

Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure

Susan Colilla, PhD,^{a*} Dan Nicolae, PhD,^b Anna Pluzhnikov, PhD,^a
Malcolm N. Blumenthal, MD,^c Terri H. Beaty, PhD,^d Eugene R. Bleecker, MD,^e
Ethan M. Lange, PhD,^f Stephen S. Rich, PhD,^f Deborah A. Meyers, PhD,^e
Carole Ober, PhD,^a Nancy J. Cox, PhD,^a and the Collaborative Study for the
Genetics of Asthma Chicago, Ill, Minneapolis, Minn, Baltimore, Md, and Winston-Salem, NC

Background: Asthma, a common and chronic disease of the airways, has a multifactorial cause involving both genetic and environmental factors. As a result, mapping genes that influence asthma susceptibility has been challenging.

Objective: This study tests the hypothesis that inclusion of exposure to environmental tobacco smoke (ETS), a potential risk factor for asthma, would improve the ability to map genes for asthma.

Methods: By using 144 white families from the Collaborative Study for the Genetics of Asthma, environmental information about exposure to ETS during infancy was incorporated into a genome-wide multipoint linkage analysis. Statistical significance of observed gene-environment interactions was assessed by means of simulation.

Results: Three regions with nominal evidence for linkage when stratified on the basis of ETS exposure were identified ($P < .01$) and showed a significant increase from the baseline lod score (1p at 97 cM, D1S1669-D1S1665; 5q at 135 cM, D5S1505-D5S816; and 9q at 106 cM, D9S910; all $P < .05$). In addition, 2 other regions, although not meeting nominal significance after stratification on the basis of ETS exposure, showed a significant increase from baseline lod score when ETS was taken into account (1q at 240 cM, D1S549; 17p at 3 cM, D17S1308; all $P < .01$).

Conclusion: These results illustrate how evidence for linkage of asthma can depend on exposure to an environmental factor, such as ETS. Future linkage analyses should include information on suspected environmental factors for asthma to help target new candidate susceptibility genes for asthma. (*J Allergy Clin Immunol* 2003;111:840-6.)

Key words: Asthma, genetics, linkage, smoking, gene-environment interaction, environmental tobacco smoke, genetic epidemiology

Asthma, like most complex diseases, has multiple genetic and environmental risk factors. Several genome-wide linkage analyses have been performed for asthma and its related phenotypes, with results suggesting that multiple genes with relatively small effects might be involved. More than 19 different chromosomal regions have been linked to asthma or its related phenotypes in more than one genome scan.¹ It has also been shown, through segregation analysis, that several asthma-related genes with small effects, along with the effect of shared environmental factors, contribute to the familial aggregation of asthma.² Because of this causative heterogeneity, detecting and localizing genes by using traditional genome-wide linkage analysis methods have been challenging.

Several environmental factors, such as environmental tobacco smoke (ETS), dust mite and pet allergens, cold air, respiratory infections, medication, and exercise, are known to exacerbate asthma symptoms,³⁻⁷ but it is less clear how important they are in the development of asthma. Childhood exposure to tobacco smoke in a household has been associated with many asthma-related phenotypes (ie, wheezing and lung function).⁸⁻¹² Recent studies suggest that maternal smoking during pregnancy (in utero tobacco smoke exposure) in particular is a significant risk factor for the development of asthma.¹³⁻¹⁵ Furthermore, some studies have suggested that an interaction between family history of asthma or atopy and exposure to tobacco smoke in early childhood (if not in utero) influences the risk for asthma.^{13,16-18} If there is a true gene-environment interaction between early exposure to tobacco smoke and any of the genetic risk factors that affect susceptibility to asthma, then appropriately accounting for exposure to ETS should improve both the power and resolution of linkage studies.

