV_1/ forced vital capacity (FVC; both pre-bronchodilator and post-bronchodilator) and BDR were performed in these eight families (pre-bronchodilator spirometry, n = 640; post-bronchodilator spirometry and BDR, n = 624). Nine additional STR markers were genotyped on chromosome 7. Secondary analyses were repeated after stratification by cigarette smoking.

Results: Among all subjects, the highest logarithm of the odds of linkage (LOD) score for FEV_1 (post-bronchodilator) was found on chromosome 7q34–35 (LOD = 2.45, including the additional markers). The highest LOD scores for FEV_1/FVC (pre-bronchodilator) and BDR were found on chromosomes 2q (LOD = 1.53) and 9p (LOD = 1.53), respectively. Among former and current smokers there was near-significant evidence of linkage to FEV_1/FVC (post-bronchodilator) on chromosome 5p (LOD = 3.27) and suggestive evidence of linkage to FEV_1 on chromosomes 3q (pre-bronchodilator, LOD = 2.74) and 4q (post-bronchodilator, LOD = 2.66).

Conclusions: In eight families of children with asthma in Costa Rica, there is suggestive evidence of linkage to FEV_1 on chromosome 7q34–35. In these families, FEV_1/FVC may be influenced by an interaction between cigarette smoking and a locus (loci) on chromosome 5p.

Spirometric measurements of pulmonary function are important markers of asthma severity and critical intermediate phenotypes for asthma research. Several groups have identified genomic regions linked to pulmonary function measurements (such as forced expiratory volume in 1 second (FEV_1) and FEV_1/ forced vital capacity (FVC) ratio) in families ascertained through probands with asthma.[1–3] The prevalence and severity of asthma are markedly variable among different Hispanic populations living in the US and Latin America, and these differences may have an underlying genetic component.[4] In the Collaborative Study on the Genetics of Asthma,[5] genome-wide linkage analysis of asthma and some of its intermediate phenotypes (eg, total serum IgE) were performed in Hispanic families from New Mexico. However, there has been no genome-wide linkage analysis of pulmonary function phenotypes in any Hispanic population.

Bronchodilators, specifically β_2-adrenergic receptor agonists, are the most widely prescribed drugs in the treatment of asthma. There is a substantial inter-individual variation in the response to inhaled β_2-agonists, and bronchodilator responsiveness (BDR) has been shown to aggregate in families,[6] consistent with a genetic component to the variation in BDR. Although multiple studies have examined genetic linkage for airway responsiveness to bronchoconstrictor agents or pre-bronchodilator and post-bronchodilator spirometry measurements, a genome-wide linkage analysis of BDR in families with asthma has not been published. The only reported linkage analysis for BDR was in families of probands with severe, early-onset chronic obstructive disease (COPD).[7]

In this study, we performed genome-wide linkage analyses of pulmonary function measurements and BDR in families of children with asthma in Costa Rica, a nation with a high prevalence of childhood asthma.[8] Most Costa Ricans live in the Central Valley, where there is a genetically isolated population of predominantly mixed Spanish and Amerindian origin.[9] Extensive genealogical records can be used to track the rapid expansion of this population from approximately 4000 founding individuals registered in the census of 1697[10] to about 2.85 million current residents of the Central Valley. The unique characteristics of the population of the Central Valley make it ideal for studies of the genetics of asthma and/or its intermediate phenotypes.

METHODS

Study subjects

Seven probands were recruited through an ongoing study of children with asthma; an eighth proband was recruited from phase II of the International Study of Asthma and Allergies in Childhood in Costa Rica.[11] Eligible probands were 6–12 years old and had physician-diagnosed asthma, ⩾2 respiratory

Abbreviations: BDR, bronchodilator responsiveness; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; LOD, logarithm of the odds of linkage; SOLAR, Sequential Oligogenic Linkage Analysis Routines; STR, short tandem repeat

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symptoms (cough, wheeze, or dyspnoea) or asthma attacks in the previous year, airway hyper-responsiveness (provocative dose of methacholine causing a 20% decline in FEV_1 (PD_{20}) $\leqslant$8.58 μmol), $\geqslant$1 sibling with physician-diagnosed asthma, and $\geqslant$6 great-grandparents born in the Central Valley of Costa Rica. All first-degree and second-degree relatives of the proband ($\geqslant$6 years old) were invited to participate. Families were further extended by including first-degree relatives of individuals with asthma.

