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Genetics and Occupational Asthma

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INTRODUCTION

Common diseases such as asthma and diabetes mellitus tend to cluster in families. The risk of developing the disease if a first-degree relative is affected is 5% to 10% greater than the prevalence of the disease in the population, but less than the 25% risk for a recessive and 50% risk for a dominant single gene disorder. The familial clustering is due not to a single gene defect, but is the outcome of multiple genes (polygenic) and their interaction with the environment.

Genetic variation between individuals, such as differences in blood groups, occurs frequently in the population. Such variants, whose frequency is stable between generations, are known as "polymorphisms." Polymorphisms are the basis of diversity within human populations and contribute not only to differences in characteristics such as height and blood pressure but also to variation in the ability to resist infection and handle environmental challenges. Such challenges include the response to agents inhaled at work, both inorganic and organic. Differences between individuals in their responses are likely to be determined, at least in part, by the functional consequences of polymorphisms of relevant genes, and their interactions with each other.

Molecular genetic studies have provided the opportunity to identify the relevant genetic polymorphisms. However, it is important to appreciate that in a multifactorial disease such as occupational asthma (OA), a single polymorphism, although increasing susceptibility to the development of asthma in those exposed to a particular agent, is unlikely to be sufficient alone, and may even not be necessary, to cause the disease. Polymorphic genes may increase susceptibility or resistance to disease but do not determine it. The identification of a single polymorphic gene alone is therefore unlikely to provide the basis for a screening test for susceptibility to OA. Knowledge of the genes involved, the function of their protein products, and of their interaction with the relevant environmental influences does however have the potential to illuminate disease mechanisms at the molecular level and provide new opportunities to treat and prevent it.

THE IMPORTANCE OF WELL-DEFINED PHENOTYPES IN GENETIC STUDIES

The genetic constitution of an organism is its genotype; the physical expression of the genotype (either as an observable characteristic or protein product) is its phenotype. Mendel inferred the patterns of inheritance in sweet peas, by studying the transmission of pairs of clearly contrasted physical characteristics (phenotypes) such as tallness versus shortness of the plant and, green versus yellow, and round versus wrinkled seeds. Similar patterns of inheritance can be inferred for human diseases such as cystic fibrosis. In such Mendelian disorders, genotype and phenotype correspond (one gene, one protein). In common diseases, such as asthma, diabetes, and hypertension, however, the correspondence is not observed because the same phenotype (e.g., asthma) is associated with different genotypes (genetic heterogeneity) or vice versa because of interaction with other genes, with environmental factors, or both.

Genetic studies of disease in man require an unequivocal definition of phenotype. This implies diagnostic criteria which are comprehensive (i.e., high sensitivity with few false negatives) and exclusive (high specificity with few false positives) to minimize misclassification bias. Genetic studies of atopy and asthma have been considerably impeded by the lack of widely accepted diagnostic criteria. Asthma is usually defined as reversible airway narrowing, whose characteristic symptoms are episodic wheeze and breathlessness. However, all that wheezes is not asthma and not all asthma wheezes; furthermore, cases of asthma may not have demonstrable reversibility at the time of testing. Another defining characteristic of asthma is airway hyperresponsiveness—an increased responsiveness of the airways to nonspecific provocative stimuli such as exercise, inhaled cold air, or histamine. Airway hyperresponsiveness, although sensitive, is not a specific characteristic of asthma, being found in some 15% of the population. These problems make a comprehensive and exclusive definition of asthma difficult. Similarly, atopy—which was defined by Pepys as the propensity to make immunoglobulin E (IgE) antibody in response to allergens encountered in everyday life—has been identified by different investigators as one or more immediate skin test responses to common inhalant allergens, an elevated total IgE in serum, or the presence of specific IgE antibody in serum to one or more common inhalant allergens. Cookson and Hopkin (1) in their genetic studies decided on a comprehensive case definition, which included one or more of any of these three; in contrast, others have focused on the inheritance of total IgE.

These difficulties and differences in case definition have made genetic studies of asthma and atopy difficult to undertake and to compare the findings of different studies.

GENETIC BASIS OF ASTHMA

Asthma and atopy show clear indications of genetic susceptibility; the frequency of disease in family members of cases is greater than in the population as a whole, and is greater in identical monozygotic (MZ) twins than in nonidentical dizygotic (DZ) twins.

Family Studies

Genetic susceptibility is suggested if a disease occurs in greater frequency in the family members of a case than in the general population. The relative risk (λ_R) is

the risk of a disease for the relative of an affected individual divided by the risk (prevalence) of the disease in the general population. λ_o and λ_s are the ratios for offspring and sibs to the population prevalence. The size of λ_R is taken to indicate the degree of concordant inheritance of genetic factors in affected relative pairs.

