

Do genetics play a role in the pathogenesis of asthma?

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Asthma is a phenotypically heterogeneous disorder associated with intermittent respiratory symptoms, bronchial hyperresponsiveness (BHR), and reversible airflow obstruction.^{1,2} Most individuals with asthma have evidence of BHR and atopy, with elevated total serum IgE, allergen-specific IgE, or skin-test reactivity to common allergens. At present, it is not clear whether asthma, BHR, and atopy are clinical manifestations of the same underlying primary defect or whether they share common pathways with distinct primary molecular origins.

A genetic component to asthma has long been suggested by studies demonstrating increased prevalences of asthma among first-degree relatives of asthmatic subjects and greater concordance rates among monozygotic compared with dizygotic twins.³⁻⁷ Such "clustering" in families is consistent with a genetic cause but may also reflect the effects of shared environmental exposures. Thus familial clustering does not prove a genetic cause. Although segregation analyses in populations of asthmatic families have suggested the likelihood of a major genetic component for susceptibility to asthma,^{8,9} direct evidence that susceptibility is genetically determined requires identifying the specific genes that confer susceptibility to asthma or to asthma-associated phenotypes, such as BHR or atopy.

This report will review the issues related to studying the genetics of complex disorders, such as asthma, the basic approaches to finding genes that underlie complex disorders, and the current status of the genetic studies of asthma.

GENETIC MODELS OF COMPLEX DISORDERS

Disorders that cluster in families more than expected by chance alone but do not follow any simple Mendelian pattern of inheritance (i.e., autosomal dominant, autosomal recessive, X-linked) are thought to have multifactorial causes and are said to be genetically "complex." Multifactorial refers to the fact that the cause includes both genetic and environmental components, and complex refers to the fact that there are likely to be several genes that influence susceptibility and that these genes may interact in a complex fashion. Most common disorders are included in this category, such as diabetes

Abbreviations used

BHR:	Bronchial hyperresponsiveness
bp:	Base pairs
CSGA:	Collaborative Study on the Genetics of Asthma
HLA:	Human leukocyte antigen
STRP:	Short tandem repeat polymorphism

mellitus, hypertension, coronary artery disease, and asthma. As a result of their multifactorial and complex causes, these disorders have several characteristics that affect the design of genetic studies and can complicate interpretation of their results.

First, these disorders are genetically heterogeneous. That is, there is likely to be more than one gene that influences susceptibility, and susceptibility in different families will be due to different combinations of susceptibility genes. Because the different genetic forms of the disease are not distinguishable at the level of the phenotype, families with different underlying molecular origins may be pooled for analysis, potentially masking the effects of particular genes. This problem can be minimized by either studying very large numbers of families or by studying inbred or genetically homogeneous populations. The larger the number of families included in a genetic analysis, the more likely it will be that a sufficient number of families with the same genetic susceptibility will be studied and that there will be sufficient power to detect particular susceptibility genes. Inbred populations are more genetically homogeneous because these individuals in the population are descendants of relatively few ancestors. As a result, fewer genes influencing susceptibility will be present in the population and should be more easily detected.

Second, not all individuals with the susceptibility genotype will have the disease. Thus the penetrance of the susceptibility genotype will be less than 100%, and, ostensibly, unaffected family members may in fact have the susceptibility allele at the disease locus. To address this problem, many study designs use affected relatives only and ignore all "unaffected" family members. Most commonly, affected sibling pairs are studied, but all affected relatives in a pedigree can be studied in this way.

Third, the definition of the phenotype in all family members is often difficult because of varied expressivity. That is, individuals with the same genetic susceptibilities may have different expressions of the disease. This includes differences in age of onset, disease severity, and the presence of associated phenotypes. Thus within a

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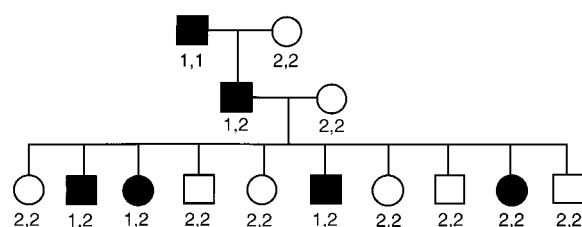


