

# Genome screen for asthma and bronchial hyperresponsiveness: Interactions with passive smoke exposure

Deborah A. Meyers, PhD,<sup>a</sup> Dirkje S. Postma, MD, PhD,<sup>b</sup> O. Colin Stine, PhD,<sup>c</sup>  
Gerard H. Koppelman, MD, PhD,<sup>b</sup> Elizabeth J. Ampleford, PhD,<sup>a</sup> Hajo Jongepier,  
MD, PhD,<sup>b</sup> Timothy D. Howard, PhD,<sup>a</sup> and Eugene R. Bleeker, MD<sup>a</sup> *Winston-Salem,  
NC, Groningen, The Netherlands, and Baltimore, Md*

**Background:** Asthma is a common respiratory disease caused by the interaction of genetic susceptibility and exposure to various environmental factors. Passive smoke exposure, characterized by parental smoking, has been shown to be a risk factor for the development of atopy and asthma. **Objective:** We sought to perform a genome-wide linkage screen for asthma and bronchial hyperresponsiveness (BHR) and to determine the influence of passive tobacco smoke exposure during childhood on the results of genetic linkage studies to investigate gene-environment interactions. **Methods:** A genome-wide linkage screen for asthma and BHR was performed in 200 families ascertained through a parent with asthma. Analyses were performed separately for the entire sample and for the smoking-exposed and nonexposed families. **Results:** For asthma and BHR, the strongest evidence for linkage was observed for chromosomes 3p and 5q. The families in which the children were exposed to passive smoking accounted for the evidence for linkage of BHR to 5q ( $P < .001$ ), but evidence for linkage to 3p was found in both sets of families. Similar results were observed for asthma. However, there was no observed difference in the frequency of asthma or BHR in the offspring from the smoke-exposed compared with the nonexposed families. **Conclusion:** The results from this study demonstrate that the influence of susceptibility genes for a common disease such as asthma might not be apparent unless there is the appropriate exposure to environmental stimuli, such as passive exposure to cigarette smoke. This approach should be useful for identification of asthma susceptibility genes. (*J Allergy Clin Immunol* 2005;115:1169-75.)

**Key words:** Smoking, bronchial hyperresponsiveness, asthma, lung function, linkage, genome-wide screen

## Abbreviations used

BHR: Bronchial hyperresponsiveness  
COPD: Chronic obstructive pulmonary disease  
CSGA: Collaborative Study on the Genetics of Asthma

Asthma is a common respiratory disease that is caused by the interaction of genetic susceptibility and exposure to various environmental factors. Published genome screens for asthma and closely associated phenotypes have reported consistent, as well as conflicting, results.<sup>1-9</sup> We have previously presented results of a genome-wide screen for atopic phenotypes in 200 families ascertained through a proband with asthma and reported evidence for linkage to several chromosomal regions, including 2q, 5q, 6p, 7q, and 12q.<sup>10,11</sup> These observations have led to studies on candidate genes in these regions, and our significant results for *CD14* and *IL13* on chromosome 5q<sup>12-14</sup> and for *CTLA4* on chromosome 2q<sup>15</sup> support the value of this family population.

Bronchial hyperresponsiveness (BHR) is an important characteristic in asthma, and longitudinal studies have shown that persistent BHR in early childhood is a risk factor for the development of asthma.<sup>16-18</sup> Therefore it is important to analyze both asthma and BHR phenotypes in family studies. In addition to genetic susceptibility, environmental factors are important in the development of allergy and asthma. Passive smoke exposure, characterized by parental smoking, has been shown to be a risk factor for the development of atopy and asthma, especially maternal smoking during pregnancy and tobacco smoke exposure during early life.<sup>19-22</sup> In addition, evidence for a significant gene-environment (passive tobacco smoke exposure) interaction has been observed in a genetic linkage study by Colilla et al.<sup>23</sup>

Our cohort of families presents a unique opportunity for further analysis of susceptibility genes for asthma and related phenotypes. First, the 200 families were ascertained through a parent with asthma who was initially studied approximately 30 to 40 years ago and reevaluated with their family members between 1990 and 2000, when the children were of similar age as the affected parent was when initially characterized. Second, the probands and their families represent a genetically homogeneous

From <sup>a</sup>the Center for Human Genomics, Departments of Pediatrics, Medicine, and Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem; <sup>b</sup>the Department of Pulmonology, University Hospital, Groningen; and <sup>c</sup>the Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore.

Supported by the Dutch Asthma Funds grant AF 95.09 and National Institutes of Health (NIH) grants R01HL48341 and R01HL66393.