First-degree relatives—parents, brothers, sisters, and children—have half their genes in common with the index case (or proband). Second-degree relatives—grandparents, aunts, uncles, nephews, and nieces—have one-quarter of their genes in common. The most commonly reported value is for λ_s but usually sibs also share a common childhood environment and $\lambda_s > 1$ may reflect a shared environment as well as a shared inheritance. An increased frequency of disease in second-degree relatives may therefore be a more reliable indication of genetic influences. The value of λ_s for cystic fibrosis is about 500, for insulin-dependent diabetes 15, and for schizophrenia 8.5. The value for asthma probably lies between two and five.

Several family studies have provided evidence that asthma and associated atopic disease, eczema and hayfever, are more frequent in the relatives of asthmatics than in the relatives of matched controls. Sibbald (2) estimated that the population frequency of asthma was between 5% and 10%. The probability of a child of an atopic asthmatic parent having asthma varied from 14% when one parent was affected to 29% when both parents were affected. The risk for the child of a nonatopic asthmatic parent to have asthma was a little greater than the risk in the general population. Atopy increased the risk of a child developing asthma to about threefold. Similarly, Jenkins et al. (3) found that the risk of asthma and associated atopic diseases in seven-year-old Tasmanian children was greater when one or both parents were asthmatic than when neither was affected. The risk of having asthma was increased to about 2.5 times when either parent had asthma and 6.7 times when both parents had asthma.

Twin Studies

Twin studies, which compare the concordance of disease in identical (MZ) twins, who are genetically identical, and nonidentical (DZ) twins who like other sibs share half their genes, can better differentiate genetic from environmental influences than family studies. Assuming that the effect of a shared familial environment is the same for MZ and DZ twins, the frequency of a disease with no genetic component will be similar in them. A disease influenced by genetic factors will be more frequent in MZ twins than in DZ twins; the larger the difference, the more likely that genetic factors are important in the development of the disease. The results of twin studies of multifactorial diseases can be expressed as "hereditability"—the proportion of the total variation of the phenotype due to genetic factors.

Twin studies have consistently shown the concordance of asthma to be higher in MZ twins than in DZ twins. In a study of 7000 Swedish twin pairs, Edfors-Lub (4) found that the concordance of asthma between MZ twins was 19% and between DZ twins was 4.8%, giving an estimated hereditability of some 15%. More recent twin studies have reported higher estimates for the hereditability of asthma. Hopper et al. (5) estimated a hereditability of 50% in a study of 3808 Australian twins. Harris et al. (6) in a study of 5684 Norwegian twins found that the risk of developing asthma in twins whose co-twin had a history of asthma (as compared to those whose co-twin did not) was increased 18 fold in MZ twins and 2.3-fold in DZ twins.

Hanson et al. (7) studied MZ and DZ twins reared together and apart; they found in either situations, a greater concordance for asthma and specific IgE [estimated by skin test and radio-allergosorbent allergy testing (RAST)] in MZ twins

than in DZ twins. Although the number of twins in this study was relatively small, the implications of its findings are considerable, suggesting a substantial genetic influence on the development of specific IgE and asthma, with little contribution from familial environment shared in childhood.

Twin studies indicate that asthma has an inherited component, but do not identify its mode of inheritance. This is usually addressed by segregation analysis in family studies, in which the occurrence of asthma is determined in terms of the degree of genetic relatedness to the index case (or proband). Families studied may be nuclear (which include only first degree relatives of the probands) or extended (which include more distant relatives and usually encompass three or more generations).

The results of such studies have been conflicting. Some have suggested that asthma is inherited as an autosomal dominant characteristic with incomplete penetrance, i.e., a single gene is responsible for asthma but not all who inherit it develop asthma (8,9). Cookson and Hopkin (1) found that 90% of atopic asthmatics had an atopic parent and suggested a dominant mode of inheritance for atopy.

Others have suggested that the inheritance of asthma is polygenic (10), implying asthma to be the outcome of an interaction between several genes. In part, these apparently striking different findings reflect differences in methods of ascertainment and phenotype definition, but probably also reflect considerable heterogeneity in the inheritance of atopy and asthma.

MOLECULAR GENETICS

Modern molecular techniques have provided the opportunity to disentangle the difficulties in genetic analysis of complex traits caused by factors such as genetic heterogeneity and polygenic inheritance. These studies allow for identification of the genes that contribute to the development of multifactorial diseases such as asthma. The ultimate purpose of these investigations is to determine the protein products of the relevant genes, their functions, and how these differ from those not at increased risk of disease. Understanding the biochemical basis of diseases such as diabetes, schizophrenia, and asthma could offer insights of therapeutic and preventative value.

Two complementary approaches have been taken: genetic linkage and investigation of candidate genes.

Genetic Linkage

The opportunity for human genetic linkage studies has been provided by recognition of the naturally occurring variation in human DNA sequences (on average, individuals differ every 200 to 500 base pairs) and the identification of a large number of polymorphic markers spaced at short intervals along human chromosomes.