All adults gave written informed consent; parental consent was obtained for participating children, who also gave written assent. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños, San José, Costa Rica and Brigham and Women's Hospital (BWH), Boston, Massachusetts.

Phenotypic assessment

Each study participant completed a questionnaire and spirometry and gave a blood sample for DNA extraction. The study questionnaire was modified from that used by the Collaborative Study on the Genetics of Asthma[12] and translated into Spanish. Two versions of the questionnaire were used: one for adolescents and adults (>12 years old) and one for children ($\leqslant$12 years old). Pack-years of cigarette smoking were calculated by multiplying the number of years of smoking by the average number of cigarettes per day, divided by 20 to convert to packs. Children ($\leqslant$12 years old) were assumed to be non-smokers.

Spirometry was performed using a Survey Tach Spirometer (Warren E Collins, Braintree, Massachusetts) according to the American Thoracic Society recommendations.[13] Height was measured to the nearest half-inch. Spirometry was performed while subjects were seated and wearing a noseclip. Up to eight attempts were made to obtain three acceptable flow-volume loops. Participants were asked to refrain from use of short-acting bronchodilator drugs for at least 4 hours before testing. Spirometry was repeated 15 min after the administration of 200 μg (two puffs) of albuterol through a spacer. The best FEV_1 and FVC were selected for both pre-bronchodilator and post-bronchodilator spirometry.

Genotyping

DNA was extracted from blood samples using Puregene Kits (Gentra Systems). A panel of 380 short tandem repeat (STR) markers was genotyped by the Genome Quebec Innovation Centre using Applied Biosystems (Foster City, California) 3700 and 3730 analysers on 671 family members. This marker panel is a modified version of the Cooperative Human Linkage Center Human Screening set/V.6[14] that also includes selected Genethon markers.[15] Marker locations were based on the deCODE map.[16] Markers were located at an average spacing of 8.2 cM.

An additional nine STR markers on chromosome 7 were genotyped at Brigham and Women's Hospital: D7S2452, D7S2533, D7S500, D7S509, D7S495, D7S2505, D7S3044 and D7S2511. Primer sequences available in the National Center for Biotechnology Information's UniSTS database (http://www.ncbi.nlm.nih.gov/) were used to design assays. Fluorescent-labelled and unlabelled primers were obtained from Invitrogen (Carlsbad, California) and Applied Biosystems. PCR product sizes were assessed on an Applied Biosystems 3100 instrument. GeneScan and GeneMapper V.3.7 software were used to assist with genotype determination, and calls were manually reviewed.

Pedigree relationships were assessed by Relpair[17]; four subjects that did not match reported relationships were excluded from analysis. Mendelian inconsistencies at individual markers for the remaining 667 individuals were assessed using PedCheck.[18]

Statistical analysis

BDR was defined as: 1) BDRbase = change in FEV_1 as a percentage of the baseline FEV_1; 2) BDRpred = change in FEV_1 as a percentage of predicted FEV_1; and 3) BDRabs = absolute change in FEV_1 (ml).[7 19] The three BDR measurements were normally distributed after $\log_{10}$ transformation.

Heritability estimation and multipoint linkage analysis of FEV_1, FEV_1/FVC and BDR measurements were performed by a variance component approach in Sequential Oligogenic Linkage Analysis Routines (SOLAR), which uses identity-by-descent sharing to estimate the additive genetic variance due to a chromosomal region.[20] Results are expressed as logarithm of the odds of linkage (LOD) scores (logarithm [base 10] of the odds of linkage versus no linkage). Covariates included age, sex, height, weight, smoking status (ever vs never) and pack-years of cigarette smoking, including quadratic terms for continuous variables. Significant covariates ($p<0.05$) were included in the linkage models. Only 19 subjects (3%) were using anti-inflammatory drugs for asthma, so drug usage was not included as a covariate. Multipoint identity-by-descent matrices were estimated by a Markov-Chain Monte Carlo algorithm implemented in the Loki program.[21] Because of potential misclassification of chronic obstructive pulmonary disease (COPD) as asthma, secondary analyses were performed in non-smokers only by removing the phenotype measurements of current and former smokers, and in smokers-only by removing the phenotypes of non-smokers.