Received for publication August 19, 2004; revised January 20, 2005; accepted for publication January 21, 2005.

Available online April 14, 2005.

Reprint requests: Deborah A. Meyers, PhD, Center for Human Genomics, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157. E-mail: dmeyers@wfubmc.edu.

0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2005.01.070

population ascertained from the northern region of The Netherlands. Third, clinical characterization, including bronchial responsiveness to histamine, was performed on all family members. Finally, because of the longitudinal data available on the probands, data were available on their smoking habits before and during the early life of their children.

The purpose of the current study was to perform a genome-wide linkage screen for asthma and BHR. In addition, we hypothesize that evidence for different susceptibility genes will be observed in families with exposure to a known environmental influence, passive exposure to tobacco smoke. This approach supports the concept that environmental influences in early life might modulate the expression of genetic susceptibility in the development of asthma.

## METHODS

### Family ascertainment

Two hundred families were ascertained through probands who were initially studied between 1962 and 1975 at Beatrixoord Hospital, Haren, the Netherlands, a regional airways disease referral center. Patients with newly diagnosed symptomatic asthma who were not experiencing a current asthma exacerbation were referred to this hospital and admitted for a standardized and comprehensive evaluation for asthma and atopy. At the time of initial testing, all probands had asthma symptoms, were hyperresponsive to histamine ( $PC_{20}$  FEV<sub>1</sub> histamine of  $<32$  mg/mL), and were younger than 45 years of age. The families of 200 of these original probands with their spouses, children, children's spouses, and grandchildren older than 6 years of age were recruited and evaluated in the 1990s.<sup>24-26</sup> The Medical Ethics Committee of the University Hospital Groningen approved this study, and all participants provided written informed consent. Additional institutional review board approval was obtained from both the University of Maryland and Wake Forest University. Among the 200 families (1183 individuals), 166 consisted of 2 generations, 33 consisted of 3 generations, and 1 consisted of 4 generations.

### Clinical evaluation

Bronchial responsiveness to histamine was measured with the 30-second inhalation histamine challenge test developed by De Vries et al<sup>27</sup> because this method was used for testing the probands during the 1960s. Doubling concentrations of histamine were inhaled (0.03-32 mg/mL in children  $<12$  years of age and 0.5-32 mg/mL in adults), and the test was stopped if there was a 20% or greater decrease in FEV<sub>1</sub>. A positive test result was defined as a  $PC_{20}$  FEV<sub>1</sub> histamine value of less than 32 mg/mL. All of the probands were hyperresponsive at initial testing 25 years previously and were classified as hyperresponsive for the linkage analyses. Skin testing to common allergens and measurements of total and specific IgE were performed on all family members.<sup>11</sup> A respiratory questionnaire was used (modified British Medical Society Respiratory Questionnaire<sup>28</sup>) to evaluate asthma characteristics, specifically respiratory symptoms, smoking history, and asthma severity and exacerbations.<sup>26</sup>

Asthma was defined with an algorithm that has been previously described in the first 92 of these 200 families.<sup>26</sup> In brief, subjects were considered to have definite asthma (class 1) if they reported characteristic asthma symptoms and if they had BHR to histamine, airway obstruction (if present) was reversible, and asthma symptoms preceded any history of cigarette smoking. Class 2 (probable asthma) represented subjects who either had BHR but experienced fewer

asthma symptoms than those in class 1 or subjects who had characteristic asthma symptoms without BHR but with airways obstruction and bronchodilator reversibility. Class 3 (uncertain) consisted of 2 groups: family members with BHR without asthma symptoms and cigarette smokers who did not meet the criteria for chronic obstructive pulmonary disease (COPD) but had airways symptoms occurring in the context of a significant history of smoking. Subjects in class 4 (COPD) had characteristics consistent with chronic obstructive lung disease (presence or absence of BHR, irreversible airways obstruction, and/or chronic bronchitis with a significant smoking history). Finally, class 5 consisted of family members with no evidence of airways disease (asthma or COPD) who did not have BHR when tested. For the linkage analysis for asthma, all probands and any family members in class 1 or 2 were considered affected.

### Genotyping

DNA was isolated from peripheral blood by using standard methods (Puregene kit; Gentra, Inc, Minneapolis, Minn). For the genome-wide screen, the Weber version 8 set of markers (Marshfield Center for Medical Genetics) was used, which spans the human genome at an average interval of approximately 10 cM and consists of 366 polymorphic autosomal markers. Multiplex PCR was performed with fluorescently labeled primers. PCR products were separated on denaturing polyacrylamide gels, and the fragments were detected with ABI 377 sequencers (Perkin Elmer Applied Biosystems, Foster City, Calif). The fragments were scanned and scored with ABI software. A modified version of the program Linkage Designer (Van Camp et al, 1997) was used to bin the alleles and check inheritance. Genotyping errors, double recombinants, and inheritance inconsistencies were detected with the LINKAGE and CRIMAP software (<http://www.ncbi.nlm.nih.gov/CBBresearch/Schaffer/fastlink.html> and <http://compugen.rutgers.edu/multimap/crimap/index.html>, respectively). Marker allele frequencies were estimated from the pedigree founders.

