Genomewide Screen for Pulmonary Function in 200 Families Ascertained for Asthma

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Changes in pulmonary function are important in determining asthma outcome. Genetic factors may influence airway obstruction in asthma. We performed a genomewide screen in 200 families of probands objectively diagnosed with asthma in the 1960s to identify chromosomal regions related to changes in pre- and postbronchodilator lung function (FEV_1 , VC, and $FEV_1\%VC$) and assess influences of early-life smoke exposure. Smoking (pack-years), age, sex, and height were covariates in variance component analyses. Significant evidence for linkage of pre- and postbronchodilator $FEV_1\%VC$ was obtained for chromosome 2q32 (LOD, 4.9, increasing to 6.03 with additional fine-mapping markers, and 3.2, respectively). Linkage existed for chromosome 5q for pre- and postbronchodilator VC (likelihood of disease [LOD], 1.8 and 2.6, respectively). Results for pre- and postbronchodilator FEV_1 were less significant (LOD, 1.5 and 1.6, chromosomes 11p and 10q, respectively). Results were not affected by passive smoke exposure. There is significant evidence for linkage of $FEV_1\%VC$ to chromosome 2q32 in families of probands with asthma, 35 cM proximal from linkage previously observed in families of probands with early-onset chronic obstructive pulmonary disease. Thus, there may be multiple genes on chromosome 2q that are important in determining presence and degree of airflow limitation in families ascertained for obstructive airway disease.

Keywords: asthma; function; genes; linkage; lung

Asthma is a common respiratory disorder that is caused by the complex interactions between susceptibility genes and exposures to a diverse group of environmental stimuli (1). Most patients with asthma have a disease that is characterized by mild, intermittent, reversible airway obstruction. However, already in childhood (2) and in severe disease, there may exist more progressive and/or less reversible airway obstruction (3, 4). Delineating the biologic mechanisms underlying persistent changes in pulmonary function is important to guide asthma therapy (5). Therefore, it is of interest to assess which genes are associated with alterations in pulmonary function in asthma.

Pulmonary function has an important role in health and disease and is an important predictor of survival in the general population (6). Family studies have suggested that 20 to 60% of the total variance in lung function may be accounted for by familial factors (7–12). Furthermore, Redline and coworkers (13) reported that monozygotic twins have a significantly higher

concordance for spirometric indices (0.72) than nonidentical twins (0.27). However, estimates of heritability have varied largely, ranging from just over 0 to 100% (10–17). Three genomewide screens in population-based families (9, 11, 18) and one in a founder population (12) have shown linkage of either FEV_1 , FEV_1 or the ratio of FEV_1 and FVC ($FEV_1\%FVC$) with chromosomal regions. Some chromosomal regions provided replication: for example, chromosome 5 (11, 12) and chromosome 4 (9, 18), a region where linkage with $FEV_1\%FVC$ in families from probands with early-onset chronic obstructive pulmonary disease (COPD) also had been found (19). Finally, a genome scan of data from Chinese patients with asthma suggested several regions of linkage (e.g., chromosome 10 and 22 for FEV_1 and chromosome 16 for FVC) (20). These results provide further evidence for a heritable component of lung function in the general population, families with asthma, and families with COPD (although with different or overlapping chromosomal regions). It is now accepted that asthma is caused by the interaction of genetic susceptibility coupled with exposure to environmental factors (1). Smoking is an important environmental stimulus affecting the development of both asthma and reduced lung function (21–28). *In utero* cigarette smoke exposure and exposure in the first few months of life appear to be important risk factors for the development of atopy and asthma (21–24) and with persistent reduced lung function (26). It therefore seems important to investigate whether a gene–smoking interaction exists with respect to alterations in lung function in asthma.