We initiated this study to examine whether incorporating ETS exposure information into a genome-wide linkage analysis would enhance the evidence for linkage in regions that harbor susceptibility genes that interact with ETS and confer risk for asthma. Moreover, if asthma has multiple independent genetic pathways for development, taking into account a known environmental risk factor for asthma might also help identify more homogeneous subgroups. In this article we describe an approach for

From ^athe Department of Human Genetics and ^bthe Department of Statistics, The University of Chicago, Chicago; ^cthe Department of Medicine, University of Minnesota, Minneapolis; ^dthe Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore; and ^ethe Center for Human Genomics and ^fthe Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem.

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*Dr Colilla is currently affiliated with the Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, Pa.

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Reprint requests: Nancy Cox, PhD, Department of Human Genetics, The University of Chicago, 920 East 58th St, Room 507, Chicago, IL 60637.

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Abbreviations used

CSGA: Collaborative Study for the Genetics of Asthma
ETS: Environmental tobacco smoke

including environmental information in a genome-wide linkage analysis of multigenerational families with asthma and test for the presence of a gene-environment interaction with ETS.

METHODS

Family ascertainment

The Collaborative Study for the Genetics of Asthma (CSGA) is a multicenter collaborative study supported by the National Heart, Lung, and Blood Institute, the purpose of which is to identify loci that contribute to the development of asthma and asthma-related phenotypes. Because only white families from the CSGA data set had a sufficiently large sample size to allow us to subset data for this method, this study only included white families ascertained from 4 centers: The University of Chicago, Johns Hopkins University, the University of Minnesota, and the University of Maryland. Families were ascertained through 2 siblings with asthma; further details about the ascertainment scheme have been described elsewhere.^{19,20} Our sample included 144 nuclear and extended families. This number is a larger number than that reported previously²¹ because of the inclusion of 15 additional white families ascertained in Minnesota. Institutional review board approval was obtained at the individual centers.

Data collection

All subjects were evaluated by using standardized protocols, with baseline spirometry performed according to American Thoracic Society criteria.^{22,23} Further details about this procedure have been published previously.^{19,20} All asthmatic patients met the following criteria: (1) age of 6 years or older; (2) at least 2 of 3 symptoms of cough, wheezing, or shortness of breath; (3) a physician's diagnosis of asthma (with no conflicting pulmonary diagnoses); (4) either bronchial hyperresponsiveness, defined as a 20% or greater decrease in FEV₁ after inhalation of 25 mg/mL or less of methacholine, or reversibility to inhaled bronchodilator, defined as a 15% or greater increase in baseline FEV₁ after inhalation of a bronchodilator (albuterol); and (5) fewer than 3 pack-years of cigarette smoking. The standard American Thoracic Society questionnaire was modified to include information about exposure to cigarette smoke as an infant. In this analysis patients were asked to respond to the following question: "Did anyone in your household smoke when you were a baby?" Persons whose response was "Yes" were considered to have been exposed to ETS. Persons whose response was "No" were considered unexposed to ETS, and a response of "Don't know" was recorded as a missing value for exposure. If a child was too young to respond to the questionnaire, the mother was asked to be a proxy respondent.

Genotyping

Using standard methods, DNA was extracted from whole blood at each of the 4 participating CSGA centers and sent to the Mamalian Genotyping Service in Marshfield, Wis, for genotyping. A total of 323 polymorphic markers from 22 autosomal chromosomes were genotyped. Additional markers were added at the individual centers to ensure that no intermarker distance was greater than 20 cM. Marker distances were based on the Marshfield genetic map (Marshfield, Wis).

Analytic methods

Each person who met the criteria for having asthma was assigned a status of either exposed or unexposed to ETS as an infant. Then affected relatives were stratified on the basis of exposure status within their family, and linkage analysis was done separately for those exposed to ETS and having asthma and for those not exposed to ETS and having asthma. For example, a family with 3 exposed affected siblings and 2 unexposed affected grandchildren would contribute 3 affected sibs to the linkage analysis of the exposed subset, and the 2 affected grandchildren would contribute to the unexposed subset analysis (Fig 1). In this example the grandparents were smokers, and therefore their children with asthma were considered exposed to ETS in the household and classified as exposed asthmatic patients. The children with asthma in this generation were considered affected in the exposed analysis, and the other individuals with asthma in the family were given an unknown affection status. Conversely, members of the next generation (grandchildren) were not exposed to ETS in the household because their parents did not smoke. Therefore the grandchildren with asthma were considered affected only in the unexposed analysis. A family had to have at least 2 affected relatives concordant on exposure to ETS to contribute information to the analysis. Eight families contained only 2 relatives with asthma who were discordant for exposure and therefore did not contribute information to either the exposed or unexposed linkage analysis. Furthermore, individuals who had an unknown exposure status were excluded from the analysis ($n = 57$ individuals).

