

Current reviews of allergy and clinical immunology

(Supported by a grant from Astra Pharmaceuticals, Westborough, Mass)

Series editor: Harold S. Nelson, MD

Genetics of asthma

Denise G. Wiesch, PhD, Deborah A. Meyers, PhD, and Eugene R. Bleecker, MD

Baltimore, Md

Asthma is a complex disease with a phenotype that has been clinically difficult to define. Associated phenotypes including bronchial hyperresponsiveness and atopy have provided useful objective alternatives in genetic and epidemiologic studies. Although asthma genes have not yet been identified, much progress has been made toward this goal. Genetic studies indicate that multiple genes are involved in the pathogenesis of this disease, and chromosomal regions likely to harbor asthma susceptibility genes have been replicated in several studies. Environmental factors, including smoking, diet, and viral respiratory infections, have also been implicated in the etiology of asthma. Directly linking these exposures as causes of asthma, however, has also proved difficult. Furthermore, interaction between susceptibility genes and environmental factors is probable and is a challenge currently being pursued by investigators worldwide. Understanding the fundamental gene-environmental interactions in the development of asthma should lead to earlier identification of susceptible individuals and more effective approaches for disease prevention. (*J Allergy Clin Immunol* 1999;104:895-901.)

Key words: Asthma, allergy, bronchial hyperresponsiveness, linkage, candidate genes, smoking, diet, viral respiratory infections, gene-environment interaction

Asthma is the most common chronic disease of childhood, affecting approximately 4.8 million children, and a total of about 14.6 million individuals in the United States.¹ Epidemiologic studies conducted during the last few decades indicate a striking variation in the occurrence of disease across nations and an overall rising prevalence rate in many developed countries.²

Although the etiology of asthma has not yet been fully delineated, it is generally believed that this disease results from exposure to environmental factors in genetically susceptible individuals. Failure to consider both environmental and genetic risk factors as well as the

Abbreviations used

BHR: Bronchial hyperresponsiveness

CSGA: Collaborative Study of the Genetics of Asthma

probable interaction between the two will likely lead to biased interpretations of risk factors and to inaccurate estimates of disease risk for a given individual. This article will review the current understanding of the genetic basis of asthma, some known environmental risk factors, and potential gene-environment interactions. Before exploring these issues, however, the clinical expression and heterogeneity of asthma and asthma-related subphenotypes will be examined.

PHENOTYPE DEFINITION

Asthma is an increasingly common disease that is characterized by variable airway obstruction and bronchial hyperresponsiveness (BHR) and is caused by acute and chronic bronchial inflammation.^{3,4} Clearly defining the disease phenotype is a central issue in genetic studies, where it may be necessary to evaluate family members who have not been previously evaluated or diagnosed. Current definitions of asthma describe its pathophysiologic basis, emphasizing the importance of inflammatory mechanisms.⁵ However, these descriptions are not helpful in differential diagnosis or early disease identification because noninvasive measures of inflammation are not readily available or well validated. Because one uniform phenotypic definition of asthma does not exist, investigators have used various approaches, including a reported physician's diagnosis of asthma, questionnaire data on relevant symptoms, the presence of BHR, or combinations of the above.⁶ Reliance on only historical information such as a prior physician's diagnosis of asthma appears to underestimate the prevalence of asthma and allergy in family and epidemiologic studies.⁷

To investigate the genetic basis of asthma, it is useful to evaluate phenotypes associated with the disease, such as BHR and total serum IgE levels, because these can be measured objectively in all individuals in a family. BHR is associated with asthma and atopy, as observed by the close association of serum IgE levels, BHR, and asthma in asymptomatic children.⁸ Because many asthmatic

From the Center for the Genetics of Asthma and Complex Diseases, University of Maryland, Baltimore, Md.

Supported by National Institutes of Health grants No. R01-HL48341 and U01 HL/AI49602.

Received for publication Aug 25, 1999; revised Sept 1, 1999; accepted for publication Sept 1, 1999.

Reprint requests: Eugene R. Bleecker, MD, Center for the Genetics of Asthma and Complex Diseases, University of Maryland, Baltimore, 108 N Greene St, Suite 119, Baltimore, MD 21201.

Copyright © 1999 by Mosby, Inc.