Genetic linkage is based on the simple principle that the closer the marker polymorphism is to the gene of interest, the less likely is separation at meiosis and the more likely that they are coinherited (linked). The genetic locus of a particular disease can be identified by the frequency of the coinheritance of the disease with a marker of known chromosomal location. If the genetic marker and the disease are unlinked (on a separate chromosome or far apart on the same chromosome) they are as likely to cosegregate as not, and the recombination fraction will be

50% or 0.5. When the marker and disease locus are on the same chromosome, the shorter the distance between them, the less likely is crossing over during meiosis, the more likely that they are inherited together, and the fewer the recombinants. The recombination fraction will fall from 50% (reflecting independent assortment) toward zero (reflecting tight linkage).

Initial linkage studies are undertaken in families with the disease, looking for cosegregation of a marker of known chromosomal location with the gene involved in the disease. Because of the large number of markers examined in a genome screen, any suggestive linkage needs to be replicated with more refined linkage mapping to identify a "candidate gene." DNA sequencing of the candidate gene allows identification of variations in the coding sequence (polymorphisms). Finally, population studies, which investigate the association between the polymorphism and the disease of interest, allows estimation of the prevalence of the polymorphism and its contribution to the disease frequency and severity.

This was the approach taken by the Oxford group in their investigation of the genetic basis of atopy. Having observed that 90% of atopic asthmatics had an atopic parent, a result they interpreted as indicating dominant inheritance, they undertook a linkage analysis within nuclear and extended families. They found significant linkage between a marker on chromosome 11q and atopy (11) (broadly defined as one or more skin-prick test reactions, specific IgE to common inhalant allergens, or an elevated serum total IgE), which they confirmed in a subsequent study of other Oxford families (12). Linkage was found to be primarily through maternal chromosomes (13). Because of its known location on chromosome 11q and plausible relevance to atopy, they postulated the β chain of the high affinity IgE receptor (Fc εR 1-β) as the candidate gene (14). Subsequent sequence analysis identified two separate polymorphisms, Leu 181 and 183, present either separately or together (15).

They subsequently studied a random population sample from the town of Busselton, in Western Australia and found that the prevalence of the Leu 181/183 polymorphism was some 4.5%. All 13 children who had inherited the polymorphism from their mothers were atopic and all but one had hay fever, asthma, or both. In contrast, none of the eight children who inherited the gene from their fathers was atopic, confirming maternal transmission of gene expression (16). A number of other studies failed to replicate these results (17,18), which in some cases may reflect the small number of families studied, but probably primarily reflects the genetic heterogenicity of atopy and asthma.

Chromosome 5q has also been extensively investigated because it contains several candidate genes relevant to asthma and atopy. These include the interleukin-4 gene cluster, which contains IL-3, -4, -5, -9, -13, and GM-CSF, the β_2 adrenoceptor gene, and the corticosteroid receptor gene. Marsh et al. (19) studied 170 individuals from 11 Amish families in the United States and found significant linkage with total IgE for five markers within the 5q 31.1 region but not for the three markers (lying just) outside this region. Postma et al. (20) studied the children and grandchildren of 84 Dutch probands with asthma. They found coinheritance of increased levels of total IgE with airway hyperresponsiveness and linkage of airway hyperresponsiveness with several markers on chromosome 5q.

These studies indicate that polymorphisms of candidate genes on chromosome 5q are probably contributing to the development of asthma and atopy, but in the absence of defined polymorphisms, it is not possible to determine their prevalence or the size of their contribution to the development or severity of disease.

Candidate Genes

The second parallel approach, applicable when the biochemical basis of the disease is understood, is to seek polymorphisms of the genes encoding potentially relevant proteins. The considerable knowledge of the cellular and molecular mechanisms of asthma, both the TH2 lymphocyte response and associated eosinophilic bronchitis, provides a remarkable array of plausible candidate genes. Investigations reported to date, particularly those relevant to OA, have investigated the association of asthma and specific IgE antibody with the highly polymorphic genes encoding the major histocompatibility complex (MHC) proteins, also known as human leukocyte antigens (HLAs) in man, which play a central role in the immune recognition of foreign proteins.

MHC molecules are expressed as heterodimeric proteins on the cell surface. Foreign peptides, derived from within the cell by the degradation of endogenous or exogenous proteins, are bound in the groove of the MHC molecule and are expressed as an MHC peptide complex which is recognized in a very specific way by the T cell receptor (TCR) on the surface of T lymphocytes. This trimolecular complex [MHC protein, foreign peptide (epitope), and TCR] is at the center of immune recognition and response. MHC proteins are divided into two classes: MHC-1 and -2. MHC Class 1 proteins (HLA-A, -B, and -C antigens in man) are expressed on the surface of all cells; MHC Class 2 (HLA-DR, -DP, and -DQ antigens in man) proteins are expressed on the surface of antigen presenting cells such as dendritic cells, B lymphocytes, macrophages, and some epithelial cells. MHC Class 1 proteins present endogenous peptides which are mostly recognized by CD8+ (cytotoxic) T lymphocytes; MHC Class 2 proteins present exogenous peptides which are recognized by CD4+ (helper) T lymphocytes, although there are exceptions, where Class 1 peptide complexes are recognized by CD4+ cells and Class 2 peptides by CD8+ cells.