We performed a genome-wide multipoint linkage analysis on the affected relatives in each exposure group by using Allegro software.²⁴ The results were compared with lod scores obtained from a genome scan of all affected relatives in the same families (which was considered the baseline lod score). We used S_{pairs} as the summary score statistic and computed lod scores at 5 equidistant points between markers along each chromosome. Regions that met a nominal significance level (ie, $P < .01$; lod ≥ 1.18) in one of the exposure groups, as well as those with a significant increase in the evidence for linkage ($P < .01$) compared with baseline values, are reported.

To determine whether there was a significant effect of grouping asthmatic patients on the basis of exposure compared with ignoring exposure information on the evidence for linkage, we randomly permuted exposure status among all asthmatic patients over 1000 simulations. Given the tendency of siblings to share exposures, the ratio of exposed to unexposed asthmatic patients within each simulation was kept constant within each group of a certain sibship size. All other affected relatives (nonsiblings) were randomly assigned an exposure, with the total proportion of exposed to unexposed patients kept consistent with the observed proportion from the original study.

Families were then stratified on the basis of exposure status for each simulated data set, and a multipoint linkage analysis was performed for a 10-cM region on each side of the position with the maximum lod score (a 20-cM region). This simulation addresses whether the resulting lod score for asthma in the exposure grouping was independent of exposure status. By randomly assigning exposure, we determined how often by chance we would achieve an lod score as large as or larger than that observed for a comparable sample size within 10 cM in either direction of the chromosomal position of interest. Empiric P values were then determined from these simulated data.

RESULTS

The average age of the asthmatic patients in the families at the time of the examination was 19.5 ± 12.8 years of age, with an average age of onset of asthma at 6.8 ± 8.2