0091-6749/99 \$8.00 + 0 1/1/102654

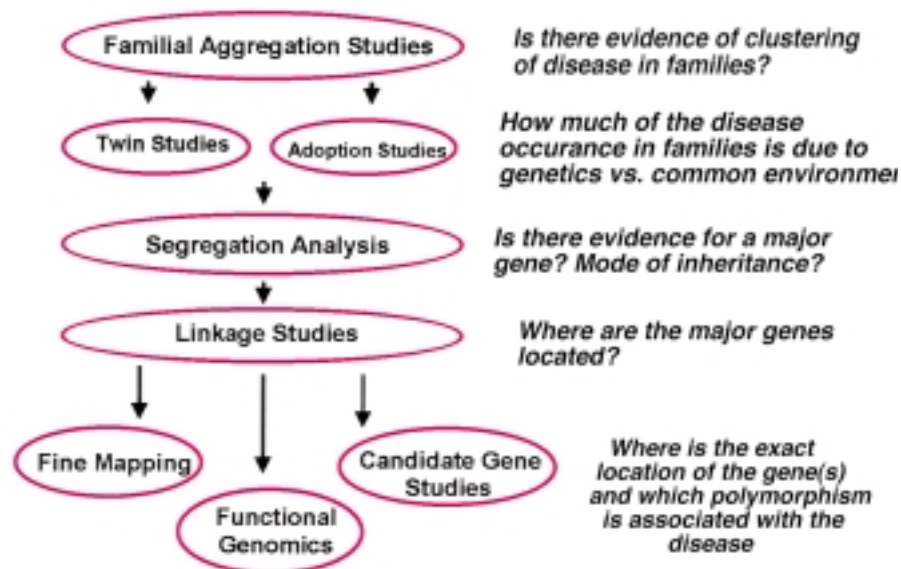


FIG 1. Overview of genetic studies.

patients are atopic, it is appropriate to study other measures of the allergic phenotype, such as total serum eosinophil counts, skin test responses to common allergens, and specific IgE measures in addition to BHR and total serum IgE levels.

GENETIC STUDIES OF ASTHMA

Evidence for a genetic component in complex diseases to eventual identification of disease genes generally progresses through several types of genetic studies (Fig 1). The following paragraphs will discuss evidence for a genetic component for asthma, chromosomal regions believed to harbor asthma susceptibility disease genes, association or candidate gene studies, and recent technology to identify multiple functional consequences of genetic variation (functional genomics).

Familial aggregation and twin studies

Familial aggregation and twin studies are useful initial steps in determining whether a trait has a measurable genetic component. Significant familial aggregation of asthma and associated phenotypes such as BHR and total serum IgE levels has been described in numerous studies.⁹⁻¹³ In addition, the presence of familial aggregation for asthma has been supported by studies reporting that an asthma phenotype is present in approximately 25% of the offspring of a parent with asthma.⁷

Higher concordance for a disease phenotype in monozygotic twins (who share 100% of their genes) compared with dizygotic twins (who share on average 50% of their genes as do other siblings) also provides important evidence of a genetic component. An increased correlation of total serum IgE levels¹⁴ and a higher concordance of asthma was seen in monozygotic twins compared with dizygotic twins.^{15,16}

Segregation analysis

Although it has limitations in complex disorders,¹⁷ segregation analysis is useful for genetic modeling. In multiple studies evidence for recessive inheritance of "high" total serum IgE levels has been found with different estimates for gene frequency and for mean levels in the "low" and "high" phenotypes. For example, in a northern Dutch population elevated total serum IgE levels appear to be inherited in a recessive pattern, with additional evidence supporting a 2-locus model.^{18,19} In a large study of Hispanic families and non-Hispanic white families, Martinez et al²⁰ reported evidence for a codominant mode of inheritance in both ethnic groups, suggesting that it is possible to distinguish "gene carriers" from "unaffected" family members despite the substantial overlap in distribution of total IgE levels.

There have been a few segregation analysis studies of other measures of the allergic or asthmatic phenotype. For bronchial responsiveness to methacholine, Townley et al²¹ were unable to exclude genetic or environmental models and concluded that the bimodal distribution of the bronchial response was not the result of segregation at a single autosomal locus. However, in our Dutch population studies evidence for dominant inheritance was observed after covarying for total serum IgE levels.²² For asthma, Martinez and Holberg²³ have reported that there is a strong familial polygenic component to asthma, suggesting the presence of several major genes.

Linkage studies—Genome-wide screens

Five recent genome-wide screens in different human populations have contributed significantly to the mapping of allergy and asthma genes. The first study by Daniels et al²⁴ was performed on families recruited from Western Australia and included phenotypic data on asth-

ma, BHR, total serum IgE, and serum eosinophil counts. A second screen by the Collaborative Study on the Genetics of Asthma²⁵ included 4 study centers in the United States that collected families from 3 ethnic groups (African Americans, Caucasians, Hispanics). The third genome screen was performed on families with asthma from the inbred Hutterite population.²⁶ The fourth screen was of asthma families ascertained from the Netherlands²⁷ and the fifth genome screen was performed in German and Swedish asthma families.²⁸ Except for the study of Daniels et al,²⁴ which used a 20 cM screening map, maps at approximately 10 cM were used in each of these studies.

