

Perspectives on the past decade of asthma genetics

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Although genetic linkage and association studies have identified more than 25 asthma or allergy susceptibility loci, replication of significant results remains a problem. Moreover, these approaches typically ignore the true complexity of these diseases, such as the role of gene-by-environment and gene-by-gene interactions. As a result, many important associations might have been missed. Recent studies demonstrate not only that such interactions exist but also that the relationship between genotype and phenotype is more complex than previously thought. (*J Allergy Clin Immunol* 2005;116:274-8.)

Key words: Asthma, allergy, genetics, gene-by-environment interactions

EXTENDING THE MENDELIAN PARADIGM TO COMPLEX DISEASES

The search for genes that influence susceptibility to common diseases remains one of the greatest challenges in human genetics. With the recent completion of the human genome project,¹ the tools are now available to fully meet this challenge and to redefine medicine in the 21st century. The ultimate goals of molecular medicine are both to identify genetically susceptible individuals and intervene before the onset of disease and to design drugs that are individualized and genotype specific. Although there have been countless successes with respect to defining the molecular basis of Mendelian (monogenic) diseases,² genetic studies of common diseases with complex causes have turned out to be considerably more challenging than originally thought. In this perspective I will provide a brief update on the status of genetic studies of asthma and allergy and then discuss some of the insights that have

Abbreviations used

AD: Atopic dermatitis
ADRB2: β_2 -Adrenergic receptor
CD14: Monocyte differentiation antigen 14
COAST: Childhood Onset of Asthma Study
FCERB1: Fc ϵ R β 1
GSTM1: Glutathione S-transferase M1
GSTP1: Glutathione S-transferase P1
HLA-G: Human leukocyte antigen G
IL4RA: IL-4 receptor, α -chain
LTA: Lymphotoxin α
LTC4A: Leukotriene C₄ synthase
NOS3: Nitric oxide synthetase 3
TIM1: T-cell immunoglobulin- and mucin domain-containing molecule 1
TLR4: Toll-like receptor 4

been gained over the past 10 years on the genetic architecture of these traits.

LINKAGE AND ASSOCIATION STUDIES IDENTIFY SUSCEPTIBILITY GENES

Fig 1 shows a common model of susceptibility to asthma and atopy, which implicates many genes and many environmental factors but implies that the effects of genes and environmental factors individually contribute to risk. However, the truth is much more complex, with genes interacting both with other genes and with environmental risk factors to confer susceptibility. In fact, few genes might have independent effects, as is typical for Mendelian diseases. Nonetheless, the approaches that have been used to find susceptibility genes, either through linkage or association studies, have for the most part considered one gene at a time (Fig 2).

Despite this overly simplistic modeling of asthma and atopy genetics, many important discoveries have been made (Fig 3). In particular, 5 genes have been identified through family linkage studies, followed by positional cloning.³⁻⁷ These genes span a wide range of functions and in all cases were either unknown or would not have been considered as candidate asthma genes before their discovery. Among more than 100 genes that have been

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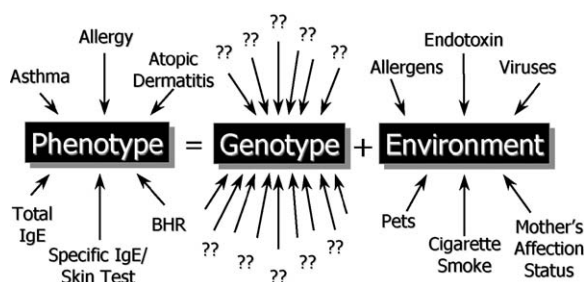


FIG 1. Common model of the genetics of complex diseases. Several related disease and quantitative phenotypes result from the effects of many loci and many environmental factors. *BHR*, Bronchial hyperresponsiveness.

interrogated through association studies (reviewed in Hoffjan et al⁸), 8 genes have been replicated in more than 5 studies, and another 13 genes have been replicated but in fewer than 5 studies. These 26 genes are likely to be true susceptibility loci but represent just the tip of the iceberg because additional positionally cloned genes are soon to be reported, and many other candidate genes will be identified and replicated. Thus one could conclude that the field of asthma genetics has been quite successful and that many genes have been identified that contribute to risk.