Log_{10} transformed BDR and raw FEV_1 measurements had acceptable kurtosis after adjustment for covariates in the final variance component models. Because of residual kurtosis (>3), FEV_1/FVC measurements were analysed by the t-distribution in SOLAR. Empirical p values for the multipoint LOD scores were estimated by comparing the observed LOD scores with the empirical distribution of LOD scores resulting from 100 000 simulations in SOLAR.

One thousand simulations were run in SOLAR to assess our statistical power to detect linkage (LOD scores ranging from $\geqslant$1 to $\geqslant$3) to a biallelic locus influencing a quantitative trait with heritability ranging from 10% to 25% in the Costa Rican pedigrees. These simulations assumed that the trait of interest was influenced by a single quantitative trait locus and that fully informative marker data were available for study subjects.

RESULTS

Study subjects

Of the 667 members of the eight participating families, 640 and 624 had spirometric measurements of lung function before and after administration of albuterol, respectively (table 1). There was marked variability among participating families in family size, percentage of former and current smokers and percentage of patients with asthma. As expected in individuals with asthma and their relatives with and without asthma, average values of FEV_1 and FEV_1/FVC were within the normal range. However, post-bronchodilator values were improved.

Analysis of FEV_1

After adjustment for significant covariates (table 2), narrow-sense heritability (h^2_N, the proportion of phenotypic variance explained by genetic factors) for pre-bronchodilator FEV_1 was 24.0% (SD 6.8%; $p = 6\times10^{-6}$). Heritability was similar for post-bronchodilator measurements of FEV_1 (h^2_N = 23.0% (SD 6.5%), $p = 3\times10^{-6}$).

Table 2 lists the highest LOD scores from the genome-wide linkage analyses of FEV_1 in all subjects, in non-smokers and in

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Table 1 Characteristics of families of children with asthma

Family	Individuals	Former and current smokers* n (%)	Asthma† n (%)	Mean (SD) FEV_1, % predicted‡		Mean (SD) FEV_1/FVC, % predicted‡		Mean (SD) bronchodilator response¶**
				Pre-BD§	Post-BD¶	Pre-BD§	Post-BD¶	
1	34	8 (23.5)	12 (35.3)	96.1 (16.8)	101.1 (13.5)	94.5 (8.7)	97.2 (8.7)	6.6 (12.1)
2	18	4 (23.5)	7 (38.9)	103.9 (24.0)	109.2 (27.4)	97.8 (6.9)	101.2 (7.0)	6.1 (10.7)
3	224	37 (16.5)	56 (25.1)	96.6 (14.8)	100.3 (14.5)	95.8 (7.0)	98.7 (7.1)	4.3 (7.2)
4	97	17 (17.5)	16 (16.5)	102.6 (17.4)	107.0 (17.0)	96.9 (8.0)	99.7 (7.8)	5.0 (7.3)
5	8	5 (62.5)	2 (28.6)	100.1 (13.2)	102.5 (13.4)	98.1 (7.7)	101.4 (5.5)	2.6 (6.8)
6	107	10 (9.4)	8 (7.5)	99.7 (16.4)	102.7 (16.0)	98.5 (6.7)	101.0 (5.7)	3.1 (7.3)
7	23	9 (39.1)	5 (21.7)	103.9 (12.4)	108.8 (11.4)	98.1 (5.6)	101.4 (4.5)	5.0 (5.3)
8	129	12 (9.3)	24 (18.6)	96.8 (13.9)	100.0 (13.1)	96.9 (8.7)	99.3 (7.5)	3.4 (7.7)
All	640	102 (16.0)	130 (20.4)	98.5 (15.8)	102.3 (15.4)	96.8 (7.6)	99.5 (7.2)	4.2 (7.7)

