Strategies for analyzing genotype-phenotype relationships in asthma

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Asthma is a genetically complex disease with a multifaceted phenotype. Different approaches including population-based and family-based methods for evaluating genotype-phenotype relationships in asthma are discussed as well as the problems that may obscure these determinations. Examples of similar efforts in cystic fibrosis and breast cancer are considered in addition to interaction between causative genes and etiologically relevant environmental exposure. (J Allergy Clin Immunol 2000;105:S482-6.)

Key words: *Genotype, phenotype, asthma, complex disease, mendelian disease, breast cancer, cystic fibrosis, case-control study, gene-environment interaction*

Abbreviations used

CFTR:	Cystic fibrosis transmembrane conductance regulator: the protein functions as a chloride channels regulate by cAMP; mutations in the gene cause cystic fibrosis
CBAVD:	Congenital bilateral absence of vasdeferens: an autosomal recessive disease with some clinical manifestations similar to cystic fibrosis.
BRCA1 and BRCA2:	Major susceptibility genes for breast cancer

As studies in the genetics of asthma continue to progress from family studies to positional cloning to eventual identification of susceptibility genes, new challenges arise in determining the exact relationship between genotype and phenotype. Genotype refers to the precise allelic makeup of an organism or cell.[1] In reference to a specific gene, the genotype is the pair of alleles that occur at the chromosomal site of the gene, which may be the same (homozygous) or different (heterozygous). Phenotype refers to the observed attribute,[1] in our case asthma or a component of asthma such as bronchial hyperresponsiveness. The phenotype is the observed manifestation of the underlying genotype and any gene-environment interactions that may occur.

Many issues surround the assessment of genotype-phenotype relationships not only for complex diseases such as asthma but also for simple mendelian diseases. In a disease in which one mutation in a gene is totally responsible for producing the disease and will always produce disease when present, there is a direct and complete correlation between genotype and phenotype. However, there are relatively few diseases that manifest themselves completely as a result of a single mutation in a single gene. Different mutations in the same disease gene may also exist that produce disease sometimes to a different degree of severity or with different clinical manifestations. Moreover, environmental factors and other modifying genes in addition to interactions between genes and environment may alter the disease phenotype. The purpose of this study was to explore possible methods for determining genotype-phenotype relationships in asthma and the problems associated with this because of potential gene-environment interactions and other issues related to the complexity of the disease. Previous efforts to delineate genotype-phenotype relationships in mendelian and nonmendelian diseases will be considered as examples.

EXAMPLES OF GENOTYPE-PHENOTYPE RELATIONSHIPS IN MENDELIAN AND NON-MENDELIAN DISEASES

Cystic fibrosis

Diseases that are referred to as "mendelian" are those that follow Mendel's laws for single gene transmission in families.[1] Cystic fibrosis is a classic mendelian disease exhibiting recessive inheritance. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) located on chromosome 7, which encodes a protein of 1480 amino acids that functions as a chloride channel regulated by cAMP.[2] More than 550 different mutations have been identified in the CFTR gene since it was first cloned in 1989. The clinical manifestations of cystic fibrosis can be attributed to impaired chloride conduction across epithelial cells in the respiratory, digestive, and reproductive tracts and include elevated sweat chloride concentration, chronic pulmonary obstruction, bacterial colonization of the airways, pancreatic enzyme insufficiency caused by blockage of the secretory ducts, and reduced fertility in men and sometimes in women.[2]

The correlation between genotype and phenotype in cystic fibrosis is complicated by the heterogeneity of both the clinical symptoms and the mutations. There is a wide variability of phenotypes between patients with

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cystic fibrosis in the clinical manifestations, the severity and rate of progression of the disease, and the age at onset.[3] There is wide variability in the CFTR mutations that can be grouped into five general classes dependent on whether they affect (1) biosynthesis, (2) protein processing and conformation, (3) chloride channel regulation, (4) chloride channel conductance or channel gating, or (5) cause the reduction of synthesized protein. The most frequent mutation, deltaF508, results in a deletion of a phenylalanine residue at codon 508 and is grouped into class 2 mutations that affect protein maturation. The first three classes of mutations are considered more severe than the later two because the later can produce some residual functional protein.[2]