Our cohort of Dutch families with asthma presents a unique opportunity to further analyze susceptibility genes for asthma and related pulmonary function phenotypes. The 200 families were ascertained through a parent (proband) who had been objectively diagnosed with asthma in the 1960s and who was reevaluated with his or her family members between 1990 and 2000. Pulmonary function testing was performed in all family members, and data are available on passive smoke exposure in the children as well as on current and past smoking habits of each individual, which allows us to evaluate this gene–environment interaction. The purposes of the current study are to report the results of a genomewide screen with linkage analyses for FEV_1 , VC, and the ratio of FEV_1/VC , and to assess the effects of passive smoke exposure and individual smoking history as interacting factors.

METHODS

Family Ascertainment

Two hundred families (1,183 individuals) were ascertained through probands who were initially studied between 1962 and 1975 at Beatrixoord Hospital, Haren, The Netherlands, a regional referral center for patients with obstructive airway diseases (28). Patients with symptomatic asthma who were not experiencing a current asthma exacerbation were referred to this hospital and admitted for a standardized, comprehensive evaluation for asthma and atopy. At the time of initial testing, all probands

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had asthma symptoms, were hyperresponsive to histamine (provocation concentration causing a fall of $\geq 20\%$ in FEV₁ [PC₂₀] histamine < 32 mg/ml), and were younger than 45 years. The families of 200 of these original probands, with their spouses, children, children's spouses, and grandchildren older than 6 years, were recruited and evaluated in the 1990s (28, 29). The Medical Ethics Committee of the University Hospital Groningen and Wake Forest University School of Medicine approved this study, and all participants provided informed consent.

Clinical Evaluation

Standardized pulmonary function testing was performed with a water-sealed spirometer according to American Thoracic Society criteria, as reported previously (4). All patients refrained from bronchodilators for the appropriate time, and all stopped inhaled steroids for 2 weeks before pulmonary function testing. Reversibility was assessed measuring spirometry before and 15 minutes after inhaling 800 μ g of salbutamol. Bronchial responsiveness to histamine was measured using the 30-second inhalation histamine challenge test developed by De Vries and coworkers (30) because this method was used for testing the probands in the 1960s. Skin testing to common allergens and measurements of total and specific IgE were performed in all family members (31).

Genotyping

DNA was isolated from peripheral blood using standard methods (Puregene kit; Gentra, Inc., Minneapolis, MN) (30). For the genome-wide screen, the Weber version 8 set of markers (Marshfield Center for Medical Genetics, Marshfield, WI) was used, which spans the human genome at an average interval of approximately 10 cM, and consists of 366 polymorphic autosomal markers. An additional seven fine-mapping markers were genotyped on 2q in our area of strongest linkage (FEV₁%VC). Multiplex polymerase chain reaction was performed using fluorescently labeled primers. Polymerase chain reaction products were separated on denaturing polyacrylamide gels, and the fragments were detected using ABI 377 sequencers (PerkinElmer Applied Biosystems, Boston, MA). The fragments were scanned and scored using ABI software. A modified version of the program Linkage Designer was used to bin the alleles and check inheritance (32). Genotyping errors, double recombinants, and inheritance inconsistencies were detected using the Linkage (33) and Cri-Map (34) software. Marker allele frequencies were estimated from the pedigree founders.

Statistical Methods

Heritability was estimated with the variance component approach and implemented using the SOLAR (Sequential Oligogenic Linkage Analysis Routines) program (35). Linkage analysis for the quantitative variables FEV₁ and VC and the ratio of FEV₁ and VC (FEV₁%VC) was performed using variance components analysis, as implemented in SOLAR, with degree of individual smoking (pack-years), age, sex, and height as covariates. The square of the FEV₁%VC was used to obtain a normal distribution for this parameter. To determine the effect of exposure to cigarette smoke in children on lung function, families were divided by smoking status of the proband. Although most probands did not have a significant

history of smoking at the time of their original evaluation in the 1960s, approximately half of the probands ($n = 95$) are now current or ex-smokers with a family history that is consistent with passive smoke exposure to their children. There was also a strong relationship in smoking status between the probands and their spouses (statistics [S] = 5.9; $p = 0.015$, McNemar's test). The genomewide screen for FEV₁, VC, and FEV₁%VC was performed for the entire sample and then separately for the smoking-exposed and nonexposed families. Results of the genomewide scan are reported where likelihood of disease (LOD) scores greater than 1.5 were identified.