THE PROBLEM OF REPLICATION

Replicating results remains the gold standard for genetic association studies, but this has proved difficult for common diseases, such as asthma, irrespective of whether the initial association was identified through candidate gene association or positional cloning studies. Even among the most replicated genes, including those shown in Fig 3, there are many negative studies (for examples, see Table 1 in Hoffjan et al⁸). In fact, there are no genes that are associated with asthma, atopy, or a related phenotype in every study reported. Moreover, even when a gene is replicated, it is often with a different phenotype (eg, a polymorphism in intron 1 of the *LTA* gene is associated with asthma in some studies and IgE in others), with different polymorphisms in the same gene (eg, the -1112C/T promoter polymorphism in the *IL13* gene is associated with atopic asthma in some studies, but the Arg130Gln polymorphism in exon 4 of the same gene is associated with asthma and atopic phenotypes in others), and even with different alleles of the same variant (eg, the -159C allele in the promoter region of the *CD14* gene is associated with atopic phenotypes in some populations, whereas the -159T allele is associated in others). This level of complexity was unexpected and has suggested that models of susceptibility that consider one locus at a time, as is the paradigm for Mendelian diseases, are not adequate for discovering and characterizing asthma and allergy susceptibility loci. Rather, models that include interactions between genes and between genes and environmental risk factors might be required to fully elucidate the genetic architectures of asthma and atopy.

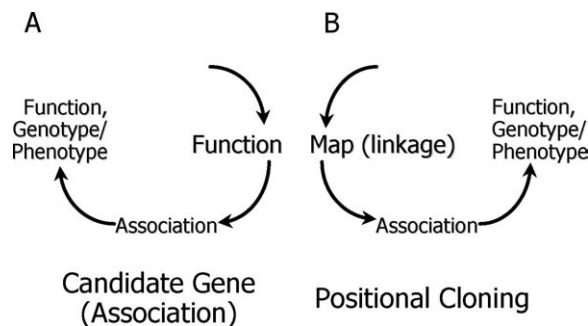


FIG 2. Strategies for identifying disease genes. **A**, With the candidate gene approach, typically used in association studies, a gene is selected on the basis of its known function (ie, functional candidate gene). Variation in that gene is then examined for associations in patients and control subjects, cohorts of individuals, or families. Ultimately, the mechanism of the association is revealed through genotype-phenotype studies and functional studies of the associated variant. **B**, In positional cloning studies initially only information on the chromosomal location is known, usually from family linkage studies. All genes in the linked region become positional candidates, and association studies are performed as described above to identify the associated gene and variation that contributes to disease risk. Once the variation is identified by means of association studies, the mechanism of the association is studied as described above.

GENE-BY-ENVIRONMENT INTERACTIONS AND ASTHMA

We and others have recently begun to examine interactions between individual genotypes and environmental exposures as a first step in developing more complex models of disease susceptibility. These models consider the possibility that specific genotypes might result in a phenotype only in certain environments or that a specific genotype might result in different phenotypes, depending on environmental exposures. Such interactions could mask associations if the study sample is heterogeneous with respect to the exposure or underlie discrepant results between samples drawn from populations that differ with respect to the exposure. A classic example of a gene-by-environment interaction is that of the Mendelian disease α_1 -antitrypsin deficiency. The risk for respiratory diseases, such as emphysema and chronic obstructive pulmonary disease, among homozygotes for the PiZ null allele (ZZ genotype) is nearly 100% in the presence of cigarette smoke exposure. In this case the exposure is thought of as a trigger of disease in genetically susceptible individuals. Other examples of gene-by-environment interactions on asthma and atopy risk have been recently reported,^{7,9-16} and these suggest that such effects might be more the rule than the exception. These studies are summarized in Table I and in all cases provide examples in which genotype-specific effects are modified by environmental exposures. Although not all of these interaction effects have been replicated, they provide the basis for future studies and for characterizing the range of effects of important environmental exposures as modifiers of disease risk.