### Statistical methods

Linkage analyses for asthma and BHR as qualitative traits were performed with multipoint parametric analysis, as implemented in GENEHUNTER-PLUS.<sup>29</sup> We assumed the autosomal dominant model previously used for our Collaborative Study on the Genetics of Asthma (CSGA) genome-wide screen,<sup>10</sup> which is consistent with the frequency of BHR and asthma in the offspring. The LOD reported is determined by means of a maximum likelihood approach used to estimate the proportion of linked families, by maximizing the admixed LOD score. Families were divided by the smoking status of the proband to determine the effect of exposure to cigarette smoke in children on asthma and BHR. Although most probands did not have a significant history of smoking at the time of their original evaluation (30-40 years ago), approximately half of the probands ( $n = 95$ ) are now current or exsmokers with a history that is consistent with passive smoke exposure in their children. There was also a significant relationship in smoking status between the probands and their spouses ( $P = .015$ , McNemar test). The genome-wide linkage screen for asthma and BHR was performed separately for the entire sample and for the smoking-exposed and nonexposed families. The M test was used to test for a difference in LOD scores between the groups of families.<sup>30</sup> Individual smoking by the offspring was not considered in the analyses. There are 72 offspring in class 1 or class 2 who have a history of smoking (mean, 4 pack-years). Forty-nine of these individuals were asthmatic before beginning to smoke cigarettes. Of the remaining 23, 20 are in families in which they were passively exposed before the age of 5 years. That leaves 3 individuals with probable asthma (class 2) in whom active smoking might be an issue. Therefore it is possible that there might be confounding effects with active smoking in a very small number of subjects ( $n = 3$ ).

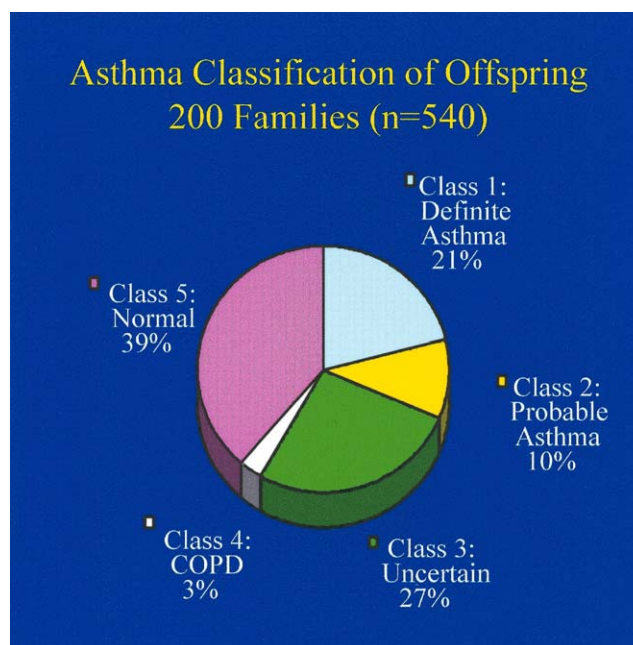


**FIG 1.** The pie chart shows the percentage of the 540 offspring with asthma, probable asthma, uncertain airways disease or COPD or who are unaffected in 200 families ascertained through a parent with asthma.

## RESULTS

### Demographics

The characteristics of the family members have been described previously.<sup>10</sup> Fig 1 shows the results of the asthma classification of the first- and second-degree offspring (n = 540) for the 200 families. Class 1 (definite asthma) was found in 21% of the offspring, whereas class 2 (probable asthma) was found in 10%, resulting in 31% of the offspring with findings consistent with a diagnosis of asthma, even though these 200 families were not ascertained for multiple family members with asthma. Class 3 or uncertain airways disease was found in 27%, and more than half of these subjects had asymptomatic BHR, resulting in 46% of all offspring with BHR. Finally, 3% of the offspring were categorized as having COPD, and 39% had no evidence of airways disease (asthma or COPD) on the basis of the absence of respiratory symptoms and BHR and normal pulmonary function.