Several studies have been done to identify common clinical features in patients with cystic fibrosis with the same genotype. A clearly observed genotype-phenotype correlation has been shown for pancreatic status in several studies.[3,4] In general, patients with cystic fibrosis with pancreatic insufficiency are homozygous for deltaF508 or compound heterozygous for "severe" mutations (ie, class 1, 2, or 3), whereas patients with pancreatic sufficiency have at least one "mild" allele (class 4 or 5). A correlation has also been observed in a recent study between severe CFTR mutations and phenotypes reflecting more severe lung disease. Patents with severe CFTR mutations had lower arterial oxygen tension, more rapid decline in pulmonary function, more episodes of airway colonization at younger ages, and more requirements for intravenous antibiotic treatment than patients with mild CFTR mutations.[5]

Patients with some of the clinical manifestations present in cystic fibrosis have been studied for the presence of CFTR mutations. For example, subjects with the autosomal recessive disease congenital bilateral absence of vas deferens (CBAVD) share similar clinical features with male subjects with cystic fibrosis. Studies show that 50% to 60% of patients with CBAVD are positive for at least one CFTR mutation and approximately 10% have two "mild" CFTR mutations. However, a substantial proportion (20% to 25%) of CBAVD patients have no known CFTR mutations.[2]

Therefore, even in a mendelian disease such as cystic fibrosis, with a known causative gene, genotype-phenotype relationships are not necessarily straightforward and may be more intricate than expected. Determination of these relationships is even more difficult in complex (nonmendelian) diseases.

Breast cancer

A complex genetic disease is any disorder that has etiologic evidence of a genetic component but does not exhibit classic mendelian dominant or recessive inheritance.[6] Several issues compound determination of genotype-phenotype relationships in complex diseases. One problem is genetic heterogeneity. Multiple genes may be necessary or sufficient to produce disease, and different genes may be acting in different populations. Incomplete penetrance is another issue. Susceptibility genes may not express themselves in all individuals. Finally, when environmental factors are involved in disease origin or progression, the potential for gene-environment interaction exists, which may also obscure the direct relationship between genotype and phenotype.

Breast cancer is an example of a complex disease in which major susceptibility genes, BRCA1 and BRCA2, have been identified. More than 235 mutations have been identified in BRCA1 and 100 in BRCA2.[7] Because complex diseases, unlike mendelian diseases, have more than a single causative gene and environmental causes, questions of genotype-phenotype correlations have been framed broadly in terms of prevalence and penetrance of the specific mutations. Many individuals with breast cancer, for example, will not have any mutations in either the BRCA1 or BRCA2 gene and have unknown causes. The genotype-phenotype questions become "How frequent is the specific mutation in patients with the disease?" (prevalence) and "Given the specific mutation, what is the risk of disease?" (penetrance). Prevalence and penetrance estimates of the different mutations vary greatly by population and method of ascertainment.[8] Screening in the general Ashkenazi Jewish population estimated the combined frequency of the three major mutations in BRCA1 and BRCA2 at approximately 2%.[9,10] Penetrance estimates were higher in families ascertained on the basis of family history, for example, the BRCA1 penetrance estimate was 64% in a recent study[11] compared with previous penetrance estimates in families used for linkage analysis (82% and 87%) in the Breast Cancer Linkage Consortium studies.[12,13] In addition, penetrances were significantly higher for the two major BRCA1 mutations than for the major BRCA2 mutation.[11] The diminished correlation between genotype and breast cancer makes potential decisions for prophylactic strategies even more difficult for women who may test positive for BRCA1 mutations and complicates the ethical issues associated with genetic testing.

WHERE ARE WE WITH ASTHMA?