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; BD, bronchodilator.
*n = 638 individuals with complete data on smoking.
†Physician-diagnosed asthma and wheezing within the past year. n = 637 subjects with complete data.
‡Based on prediction equations for Mexican-Americans in Hankinson *et al*.[22]
§n = 640.
¶n = 624. Sixteen individuals did not have post-bronchodilator spirometry.
**As a percentage of baseline FEV_1 (BDRbase).

smokers only; full results are available in Supplementary table 1 (available online at http://thorax.bmj.com/supplemental). In the genome-wide linkage analysis of pre-bronchodilator FEV_1 in all subjects, the highest LOD score (1.63 at 127 cM) was found on chromosome 6q. Four additional genomic regions (chromosomes 4q, 6p, 7p and 16q) showed modest evidence of linkage (LOD >1) to pre-bronchodilator FEV_1. After excluding the phenotypic data of former and current smokers from the analysis, there was only modest evidence of linkage to pre-bronchodilator FEV_1 on chromosomes 4q and 9q. In smokers-only, the highest LOD score was on chromosome 3q (LOD = 2.74 at 241 cM). Chromosomes 4q and 8p also showed suggestive evidence of linkage.

In the genome-wide linkage analysis of post-bronchodilator FEV_1 in all subjects, there was suggestive evidence of linkage to chromosome 7q (LOD = 2.13 at 150 cM)[23] and modest evidence of linkage (LOD >1) to chromosomes 1p, 2p, 15q and 16q. Nine additional STR markers were genotyped on chromosome 7q, the region with the highest LOD score in the analyses of all subjects. With the additional markers, the LOD score for post-bronchodilator FEV_1 increased to 2.45 (at 152 cM) in all subjects (fig 1). Among non-smokers only, chromosomes 1p, 7q and 14q showed modest evidence of linkage to post-bronchodilator FEV_1. After inclusion of additional markers on chromosome 7q, there was suggestive evidence of linkage (LOD = 2.14) in non-smokers. Chromosomes 2p, 4q (LOD = 2.66 at 138 cM), 8p and 14q all showed suggestive evidence of linkage in smokers only.

Analysis of FEV_1/FVC

The estimated heritability was 19.9% (SD 7.8%; $p = 2\times10^{-4}$) for pre-bronchodilator values of FEV_1/FVC ratio and 15.4% (SD 6.6%; p = 0.001) for post-bronchodilator values. Table 2 and Supplementary table 2 (available online at http://thorax.bmj.com/supplemental) show the results of the genome-wide linkage analysis of FEV_1/FVC. In the genome-wide linkage analysis of pre-bronchodilator FEV_1/FVC in all subjects, the highest LOD score was on chromosome 2q (LOD = 1.53 at 245 cM). There was also modest evidence of linkage to pre-bronchodilator FEV_1/FVC on chromosomes 4q and 7q. Among non-smokers there was modest evidence of linkage to pre-bronchodilator FEV_1/FVC on chromosomes 2p, 3q, 4q (LOD = 1.50 at 95 cM), 6p, 7q and 13q. In smokers only the highest LOD score was on chromosome 9q (LOD = 1.52 at 72 cM).

For post-bronchodilator measurements of FEV_1/FVC, the highest LOD score in all subjects was on chromosome 7p (LOD = 1.40 at 74 cM). There was also modest evidence of linkage to post-bronchodilator FEV_1/FVC on chromosomes 1q, 3p, 4q and 20q. Among non-smokers there was modest evidence of linkage to post-bronchodilator FEV_1/FVC on chromosomes 2p, 3p, 4q, 5q, 6p, 7p and 18q. In smokers only the LOD score of 3.27 on chromosome 5p (49 cM) approached genome-wide significant evidence of linkage.[23]

Analysis of bronchodilator responsiveness

The three measures of bronchodilator responsiveness (log_{10} transformed) were significantly heritable (BDRbase: $h^2_N = 10.5\%$ (SD

Table 2 Genome-wide linkage analysis for FEV_1 and FEV_1/FVC in all subjects, non-smokers and smokers only