Evidence for linkage to multiple chromosomal regions was reported in each study. Perhaps most interesting are those regions showing evidence for linkage by multiple groups ("replicated" regions), which include chromosomes 5q, 6p, 11q, 12q, and 13q. However, there are other regions that have been reported in at least 2 populations (1, 2q, 3, 14, 9, 16, and 17q). "Novel" locations observed only in one study so far include chromosomes 7p, 10q, 19, 20, and 21.

Chromosome 5q. Linkage to chromosome 5q was reported initially with use of a candidate gene approach because there are multiple genes on 5q that may be important in the regulation of IgE and the development or progression of inflammation associated with allergy and asthma. Linkage to 5q has been observed in the Dutch population for a range of phenotypes from asthma and BHR to total serum IgE levels.²⁹⁻³² In the inbred and genetically isolated Amish population, linkage to 5q in the region of several of the cytokines was observed for regulation of total serum IgE levels.³⁰ In the Collaborative Study of the Genetics of Asthma (CSGA) Caucasian families, evidence for linkage to this region of 5q was observed for the asthma phenotype.²⁵ Strong evidence for linkage to 5q (D5S1480) was also seen in the Hutterites for the "loose" definition of asthma, which included subjects with asthma, BHR, or asthma symptoms.²⁶

Chromosome 11. Evidence for linkage of a broadly defined allergic phenotype to markers on chromosome 11q was detected at the early stages of a genome-wide screen.³³ These investigators later postulated that sequence variants in the high-affinity IgE receptor (FcεR) gene may increase the risk for development of allergy and possibly even asthma.³⁴ The postulated sequence variant (Leu181) has not been observed in several other studies, including the Dutch population.³⁵ However, from genome-wide screens there is continued evidence for linkage of the atopic phenotype to this region²⁴ and for the asthma phenotype in the CSGA African Americans.²⁵

Chromosome 12q. Evidence for linkage to 12q for phenotypes ranging from BHR to allergy was first observed with use of a candidate gene³⁶ and a genome screen approach.²⁵ Evidence for linkage for both asthma and total IgE levels was reported in an Afro-Caribbean population and for total IgE levels in the Amish population.³⁶ In the CSGA, evidence for linkage was observed

for the asthma phenotype in Caucasians and Hispanics but not in African Americans.²⁵ In an additional candidate gene study that used randomly ascertained families and a sample of multiplex families ascertained for asthma, evidence for linkage was observed for both asthma and allergic phenotypes.³⁷ Several candidate genes map to this wide region, including IFN- γ , nitric oxide synthase, and mast cell growth factor, but variants in these genes have not been fully evaluated.

Chromosome 13. For the asthma phenotype, evidence for linkage was observed to 13q21.3-qter from the genome screen in the CSGA Caucasian families.²⁵ Evidence for linkage of the atopic phenotype (but not BHR) was reported by Daniels et al.²⁴

There are multiple regions of the genome likely to contain susceptibility genes, as seen from studies in different populations. However, the evidence for any given linkage varies across populations, probably because of founder effects, admixture, and possibly varying environmental influences. In addition, new linkages are being reported and need to be confirmed in additional populations. It is clear from these studies, however, that there are multiple susceptibility genes for asthma and allergy.

Candidate Gene Association Studies

Although linkage studies are performed in families, association studies are generally performed in samples of unrelated individuals (affected and unaffected). They may be performed to determine the increased risk of disease as a result of the presence of a susceptibility allele or to investigate the possible role of a "severity" allele that may affect progression of the disease or even response to treatment. The choice of controls is often difficult because the subjects need to be matched for any variable that may influence the results, for example, age, sex, and ethnic background.¹⁷ It is important to determine a priori the rationale for performing such an association study because there are many potential candidate genes for common disorders. Candidate genes may be positional in nature (in a linked region) or functional where there is a known effect of the gene on biologic function.

An example of a candidate gene that may affect severity and where there are known functional variants is the β_2 -adrenergic receptor on 5q. It has been reported that individuals who are homozygous for Gly16 are more likely to have more severe asthma (corticosteroid dependent or with nocturnal symptoms).³⁸ In addition, a recent study suggests that polymorphisms in the β_2 -adrenergic receptor gene may be important in specific responses to therapy (pharmacogenetics).³⁹ Additional studies where individuals are genotyped before treatment so that well-matched groups are included is important in pharmacogenetic investigations.