### Genome screen for BHR and asthma

For BHR, the strongest evidence for linkage was observed for chromosomes 5q (LOD = 2.87 at D5S816) and 3p (LOD = 2.44 for D3S1766; Fig 2). For asthma, the highest LOD scores were observed for the same regions on chromosomes 3p and 5q, with the highest LOD score on chromosome 3p (LOD = 2.57 at D3S2409) and an LOD score of 1.94 for chromosome 5q (D5S816; Fig 3).

### Effect of passive smoke exposure

There were no significant differences observed between the clinical characteristics in the children from the smoke-exposed families compared with the offspring from the

nonexposed families (Table I). For example, 50.4% (n = 268) of the children in the 95 families with passive smoke exposure were hyperresponsive (Table I). In the 105 families classified as not exposed to tobacco smoke, similar results were observed, and 49.8% (n = 272) of the children were hyperresponsive (*P* = not significant).

The families in which the children were exposed to passive smoking accounted for all the evidence for linkage of BHR to chromosome 5q; the LOD score was 3.77 for chromosome 5q in the passive smoke-exposed families and 0.18 in the nonexposed families (*P* < .001, M test). For BHR, evidence for linkage to 3p was found in both sets of families but was higher in those with smoke exposure; the LOD score was 2.17 for the passive smoke-exposed families, and the LOD score was 1.12 for the nonexposed families (*P* > .05, M test). Similar results were observed for asthma. For chromosome 5q, the LOD score was 2.54 in the tobacco-exposed families and 0.15 in the nonexposed families. The LOD score on chromosome 3p was 2.63 in the smoke-exposed families and 0.71 in the nonexposed families. The only additional region that showed differences for tobacco smoke-related gene-environment interaction was chromosome 19, with LOD scores of 2.17 for asthma and 1.94 for BHR in the nonexposed families (both at D19S714), with no evidence for linkage in the exposed families (LOD = 0.1; *P* > .05, M test). To test the robustness of our classification scheme, the analysis was repeated by classifying all nuclear families in which at least one child was exposed to passive smoke from either parent before the age of 5 years as a family with passive smoke exposure. Similar results were observed; for chromosome 5q, the LOD score was 3.4 at

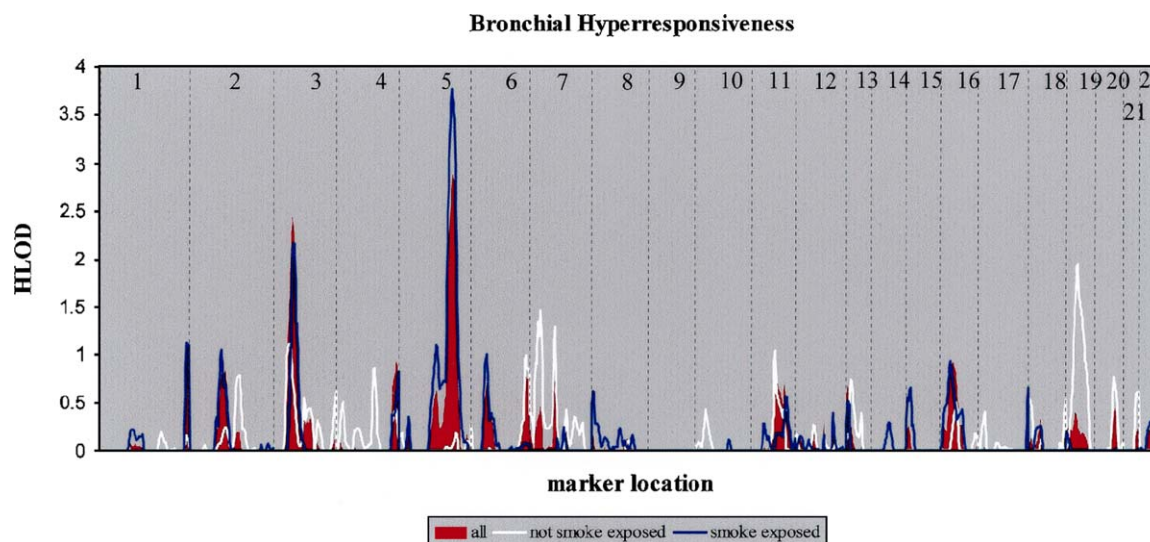


**FIG 2.** The results of the genome-wide screen for BHR are shown for each chromosome, with the LOD score from the total 200 Dutch families and from the families classified with and without passive cigarette smoke exposure to the children in early life.