Phenotype	Bronchodilator	Subjects	Covariates	Chromosome	cM	LOD	p Value
FEV_1	Pre	All	Age, age^2, ht, ht^2, wt^2, gender, smoker	6	127	1.63	0.004
		Non-smokers	Age, age^2, ht, ht^2, wt^2, gender	9	153	1.09	0.014
		Smokers	Age, age^2, ht, gender	3	241	2.74	<0.001
	Post	All	Age, age^2, ht, ht^2, wt^2, gender	7	150	2.13	0.002
		Non-smokers	Age, age^2, ht, ht^2, wt^2, gender	7	150	1.73	0.003
		Smokers	Age, age^2, ht, gender	4	138	2.66	<0.001
FEV_1/FVC	Pre	All	Age	2	245	1.53	0.004
		Non-smokers	Age, ht	4	95	1.50	0.004
		Smokers	Age, age^2	9	72	1.52	0.002
	Post	All	Age, age^2, ht, wt	7	74	1.40	0.007
		Non-smokers	Age, ht, wt	18	117	1.46	0.006
		Smokers	Age, age^2, packs	5	49	3.27	<0.001

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; ht, height; smoker, ever smoker; packs, pack-years; wt, weight.
Age^2, ht^2 and wt^2 represent the quadratic terms for age, height and weight, respectively.
The maximum LOD score for each analysis is reported. Full results are available in supplementary tables 1 and 2.

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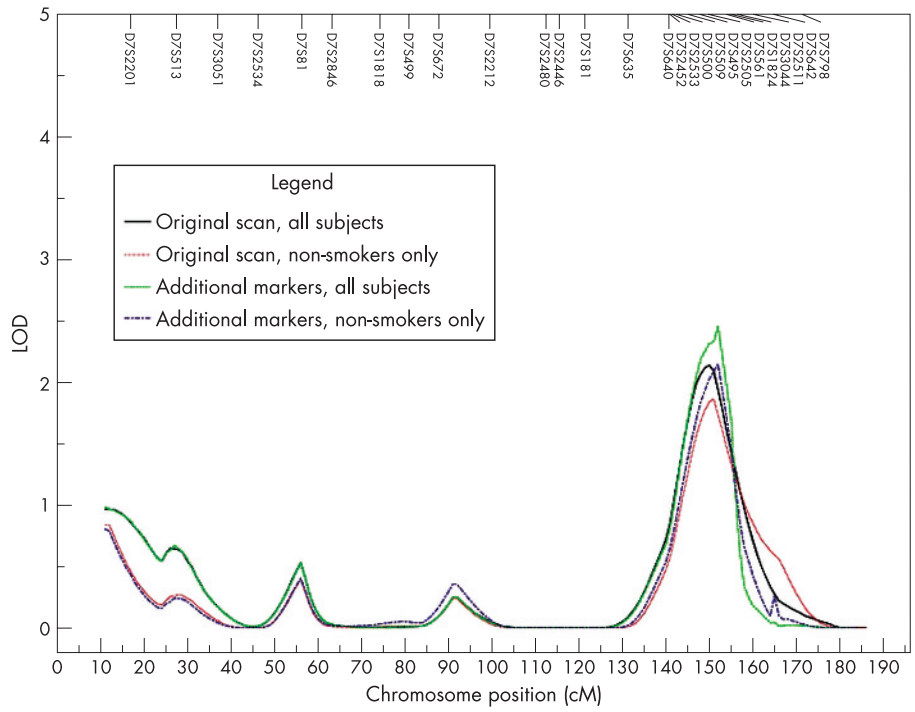


Figure 1 Linkage analysis on chromosome 7 for post-bronchodilator FEV_1 in all subjects and in non-smokers only using short tandem repeat (STR) markers from the initial genome scan and including nine additional STR markers.