Another example of association studies are 2 recent studies of the IL-4 receptor and 2 functional polymorphisms. In the first study an association with atopy defined as an elevation in total serum IgE levels and a gain of function mutation was observed.⁴⁰ In the second

study an association was observed with atopic asthma and a different polymorphism in the gene.⁴¹ Polymorphisms within a gene are often in linkage disequilibrium because of close physical spacing and it is difficult to determine which polymorphism(s) in a candidate is responsible for a modest effect that may be seen.

Functional genomics

Along with association studies and positional cloning, functional genomics represents another type of genetic approach with the potential for identifying asthma disease genes. This recent advance in molecular technology permits the identification of thousands of genes simultaneously and quantification of specific differential expression under varying physiologic conditions or between cases and controls. For example, studies are now underway comparing genes that are differentially expressed in allergic individuals before and after allergen challenge with the hope of pinpointing potential disease genes.

ENVIRONMENTAL RISK FACTORS FOR ASTHMA

An environmental component in the pathogenesis of asthma is well accepted, as is the potential for gene-environment interaction. The following is a review of some of the important environmental risk factors believed to contribute the asthma phenotype.

Diet

Several epidemiologic studies have investigated diet as a potential risk factor for asthma, although results have been inconsistent. Breast-feeding has been shown to be both protective and to increase the risk of asthma in different studies.⁴²⁻⁴⁴ Dietary intake of vitamin C was shown to be protective in studies where reduced plasma levels of vitamin C were observed in asthmatic patients.⁴⁵⁻⁴⁷ In addition, fatty acid composition of the diet, in particular the relative amounts n-3 and n-6 polyunsaturated fatty acids found in fish oil, may be associated with asthma.⁴⁸ Other epidemiologic studies have also found an association between fish consumption and asthma, BHR, and chronic nonspecific lung disease.⁴⁹⁻⁵¹ Dietary sodium has been shown to relate to increased bronchial responsiveness to histamine challenge.⁵²⁻⁵⁴ However, other epidemiologic studies have not shown a relationship between urinary sodium levels and methacholine airway responsiveness.⁵⁵⁻⁵⁷

Viral respiratory infections

Viral respiratory infections occur frequently in childhood and are known to provoke wheezing in asthmatic as well as nonasthmatic children.⁵⁸ It has been hypothesized that viral infections enhance airway inflammation⁵⁸ and airway hyperresponsiveness,⁵⁹ and follow-up studies of children suggest that these illnesses may predispose to the development of asthma.⁶⁰⁻⁶³

The role of viral antigens in the etiology of asthma is uncertain, and opposing hypotheses exist relating to T-

helper lymphocytes, which are classified as T_H1 or T_H2 cells depending on the cytokines they produce.⁶⁴ Some postulate that the rising prevalence of asthma seen in developed countries may actually be a result of the overall decrease in viral infections in childhood because of improved immunization efforts.^{3,65,66} Because viral antigens have been shown to stimulate the T_H1 inflammatory response in airways and asthma has been associated with the T_H2 profile, decline in childhood respiratory infections may cause activation of T_H2 cells and susceptibility to asthma.

Smoking

There are substantial data showing that tobacco smoking increases airway responsiveness, perhaps by inducing airway inflammation,^{67,68} although it has not been directly linked to asthma as a cause. Smokers also tend to report wheezing more frequently than nonsmokers do, and wheezing tends to decline after cessation. The increased airway responsiveness in active smokers also tends to abate after smoking cessation.⁶⁹

Maternal smoking

There is also evidence suggesting that in utero exposure to tobacco smoke components may affect airway responsiveness after birth. On the basis of histamine challenge data from 63 healthy infants at a mean age of 4.5 weeks, parental smoking and a family history of asthma were associated with an increased level of airway responsiveness.⁷⁰ In a similar prospective study, children whose mothers smoked during pregnancy had decreased pulmonary function shortly after birth.⁷¹

Maternal-fetal interactions

Clinically and historically, pregnancy is considered to be a state of altered maternal cellular immunity. These changes in cellular immunity are felt to be necessary for the mother to tolerate a naturally occurring allograft, a fetus expressing foreign paternal MHC molecules. Although it is speculated that an "immunologic barrier" protects the fetus from rejection by maternal lymphocytes, the mechanism of this protection is largely unknown and the subject of recent research.

The depression of maternal cellular immunity (or T_H1 immune responses) has led to the hypothesis that a switch to an up-regulated T_H2 immune response occurs during pregnancy and represents an "allergic" phenotype.⁷² A pregnant woman with allergy or asthma may influence the expression of these traits in her offspring by the transmission of susceptibility genes as well as by providing further upregulation of the allergic T_H2 phenotype during gestation.⁷³ In addition, environmental exposures to allergens and tobacco smoke may amplify allergic responses in both the mother and child.