MHC proteins are encoded on the short arm of chromosome 6. HLA-A, -B, and -C each have one gene on each chromosome. HLA-DR has up to four genes and HLA-DP and -DQ each have two genes encoding α and β chains, a total of 12 genes for the HLA system on each chromosome allowing a potential 24 different HLA types in any individual. HLA genes are also highly polymorphic with more than 200 variants of these 12 genes, which are numbered in sequence (e.g., HLA-A1, -A2, -A3, etc.) providing billions of potential combinations of the 200 genes that are unique to each individual (other than identical twins who share the same HLA type).

MHC proteins have evolved as a mechanism of self-defence against infective agents, which are themselves continually changing and adapting. HLA genes are reshuffled in each generation and the constantly changing resistance against infection in the reshuffling process has been proposed as the major evolutionary benefit of sex.

Because MHC proteins bind epitopes in the molecular groove (Bjorksten groove), created by the β-pleated sheet of the heterodimer, polymorphisms in the genome determine the capacity to bind specific epitopes and present them to T cells. Epitopes that are not presented will not activate (and therefore effectively bypass) the immune response.

Allergens and low–molecular weight chemical haptens, which bind to host proteins, are taken up by dendritic cells, degraded into oligopeptides which are bound in the groove of MHC-2 proteins, expressed on the cell surface, and recognized by CD4+ T lymphocytes possessing the relevant TCR. Variation in the immunological response to inhaled allergens and haptens may therefore in part be

determined by differences in HLA type. For this reason, studies of the molecular genetics in OA have to date focused on searching for associations between MHC Class 2 (HLA-DR, -DP, and -DQ) genes and the development of specific IgE antibody and asthma in those who are exposed to allergens and haptens in the workplace.

Before the advent of molecular techniques, HLA typing was undertaken serologically. The ability to distinguish HLA types by molecular methods—probing with sequence-specific oligonucleotides (SSOs) after amplification by polymerase chain reaction (PCR) or, more specifically, using sequence-specific primers (SSPs)—has increased considerably the number of different HLA types recognized and the accuracy of their identification. In addition, knowledge of the amino acid sequence of different HLA molecules has allowed the identification of specific amino acid substitutions in HLA molecules, which may be associated with susceptibility or resistance to a particular disease [see isocyanate-induced asthma and chronic beryllium disease (CBD) below].

Studies relating to the associations of disease with a particular HLA haplotype have usually been made by comparing the frequency of the HLA haplotype in patients with the disease, with the frequency in an appropriately matched referent group. The relative risk between the two groups can be expressed as an odds ratio and appropriate statistical tests can be applied. A significant association between the disease and HLA type may imply:

A Cause and Effect Relationship

The particular HLA type is a genetic determinant of the disease whose importance is reflected in the size of the odds ratio. An odds ratio in excess of 100 (e.g., ankylosing spondylitis and HLA-B27) implies the particular HLA type as a major genetic determinant of the disease. Odds ratios of this size are rare and weaker associations more usual. In these circumstances, the contribution of the particular HLA to the disease, although real, may be of less importance than other genes or environmental factors. The effect of multiple gene polymorphisms, however, can be cumulative.

Linkage Disequilibrium

Linkage disequilibrium takes place when two genes occur together more frequently than would be expected by chance. This is more likely to occur with HLA polymorphisms which can confer a selective advantage against infectious disease. For example, HLA-A1 and -B8 occur more frequently than would be expected by chance in North Europeans, possibly because this combination conferred protection against plague. An HLA type which is associated with a disease may, therefore, only be a marker for a polymorphism of another HLA gene with which it is in linkage disequilibrium.

Confounding

The association of the disease with HLA type reflects the association of HLA type with a particular ethnic or geographical group, which differs between the disease group and the referent group. As an illuminating example of such confounding, Lander and Schork (21) highlighted the association of HLA-A1 with the ability to use chop sticks in the population of San Francisco, a relationship more likely to reflect the higher prevalence of this polymorphism in Chinese than in Caucasians, than immunological determinism of agility in handling chopsticks. Referent groups

need to be matched with the disease group or be capable of adjustment for factors which may confound HLA relationships that include social, geographical, and ethnic background.

Overestimating the High Probability of a Chance Association in Multiple Comparisons

The majority of studies which have explored associations between the HLA types and the disease have been "fishing expeditions" undertaken without a specific prior hypothesis. The probability of an association occurring by chance among the multiple comparisons made with different HLA types is predictably high. This problem can be overcome in at least two ways.

a. Applying Bonferroni's correction the P value of each test is multiplied by the number (λ) of comparisons made ($P' = \lambda P$). For example, where $P = 0.02$ and five comparisons have been made, Bonferroni's correction: $P = 0.02 \times 5 = 0.1$.
b. The results of the fishing expedition are regarded as hypothesis generating, from which a specific hypothesis informed by the results of the initial study may be tested in a hypothesis testing study in a second independent population.