6.1%), p = 0.01; BDRpred: h^2_N = 10.4% (SD 6.1%), p = 0.01; BDRabs: h^2_N = 8.0% (SD 5.6%), p = 0.04), although the heritability estimates were lower than those of the pulmonary function measures. No covariates were significant in the variance component models of the three BDR outcomes in all subjects and in non-smokers. In the genome-wide linkage analysis of BDRbase (log_{10} transformed), the highest LOD score was found on chromosome 4q (LOD = 1.26 at 70 cM; table 3 and supplementary table 3 (available online at http://thorax.bmj.com/supplemental)). A LOD score >1 was found in this location in the analysis of BDRpred. In the analyses of BDRpred and BDRabs, the highest LOD scores were found on chromosome 9p at 49 cM (BDRpred: LOD = 1.25, BDRabs: LOD = 1.53). Despite the reduced sample size, the LOD scores were increased on chromosomes 4q and 9p in analyses restricted to non-smokers only. In non-smokers only, a region on chromosome 10p had LOD scores >1 for all three BDR phenotypes. In smokers, regions on chromosomes 12q and 16q had LOD scores >1 for all three BDR measures.

Statistical power

Our statistical power to detect a LOD score ⩾1 was 42% for traits with h^2_N = 10% (eg, BDRbase) and 98% for traits with h^2_N = 25% (eg, pre-bronchodilator FEV_1). For LOD scores ⩾2, our power ranged from 13.6% for traits with h^2_N = 10% to 87% for traits with h^2_N = 25%. Finally, our power to detect a LOD score ⩾3 ranged from 4% for traits with h^2_N = 10% to 67% for traits with h^2_N = 25%.

DISCUSSION

In families of schoolchildren with asthma in Costa Rica, we found significant genetic contributions (heritability) to inter-individual variation in measures of pulmonary function and BDR. The heritability estimates of FEV_1 were similar in magnitude to previous studies,[24] but the heritabilities of FEV_1/FVC and BDR were lower than have been reported.[7] Because of low heritability, our study had limited statistical power to detect linkage to BDR. However, we had adequate statistical power to detect and found genome-wide suggestive evidence of linkage[23] to post-bronchodilator FEV_1, on chromosome 7q34–35, which was improved after inclusion of additional STR markers.

Although no significant or suggestive evidence of linkage was found in the other genome-wide linkage analyses in all subjects, we uncovered potential regions of interest, including chromosome 9p for BDR and chromosome 2q for FEV_1/FVC. Despite a small number of smokers and correspondingly limited power, the evidence for linkage for FEV_1/FVC (post-bronchodilator) on chromosome 5p approached genome-wide significance.

Several authors have reported genome-wide linkage analyses for spirometric measures of pulmonary function in families ascertained through probands with asthma; however, the present study is the only genome-wide linkage analysis of FEV_1 and FEV_1/FVC in a Hispanic population. In a study of 2551 members of 533 families in China, Xu *et al*[1] found the strongest evidence for linkage to FEV_1 on chromosomes 10p and 22q. In 591 individuals in 202 Australian families, Ferreira *et al*[2] showed suggestive evidence of linkage to FEV_1 on chromosomes 5q, 8p, 12q, 17q and 20q and to FEV_1/FVC on chromosomes 4q, 9q and 12q. Postma *et al*[3] showed different regions of linkage to FEV_1 in genome-wide analyses of all subjects, smokers and non-smokers among 1183 members of 200 Dutch families. They reported genome-wide significant evidence of linkage to FEV_1/FVC (both pre-bronchodilator and post-bronchodilator) on chromosome 2q.

There have been no previous reports of suggestive or significant evidence of linkage to FEV_1 on chromosome 7q. However, the genome-wide linkage analyses of pulmonary function described above were completed in populations of European and Asian descent. A unique aspect of our study is that we were able to recruit large extended pedigrees of children with asthma because of detailed genealogical records and low migration out of the relatively genetically isolated

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Table 3 Genome-wide linkage analysis for bronchodilator responsiveness in all subjects, non-smokers and smokers only