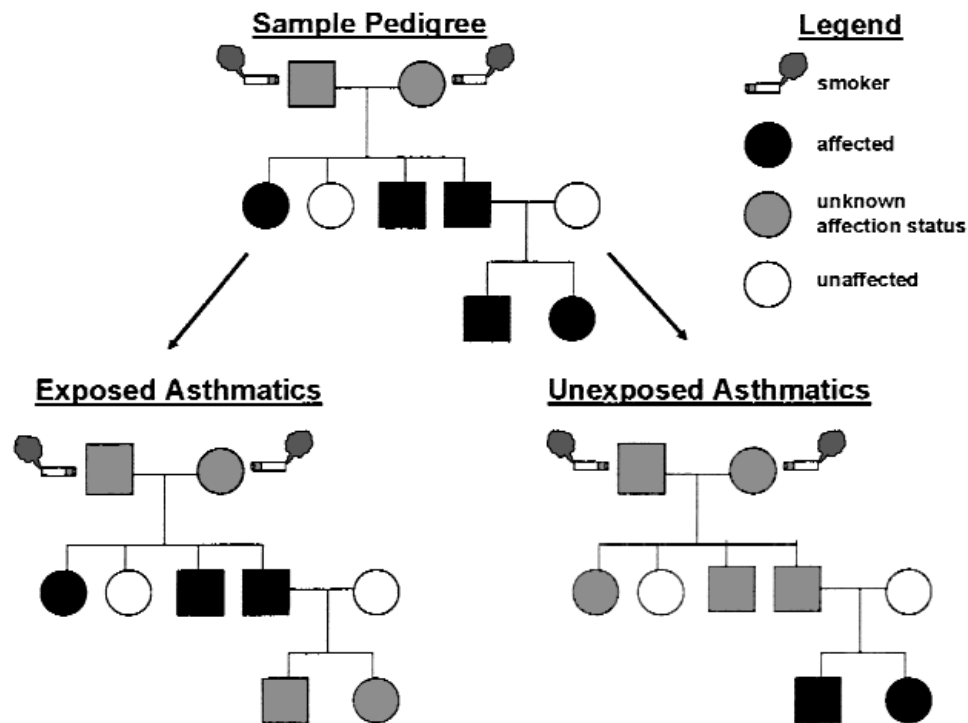


FIG 1. Schematic diagram of how affected individuals in a family were stratified into exposed and unexposed groups.

years, as reported previously.^{19,20} The asthmatic patients were predominantly atopic, with approximately 80% having at least one positive skin test response. Thirty-eight percent of the individuals affected with asthma reported that they were exposed to ETS as an infant, yielding 51 informative families (191 affected individuals) with at least 2 related asthmatic individuals exposed to ETS and 77 families (243 affected individuals) with at least 2 asthmatic individuals who were not exposed to ETS.

The genome-wide linkage analysis results are presented on the basis of ETS exposure for the 22 autosomal chromosomes in Fig 2. Although none of the lod scores for the baseline linkage analysis using all asthmatic patients achieved a genome-wide level of significance,²⁵ there were several regions with either nominally significant evidence for linkage ($P < .01$) when affected patients were separated on the basis of exposure or in which there was a significant increase ($P < .01$) in the evidence for linkage relative to baseline. The genome-wide lod scores are available on the CSGA Web site.

Regions that had either nominal evidence for linkage in an exposure group or were significantly different from baseline lod score are shown in Table I, with their corresponding centimorgan position and nearest markers. The lod score in the exposed group ($n = 51$ families) increased to 1.29 for a region on chromosome 1p at 97 cM between markers D1S1669 and D1S1665 (from an lod score of 0.54 using all asthmatic patients in 144 families). The lod score for the unexposed group of asthmatic patients at this position was zero ($n = 77$ families). The 2-fold increase in lod score on 1p in the ETS-exposed

group in approximately one third of the total families was nominally significant (empiric $P = .034$) and is consistent with an interaction between ETS and a gene in this region. The linkage peak in the exposed group also appears narrower than the peak found in all asthmatic patients in this region.

A 4-fold change in lod score was also seen on 5q at 135 cM, near markers D5S1505 and D5S816, where the lod score increased from 0.34 for all asthmatic patients to 1.23 in the exposed group. This region also had an lod score of zero at this position in the unexposed asthmatic patients. The increase in the lod score on 5q approached statistical significance, as judged by the empiric P value of .051 for a 20-cM region around 135 cM. Again, what was originally a broad peak on 5q for all asthmatic patients became narrower in the exposed group, despite the fact that only approximately one third of families contributed information.

A nominally significant increase in lod score was also found for a region on 9q (D9S910) at 106 cM ($P = .009$) but only in the asthmatic patients who were not exposed to ETS as an infant. The lod score was 0.27 for all asthmatic patients combined but increased to 1.18 in the unexposed group (77 families), whereas the lod score for the exposed group decreased to zero.

Similar results were found for markers near the HLA region (38 cM) on 6p, but because the original lod score was 1.10 in this region, the increase in lod score to 1.45 for the unexposed group was not significant ($P = .17$). The lod score decreased to 0.25 in the exposed group of affected patients at this location, but this change was not