RESULTS

Demographics

The characteristics of the family members are reported for the probands, their spouses, and their first- and second-degree offspring (Table 1). As reported previously (31), all probands had initially symptomatic asthma with reversible airway obstruction accompanied by airway hyperresponsiveness. The probands showed evidence of airway obstruction with a postbronchodilator FEV₁%VC of 64.7 and a reduced FEV₁%predicted of 82.4. On average, spouses and offspring had normal pulmonary function.

FEV₁

Heritability was estimated at 0.33 and 0.29 for pre- and post-bronchodilator FEV₁, respectively (Table 2). LOD score values greater than 1.5 were observed for chromosomes 11p and 10q; specifically, prebronchodilator FEV₁ with an LOD of 1.50 to D11S1392 and postbronchodilator FEV₁ with an LOD of 1.64 to D10S677 (Figure 1). Individual smoking was not a significant covariate in these models. Passive smoke exposure in early childhood did not explain the observed linkages: all LOD scores were less than 1.5 in the above-mentioned regions in the families with smoke exposure in the offspring. In families without passive smoke exposure, an LOD score of 1.55 was found for prebronchodilator FEV₁ with chromosome 5q (marker D5S820) and 1.59 for postbronchodilator FEV₁ with chromosome 3 (marker D3S2387).

VC

Heritability was estimated at 0.29 and 0.32 for pre- and postbronchodilator VC, respectively. LOD score values greater than 1.5 were observed for chromosome 5q; with an LOD equal to 1.80 for the prebronchodilator VC at D5S816 and 2.58 for the postbronchodilator VC at the same map position (Figure 1, Table 2). Age, sex, and height were significant covariates in the resulting model, but individual smoking was not. No LOD scores greater than 1.5 were observed in the families without passive smoke exposure. In

TABLE 1. CLINICAL CHARACTERISTICS OF 200 DUTCH FAMILIES OF PROBANDS WITH ASTHMA

	Probands	Spouses	Children	Grandchildren
No.	200	201 [†]	540	186
Age, yr*	52.1 (8.4)	51.0 (9.2)	24.3 (9.2)	12.1 (5.6)
Male, %	62.0	37.8	45.0	49.3
Total IgE, kU/L*	92.9 (1.0–2,880)	26.2 (0.5–1,940)	64.1 (0–3,360)	62.4 (0.5–3,710)
Positive skin test	81.9	31.0	54.1	38.0
FEV ₁ % predicted, prebr	69.6	98.4	93.7	90.5
FEV ₁ % predicted, postbr	82.4	103.9	100.2	97.0
FEV ₁ %VC prebr, %	59.7	76.7	81.0	82.6
FEV ₁ %VC postbr, %	64.7	79.4	85.2	86.7
≥ 5 pack-yr smoking, %	47.5	58.2	24.6	7.3

Definition of abbreviations: postbr = postbronchodilator; prebr = prebronchodilator.

Total IgE = geometric mean.

* Mean values with range.

[†] One proband married twice; both spouses participated in the study.

TABLE 2. OVERVIEW OF LOD SCORES ≥ 1.5 WITH LOCATIONS AND MARKERS IN THE GENOME SCREEN OF LUNG FUNCTION PARAMETERS IN FAMILIES OF PROBANDS WITH ASTHMA