Several issues exist in determining genotype-phenotype relationships in asthma, some of which are similar to other complex diseases and some of which are unique. A major problem in genetic studies of asthma has been the uncertainty in the phenotype.[14] However, components of the asthma phenotype that are more objectively defined, such as bronchial hyperresponsiveness, can be used as phenotypic outcomes. In addition, studies suggest that multiple genes may be involved in asthma and the frequencies of these genes may vary in different populations.[15] Unlike breast cancer, however, no major asthma susceptibility genes have been identified. Nonetheless, several candidate genes exist in regions of interest identified by recent linkage studies,[15,16] and genotype-phenotype relationships can be examined in those candidates that are polymorphic. Asthma also has a strong environmental component, and different populations

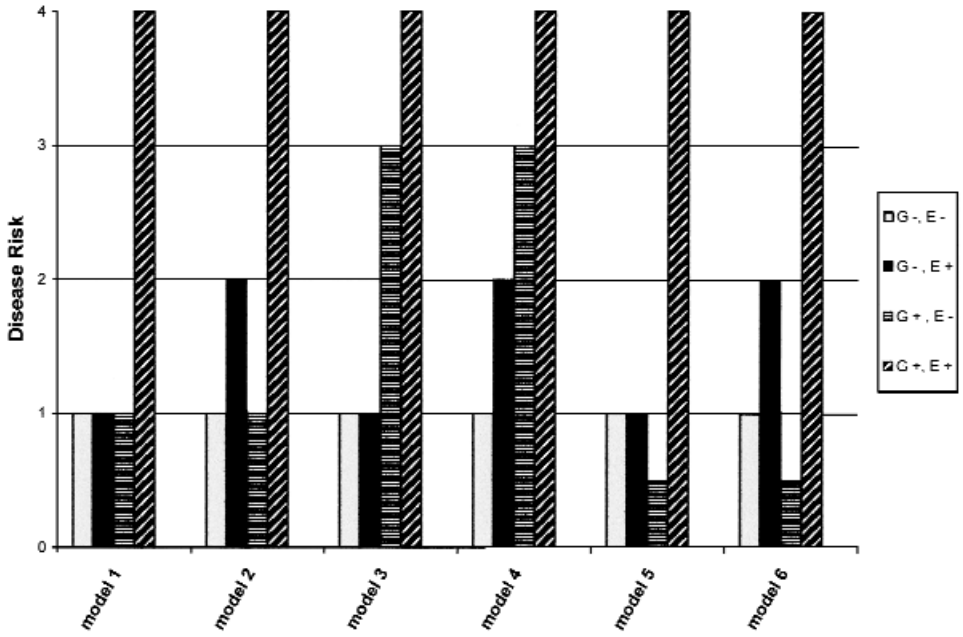

FIG 1. Six models of gene-environment interaction showing the effect of combinations of the presence and absence of a genetic (G+, G–) and an environmental (E+, E–) factor on disease risk.

may experience different environmental exposures. Thus potential gene-environment interactions need to be considered when determining genotype-phenotype correlations.

GENE-ENVIRONMENT INTERACTIONS

Current concepts of asthma pathogenesis propose that onset of the disease and its clinical course are determined by gene-environment interactions; that is, those persons who develop asthma are both genetically susceptible and receive an appropriate environmental stimulus. The underlying hypothesis is that individuals with different asthma-related genotypes will have different sensitivities to environmental exposures. Several different models for gene-environment interactions have been suggested (Fig 1, adapted from Reference 17). The first is that both the presence of a given asthma susceptibility genotype and an environmental exposure are necessary to produce an excess risk of disease. The second model shows an environmental exposure causing an increased risk of disease in all individuals but a much greater risk in individuals with the susceptible genotype. A third possible pattern of gene-environment interaction is that the environmental exposure will only increase the risk of disease in individuals with the susceptible genotype. In the fourth model, both the environment and the genotype produce excess risk. Finally, when there is a protective effect of the genotype, depending on the presence or absence of the environment, the last two models of gene-environment interaction are possible. Failure to consider gene-environment interactions, if they are truly present in asthma, could lead to spurious inferences about genotype-phenotype associations.