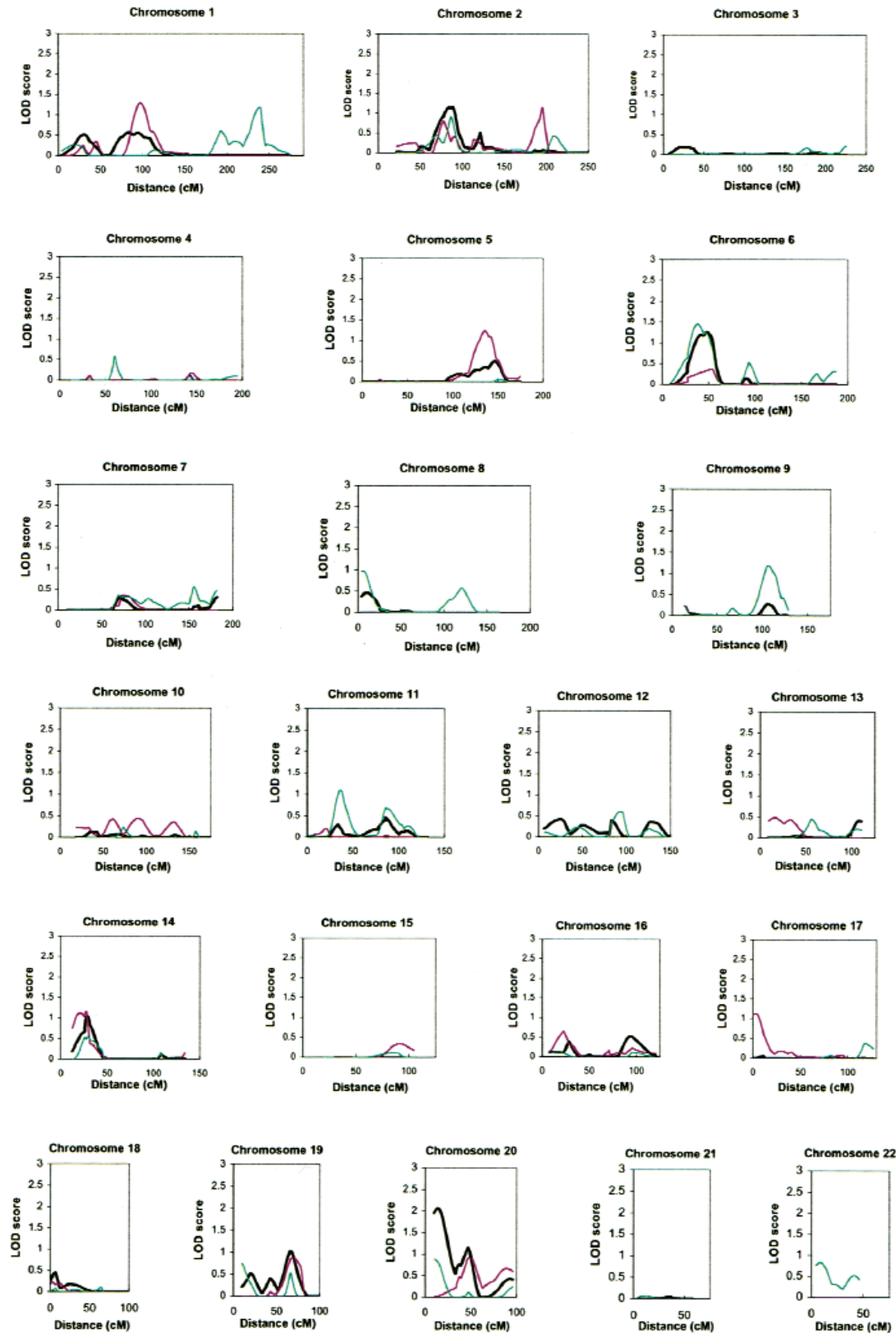


FIG 2. Results from genome-wide linkage analysis of asthma in CSGA white families on the basis of ETS exposure. The *thick black line* represents the lod scores from all asthmatic patients, the *pink line* is for exposed asthmatic patients, and the *green line* represents unexposed asthmatic patients.