GENE-ENVIRONMENT INTERACTIONS

Current concepts of asthma pathogenesis postulate that onset of the disease and its clinical course are deter-

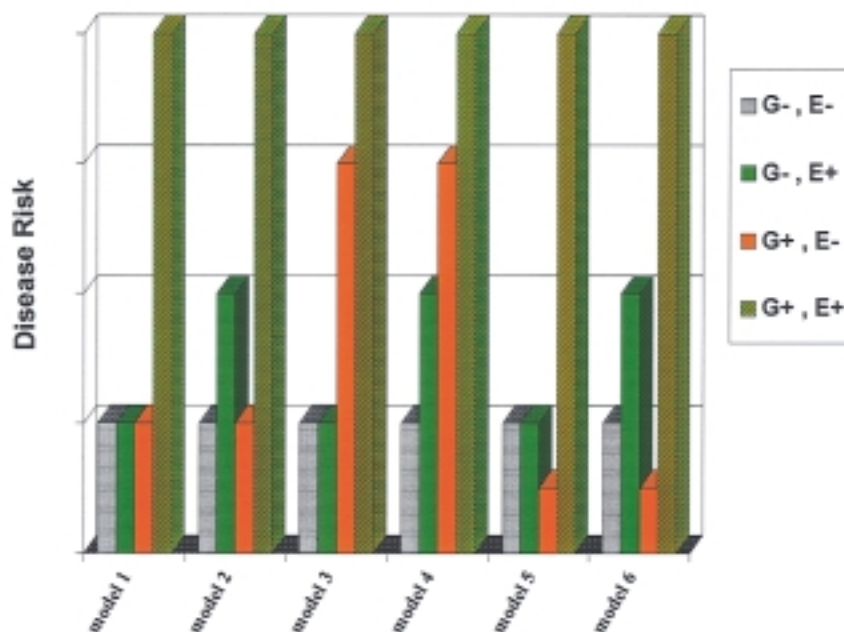


FIG 2. Potential models of gene-environment interaction.

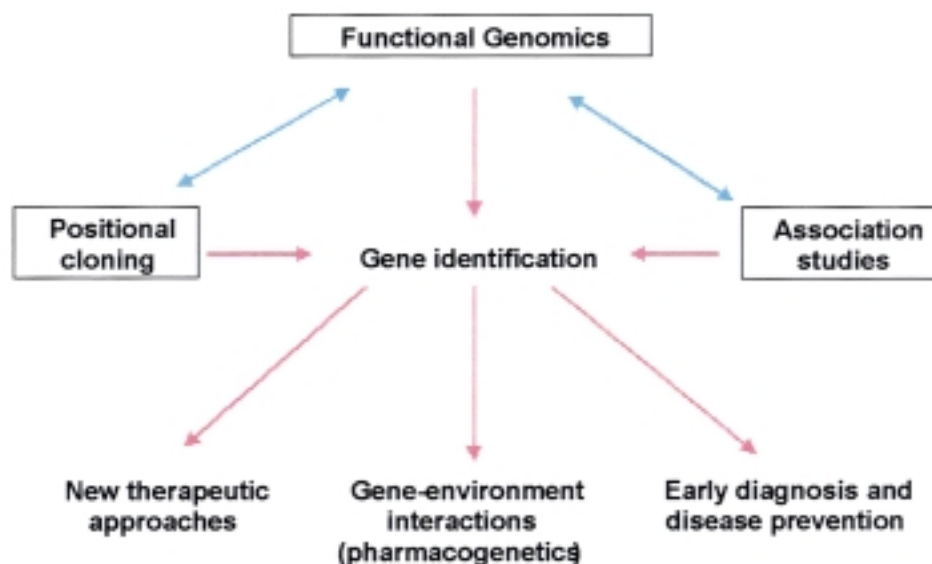


FIG 3. Public health significance of genetics studies of asthma.

mined by gene-environment interactions, that is, those persons in whom asthma develops are both genetically susceptible and receive an appropriate environmental stimulus. Across the range of asthmatics in the population, the relative influence of genes and environmental factors probably varies. Gene-environment interactions can be assessed in case-control and cohort studies as well as in family-based genetic studies. The underlying hypothesis is that individuals with different asthma-related genotypes will have different sensitivities to environmental exposures. Khoury et al⁷⁴ suggested several possible patterns for gene-environment interaction (Fig 2). One is that both the presence of a given asthma suscepti-

bility gene and an environmental exposure are necessary to produce excess risk of disease. The second pattern is that an environmental factor could produce an increased risk of disease in all individuals but a much greater risk in individuals with the susceptible genotype. The third possible pattern of gene-environment interaction is that the environmental exposure alone will not produce increased risk, but in individuals with the susceptible genotype the presence of the environmental factor will increase the risk of asthma more than the genotype alone. The fourth pattern is that both the genotype and the environmental exposure produce increased risk. The fifth and sixth patterns occur when there is an opposite effect of

the genotype, depending on whether the environmental factor is present.⁷⁴ As underlying asthma susceptibility genes are discovered, interactions with environmental factors known to be associated with asthma can be assessed.