Trait	Method	Set	Model					Position		Chromosomal Marker [‡]
			Age	Sex	Height	Pack-yr*	H ² R	cM [†]	LOD	
FEV ₁	Pre	All	+	—	+	—	0.33	57	1.50	D11S1392
		ns	+	+	+	—	0.47	159	1.55	D5S820
		smk	+	—	+	—	0.18	123	1.21	D11S4464
	Post	All	+	+	+	—	0.29	118	1.64	D10S677
		ns	+	+	+	—	0.34	6	1.59	D3S2387
		smk	+	—	+	—	0.23	153	1.46	D3S1764
VC	Pre	All	+	+	+	—	0.29	140	1.80	D5S816
		ns	+	+	+	—	0.31	261	0.97	D2S125
		smk	+	+	+	—	0.25	156	1.32	D7S2195
	Post	All	+	+	+	—	0.32	140	2.58	D5S816
		ns	+	+	+	—	0.30	140	1.08	D5S816
		smk	+	+	+	—	0.32	156	1.75	D7S2195
FEV ₁ %VC	Pre	All	+	+	—	—	0.44	195	4.92	D2S1384
		ns	+	+	—	+	0.60	56	2.21	D14S587
		smk	+	+	—	+	0.10	3	1.26	D21S1432
	Post	All	+	+	—	+	0.36	195	3.17	D2S1384
		ns	+	+	—	+	0.54	147	1.81	D5S1480
		smk	+	+	—	+	0.31	196	0.79	D2S1384

Definition of abbreviations: H²R = heritability; LOD = likelihood of disease; post = postbronchodilator measurement; pre = prebronchodilator measurement; smk = smoking; ns = nonsmoking.

Results are stratified for effect of passive smoke exposure. +, significant covariate in the model; —, not a significant covariate in the model.

* Pack-years smoking.

[†] cM position of linkage peak based on Marshfield Foundation genetic map.

[‡] Closest marker to linkage peak.

the families with smoke exposure in the offspring, an LOD of 1.75 for postbronchodilator VC on chromosome 7 (marker D7S2195) was observed (Table 2).

FEV₁%VC

Heritability was estimated at 0.44 and 0.36 for pre- and postbronchodilator FEV₁%VC, respectively. Age and sex were significant covariates ($p < 0.001$), but height did not significantly contribute; individual pack-years of smoking contributed to postbronchodilator FEV₁%VC only. The genomewide screen for FEV₁%VC showed strong evidence for linkage to chromosome 2q, with an LOD score of 4.92 for prebronchodilator ratio and 3.17 for postbronchodilator ratio, both at D2S1384 (Figure 2, Table 2). An additional seven marker microsatellites were genotyped in the region of linkage on 2q with the highest LOD score, and the LOD increased to 6.0249 at D2S1391 for prebronchodilator FEV₁%VC. The 1.5 LOD support interval goes from midway between D2S1776 and D2S1391 (centromeric) to midway between D2S1384 and D2S434 (telomeric). Passive smoking did not contribute to the linkage observed with pre- and postbronchodilator FEV₁%VC. In the families without passive smoke exposure in the children, there were four regions with LODs greater than 1.5 for prebronchodilator FEV₁%VC, with the highest LOD being observed on chromosome 14 (LOD score, 2.21 at D14S587). Furthermore, there were three regions with LOD scores greater than 1.5 for postbronchodilator FEV₁%VC, with the highest LOD on chromosome 5 (1.81 at D5S1480). LOD scores greater than 1.00 of all lung function variables are shown in Table E1 in the online supplement.

DISCUSSION

The results of this study report a high level of evidence for linkage of airway obstruction as assessed by FEV₁%VC to chromosome 2q32 in families ascertained through a proband diagnosed with asthma in the 1960s. The LOD scores were 4.9 and 3.2 for pre- and postbronchodilator FEV₁%VC, respectively,

approximately 6 cM from the region where we have previously observed linkage with total serum IgE in the same families (27). Furthermore, the addition of seven fine-mapping markers increased the LOD score for postbronchodilator FEV₁%VC to 6.03. These findings strongly support the presence of gene(s) that modulate airway obstruction in this region of chromosome 2q. Another important region for linkage of lung function variables was chromosome 5q32. LOD scores of pre- and postbronchodilator VC were 1.8 and 2.6, respectively. Interestingly, this was in the same chromosomal region we found suggestive evidence for linkage with postbronchodilator FEV₁%VC (LOD score, 1.77) in families with children not exposed to cigarette smoke. Finally, the knowledge of personal smoking habits of all participants and of passive smoke exposure during early life in the offspring of the probands with asthma allowed us to evaluate the effects of this environmental factor on linkage of lung function parameters. Thus, we showed that these linkage results were not influenced by active and passive smoke exposure.