Phenotype*	Subjects	Covariates	Chromosome	cM	LOD	p Value
BDRbase	All	None	4	70	1.26	0.008
	Non-smokers	None	10	31	1.59	0.002
	Smokers	Gender	16	66	1.31	0.019
BDRpred	All	None	9	49	1.25	0.007
	Non-smokers	None	10	31	1.69	0.002
	Smokers	Gender	16	66	1.22	0.018
BDRabs	All	None	9	49	1.53	0.003
	Non-smokers	None	9	49	1.72	<0.001
	Smokers	Gender	16	66	1.60	0.012

The maximum LOD score for each analysis is reported. Full results are available in supplementary table 3.
*Definitions of BDR phenotypes[7]:
BDRbase = ($FEV_{1post\text{-}bronchodilator}$-$FEV_{1pre\text{-}bronchodilator}$)/$FEV_{1pre\text{-}bronchodilator}$ ×100%.
BDRpred = ($FEV_{1post\text{-}bronchodilator}$-$FEV_{1pre\text{-}bronchodilator}$)/$FEV_{1predicted}$ ×100%.
BDRabs = ($FEV_{1post\text{-}bronchodilator}$-$FEV_{1pre\text{-}bronchodilator}$).
All three BDR variables were log_{10} transformed for analysis.

population of the Central Valley of Costa Rica. Our statistical power to detect linkage to lung function measures may have been increased by inclusion of extended pedigrees (which offer more power for linkage analysis of quantitative traits than sib-pair studies with the same sample size)[25] and by founder effects leading to relatively few susceptibility genes for asthma-related traits in Costa Rica. On the other hand, genetic heterogeneity in determinants of lung function among the Spanish and Amerindian founders of the population of the Central Valley may have hindered our statistical power. Although the characteristics of the Costa Rican population may limit the generalisability of our results, it should be noted that the G protein coupled receptor-154 (*GPR154*) was first identified as a potential asthma-susceptibility gene in a genetically isolated population in Finland[26] and then shown to be relevant in other European nations.[27 28] Thus, some of our results may be relevant across ethnic groups and others may be more relevant to Costa Ricans and other Hispanic groups of predominant Spanish and Amerindian ancestry.

Genome-wide linkage analyses of pulmonary function have also been performed in families from the general population[24 29–31] and in families of probands with severe, early-onset COPD.[32] Several of the regions of interest (LOD ⩾1.5) in our study are similar to the findings in those studies (table 4), suggesting that some genomic regions are likely to contain genetic variants that influence pulmonary function in normal individuals, in patients with COPD and in those with asthma. Some of these regions may be relevant across different ethnic groups as well. In our analysis, the highest LOD score for FEV_1/FVC was found on chromosome 2q. This region overlaps the linkage peaks for FEV_1/FVC in families from the general population in Utah[31] and in families from the Boston Early-Onset COPD Study.[32] The FEV_1/FVC linkage found in Dutch asthma families is also located on chromosome 2q, but closer to the centromere.[3]

The highest LOD score in any of the genome scans was found for FEV_1/FVC (post-bronchodilator) on chromosome 5p13 in an analysis limited to former and current smokers. Despite the limited sample size, the LOD score of 3.27 approached genome-wide significance,[23] possibly identifying a locus (or loci) for smoking-related air flow obstruction in families with a genetic predisposition to asthma, consistent with the Dutch hypothesis, which proposes a common origin for asthma and COPD. Several cadherin genes (CDH-6, 9 and 10) are located on chromosome 5p13. E-cadherin (CDH1), another member of the cadherin family, is a cell adhesion molecule involved in epithelial permeability in allergic asthma.[34] The importance of other cadherin genes in asthma and COPD is unknown.

Although other studies of asthma have analysed both pre-bronchodilator and post-bronchodilator spirometry, the only previous genome-wide linkage analysis of BDR as a distinct phenotype is from the Boston Early-Onset COPD Study.[7] An analysis limited to chromosome 12q examined BDR in families from the Childhood Asthma Management Program Study[35]; the present study is the first reported genome-wide linkage analysis of BDR in families of subjects with asthma. Similar to the Boston Early-Onset COPD Study, we found significant heritability of BDR, although the heritability estimates in our asthma families (h^2_N range 8.0–10.5% for the three BDR measures) are lower than those found in the COPD families (h^2_N range 10.1–26.3%). As in our study, no significant linkage for BDR measurements was found in the Boston Early-Onset COPD Study, although regions on chromosomes 3q and 4q had LOD scores of ⩾1.5.