ASSOCIATION STUDIES IN COMPLEX DISEASES

Linkage analyses and association studies are the most widely used methods to identify genetic determinants of complex diseases. Linkage analyses identify genes responsible for diseases with simple Mendelian inheritance, such as cystic fibrosis. However, the application of linkage analysis to complex disorders is very difficult because of genetic heterogeneity, incomplete penetrance, and gene-environment interactions. Association studies are usually performed to study the genetics of multifactorial diseases. The case–control study is the most widely used design for detecting common disease alleles with modest risk, in which the differences in allele or genotype frequencies between cases and controls are evaluated. However, the characteristics of complex diseases also limit the power of association studies. The general limitations for such studies are population stratification, small sample sizes, modest genetic effects, gene—environment interactions, the assessment of statistical significance, and multilocus effects. In addition, the difficulties in quantifying and characterizing exposure characteristics (length of exposure, onset of symptoms, etc.), intermediate phenotypes (such as IgE levels or airway responsiveness in the case of asthma), and multiple outcomes make association studies of complex traits a challenge (22–25).

In multifactorial diseases, the risk is often confounded by interactive and additive effects of genes and by interactions between genes and environmental or occupational factors as has been shown in the case of silicosis (26). Silicosis, an interstitial lung disease resulting from inhalation of crystalline silica, is characterized by chronic inflammation leading to severe pulmonary fibrotic changes. Proinflammatory cytokines, such as tumor necrosis factor (TNF) α and IL-1, have been implicated in the formation of these lesions and a strong association was found between the disease severity and TNFα–238 variant. Gene–gene interactions, including IL-1α +4845 plus TNFα–238 and IL-1RA +2018 plus TNFα–308, were associated with

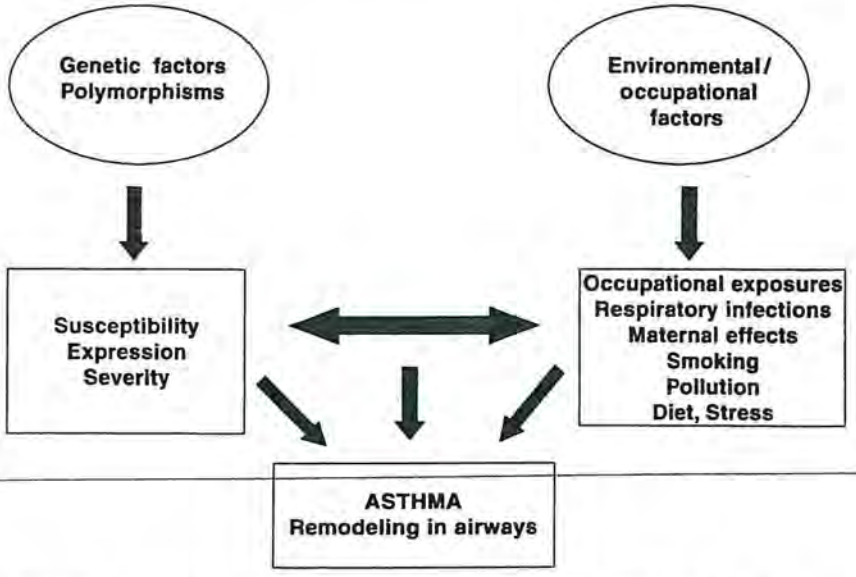

Figure 1 Gene–environment interactions in the development of asthma.

altered risk for silicosis ($P = 0.04$ for both). Three-way interaction analysis between each gene–gene interaction and exposure showed that the prevalence of silicosis increases with increasing exposure, except in the case where both IL-1α +4845 and TNFα–308 minor variants are present (26,27). Figure 1 illustrates a similar interaction model for asthma. Susceptibility genes are assumed not to confer a major risk for the disease; rather they act either alone or in combination with other genes to modify disease progression, severity, or resolution after exposure to triggering factors. The interaction between genes and exposure usually determine the onset or expression of disease. Gene–environment interactions may be measured by the different effects of an exposure on disease risk among individuals with different genotypes, or by the different effects of a genotype on disease risk among individuals with different exposures (28,29). In OA, the main factors that affect the onset of the symptoms are the types and intensity of allergen exposure. The higher the level of exposure, the more likely sensitization will develop and the less the genetic influence. The genetic variations do not cause qualitative differences in the response, but rather induce a shift in the dose–response relationship (30). OA may be a good model for studying gene–environment interactions because the exposure, the time that individual became sensitized, and the interval between exposure and onset of clinical symptoms can be observed (31).