TABLE I. Chromosomal regions with nominal evidence for linkage in an exposure group or with a significant change in lod score compared with baseline values

Region	Maximum lod location (cM)	Nearest markers	Lod scores (<i>P</i> value)		
			All asthmatic patients (n = 144 families)	Exposed asthmatic patients (n = 51 families)	Unexposed asthmatic patients (n = 77 families)
1p	97	D1S1669, D1S1665	0.54	1.29 (.034)*	0.00
1q	240	D1S1663, D1S549	0.00	0.00	1.17 (.002)
5q	135	D5S1505, D5S816	0.34	1.23 (.051)	0.00
6p	38	D6S259, D6S1281	1.10	0.26 (.658)	1.45 (.170)
9q	106	D9S910, D9S938	0.27	0.00	1.18 (.009)
17p	3	pter, D17S1308	0.00	1.12 (.016)	0.00

**P* values for testing the significance of the increase in lod score was determined on the basis of 1000 simulations permuting exposure status among the affected individuals.

significant ($P = .66$), providing little evidence that ETS modifies expression of any asthma susceptibility loci in this region.

Other regions that had significant increases in lod scores after stratification over baseline included a region on 1q for families with unexposed affected patients and a region on 17p for families with exposed affected patients (Table I). Simulation results for 1q (D1S549), which had an lod score of 1.17 in families with unexposed affected patients, were significant at a P value of .002 when permuting exposure status among families for the 20-cM region around 240 cM. Similarly, a region on 17p (D17S1308) at 3 cM was also significant in the families with exposed affected patients ($P < .016$), achieving an lod score of 1.12.

DISCUSSION

Although gene-environment interaction is widely believed to contribute to the complexity of the transmission of asthma and other common disorders in families, information on environmental risk factors have rarely been incorporated into linkage studies. Indeed, asthma is one of the few complex disorders for which environmental risk factors are sufficiently well characterized to merit examination in the context of genetic studies. Our approach to mapping loci affecting asthma susceptibility through a gene-environment interaction involves stratifying affected individuals by means of environmental exposure (ETS) in a genome-wide linkage analysis. In this analysis we found regions showing nominally significant increased lod scores when accounting for exposure (or nonexposure) to ETS.

Linkage results from regions on 1p, 5q, and 17p showed significant changes in lod score in the exposed groups when ETS was taken into account. Both 1p and 5q have shown linkage to asthma and asthma-related phenotypes previously.²⁶⁻³⁵ Although none of these lod scores achieved the criteria for genome-wide statistical significance at these 2 regions, it should be noted that when grouping asthmatic patients on the basis of exposure, the number of informative families was reduced from 144 to 51 families in the exposed group, and yet the lod score significantly increased in this region. Because

stratifying asthmatic patients on the basis of an environmental exposure will reduce the number of affected relatives and the number of families in the analysis, lod scores would not necessarily be expected to increase (or decrease) significantly unless a gene-environment interaction exists. Therefore we interpret the significant increase in the lod score on 1p, 5q, and 17p in the exposed groups to indicate that a gene-environment interaction might exist between ETS and unobserved susceptibility genes in these regions.

Inclusion of environmental information in a linkage analysis might also improve the resolution of a linkage peak if a gene-environment interaction is involved in disease susceptibility. In our analysis the lod score peaks were narrower in the exposed group compared with in the overall analysis for both regions on 1p and 5q, despite the smaller number of affected individuals and families. Therefore inclusion of environmental risk factor information has the potential to help localize susceptibility genes to a smaller chromosomal region when a gene-environment interaction exists.

The gene-environment interaction with exposure to ETS for both the 1p and 5q regions is particularly interesting because a previous analysis in the CSGA families detected a gene-by-gene interaction between chromosomes 1 (D1S2134) and 5 (D5S1480),²¹ which is close to the highest linkage peaks on these chromosomes that were found in the current analysis. It is possible that such a gene-gene interaction could actually represent the effects of 2 genes in similar pathways for asthma susceptibility in which ETS exposure might be relevant. Dissecting the complexities of the relationship between genes on 1p and 5q and exposure to ETS will require considerable further investigation.

Wang et al³⁶ recently reported the results of an association study suggesting an interaction between variation at the β -adrenergic receptor on 5q and smoking. When our analysis included all families, the maximum lod score peaked near the location of the β -adrenergic receptor at 146 cM. However, the results for the analysis of the exposed affected individuals showed a maximum peak at the centromeric end of the cytokine cluster, near the *IL4* and *IL13* genes. Evidence for linkage to asthma and its related phenotypes has been observed over a broad region

of chromosome 5q, and it is possible that multiple genes in this region affect susceptibility. Using information on environmental exposure might be one way of improving the resolution of linkage mapping within a region.