FIG. 1. A disease gene is segregating in the family (affected individuals are indicated by shaded symbols). Family members have been typed for a marker locus at which two alleles (1 and 2) are segregating. Genotypes at the marker locus are shown under the symbols. If the marker locus and the disease locus were segregating independently (i.e., no linkage), the father in generation II would transmit allele 1 and allele 2 in equal proportions to his affected and unaffected children. However, in this family, allele 1 is transmitted from the father to three of four affected children and allele 2 is transmitted to six of six unaffected children. This is consistent with evidence for linkage but requires studying additional families to prove linkage. The affected daughter in generation III with a 2,2 genotype represents a recombination event in the father; that is, a crossover occurred during paternal meiosis between the disease locus and the marker locus so that the disease allele was transferred to the chromosome with allele 2 at the marker locus. On the basis of the genotypes in this sibship, the estimated recombination fraction (θ) between the disease and marker locus is 0.10 (i.e., 1 in 10 meioses).

single family, individuals may have both very severe and very mild forms of asthma, or some individuals may only be atopic. Whether or not these phenotypes are expressions of the same genetic susceptibilities is not known a priori, and it may be difficult to classify individuals with respect to affection status. To minimize this potential problem, it is always safest to include in the analysis only individuals with unambiguous phenotypes.

Fourth, phenocopies are individuals that have a disease phenotype that is indistinguishable from the other affected relatives, but the underlying cause differs. This can result from a particular family member having a form of the disease that is a result of environmental exposures (e.g., occupational asthma) or from different susceptibility alleles that are present in other family members. The latter should not be unusual in families with diseases that are common in the population because susceptibility alleles can be introduced in the family through more than one ancestor. Nothing can be done to minimize this potential problem when designing genetic studies, but it can be taken into account in certain analytical approaches by allowing individuals with the nonsusceptible genotype to have a penetrance greater than 0%.

Last, the effects of gene-environment interactions are unknown. At the present time, the effects of environmental exposures are largely ignored in genetic studies. However, once susceptibility genes are identified, it may become possible to study the effects of specific environmental exposures on triggering or exacerbating the disease expression.

FINDING DISEASE GENES

The basic approach to finding disease genes is to find a marker allele (described below) that is coinherited with the disease in families. The underlying premise is that among the 3 billion base pairs (bp) of DNA in the human genome, one or more susceptibility alleles are segregating in families with affected individuals. When a marker allele and the susceptibility allele are on different chromosomes or far apart on the same chromosome, they will segregate independently in families (according to Mendelian principles), and the marker allele will appear in affected and unaffected family members with equal frequency. However, if the marker allele is close to the susceptibility allele on a chromosome, the marker and the disease will cosegregate in families, providing evidence for linkage. Thus in the presence of linkage, the marker allele will occur in affected family members more often than it will occur in unaffected members. If this pattern of cosegregation is present in many families or in many affected relative pairs, this will constitute evidence for linkage (Fig. 1).

The marker loci used in linkage studies are called short tandem repeat polymorphisms (STRPs). STRPs (also known as microsatellites) are tandemly repeated DNA sequences of either 2 bp (dinucleotide), 3 bp (trinucleotide), or 4 bp (tetranucleotide). At a particular STRP locus, the number of tandem repeats can vary considerably from chromosome to chromosome; most individuals will have two different size alleles inherited from each parent (Fig. 2). This is particularly useful in linkage studies because it allows the tracking of markers on specific parental chromosomes to each offspring. In addition, STRPs are abundant in the genome, so markers that are relatively evenly distributed among the chromosomes can be used in linkage studies. The more marker loci used, the more likely it is that at least one will cosegregate with the disease in families. The chromosomal locations of the STRPs are known, so cosegregation (or linkage) of a disease phenotype with an STRP indicates that the susceptibility locus is in relatively close proximity to the marker locus on the chromosome. Once linkage to a marker locus is established, the relative location of the susceptibility locus is known, and additional studies in the region of the marker locus are conducted to refine the location of the disease locus and (eventually) allow the identification of the disease gene. At the present time, linkages of asthma to many chromosomal locations have been reported, but no specific "asthma" genes have yet been identified.