Occupational Asthma

Investigation of the genetic influences and of genetic–environmental interactions in the development of asthma and atopy in the general community is beset by considerable difficulties. The problems of phenotype definition have already been described. In addition, there have to date been few identified polymorphisms associated with atopy or asthma. There is also a lack of accurate measures of exposure to relevant allergens in defined populations, necessary to assess exposure–response relationships and from these, genetic–environmental interactions.

OA overcomes many of these hurdles. Unambiguous case definition (phenotype) can be made on the basis of inhalation tests (for asthma) or serological tests for specific IgE or skin-prick test responses (for immunological sensitization). The disease occurs in identifiable and circumscribed populations, in well-defined and measurable circumstances of exposure.

The majority of reported cases of OA fulfill the criteria for a specific hypersensitivity response, implying the development of an immunological reaction to the responsible initiating agent. For most of the causes of hypersensitivity-induced OA, there is accompanying evidence of specific IgE antibody. This implies specific activation of TH2 lymphocytes with IL-4 and -5 generation. Specific IgE antibody has been identified for all high-molecular-weight proteins and for some low-molecular-weight chemicals (e.g., acid anhydrides, complex platinum salts, and reactive dyes). However, asthma caused by low-molecular-weight chemicals, such as isocyanates and plicatic acid, has been accompanied by specific IgE antibody, inconsistently, in a minority of cases. Examination of the airway mucosa in bronchial biopsies from patients with asthma induced by isocyanates and plicatic acid, however, has shown the presence of activated lymphocytes and eosinophils—a pattern of cellular infiltration characteristic of allergic asthma. Asthma in these cases may be the outcome of activated IL-5 but not IL-4 secreting TH2 lymphocytes.

HLA and High-Molecular-Weight Allergens

HLA association studies are likely to be most fruitful when studying immunological responses or diseases initiated by proteins with a limited number of epitopes. HLA molecules bind to and present single epitopes to T lymphocytes. Multiple epitopes are likely to be expressed by different HLA molecules diluting the strength of any individual HLA–epitope associations that may be important in disease etiology.

In their studies of the relationship of HLA types with specific IgE to purified short ragweed pollen allergens, Marsh et al. (32) found the strongest associations of the low-molecular-weight allergens—HLA-DR2.2 with specific IgE to Amb aV and HLA-DR5 and Amb a V1 (33). The associations with high-molecular-weight allergens were weaker, probably reflecting the greater epitope density on high-molecular-weight proteins.

Possibly for this reason the majority of published studies of HLA associations in OA have been investigations of low-molecular-weight chemical sensitizers, which act as haptens and are probably a constituent of the epitope, potentially minimizing the number of epitopes, and therefore different HLA molecules, engaged in the immune response.

The majority of studies of OA caused by high-molecular-weight allergens have not found strong relationships. Three association studies have been reported of laboratory animal allergy and HLA. One study found the prevalence of HLA-DR4 and -B15 in 27 cases of laboratory animal allergy, which was about double that of normal controls, and of those drawn from the same workforce (34). In another study, Kerwin et al. (35) reported HLA restriction of human T lymphocyte responses, from nine mouse allergic patients, to mouse allergens (Mus m 1). There was an excess of HLA-DR4, -DR11, and -DRW17 in the nine cases as compared to 100 controls tested in the same laboratory. Both of these studies included only a small number of cases (27 and 9), and the first investigated the association of HLA with specific IgE to a complex mixture of high-molecular-weight protein allergens present in rat urine.

Table 1 Laboratory Animal Allergy. HLA: Sensitization and Symptoms (OR 95% CI[a])

	Sensitization	Work-related chest symptoms	Sensitization + work-related chest symptoms
HLA-DR7	1.82 (1.12–2.97)	2.96 (1.64–5.37)	3.81 (1.90–7.65)
HLA-DR3	0.55 (0.31–0.97)	0.71 (0.35–1.45)	0.57 (0.24–1.32)

[a]Adjusted for atopy, exposure, and site.
Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio.
Source: From Ref. 36.

The third and considerably the largest reported study of HLA associations in laboratory animal allergy was of 109 cases with specific IgE to rat urine proteins and of 397 referents from six pharmaceutical companies in the United Kingdom. The cases and referents had worked with rats for a similar period, an average of eight years. Jeal et al. (36) found an increasingly strong association between HLA-DR7 and specific IgE to rat urine protein, work-related respiratory symptoms and work-related respiratory symptoms with specific IgE to rat urine protein. Individuals with specific IgE to rat urine protein were more likely than referents to be HLA-DR3 negative (Table 1).

Although the authors recognize that the findings should be considered as hypothesis generating, needing testing in a separate population, they do have biological plausibility. The lipocalin rat allergen (Rat n 1) binds with high affinity to hydrophobic ligands. Of seven amino acid residues in which HLA-DR3 and -DR7 differ in their hydrophobicity, six are hydrophobic in HLA-DR7 and hydrophilic in HLA-DR3. Pocket nine of the peptide-binding groove of the HLA molecule has three polymorphic residues (positions 9, 37, and 57), all of which are hydrophobic in HLA-DR7 and hydrophilic in HLA-DR3. Binding affinity for Rat n 1 could be increased by hydrophobic residues in pocket nine of HLA-DR7 and reduced by hydrophilic residues of HLA-DR3, plausibly, at least in part, explaining these differential HLA associations with allergy to rat urine proteins.