Stratifying affected individuals on the basis of exposure to an environmental risk factor in a genome-wide linkage analysis could well reduce heterogeneity for a complex phenotype, thus making the analysis more powerful to detect susceptibility loci. For example, the most significant change in lod score occurred for a region on 9q (D9S910) in the unexposed group of asthmatic patients. This region has shown linkage to asthma in at least 2 other studies.^{26,34} The increased evidence for linkage from analysis of unexposed asthmatic patients is consistent with the hypothesis that some loci act independently of ETS to cause asthma. Thus by removing asthmatic patients exposed to ETS, a more homogenous subgroup of affected individuals might be identified in the unexposed group. Given the heterogeneous nature of the asthma, it is quite possible that different sets of genes might influence risk in families with specific environmental exposures than in families lacking such exposure.

The 2 other regions that showed significant changes in lod score when stratifying on the basis of ETS were 1q (unexposed group) and 17p (exposed group). The region on 1q has shown evidence of linkage to asthma, bronchial hyperresponsiveness, or both in the Hutterite population,³¹ which, interestingly, is a population of non-smokers. The 17p region, which is close to the p-terminal end of the chromosome, has not previously been reported to show linkage to asthma. More investigation will be needed to determine whether novel asthma susceptibility loci exist in these regions and how dependent their linkage might be on environmental factors.

Other nonparametric linkage methods have accounted for covariate effects on linkage by using logistic regression of identical by descent sharing among affected relatives or a variance components method.³⁷⁻⁴² Such methods have constraints on which covariates can be included in the analysis. The stratification approach described here is a simpler way to use environmental exposure information in a multipoint linkage analysis of a dichotomous complex trait in multigenerational family data.

However, as with other studies testing for gene-environment interaction, this approach has its limitations. First, to have sufficient power, one can only examine the effects of common environmental factors (unless the sampling of affected individuals is designed to collect similar numbers of families with exposed and unexposed affected individuals). Second, because this approach stratifies the affected individuals on the basis of exposure, the sample size requirements will be larger than what is commonly collected for a linkage analysis to have sufficient power to test for an interaction. Although we were able to detect significant changes in lod scores when stratifying on the basis of a common dichotomous exposure, the lod scores were still relatively modest. However, not only was the ETS exposure measured retrospectively and therefore subject to recall bias, we were

also not able to distinguish between an effect of in utero tobacco smoke exposure caused by maternal smoking during pregnancy and a more general exposure to tobacco smoke in the household after birth. These factors increase the potential for misclassification of exposure in this study, which would likely bias toward the null. Despite these potential biases, significant increases in the lod score were observed between the different exposure groups. Finally, given that this analysis was a secondary analysis and not corrected for multiple comparisons, these results should be considered exploratory and require replication in an independent sample.

In summary, we present the results of the first genome-wide screen for asthma that incorporated information on a potential environmental risk factor. Lod scores in 5 regions increased significantly, 3 in the exposed group and 2 in the unexposed group. These results are consistent with the hypothesis that genes in 3 chromosomal regions (1p, 5q, and 17p) might interact with ETS to confer risk, whereas genes in 2 other regions (1q and 9q) might confer susceptibility through a pathway independent of ETS exposure. Furthermore, including information on ETS exposure in a genome-wide linkage analysis not only increased the lod score in 5 regions but also appears to have increased the resolution in 2 regions (1p and 5q) and narrowed the peak region.

Finally, by creating more homogenous phenotypes, the power to detect linkage might be increased, even when a smaller number of exposed and unexposed families are used. Given the multifactorial nature of complex disease, this approach might be particularly useful for mapping genes for common diseases, such as asthma, in which environmental risk factors are established and multiple susceptibility genes are likely involved.

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