STUDY DESIGNS TO DETERMINE GENOTYPE-PHENOTYPE RELATIONSHIPS IN ASTHMA

Study design I. Case-control: Population-based versus family-based

Case-control designs can be used in analyzing genotype–phenotype relationships in asthma susceptibility genes or polymorphic candidate genes. Sampling may be of asthma cases and appropriately selected control subjects from either the general population (unrelated control subjects) or from family members. The different sampling schemes have advantages and disadvantages. Results from population-based studies may be more relevant to the general population of asthmatic subjects. In addition, it may be easier to study gene-environment interactions because of the potential range of variability in environmental exposures between unrelated individuals. However, confounding caused by other factors is a major problem making the choice of control subjects critically important. Family-based studies have the advantage of having better control of such confounding factors including other background genes, race, and environmental exposures. Although gene-environment interactions may be difficult to examine because of limited variability, it may be easier to look directly at the effect of the genotype on the presence or absence of disease in family-based studies.

Study questions will also vary depending on whether previously determined asthma susceptibility genes are chosen or polymorphic candidate genes. As with the breast cancer genes, case-control studies of asthma susceptibility genes could address the risk and penetrance of specific mutations, assuming that more than one muta-

tion exists. Frequencies of specific mutations may be compared between asthma cases and control subjects and estimates of risk obtained (odds ratio). Penetrance estimates could be generated in family-based studies by looking at the proportion of relatives (of cases) with the mutation that also had asthma. In addition, gene-environment interactions could be determined by observing differences in odds ratios obtained in cases and control subjects with and without a suspected environmental exposure.[17]

When genotype-phenotype relations are studied in polymorphic candidate genes, the case-control approach can answer basic questions of susceptibility to asthma in addition to gene-environment interactions by using standard logistic regression models. However, the potential statistical problem of multiple comparisons may exist in these types of studies if multiple polymorphisms exist with equal prior probability of being associated with asthma. Candidate genes with the fewest polymorphisms, or a few "suspect" polymorphisms, may have the most power in detecting associations with asthma because fewer comparisons would be made.

Study design II. Subjects with asthma: Population-based versus family-based

Genotype-phenotype studies in samples of subjects with asthma are useful to examine the modifying effects of mutations on phenotype. Asthma may be well suited for this approach given the wide variability of phenotype even among family members and the multiple phenotypic components that define the disease. For example, bronchial hyperresponsiveness and inflammation are components of asthma; however, the degree of hyperresponsiveness and the level of serum IgE varies greatly among individual asthmatic subjects. Therefore, mutations in genes that may be controlling only a portion of the asthma phenotype may be detectable by studying phenotypic variability among asthmatic subjects.

Population-based and family-based sampling can be done with this type of study design as well. As with case-control designs, population-based samples of asthmatic subjects may give results that are more relevant to the general population of people with asthma, and environmental variance should be greater than in family-based samples allowing for determination of gene-environment interactions. Family-based studies of asthmatic siblings will have the advantage of minimizing other genetic effects with perhaps a more specific determination of genotype-phenotype correlations.

Like the studies done in patients with cystic fibrosis, specific mutations in identified susceptibility genes could be examined by dividing subjects according to their genotype and observing whether any subphenotypes, such as serum IgE level or degree of bronchial hyperresponsiveness, differed among the groups. Subjects could also be divided into groups on the basis of similar subphenotypic components of asthma, and the frequencies of the mutations among the groups could be explored. Candidate genes could be studied in a similar manner. This approach may prove to be attractive for identifying minor genes or genes that affect, for example, asthma severity rather than susceptibility. Minor genes may be undetectable in linkage studies or case-control studies if they merely modify the disease instead of determining if asthma is present or not.

CONCLUSIONS

The future of genetic studies of asthma points to the direct analysis of mutations in susceptibility and candidate genes and subsequent delineation of genotype-phenotype relations.[18] Currently, promising candidate genes exist in chromosomal regions identified from linkage studies. In addition, the potential of identifying genes that confer only a slight increase in risk or influence the progression and treatment of the disease through studies on asthmatic subjects promises to advance our understanding of the pathogenesis of asthma and the role of different genes in modifying phenotype. Appreciation of the role of the specific mutations and their interaction with relevant environmental exposures in phenotypic outcomes will undoubtedly prove consequential in developing preventive and clinical therapies, the ultimate goal of asthma genetics studies.

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