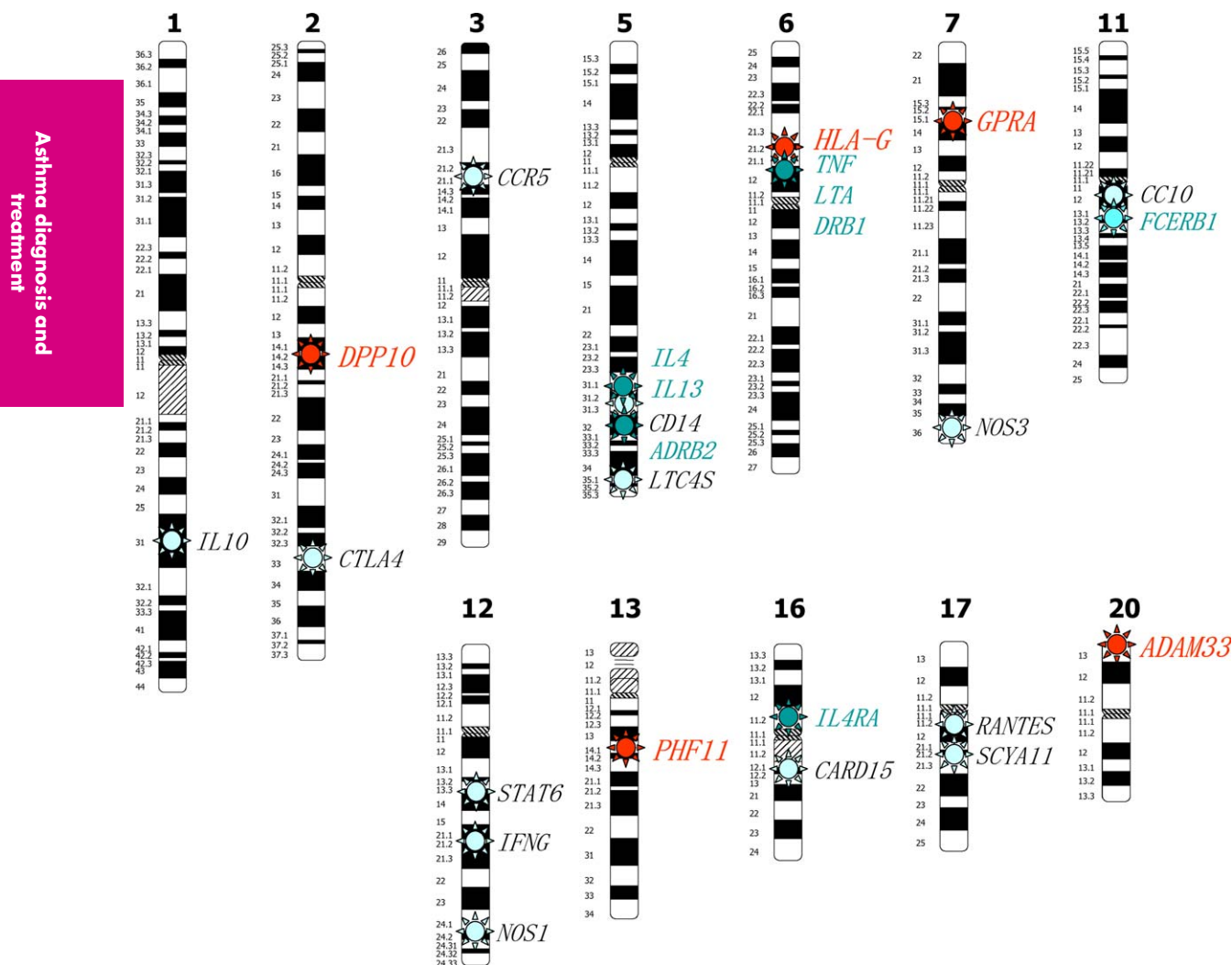


FIG 3. Approximate locations of asthma and atopy genes on human chromosomes. Five genes identified through linkage followed by positional cloning studies are shown in red. Twenty-one genes that were identified through association studies and replicated in subsequent studies are also shown (summarized from Hoffjan et al⁸). Eight genes that have been replicated in more than 5 studies are shown in blue, and 13 genes that have been replicated but in fewer than 5 studies are shown in black.

THE IMPORTANCE OF EARLY-LIFE EXPOSURES

Epidemiologic studies have identified many environmental factors that influence risk for asthma and allergic disease, such as maternal asthma, birth order, and sibship size and early-life exposure to viral infections, endotoxin (LPS), day care, pets, and allergens. Yet few studies to date have examined how exposure to environmental risk factors during development modifies genotype-specific risks for asthma and allergic disease. The Childhood Onset of Asthma (COAST) Study is a prospective birth cohort study of high-risk children designed to evaluate the role of genes and environment on the development of immune responsiveness and allergic phenotypes.¹⁷ The first studies in this cohort to examine gene-by-environ-

ment interactions were recently reported, providing some intriguing examples of interactions. A study of the effects of dog ownership on the development of immune responsiveness and atopy in infancy revealed a protective effect of having a dog in the house at the time the child was born: only 30% of infants had atopic dermatitis (AD) if a pet was present in the home compared with 51% of infants in homes without a dog ($P < .0001$).¹⁴ However, this difference was even more striking among children with the -159TT genotype at the locus encoding the receptor for LPS, *CD14*: only 5% of TT children exposed to a dog had AD compared with 43% of unexposed TT children ($P = .04$). In this example, even though both the polymorphism (*CD14* -159C/T) and the environmental exposure (dog) independently influenced risk for AD, the interaction between the 2 was