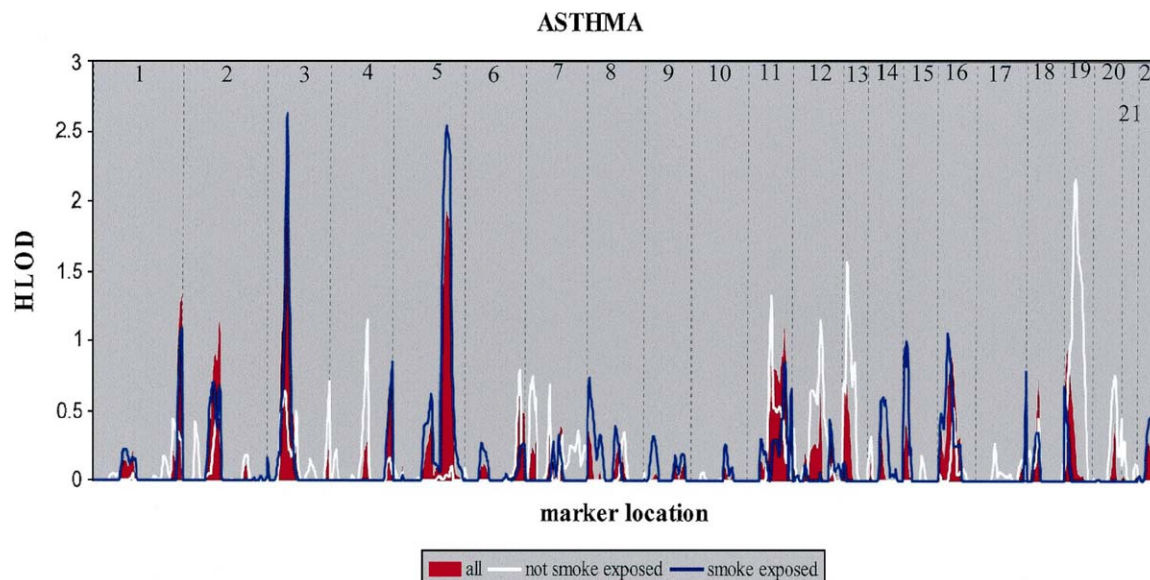


**FIG 3.** The results of the genome-wide screen for asthma are shown for each chromosome, with the LOD score from the total 200 Dutch families and from the families classified with and without passive cigarette smoke exposure to the children in early life.

the same chromosomal location as before in the exposed families and 0.2 in the nonexposed families.

## DISCUSSION

This study reports the results of linkage analysis for asthma and bronchial responsiveness by using a well-characterized, homogeneous asthmatic family population. The families were not ascertained through multiple affected siblings but through a single proband (a parent)

with asthma, and analysis of the offspring showed a high frequency of asthma phenotypes (31% with asthma or probable asthma [classes 1 and 2] and 46% with BHR), supporting an important hereditary component for these phenotypes. In addition, with a history of passive exposure to cigarette smoke during early life in the children in approximately half of these families, the effects of this environmental factor that is related to asthma susceptibility could be investigated. No significant differences in the clinical characteristics were observed for the offspring from the smoke-exposed versus nonexposed families.

However, it was shown that passive smoke exposure in early childhood represents an important gene-environment interaction for a gene or genes on chromosome 5q. The evidence for linkage for BHR for chromosome 5q was observed in the passive smoke-exposed families ( $P < .001$ ), providing an important replication of a similar study by Collila et al,<sup>23</sup> which showed a similar but smaller effect in 144 US families. These results demonstrate that exposure to environmental factors early in life modulate the expression of underlying genetic susceptibility for asthma-related phenotypes. It is difficult to accurately measure environmental exposures retrospectively, and therefore it is not possible to state that these results are only a result of exposure to tobacco smoke; they might be also due to an increase in other environmental exposures that could be correlated with exposure in early life to tobacco smoke (eg, viral infections). Asthma susceptibility is due to the effect of multiple genes, but an environmental exposure, such as passive smoke exposure, increases the importance of the effects of the gene or genes on chromosome 5q on asthma susceptibility.

There are several important features of our findings that warrant further discussion. First, results of the genome-wide screen for asthma and BHR show evidence for linkage on chromosomes 3p and 5q. Linkage to chromosome 5q for asthma or related phenotypes has been reported by multiple investigators.<sup>3,4,31-34</sup> In the first 92 of the 200 families of this study, evidence for linkage to chromosome 5q was observed for total serum IgE levels and BHR.<sup>24,25</sup> Later, when all 200 families were recruited, evidence for linkage for total serum IgE levels was observed, although chromosome 5q was not the region with the strongest evidence for linkage.<sup>10</sup> Evidence for linkage of total serum IgE levels was also observed in an inbred, nonsmoking Amish population<sup>31</sup> and was strongest in the *IL4*, *IL13* cytokine region of chromosome 5q, whereas our evidence for linkage was more distal on chromosome 5q. Thus it is likely that several linkage peaks are present because of multiple genes, the effects of which are related to atopy and BHR, and both phenotypes are strongly associated with asthma. Thus linkage peaks in this chromosomal region might be dependent on the specific phenotype being analyzed, as well as the characteristics and ascertainment of the population under study.