HLA and Low-Molecular-Weight Chemical Sensitizers

Low molecular chemical haptens have, at least in theory, the advantage for HLA association studies that if they are a constituent part of the "foreign" epitope, the potential for dilution of association by multiple epitopes is diminished. Certainly, the majority of association studies of HLA with the development of specific IgE or asthma have been reported for cases caused by low-molecular-weight chemicals.

Acid Anhydrides. Young et al. (37) reported an association between HLA-DR3 and the presence of specific IgE antibody to albumin conjugates of trimellitic anhydride (TMA) but not the closely related phthalic anhydride (PA). The 30 cases were chosen from acid anhydride workers identified from factory surveys or from clinical referrals as having specific IgE antibody to TMA, PA, or tetrachlorophthalic anhydride (TCPA). Twenty-eight (93%) cases had rhinitis, asthma, or both. The 30 referents were matched on type and duration of acid anhydride exposure such that they had an equal opportunity for developing specific IgE to anhydrides. Referents did not have specific IgE (identified by skin-prick test or RAST) to acid anhydrides or symptoms of rhinitis or asthma. Referents were also matched for atopy and smoking habit.

Table 2 HLA-DR3 Frequency in Cases and Referents Exposed to the Acid Anhydrides TMA, PA, and TCPA

Acid anhydride exposure	Cases DR3+	Referents DR3+	Odds ratio	*P*
TMA	8/11	2/14	16	<0.05
PA	2/12	2/14		
TCPA	5/7	0/0		
Total	15/30 (50%)	4/28 (14%)		

Abbreviations: HLA, human leukocyte antigen; PA, phthalic anhydride; TMA, trimellitic anhydride; TCPA, tetrachlorophthalic anhydride.
Source: From Ref. 37.

The frequency of HLA-DR3 in cases and referents exposed to the three anhydrides is shown in Table 2. After correction for multiple comparisons, HLA-DR3 was found significantly more frequently in the cases of TMA sensitization with an odds ratio of 16. Cases of PA sensitization, however, were no more likely to be HLA-DR3 positive than their referents. The proportion of TCPA cases that were HLA-DR3 positive (5/7) was similar to that of TMA (8/11), but unfortunately no referents were obtained for comparison.

The results of the study suggested HLA type to be important in determining the development of specific IgE to TMA, possibly TCPA, but not PA. The study, however, had no specific prior hypothesis and the association of specific IgE to TMA with HLA-DR3 requires testing in another population.

Isocyanates (and Beryllium). Isocyanate-induced asthma differs from the majority of cases of acid anhydride-induced asthma in the lack of a consistent accompaniment of specific IgE antibody. Although the explanation for the lack of specific IgE antibody to isocyanates may be technical, it suggests a possible difference in the nature of the immunological response underlying the development of isocyanate-induced asthma. Nonetheless, the finding of activated lymphocytes and eosinophils in bronchial biopsies from cases suggests an immunological basis for the disease.

Bignon et al. (38) investigated HLA specificity in isocyanate-induced asthma and found an association of disease with HLA-DQB*0503 and an inverse relationship with HLA-DQB*0501 (Table 3). In a subsequent study, Balboni et al. confirmed these results in another population of isocyanate workers and observed that in the 57 position of HLA-DQB1, aspartic acid was present in HLA-DQB1*0503 and valine in HLA-DQB1*0501 (Table 4) (39). The observations suggested that the amino acid

Table 3 Isocyanate-Induced Asthma and HLA DQB1*0501 and *0503

	Isocyanate asthma	Referents	RR	*P*
HLA-DQB1	56	32		
*0501	1 (2%)	5 (16%)	0.14	<0.03
*0503	7 (13%)	0 (0)	9.8	<0.04

Abbreviations: HLA, human leukocyte antigen; RR, relative risk.
Source: From Ref. 38.

Table 4 Isocyanate-Induced Asthma and HLA-DQB Asp 57

HLA-B Asp 57 haplotypes	Isocyanate asthma	Referents
Asp 57+ homozygotes	17 (57%)	44 (32%)
Asp 57 heterozygotes	12 (40%)	73 (53%)
Asp 57– homozygotes	1 (3%)	21 (15%)
Total	30	138

Abbreviation: HLA, human leukocyte antigen.
Source: From Ref. 39.

present in residue 57 of HLA-DQB1 could be an important determinant of susceptibility to isocyanates in exposed workforces.