The ability to detect linkage to measures of BDR may be limited by the day-to-day variability in BDR that is inherent in

Table 4 Overlapping regions of linkage for pulmonary function phenotypes

	Present study		Previous studies		
Chromosome	Phenotype	LOD score (cM)	Author, year	Phenotype/Population	LOD score (cM)
2	FEV_1/FVC, pre-BD	1.53 (245)	Palmer, 2003[7]	FEV_1/FVC, post-BD/Early-onset COPD	4.42 (222)
			Malhotra, 2003[31]	FEV_1/FVC, pre-BD/General population	2.36 (216–251)*
			Postma, 2005[3]	FEV_1/FVC, pre-BD/Asthma	4.92 (195)
4	FEV_1/FVC, pre-BD	1.50 (95)	Wilk, 2003[30]	FEV_1/FVC, pre-BD/General population	1.64 (89.3)
			Ferreira, 2005[2]	FEV_1/FVC, pre-BD/Asthma	2.07† (94)
7	FEV_1, pre-BD	1.53 (11)	Bouzigon, 2004[33]	FEV_1 % predicted, pre-BD/Asthma	2.17 (17.7)

Chromosomal regions with LOD score ⩾1.5 in the present study (all subjects) and in at least one previous study are shown.
*1−LOD drop support interval.
†−log_{10} (p Value).

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asthma. It is not clear which definition of BDR is most useful in genetic studies, so we used three commonly accepted measures.[7] The fact that many of the regions with LOD scores >1 were similar across the analyses of two or all three BDR variables implies that these three definitions are likely to reflect the same underlying phenotype. The concordance of results is not perfect, as some regions were found in only one of the analyses of the BDR variables. The heritability estimates for the BDR measures were lower than those of the spirometric traits, although all heritabilities were statistically significant. This may also reduce the ability to detect significant linkage for BDR.

Additionally, as all subjects did not have post-bronchodilator spirometry, the power in the analyses of post-bronchodilator traits and BDR may be reduced. However, this power reduction should be minimal, as only 16 subjects did not complete the post-bronchodilator measurements. Most patients with asthma (especially children) will have pulmonary function test values within the normal range; our study is no exception (table 1). The limited phenotype range may also limit power in genetic studies. Despite this, we were able to find suggestive evidence for linkage to FEV_1.

Post-bronchodilator spirometry is less likely to have substantial day-to-day variability in asthma, as the post-bronchodilator values generally reflect underlying lung function.[7] In our analysis, the strongest linkage evidence was for post-bronchodilator FEV_1. However, pre-bronchodilator spirometry may be more variable within an individual subject and may be more reflective of asthma symptoms and severity. Because the analyses of pre-bronchodilator and post-bronchodilator spirometry may yield different information on lung development, asthma severity and asthma susceptibility, we chose to perform genome scans on both pre-bronchodilator and post-bronchodilator phenotypes.

Even though we did not find genome-wide significant evidence of linkage for pulmonary function or BDR, the suggestive evidence of linkage to FEV_1 on chromosome 7q warrants further investigation. Several plausible asthma candidate genes are located in this region, including the T cell receptor, β subunit (*TRB@*) and endothelial nitric oxide synthase (*NOS3*). Polymorphisms in *NOS3* have been associated with asthma in some studies[36 37] but not in others.[38 39] As in any genetic analysis, our findings may be due to chance or to causal genetic variants. Although our results were adjusted for multiple testing in the setting of a genome-wide linkage analysis of a single phenotype, we did not adjust for testing of multiple traits because of correlation among lung function phenotypes. Because current methods for association studies cannot be used in a small number of extended pedigrees, we plan to assess our findings further by testing for an association between variants in candidate genes on chromosome 7q34–35 and FEV_1 in nuclear families of children with asthma in Costa Rica.

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Supplementary tables 1, 2 and 3 available online at http://thorax.bmj.com/supplemental

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