CONCLUSION

Susceptibility to development of asthma is the result of multiple environmental risk factors and multiple genes. Genetic studies of asthma continue to provide insight into the pathophysiologic mechanisms of the disease. This should ultimately lead to new and more effective therapeutic interventions, new diagnostic methods for presymptomatic diagnosis, the development of strategies for disease prevention in susceptible individuals, and delineation of the interaction between genotype and the response to specific treatments (pharmacogenetics) (Fig 3). Family studies have already provided insight on disease expression and characterization of the asthmatic phenotype.

Several environmental factors have been implicated in the etiology of asthma, including active and passive exposure to tobacco smoke, viral respiratory infections in childhood, and dietary constituents. In addition, a fetus may be predisposed to disease not only by transmission of susceptibility genes but also by the maternal immunologic environment of an asthmatic or allergic mother during gestation.

Although asthma susceptibility genes have yet to be reported, a great deal of progress has been made in recent years. Moreover, molecular advances including completion of the human genome sequence, identification of vast numbers of sequence variants, and new techniques to identify multiple functional consequences of genetic variation offer promising new avenues for unraveling the genetic and environmental factors that are important in the pathogenesis of common diseases such as asthma.

REFERENCES

- Adams PF, Marano MA. Current estimates from the National Health Interview Survey, 1994. *Vital Health Stat* 1995;10-94.
- Wiesch DG, Samet JM. Epidemiology and natural history of asthma. In: Middleton E, Ellis EF, Yunginger JW, Reed CE, Franklin Adkinson N, Busse WW, editors. *Allergy: principles and practice*. St Louis: Mosby; 1998. p 799-815.
- Holgate ST. Asthma genetics: waiting to exhale. *Nat Genet* 1997;15:227-9.
- National Heart, Lung, and Blood Institute, National Institutes of Health. Guidelines for the diagnosis and management of asthma. Washington: US Government Printing Office; 1991. Publication No.: 91-3042.
- National Heart, Lung, and Blood Institute, National Institutes of Health. Guidelines for the diagnosis and management of asthma. Washington: US Government Printing Office; 1995. Publication No.: 95-3659.
- Wiesch DG, Meyers DA, Bleecker ER, Samet JM. Classification of the asthma phenotype in genetic studies. In: Liggett SB, Meyers DA, editors. *The genetics of asthma*. New York: Marcel Dekker; 1996. p 421-39.
- 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.
- Sears MR. Epidemiological trends in bronchial asthma. In: Kaliner MA, Barnes PJ, Persson CGA, editors. *Asthma, its pathology and treatment*. New York: Marcel Dekker; 1991. p 1-49.
- Luoma R, Koivikko A. Occurrence of atopic diseases in three generations. *Scand J Soc Med* 1982;10:49-56.
- Higgins M, Keller J. Familial occurrence of chronic respiratory disease and familial resemblance in ventilatory capacity. *J Chron Dis* 1975;28:239-51.
- Sibbald B, Turner-Warwick M. Factors influencing the prevalence of asthma among first degree relatives of extrinsic and intrinsic asthmatics. *Thorax* 1979;34:332-7.
- Hopp RJ, Bewtra A, Biven R, Nair NM, Townley RG. Bronchial reactivity pattern in nonasthmatic parents of asthmatics. *Ann Allergy* 1988;61:184-6.
- Longo G, Strinati R, Poli F, Fumi F. Genetic factors in nonspecific bronchial hyperreactivity. *Arch Pediatr Adolesc Med* 1987;141:331-4.
- Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. *J Allergy Clin Immunol* 1984;73:265-70.
- Edfors-Lubs M. Allergy in 7000 twin pairs. *Acta Allergol* 1971;26:249-85.
- Duffy DL, Nicholas MG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 1990;142:1351-8.
- Lander ES, Schork NJ. Genetic dissection of complex traits. *Science* 1994;265:2037-48.
- Meyers DA, Postma DS, Panhuysen CI, 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.
- Xu J, Levitt RC, Panhuysen CIM, Postma DS, Taylor EW, Amelung PJ, et al. Evidence for two unlinked loci regulating total serum IgE levels. *Am J Hum Genet* 1995;57:425-30.
- Martinez F, Holberg C, Halonen M, Morgan WJ, Wright A, Taussig LM. Evidence for mendelian inheritance of serum IgE levels in Hispanic and non-Hispanic white families. *Am J Hum Genet* 1993;55:555-65.
- Townley RG, Bewtra A, Wilson AF, Hopp RJ, Elston RC, Nair N, et al. Segregation analysis of bronchial response to methacholine inhalation challenge in families with and without asthma. *J Allergy Clin Immunol* 1986;77:101-7.
- Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Panhuysen CIM, Xu J, et al. Genetic susceptibility to asthma: bronchial hyperresponsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333:894-900.
- Martinez FD, Holberg CJ. Segregation analysis of physician diagnosed asthma in Hispanic and non-Hispanic white families. *Respir Sci* 1995;14:2-8.
- 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.
- Collaborative Study of the Genetics of Asthma. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat Genet* 1997;15:389-97.
- Ober C, Cox N, 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.
- Stine OC, Postma DA, Howard T, Koppelman GH, Bergin JS, Zheng S, et al. Genome screen for asthma and related phenotypes in a restricted Dutch population [abstract]. *Am J Respir Crit Care Med* 1998;157:A855.
- 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.
- Bleecker ER, Amelung PJ, Levitt RC, Postma DS, Meyers DA. Evidence for linkage of total serum IgE and bronchial hyperresponsiveness to chromosome 5q: a major regulatory locus important in asthma. *Clin Exp Allergy* 1995;25:84-8.
- Marsh DG, Neely JD, Breazeale DR, Ghosh B, Friedhoff LR, Ehrlich-Kautzy E, et al. Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum IgE concentrations. *Science* 1994;264:1152-6.
- 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.
- Postma DS, Bleecker ER, 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.
- Cookson WOCM, Sharp PA, Faux JA, Hopkin JM. Linkage between

- immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989;1:1292-5.
34. Shirakawa T, Enomoto T, Shin-ichiro S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-9.
35. Amelung PJ, Panhuysen CIM, Postma DS, Levitt RC, Koeter GH, Francomano CA, et al. Atopy and bronchial hyperresponsiveness: exclusion of linkage to markers on chromosomes 11q and 6p. *Clin Exp Allergy* 1992;22:1077-84.
36. Barnes KC, Neely ND, Duffy DL, Freidhoff LR, Breazeale DR, Schou C, et al. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. *Genomics* 1996;37:41-50.
37. Dewar JC, Wilkinson J, Wheatley A, Thomas NS, Doull I, Morton NE, et al. The glutamine 27 β_2 -adrenergic receptor polymorphism is associated with elevated IgE levels in asthmatic families. *J Allergy Clin Immunol* 1997;100:261-5.
38. Reihsaus E, Innis M, MacIntyre N, Liggett SB. Mutations in the gene encoding for the beta2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1993;8:334-9.
39. Drazen J. Clinical pharmacology of leukotriene receptor antagonists and 5-lipoxygenase inhibitors. *Am J Respir Crit Care Med* 1998;157(Suppl):S233-7.
40. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the a subunit of the interleukin-4 receptor. *N Engl J Med* 1997;337:1720-5.
41. Mitsuyasu H, Izuhara K, Mao Q, Gao PS, Kawai M, Sasaki S, et al. Ile50Val variant of IL4R-alpha upregulates IgE synthesis and associates with atopic asthma. *Nat Genet* 1998;19:119-20.
42. Martin AJ, Landau LI, Phelan PD. Natural history of allergy in asthmatic children followed to adult life. *Med J Aust* 1981;2:470-4.
43. Zeiger RS, Heller S, Mellon MH, Forsythe AB, O'Connor RD, Hamburger RN, et al. Effect of combined maternal and infant food-allergen avoidance on development of atopy in early infancy: a randomized study. *J Allergy Clin Immunol* 1989;84:72-89.
44. Miskelly FG, Burr ML, Vaughan-Williams E, Fehily A, Butland BK, Merrett TG. Infant feeding and allergy. *Arch Dis Child* 1988;63:388-93.
45. Akinkugbe FM, Ette SI. Role of zinc, copper, and ascorbic acid in some clinical paediatric problems. *J Trop Pediatr* 1989;33:337-42.
46. Aderele WI, Ette SI, Oduwale O, Ikpeke SJ. Plasma vitamin C (ascorbic acid) levels in asthmatic children. *Afr J Med* 1985;14:115-20.
47. Olusi S, Ojutiku OO, Jessop WJE, Iboko MI. Plasma white blood cell ascorbic acid concentrations in patients with bronchial asthma. *Clin Chem Acta* 1979;92:161-6.
48. Schwartz J, Weiss ST. The relationship of dietary fish intake to level of pulmonary function in the first National Health and Nutrition Examination Survey (NHANESI). *Eur Respir J* 1994;7:1821-4.
49. Miedema I, Feskens EJM, Heederik D, Kromhout D. Dietary determinants of long term incidence of chronic nonspecific lung disease. *Am J Epidemiol* 1993;138:37-45.
50. Peat JK, Salome CM, Woolcock AJ. Factors associated with bronchial hyperresponsiveness in Australian adults and children. *Eur Respir J* 1992;5:921-9.
51. Hodge L, Salome CM, Peat JK, Haby MM, Wei XA, Woolcock AJ. Consumption of oily fish and childhood asthma risk. *Med J Aust* 1996;164:137-40.
52. Javaid A, Cushley MJ, Bone MF. Effect of dietary salt on bronchial reactivity to histamine in asthma. *BMJ* 1988;297:454.
53. Burney PGJ, Neild JE, Twort CHC. The effect of changing sodium on the bronchial response to histamine. *Thorax* 1989;44:36-41.