We have previously reported that there is evidence of linkage for total serum IgE levels in this same region on chromosome 2q32 as for FEV₁%VC. The maximum LOD score for IgE was 3.16 near marker D2S2314 (36, 37), whereas the maximum LOD score for FEV₁%VC was 4.92 near marker D2S1384. This suggests that there may be gene(s) related to both atopy and pulmonary function located in this region. Interestingly, we found a significant but modest correlation ($r = 0.33$, $p < 0.01$) between FEV₁%VC and total serum IgE levels in all family members, suggesting the presence of similar genes affecting IgE and FEV₁%VC. Alternatively, because the explained variance is small, different genes or multiple genes may interact in various ways with each other or with environmental stimuli to regulate the expression of these two asthma phenotypes.

In performing genetic studies of parameters related to pulmonary function, it is crucial to understand the ascertainment, clinical characterization, and type of family population that are studied to compare results. In this study, we are not only evaluat-

Figure 1. Genomewide screen for major genes regulating pre- and postbronchodilator FEV₁ and VC values in 200 Dutch families from a proband with asthma. (A) Prebronchodilator FEV₁; (B) postbronchodilator FEV₁; (C) prebronchodilator VC; (D) postbronchodilator VC.

Figure 1. Continued

ing decreases in lung function due to normal aging only but also those due to the presence of obstructive airway disease primarily caused by asthma. Therefore, we would expect our results to be more similar to those reported by Silverman and coworkers (19), who ascertained families through a proband with early-onset COPD, rather than similar to those obtained by population-based family studies (9, 11, 12, 18). Indeed, three (9, 12, 18) of these four articles did not report strong evidence for linkage of $FEV_1\%FVC$ (no significance [12, 18] or highest $LOD = 1.4$ on 6q [9]) in their genome screen, whereas Silverman and colleagues (19) demonstrated strong evidence for linkage of both pre- and postbronchodilator $FEV_1\%FVC$ to chromosome 2q. Furthermore, Allen and coworkers (38) found an association with asthma on 2q14.1 and, more specifically, of *DPP10*, a gene encoding a homolog of dipeptidyl peptidases (DPP), which cleaves terminal dipeptides from cytokines and chemokines. The highest LOD score for $FEV_1\%VC$ we found was approximately 35 cM proximal to the peak reported by Silverman and colleagues (19) and approximately 60 cM distal to the *DPP10* locus. Because the latter two studies reported only genomewide screen markers and used the FVC, whereas we used slow vital capacity, it is difficult to determine whether these represent actual different linkage peaks. Moreover, it was not stated whether pre- or postbronchodilator values for spirometry were used by Silverman and colleagues. A later publication (39) showed that post-bronchodilator $FEV_1\%FVC$ was significantly associated with the same location. Thus, the remaining obstruction after inhalation of a bronchodilator was linked, which the authors suggest reflects the presence of fixed airflow limitation that is characteristic of COPD. We extend this observation in that post-bronchodilator $FEV_1\%VC$ is also associated with genes on chromosome 2q in families of a proband with asthma. Although the linkage peaks observed in the COPD study and this study appear to be distinct, it is possible that they represent linkage to the same locus. In this case, the similar linkage region for both $FEV_1\%VC$ and $FEV_1\%FVC$ in families with asthma and COPD may lend support to the hypothesis that the same gene(s) could be important in the development of airway obstruction in both of these airway diseases (40).