TABLE I. Examples of gene-by-environment interaction effects on asthma and atopic disease

| Gene | Environmental Exposure | Phenotype | Comment | Reference |
|---------------|---|---|--|-------------------------------|
| <i>LTC4S</i> | Aspirin exposure | Asthma | −444C allele is increased among individuals with aspirin-induced asthma compared with individuals with aspirin-tolerant asthma | Sanak et al ⁹ |
| <i>ADRB2</i> | Cigarette smoke | Asthma | Increased risk of asthma among smokers with Arg16 genotype but not among nonsmokers | Wang et al ¹⁰ |
| <i>ADRB2</i> | Physical activity | Asthma | Increased risk of asthma among sedentary women with Gly16 genotype but not among active women | Barr et al ¹¹ |
| <i>TIM1</i> | HAV | Atopy | HAV protects against atopy in individuals with a 6-amino-acid insertion at residue 157 (157insMTTTP) but not in individuals without the insertion | McIntire et al ¹² |
| <i>TLR4</i> | Endotoxin levels | Asthma | At high levels of endotoxin exposure, carriers of the Gly299 and Ile399 alleles have reduced risk for asthma compared with other genotypes and other exposure groups | Werner et al ¹³ |
| <i>CD14</i> | Dog ownership at birth | AD | −159TT genotype is protective against AD in the first year among children with a dog in the home at birth | Gern et al ¹⁴ |
| <i>GSTM1</i> | Diesel exhaust particles | IgE and histamine response | Enhanced responses among <i>GSTM1</i> -null individuals but not among individuals with other genotypes | Gilliland et al ¹⁵ |
| <i>GSTP1</i> | Diesel exhaust particles | IgE and histamine response | Enhanced responses among individuals with the Ile105 allele but not among individuals without this allele | Gilliland ¹⁵ |
| <i>NOS3</i> | Day-care exposure in the first 6 mo of life | Change in <i>TH2</i> cytokine (IL-5 and IL-13) response in first year of life | Asp298 homozygosity associated with smallest changes in T_H2 responses among children attending day care and largest changes among children not attending day care | Hoffjan et al ¹⁶ |
| <i>FCERB1</i> | Day-care exposure in the first 6 mo of life | IL-5 response at 1 y of age | Gly237 allele associated with decreased IL-5 responsiveness among children attending day care and increased responsiveness among children not attending day care | Hoffjan et al ¹⁶ |
| <i>IL4RA</i> | Day-care exposure in the first 6 mo of life | IFN- γ response at 1 y of age | Val50 homozygosity associated with lowest response among children attending day care and highest response among children not attending day care | Hoffjan et al ¹⁶ |
| <i>HLA-G</i> | Maternal BHR | Asthma-BHR in child | −964G allele is associated with asthma in children of mothers with BHR; −964A allele is associated with atopy and asthma among children of mothers without BHR | Nicolae et al ⁷ |

HAV, Hepatitis A; BHR, bronchial hyperresponsiveness.

significant ($P = .0071$), indicating that the risk associated with the TT genotype differs in different exposure groups. Interactions between *CD14* genotype and levels of endotoxin exposure have been suggested as an explanation for the discrepant results of association studies with this polymorphism,¹⁸ as discussed earlier, and these data support that hypothesis.

In a second study in this cohort, the effects of day-care attendance in the first 6 months of life on cytokine response profiles and allergic phenotypes were examined.¹⁶ Seventy-two polymorphisms in 35 genes were selected because of their putative role in immune re-

sponses or asthma and genotyped in 99 COAST children who attended day care and 109 COAST children who did not. Interestingly, neither day-care attendance nor genotype at these loci by themselves significantly influenced any of the first-year phenotypes examined. However, highly significant interaction effects ($P < .001$) were demonstrated with genotypes at 3 loci: *NOS3*, *FCERB1*, and *IL4RA*. In each case the effects of a particular genotype on the phenotype were opposite depending on whether the child attended day care (ie, the same genotype was associated with the highest cytokine responses or protection from disease among children attending day care

but the lowest cytokine responses or risk for disease among children not attending day care). That is, the genotype effects at these loci were modified by the environment such that the same genotype was associated with protection from or risk for a phenotype depending on this early-life exposure! In the pooled sample (not stratified by day-care attendance) there were no detectable differences between genotypes (summarized in Table I). Interestingly, the interaction effects with the *FCERB1* and *IL4RA* genes were likely accounted for by the increased number of viral infections among children attending day care; however, the interaction effects with *NOS3* were independent of viral infections, suggesting that risk factors other than viruses but that are correlated with day-care exposure interact with the *NOS3* genotype to determine risk. Complex interactions such as these could underlie some of the association studies in which one allele of a polymorphism is associated in some populations and the other allele of the same polymorphism is associated with the same phenotype in others.

CONCLUDING REMARKS

The mechanisms underlying these interactions are not yet known. Nonetheless, these studies and others are beginning to reveal the true complexities of the genetics of asthma and allergy. The next phase of genetic investigation should continue to unravel the nature and overall importance of gene-by-environment and gene-by-gene interactions on the development of asthma and allergic phenotypes on disease progression and severity and on the response to therapeutic interventions. Thus the next 10 years of asthma genetic research will begin to meet the goals of the new molecular medicine.

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