On chromosome 5q, we observed stronger evidence for linkage to BHR than to asthma in these Dutch families. This is an interesting finding because although all probands with asthma had evidence of BHR, there were an additional 15% of the offspring who had BHR without symptoms of asthma (asymptomatic BHR) who could not be classified as having clinical asthma. Thus the linkage on chromosome 5q for BHR represents a broader category that included both family members with diagnosed and symptomatic asthma and those potentially at risk for developing asthma (asymptomatic BHR). Epidemiologic studies have provided evidence that BHR in early childhood is a risk factor for the development of asthma and that asymptomatic BHR, even later in life, is a risk factor for the development of asthmatic symptoms.<sup>16-18,35</sup>

**TABLE I.** Clinical characteristics of offspring in the smoke-exposed and nonexposed families

|                                           | Nonexposed families | Smoke-exposed families |
|-------------------------------------------|---------------------|------------------------|
| Sex, M/F                                  | 119/149             | 125/147                |
| Age, y (mean [SD])                        | 22.9 (9.0)          | 25.6 (9.3)             |
| Total IgE, IU (geometric mean [range])    | 67.6 (1-2560)       | 58.88 (1-3360)         |
| ≥1 positive skin test result              | 55.3%               | 51.9%                  |
| Predicted FEV <sub>1</sub> (mean)         | 92.42%              | 94.87%                 |
| Reversibility (≥15% of baseline)          | 13.1%               | 8.9%                   |
| FEV <sub>1</sub> /VC ratio (mean)         | 81.2                | 80.7                   |
| BHR positive (PC <sub>20</sub> ≤32 mg/mL) | 49.8%               | 50.4%                  |

VC, Vital capacity.

Additional follow-up studies would be needed to determine the proportion of offspring with asymptomatic BHR that have asthma over time.

Strong evidence for linkage of asthma and BHR to chromosome 3p was observed in a region in which we did not observe linkage to allergic phenotypes.<sup>10,11</sup> In addition, this region had been observed in the first genome screen in the Hutterites but was not replicated in their later study.<sup>3,4</sup> This might either represent a susceptibility gene with a higher frequency in this homogenous population because of a founder effect or reflect differences in phenotypic assessment; for example, histamine inhalation challenge was used to test for BHR in our study, whereas methacholine inhalation challenge has been used in most published genome screens.

Previous studies have shown that exposure to passive smoking is an environmental stimulus and risk factor for the development of asthma.<sup>36,37</sup> Young et al<sup>38</sup> measured lung function in the first year of life and showed that *in utero* exposure to cigarette smoke was associated with reduced lung function. Persistent decreases in lung function in conjunction with *in utero* smoke exposure are especially evident in children in whom asthma has developed.<sup>39</sup> Furthermore, lower lung function in childhood constitutes a risk factor for the development of BHR and potentially asthma. Thus exposure to tobacco smoke represents an important environmental factor that can be used in family studies in which only historical information is available on other early-life influences, such as bacterial and viral respiratory infections and the type and intensity of allergen exposures.

In this study we did not observe a difference in the frequency of asthma or BHR or differences in lung function between the children from smoke-exposed compared with nonexposed families (Table I). However, it is important to remember that the primary purpose of our study was to perform a family-based linkage analysis. Therefore the families were classified as exposed or nonexposed for the genetic analysis, and detailed history on exposure for each child was not available and not required to perform the genetic analyses. In addition, this study was designed as a genetic study and not an epidemiologic

study, and therefore the lack of difference in clinical characteristics between the 2 sets of children should be interpreted with caution.

However, we observed significant results for the effects of passive exposure to cigarette smoke on the genome screen linkage results, especially for chromosome 5q. This same effect for chromosome 5q was observed in a similar study on the 144 CSGA white families, in which the LOD increased from 0.34 in the total data set to 1.23 ( $P < .01$ ) in the 51 smoke-exposed families.<sup>23</sup> Our results provide an important replication of their early findings and provide substantial additional evidence for a significant gene-environment interaction involving early-life exposure to tobacco smoke. It is very important to replicate genetic studies, such as linkage and association studies, because the prior probability of a true effect is low as a result of the number of chromosomes and genes in human subjects.<sup>40</sup> One might hypothesize that there are susceptibility genes for BHR on chromosome 5q that require early-life exposure to environmental factors, such as tobacco smoke, for their expression and the subsequent development of asthma.