Silverman and coworkers reported that personal smoking was a significant covariate for the linkage of postbronchodilator

$FEV_1\%FVC$ (19), possibly due to the fact that the families were ascertained through a family member with extensive smoking history. Linkage for the prebronchodilator ratio was not influenced in our population by passive tobacco exposure. This is of interest because of the different characteristics of airway obstruction caused by asthma and COPD in these two populations. It is possible that both studies support the presence of similar genes that cause airway obstruction and modulate pulmonary function in these two disorders. However, this requires further fine mapping and gene identification in families with asthma and with COPD.

In contrast to the results on $FEV_1\%VC$, linkage of pre- and postbronchodilator FEV_1 , a major determinant for alterations in $FEV_1\%VC$, showed more variability and less significant results, with LOD scores in the range of 1.50 to 1.64. FEV_1 is highly influenced by airway obstruction, whereas VC is less affected. FEV_1 and VC are only modestly related in the general population, suggesting interindividual differences in airway size relative to lung volume (41). Thus, it is not surprising that different linkage positions in the genome have been identified for the various ways of expressing airflow limitation. $FEV_1\%VC$ is considered to be a measure of obstructive airway disease (42). It is therefore of interest that we found the strongest evidence for linkage with $FEV_1\%VC$ and not with FEV_1 , the most frequently used lung function parameter to assess severity in asthma and COPD. For FEV_1 in the families with early-onset COPD, the highest LOD score was observed on chromosome 12p, where the LOD increased to 2.43 at 37 cM after the addition of fine-mapping markers (19). In the general Framingham population (9), the highest LOD was observed on chromosome 6 (LOD , 2.4). Neither of these regions was observed in the current study. This may signify differences in the recruitment of populations, age, ethnic background, or other interacting factors, including the presence of concomitant allergic factors in our families with asthma. The same may be true for the observed linkage of lung function with chromosome 5q in our families with asthma. This linkage was found in the same region with which we have previously found linkage with bronchial hyperresponsiveness (29). This is not surprising because bronchial hyperresponsiveness to a direct stimulus like histamine is determined partially by prechallenge lung function. Whether this implies that the ob-

Figure 2. Genomewide screen for major genes regulating pre- and postbronchodilator FEV₁%VC values in 200 Dutch families from a proband with asthma. No differences in y-axis scale for pre- and postbronchodilator FEV₁%VC. (A) Prebronchodilator FEV₁%VC; (B) postbronchodilator FEV₁%VC.

served linkage reflects the same gene(s) for bronchial hyper-responsiveness as for airway obstruction requires investigation.

In summary, we have shown for the first time that significant linkage exists of FEV₁%VC to a region on chromosome 2q32 in families of a proband diagnosed with asthma in the 1960s. Other investigators have reported linkage with FEV₁%FVC in an adjacent chromosomal region in a population of families of probands with early-onset COPD. This suggests that there may

exist a general underlying mechanism of impaired lung function that may be independent of the obstructive airway disease under study. We could not find linkages in pulmonary function that were previously reported in general populations. This may well be due to the fact that we investigated a population ascertained through a parent with asthma and many of the offspring in these families also had this disease. Epidemiologic data suggest an effect of maternal smoking during pregnancy on the development