Genetic studies of complex diseases can differ with respect to study design as well as mapping approaches. Study designs will vary with respect to type of family members studied (extended or nuclear families versus affected relative pairs) and the methods by which the families are ascertained. For example, studies that ascertain families through a single affected parent could yield different results than studies that ascertain families through a pair of affected siblings. Similarly, the definition of the phenotype in the individual(s) through which

the family was ascertained (this individual is called the “proband”) can also influence the outcome of the study. Thus studies that ascertain families through atopic probands may identify different regions that show evidence for linkage to asthma than studies that ascertain families through asthmatic probands. This is because asthma susceptibility genes that are present in families heavily weighted for atopy may differ from the asthma susceptibility genes in families less heavily weighted for atopy.

Mapping designs may also differ. The two basic mapping approaches used to study complex disorders are the candidate gene approach and the genome-wide search. In the candidate gene approach, a number of genes are identified that are hypothesized to be disease susceptibility loci based on their known function. STRPs that are either within or around the candidate gene are used to test for linkage in families. This approach is powerful when the disease gene is one of the candidates selected. However, because there are an extraordinary number of potential candidate genes for a disease as complex as asthma and because only a small proportion of the approximately 50,000 to 100,000 human genes have been identified, the candidate gene approach is of limited utility. In contrast, in a genome-wide search there is no *a priori* hypothesis regarding specific genes; rather, STRPs that span the human genome are used to identify all loci with detectable effects on the genotype. The latter is the more powerful of the two approaches, but obviously more costly and labor intensive. The remaining sections of this report will provide an overview of the results of the genetic studies of asthma.

CANDIDATE GENE STUDIES

Until recently, all genetic studies of asthma and atopy used a candidate gene approach. With the use of STRPs or other polymorphisms in or near candidate genes, five regions have been identified that show evidence for linkage to asthma or atopy in one or more samples. These regions include the cytokine gene cluster on chromosome 5q,¹⁰⁻¹³ the human leukocyte antigen (HLA) complex on chromosome 6p,¹⁴⁻¹⁸ the β -subunit of the Fc ϵ receptor on chromosome 11q,¹⁹⁻²² the region on chromosome 12q that contains the interferon- γ gene,²³ and the T-cell receptor α/δ locus on 14q.²⁴ The evidence for linkage in each of these regions has been reviewed elsewhere.²⁵ However, of particular note is that not all studies have been able to replicate the evidence for linkage with genes in these regions. This could be due to genetic heterogeneity, differences in study designs and definitions of affected individuals, or false-positive findings (i.e., type 1 errors). It is often difficult (or even impossible) to know which of these explanations is the truth when linkage studies cannot be replicated in independent samples. In fact, the only proof that any of these findings represent true linkages is to find the susceptibility gene itself, a goal that has not yet been reached.

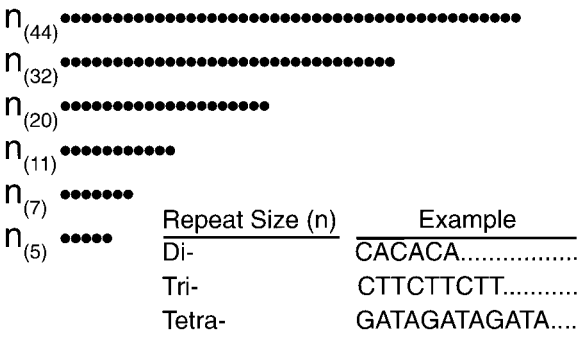


FIG. 2. Short tandem repeat polymorphisms (STRPs) are tandemly repeated DNA sequences of either 2 bp (dinucleotide), 3 bp (trinucleotide), or 4 bp (tetranucleotide). Repeat units are shown as filled circles. Examples of repeat units are shown in the lower right corner of the figure. These loci are abundant in the human genome and can be used as “markers” in linkage studies. The number of repeats at a particular locus can vary (from 5 to 44 in the figure), and most individuals have two different-sized alleles at many STRP loci. STRPs that are used in linkage studies typically have more than 10 alleles, and commonly more than 75% of individuals are heterozygous for the alleles at the locus.