A final observation is that BHR and asthma showed suggestive evidence for linkage with chromosome 19p in families with children without passive smoke exposure in childhood. This region was reported in at least one other genome-wide screen; in the CSGA white families, an LOD of 1.02 was observed approximately 15 cM distal to the region we observed.<sup>6</sup> However, this region was not observed when the families were divided on the basis of passive tobacco smoke exposure.<sup>23</sup>

Genetic approaches are useful in dissecting the complex genetic relationships between susceptibility to asthma, BHR, and atopy. Significant progress has been made in the last decade, although it still remains a significant challenge to identify genes responsible for complex multifactorial disorders, such as asthma, COPD, and atopy. As these genes are delineated, the specific role of each gene in the development of asthma must be understood in conjunction with environmental influences. Our findings and those of Collila et al<sup>23</sup> provide an important example of how environmental influences, such as exposure to tobacco smoke early in life, might interact with individual genetic susceptibility to asthma development and related phenotypes. The results emphasize how changes in environmental exposures might increase the risk that individuals with an underlying genetic susceptibility will have disease. We propose that these findings are important in understanding how subtle changes in the environment might interact with genetic profiles to change the prevalence and expression (severity) of common diseases, such as asthma and atopy.

## REFERENCES

- Daniels SE, Bhattacharya S, James A, Leaves NI, Young A, Hill MR, et al. A genome-wide search for quantitative loci underlying asthma. *Nature* 1996;383:247-50.
- Wjst M, Fischer G, Immervoll T, Jung M, Saar K, Rueschendorf F, et al. A genome-wide search for linkage to asthma German Asthma Genetics Group. *Genomics* 1999;58:1-8.
- Ober C, Cox NJ, Abney M, Dirienzo A, Lander ES, Changyaleket B, et al. Genome-wide search for asthma susceptibility loci in a founder population. *Hum Mol Genet* 1998;7:1393-8.
- Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma susceptibility alleles in a founder population. *Am J Hum Genet* 2000;67:1154-62.
- Dizier MH, Besse-Schmittler C, Guiloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J. Genome screen for asthma and related phenotype in the French EGEA study. *Am J Respir Crit Care Med* 2000;162:1812-8.
- Xu J, Meyers DA, Ober C, Blumenthal MN, Mellen B, Barnes KC, et al. Genomewide screen and identification of gene-gene interactions for asthma-susceptibility loci in three U.S. populations: Collaborative Study on the Genetics of Asthma. *Am J Hum Genet* 2001;68:1437-46.
- Xu J, Fang Z, Wang B, Chen C, Guang W, Jin Y, et al. A genome-wide search for quantitative-trait loci underlying asthma. *Am J Hum Genet* 2001;69:1271-7.
- Laitinen T, Daly MJ, Rioux JD, Kauppi P, Laprise C, Petays T, et al. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. *Nat Genet* 2001;28:89-91.
- Hakonarson H, Bjornsdottir US, Halapi E, Palsson S, Adalsteinsdottir E, Gislason D, et al. A major susceptibility gene for asthma maps to chromosome 14q24. *Am J Hum Genet* 2002;71:483-91.
- Xu J, Postma DS, Howard TD, Koppelman GH, Zheng SL, Stine OC, et al. Major genes regulating total serum immunoglobulin E levels in families with asthma. *Am J Hum Genet* 2000;67:1163-73.
- Koppelman GH, Stine OC, Xu J, Howard TD, Zheng SL, Kauffman HF, et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. *J Allergy Clin Immunol* 2002;109:498-506.
- Koppelman GH, Reijmerink NI, Stine OC, Howard TD, Meyers DA, Postma DS, et al. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001;163:965-9.
- Howard TD, Whittaker PA, Zaiman AL, Koppelman GH, Xu J, Hanley MT, et al. Identification and association of polymorphisms in the interleukin 13 gene with asthma and atopy in a Dutch population. *Am J Respir Cell Mol Biol* 2001;25:377-84.
- Howard TD, Koppelman GH, Xu J, Zheng S, Postma DS, Meyers DA, et al. Gene-gene interaction in asthma: IL4RA and IL 13 in a Dutch population. *Am J Hum Genet* 2002;70:230-6.
- Howard TD, Postma DS, Koppelman GH, Zheng SL, Wysong AK, Xu J, et al. Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol* 2002;110:743-51.
- Burrows B, Sears MR, Flannery EM, Herbison GP, Holdaway MD. Relationships of bronchial responsiveness assessed by methacholine to serum IgE, lung function, symptoms, and diagnoses in 11-year-old New Zealand children. *J Allergy Clin Immunol* 1992;90:376-85.
- Peat JK, Toelle BG, Salome CM, Woolcock AJ. Predictive nature of bronchial responsiveness and respiratory symptoms in a one year cohort study of Sydney school children. *Eur Respir J* 1993;6:662-9.
- Carey VJ, Weiss ST, Tager IB, Leeder SR, Speizer FE. Airways responsiveness, wheeze onset, and recurrent asthma episodes in young adolescents. The East Boston Childhood Respiratory Disease Cohort. *Am J Respir Crit Care Med* 1996;153:356-61.
- Ehrlich RI, Du Toit D, Jordaan E, Zwarenstein M, Potter P, Volmink JA, et al. Risk factors for childhood asthma and wheezing. Importance of maternal and household smoking. *Am J Respir Crit Care Med* 1996;154:681-8.
- Li YF, Gilliland FD, Berhane K, McConnell R, Gauderman WJ, Rappaport EB, et al. 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;162:2097-104.
- Lux AL, Henderson AJ, Pocock SJ. Wheeze associated with prenatal tobacco smoke exposure: a prospective, longitudinal study. ALSPAC Study Team. *Arch Dis Child* 2000;83:307-12.
- Lanphear BP, Aligne CA, Auinger P, Weitzman M, Byrd RS. Residential exposures associated with asthma in US children. *Pediatrics* 2001;107:505-11.
- Collila S, Nicolae D, Pluzhnikov A, Blumenthal MN, Beaty TH, Meyers DA, et al. Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. *J Allergy Clin Immunol* 2003;111:840-6.