of asthma and atopy. Our results do not suggest an interactive effect of a genetic background and smoke exposure *in utero* on the development of lung function impairment. We hypothesize that gene(s) on chromosome 2q32, as well as in other linked chromosomal regions reported in this study, may be important in the development of airway remodeling and the severity of airway obstruction in asthma. Further identification of the genes in the regions identified in the current study may help us to better understand some of the underlying biology of the severity of asthma.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Koppelman GH, Los H, Postma DS. Genetic and environment in asthma: the answer of twin studies. *Eur Respir J* 1999;13:2-4.
- Weiss ST, Tosteson TD, Segal MR, Tager IB, Redline S, Speizer FE. Effects of asthma on pulmonary function in children: a longitudinal population-based study. *Am Rev Respir Dis* 1992;145:58-64.
- Vonk JM, Jongepier H, Panhuysen CI, Schouten JP, Bleecker ER, Postma DS. Risk factors associated with the presence of irreversible airflow limitation and reduced transfer coefficient in patients with asthma after 26 years of follow up. *Thorax* 2003;58:322-327.
- Panhuysen CIM, Bleecker ER, Koeter GH, Meyers DA, Postma DS. Characterization of obstructive airways disease in family members of probands with asthma: an algorithm for the diagnosis of asthma. *Am J Respir Crit Care Med* 1998;157:1734-1742.
- GINA guidelines. Available from: www.ginasthma.com [accessed October 2004].
- Weiss ST, Segal MR, Sparrow D, Wagner C. Relation of FEV₁ and peripheral blood leucocyte count to total mortality: The Normative Aging Study. *Am J Epidemiol* 1995;142:499-503.
- Coultas DB, Hanis CL, Howard CA, Skipper BJ, Samet JM. Heritability of ventilatory function in smoking and nonsmoking New Mexico Hispanics. *Am Rev Respir Dis* 1991;144:770-775.
- Wilk JB, Djousse L, Arnett DK, Rich SS, Province MA, Hunt SCC, Crapo RO, Higgins M, Myers RH. Evidence for major genes influencing pulmonary function in the NHLBI Family Heart Study. *Genet Epidemiol* 2000;19:81-94.
- Joost O, Wilk JB, Cupples LA, Harmon M, Shearman AM, Baldwin CT, O'Connor GT, Myers RH, Gottlieb DJ. Genetic loci influencing lung function: a genome-wide scan in the Framingham study. *Am J Respir Crit Care Med* 2002;165:795-799.
- Palmer LJ, Knuiman MW, Divitini ML, Buron PR, James AL, Bartholomew HC, Ryan G, Musk AW. Familial aggregation and heritability of adult lung function: results from the Busselton Health Study. *Eur Respir J* 2001;17:696-702.
- Malhotra A, Pfeiffer AP, Ryujin DT, Eisner T, Kanner RE, Lappert MF. Further evidence for the role of genes on chromosome 2 and chromosome 5 in the inheritance of pulmonary function. *Am J Respir Crit Care Med* 2003;168:556-561.
- Ober C, Abney M, McPeck MS. The genetic dissection of complex traits in a founder population. *Am J Hum Genet* 2001;69:1068-1079.
- Redline S, Tishler PV, Rosner B, Lewitter FI, Vandenburgh M, Weiss ST, Speizer FE. Genotypic and phenotypic similarities in pulmonary function among family member of adult monozygotic and dizygotic twins. *Am J Epidemiol* 1989;129:827-836.
- Astemborski J, Beaty T, Cohen B. Variance components analysis of forced expiration in families. *Am J Med Genet* 1985;21:741-753.
- Hubert H, Fabsitz R, Feinleib M, Gwinn C. Genetic and environmental influences on pulmonary function in adult twins. *Am Rev Respir Dis* 1982;125:409-415.
- Gibson J, Martin N, Oakeshott J, Rowell D, Clark P. Lung function in an Australian population: contribution of polygenic factors and the Pi locus to individual differences in lung function in a sample of twins. *Ann Hum Biol* 1983;10:547-556.
- Kawakami Y, Shida A, Yamamoto H, Yoshikawa T. Pattern of genetic influence on pulmonary function. *Chest* 1985;87:507-511.
- Wilk JB, De Stefano AL, Arnett DK, Rich SS, Djousse L, Crapo RO, Leppert MF, Province MA, Cupples A, Gottlieb DJ, et al. A genome-wide scan of pulmonary function measures in the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Respir Crit Care Med* 2003;167:1528-1533.