GENOME-WIDE SEARCHES

Two genome-wide searches in atopic and asthmatic families, respectively, have been completed. In the first, the Oxford group²⁶ studied 172 sib pairs from Australian families with atopic and nonatopic members. Twelve percent of individuals within these families were asthmatic. Asthma and atopy were studied as well as four quantitative phenotypes: total serum IgE, a skin test index, blood eosinophilia, and BHR (slope). A genome-wide screen with 294 STRPs revealed some evidence for linkage with various atopic phenotypes in six chromosomal regions: 4q34-35 and eosinophil count ($p < 0.0001$), 6p21 (the HLA region) and eosinophil count ($p < 0.0001$), 7p13 and BHR ($p < 0.0005$), 11q13 (Fc ϵ receptor region) and skin test index ($p < 0.00005$), 13q14.1-14.2 and atopy ($p < 0.005$), and 16q21-24 and total IgE ($p < 0.005$). Thus two regions that had previously shown evidence for linkage in candidate gene studies (6p21 and 11q13) and four novel regions (4q34-35, 7p13, 13q14.1-14.2, and 16q21-24) were identified in this study. Linkages in four regions (4q34-35, 11q13, 13q14.1-14.2, and 16q21-24) were replicated in a second sample that had participated in previous genetic studies by the Oxford group.¹⁹ Because the probability of a type 1 error (i.e., false-positive) in a genome-wide screen is high, it is likely that only some of the novel regions reported in this study represent true linkages. Thus replication of these results in independent samples will strengthen the evidence for linkage and increase the likelihood that genes influencing atopy reside within these regions.

A second genome screen by the US Collaborative Study on the Genetics of Asthma (CSGA) has been completed. In this study, 140 families were ascertained through two asthmatic sib pairs from three racial/ethnic groups (African-American, Caucasian, and Hispanic).²⁷

Each ethnic group was analyzed separately. Linkage to the quantitative phenotype "asthma" was examined using 360 STRPs. Evidence for linkage was reported for four regions previously identified by candidate gene searches: the cytokine cluster on 5q31-33 in Caucasians ($p = 0.0187$), the HLA region in 6p21 in Caucasians ($p = 0.0129$), the interferon- γ region on 12q15-24.1 in Caucasians and Hispanics ($p = 0.0042$), and the T-cell receptor α/δ locus on 14q11.2 in Caucasians ($p = 0.0062$). In addition, seven novel regions showed evidence for linkage to asthma: 5p15 and 17p11.1-11.2 in African-Americans ($p = 0.0008$ and 0.0015 , respectively); 11p15, 13q21.3-qter, and 19q13 in Caucasians ($p = 0.0089$, 0.0014 , and 0.0013 , respectively); and 2q13 and 21q12 in Hispanics ($p = 0.0005$ and 0.0040 , respectively). Only one of these regions (13q) overlaps with the regions identified in the Oxford genome screen, suggesting that the other regions identified by the CSGA may be more specifically associated with asthma than with atopy and that genes on chromosome 13q may influence atopy as well as asthma. Studies of additional families and phenotypes in the CSGA and studies by other investigators should help clarify the role of these novel regions in susceptibility to both asthma and atopy.

CONCLUSIONS

Evidence from candidate gene studies as well as from genome-wide searches suggests that asthma is indeed a genetic disorder. Loci in many chromosomal regions may influence susceptibility to asthma and atopy. These studies suggest that the relative importance of specific asthma-susceptibility genes and the effects of environmental exposures or modifying genes on disease expression may vary across ethnic groups and perhaps even between different Caucasian samples. Furthermore, these results are consistent with the hypothesis that susceptibility to asthma may be influenced by many genes with relatively small effects rather than a few genes with major effects. Identification of the specific asthma genes that influence asthma phenotypes will shed light on the molecular pathways that influence this complex disorder and provide a better understanding of the pathogenesis of asthma and atopy.

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