24. Meyers DA, Postma DS, Panhuysen CIM, Xu J, Amelung PJ, Levitt RC, et al. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994;23:464-70.
25. Postma DS, Bleecker EER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI, et al. Genetic susceptibility to asthma—bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333:894-900.
26. Panhuysen CI, Bleecker ER, Koeter GH, Meyers DA, Postma DS. Characterization of obstructive airway disease in family members of probands with asthma. An algorithm for the diagnosis of asthma. *Am J Respir Crit Care Med* 1998;157:1734-42.
27. 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 asthma and bronchitic patients. *Int Arch Allergy* 1962;20:93-101.
28. Van der Lende R, Orie NG. The MRC-ECCS questionnaire on respiratory symptoms (use in epidemiology). *Scand J Respir Dis* 1972;53:218-26.
29. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analyses: a unified multipoint approach. *Am J Hum Genet* 1996;58:1347-63.
30. Morton NE. The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type. *Am J Hum Genet* 1956;8:80-91.
31. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlick-Katuzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
32. Noguchi E, Shibasaki M, Arinami T, Takeda K, Maki T, Miyamoto T. Evidence for linkage between asthma/atopy in childhood and chromosome 5q31-q33 in a Japanese population. *Am J Respir Crit Care Med* 1997;156:1390-3.
33. Martinez FD, Solomon S, Holberg CJ, Graves PE, Baldini M, Erickson RP. Linkage of circulating eosinophils to markers on chromosome 5q. *Am J Respir Crit Care Med* 1998;158:1739-44.
34. Yokouchi Y, Nukaga Y, Shibasaki M, Noguchi E, Kimura K, Ito S. Significant evidence for linkage of mite-sensitive childhood asthma to chromosome 5q31-q33 near the interleukin 12B locus by a genome-wide search in Japanese families. *Genomics* 2000;66:152-60.
35. Wright AL. Epidemiology of asthma and recurrent wheeze in childhood. *Clin Rev Allergy Immunol* 2002;22:33-44.
36. Martinez FD, Cline M, Burrows B. Increased incidence of asthma in children of smoking mothers. *Pediatrics* 1992;89:21-6.
37. Gilliland FD, Li YF, 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;163:429-36.
38. Young S, Sherrill DL, Arnott J, Diepeven D, LeSouef PN, Landau LI. Parental factors affecting respiratory function during the first year of life. *Pediatr Pulmonol* 2000;29:331-40.
39. Gilliland FD, Berhane K, Islam T, Wenten M, Rappaport E, Avol E, et al. Environmental tobacco smoke and absenteeism related to respiratory illness in school children. *Am J Epidemiol* 2003;157:861-9.
40. Hirschhorn JN, Altshuler. Once and again—issues surrounding replication in genetic association studies. *J Clin Endocrinol Metab* 2005;87:4438-41.