- Silverman EK, Palmer LJ, Mosley JD, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, et al. Genomewide linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic obstructive pulmonary disease. *Am J Hum Genet* 2002;70:1229-1239.
- Xu X, Fang Z, Wang B, Chen C, Guang W, Jin Y, Yang J, Lewitzky S, Aelony A, Parker A, et al. A genome-wide search for quantitative loci underlying asthma. *Am J Respir Crit Care Med* 2001;69:1271-1277.
- Li YF, Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Rappaport EB, Peters JM. Effects of in utero and environmental tobacco smoke exposure on lung function in boys and girls with and without asthma. *Am J Respir Crit Care Med* 2000;162:2097-2104.
- Sherill D, Sears MR, Lebowitz MD, Holdaway MD, Hewitt CJ, Flannery EM, Herbison GP, Silva PA. The effects of airway hyperresponsiveness, wheezing, and atopy on longitudinal pulmonary function in children: a 6 year follow-up study. *Pediatr Pulmonol* 1992;13:78-85.
- Gilliland FD, Li Y, Peters JM. Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med* 2001;163:429-436.
- Young S, Sherrill DL, Arnott J, Diepeveen D, LeSouef PN, Landau LI. Parental factors affecting respiratory function during the first year of life. *Pediatr Pulmonol* 2000;29:331-340.
- Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Lesouef PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. *Am J Respir Crit Care Med* 2001;163:37-42.
- Boezen HM, Vonk JM, Van Aalderen WMC, Brand PLP, Gerritsen J, Schouten JP, Boersma ER. Perinatal predictors of respiratory symptoms and lung function at a young adult age. *Eur Respir J* 2002;20:383-390.
- Gilliland FD, Berhane K, Li Y-F, Rappaport EB, Peters JM. Effects of early onset asthma and *in utero* exposure to maternal smoking on childhood lung function. *Am J Respir Crit Care Med* 2003;167:917-924.
- Meyers DA, Postma DS, Panhuysen CIM, Xu J, Amelung PJ, Levitt RC, Bleecker ER. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994;23:464-470.
- Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI, Meyers DA, Levitt RC. Genetic susceptibility to asthma: bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333:894-900.
- de Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. *Int Arch Allergy* 1962;20:93-101.
- Koppelman GH, Stine OC, Xu J, Howard TD, Zheng SL, Kauffman HF, Bleecker ER, Myers DA, Postma DS. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol* 2002;109:498-506.
- Van Camp G, Balemans W, Willems PJ. Linkage Designer and Linkage Reporter software for automated gene localization studies [technical tip]. *Trends Genet* 1977;13:82.
- Lathrop GM, Loalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-3446.
- Lander ES, Green P. Construction of multilocus genetic linkage maps in humans. *Proc Natl Acad Sci USA* 1987;84:2363-2367.
- Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998;62:1198-1211.
- Xu J, Postma DS, Howard TD, et al. Major genes regulating total serum immunoglobulin E levels in families with asthma. *Am J Hum Genet* 2000;67:1163-1173.
- Howard TD, Postma DS, Hawkins GA, Koppelman GH, Zheng SL, Wysong AK, Xu J, Meyers DA, Bleecker ER. Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002;110:743-751.
- Allen M, Heinzmann A, Noguchi E, Abecasis G, Brochholme J, Ponting CP, Bhattacharayya S, Tinsley J, Zhang Y, Holt R, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat Genet* 2003;35:1-6.
- Palmer LJ, Celedon JC, Chapman HA, Speizer FE, Weiss ST, Silverman EK. Genome-wide linkage analysis of bronchodilator responsiveness and postbronchodilator spirometric phenotypes in chronic obstructive pulmonary disease. *Hum Mol Genet* 2003;12:1199-1210.
- Sluiter HJ, Koeter GH, De Monchy JGR, Postma DS, de Vries K, Orie NG. The Dutch hypothesis (chronic non-specific lung disease) revisited. *Eur Respir J* 1991;4:479-489.
- Chen Y, Rennie DS, Lockinger LA, Dosman JA. Major genetic effect on forced vital capacity: the Humboldt Family Study. *Genet Epidemiol* 1997;14:63-76.
- Rybicki BA, Beaty TH, Cohen BH. Major genetic mechanisms in pulmonary function. *J Clin Epidemiol* 1990;43:667-675.