54. Burney PGJ. Asthma mortality in England and Wales: evidence for a further increase, 1974-1984. *Lancet* 1986;2:323-6.
55. Sparrow D, O'Connor GT, Rosner B, Weiss ST. Methacholine airway responsiveness and 24-hour urine excretion of sodium and potassium. *Am Rev Respir Dis* 1991;144:722-5.
56. Britton J, Pavord I, Richards K. Dietary sodium intake and the risk of airway hyperreactivity in a random adult population. *Thorax* 1994;49:875-80.
57. Pistelli R, Forastiere F, Corbo GM. Respiratory symptoms and bronchial responsiveness are related to dietary salt intake and urinary potassium excretion in male children. *Eur Respir J* 1993;6:517-22.
58. Busse WW, Lemanske RFJ, Dick EC. The relationship of viral respiratory infections and asthma. *Chest* 1992;101:385-8s.
59. Lemanske RFJ, Dick EC, Swenson CA. Rhinovirus upper respiratory infection increases airway reactivity in late asthmatic reactions. *J Clin Invest* 1989;83:1-10.
60. McConnochie KM, Hall CB, Barker WH. Lower respiratory tract illness in the first two years of life: epidemiologic patterns and costs in a suburban pediatric practice. *Am J Public Health* 1988;78:34-9.
61. Hall CB, Hall WJ, Gala CL, McGill FB, Leddy JP. Long-term prospective study in children after respiratory syncytial virus infection. *J Pediatr* 1984;105:358-64.
62. Henry RL, Hodges IGC, Milner AD, Stokes GM. Respiratory problems 2 years after acute bronchiolitis in infancy. *Arch Dis Child* 1983;58:713-6.
63. Pullan CR, Hey EN. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. *BMJ* 1982;284:1665-9.
64. Gem JE, Busse WW. Role of T cells in virus-induced asthma. In: Liggett SB, Meyers DA, editors. *The genetics of asthma*. New York: Marcel Dekker; 1996. p 39-66.
65. Cookson WOC, Moffatt MF. Asthma: an epidemic in the absence of infection? *Science* 1997;275:41-3.
66. Shirakawa T, Enomoto T, Shin-ichiro S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-9.
67. Tashkin DP, Altose MD, Bleecker ER, Connett JE, Kanner RE, Wong Lee W, et al. The lung health study: airway responsiveness to inhaled methacholine in smokers with mild to moderate airflow limitation. *Am Rev Respir Dis* 1992;145:301-10.
68. Kauffmann F, Frette C, Annesi I, Oryszczyn MP, Dore M, Neukirch F. Relationships of haptoglobin level to FEV1, wheezing, bronchial hyperresponsiveness and allergy. *Clin Exp Allergy* 1991;21:669-74.
69. US Department of Health and Human Services. A report of the Surgeon General: the health benefits of smoking cessation. Washington: US Government Printing Office; 1990.
70. Young S, Le Souef PN, Geelhoed GC, Stick SM, Turner KJ, Landau LI. The influence of a family history of asthma and parental smoking on airway responsiveness in early infancy. *N Engl J Med* 1991;324:1168-73.
71. Hanrahan JP, Tager IB, Segal MR, Tosteson TD, Castile RG, Van Vunakis H, et al. The effect of maternal smoking during pregnancy on early infant lung function. *Am Rev Respir Dis* 1992;145:1129-35.
72. Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol* 1993;151:4562-73.
73. Morgan K. The deposition and clearance of dust from the lungs. In: Morgan KC, Seaton A, editors. *Occupational lung disease*. Philadelphia: WB Saunders; 1975.
74. Khoury MJ, Beaty TH, Cohen BH. Fundamentals of genetic epidemiology. New York: Oxford University Press; 1993.