The importance of a specific amino acid substitution in an HLA molecule in determining the ability of an individual to present a hapten has also been suggested by Richeldi et al. (40) in relation to CBD. CBD, a disease very similar in its clinical manifestations to sarcoidosis, is characterized by the presence of hypersensitivity granuloma and proliferation of T lymphocytes from blood and bronchoalveolar lavage when incubated with beryllium salts. Richeldi et al. (40) demonstrated a strong association between the presence of glutamic acid in position 69 of the β1 chain of the HLA-DPB1 molecule and development of CBD in exposed workers. Of the 32 cases of CBD, 31 (97%) were HLA-DPB1* Glu 69 positive as compared to 27% of the 44 referents without CBD.

Complex Platinum Salts (and Beryllium Again). Complex platinum salts, of which ammonium hexachloroplatinate (ACP) is the most important, are essential intermediates in platinum refining. ACP is a potent cause of asthma, which is associated with an immediate skin-prick test response in the majority of cases.

In a case-referent study of the male workforce of a platinum refinery in South Africa, Newman Taylor et al. (41) found an excess of HLA-DR3 and a deficit of HLA-DR6 in skin-test positive cases as compared to referents, matched for intensity and duration of exposure and ethnic background. Stratifying those employed into "high" and "low" exposure jobs, the relative risk of a case being HLA-DR3 positive or HLA-DR6 negative was greater in the low than in the high exposure groups (Table 5).

These results suggest that in those occupationally exposed to ACP, genetic susceptibility is an important determinant of the development of sensitization

Table 5 HLA and Exposure Intensity vs. Sensitization to Complex Platinum Salts

	Cases, *n* (%)	Referents, *n* (%)	OR (95% CI)
HLA-DR3			
All	18 (41)	15 (26)	2.3 (1.0–5.6)
Low exposure	6 (55)	4 (22)	Infinite
High exposure	12 (36)	11 (28)	1.6 (0.6–4.1)
HLA-DR6			
All	16 (36)	34 (60)	0.4 (0.2–0.8)
Low exposure	2 (18)	12 (67)	0.1 (0.02–1.1)
High exposure	14 (42)	22 (56)	0.5 (0.2–1.3)

Abbreviations: HLA, human leukocyte antigens; OR, odds ratio; CI, confidence index.
Source: From Ref. 41.

and although the absolute risk of becoming a case is greater in the more heavily exposed, among those who were HLA-DR3 positive or -DR6 negative, the relative risk of becoming sensitized to ACP was markedly greater in those with lower exposure to ACP.

The other study that has investigated genetic environmental interactions is that reported by Richeldi et al. (42) of the risks of developing CBD in a factory workforce exposed to beryllium, in relation to intensity of exposure (using job title as a surrogate measure) and HLA-DPB1 Glu 69 (Table 6). On exposure to beryllium, 6 out of 127 cases (4.7%) developed CBD, the majority of cases (5/6) occurring among the machinists in the high-exposure group (ca. 0.9 $\mu g/m^3$). Five cases occurred among the 41 HLA-DPB1 Glu 69 positive individuals (12.2%) and one case among 86 HLA-DPB1 Glu 69 negative individuals (1.2%), a 10-fold increased risk for HLA-DPB1 Glu 69 positive individuals. Because the number in the study population was small, it is difficult to interpret genetic environmental interactions with confidence, only one case of CBD occurring in a nonmachinist who was HLA-DPB1 Glu 69 positive. The results, however, indicate an exposure–response relationship overall and in the Glu 69 positive group. In contrast to the findings in the ACP population, the relative risk of developing CBD in Glu 69 positive individuals was greater at higher levels of exposure to beryllium.

Implications of HLA Associations

These HLA associations have clear biological implications. They provide substantial evidence for specific immunological response in the development of OA initiated by low-molecular-weight chemical sensitizers. This is of particular importance for isocyanate-induced asthma, where the absence of demonstrable specific IgE antibody has led to suggestions that the disease is not immunologically mediated. The clear evidence for HLA association, taken with the finding of infiltration by activated lymphocytes and eosinophils in bronchial biopsies, provides coherent evidence for an immunological mechanism. In the case of acid anhydrides and complex platinum salts, where there is evidence for an exposure–response relationship, modified particularly by smoking but also by atopy, the association of sensitization with HLA type identifies a further and important risk factor, whose magnitude may vary with the level of exposure.

Genetic markers, such as HLA polymorphisms, hold out the hope that they will allow identification of susceptible individuals. However, to date, as with asthma caused by agents such as laboratory animals and platinum salts, and atopy, the association is not strong enough to be used as a discriminatory preemployment tool.

Table 6 Beryllium Exposure

	Machinist (0.9 $\mu g/m^3$)	Non-machinist (0.3 $\mu g/m^3$)	Total
HLA-DPB1 Glu 69 +ve	4/16 (25%)	1/25 (4%)	5/41 (12.2%)
HLA-DPB1 Glu 69 −ve	1/31 (3.2%)	0/55 (0%)	1/86 (1.2%)
Total	5/47 (10.6%)	1/80 (1.3%)	6/127 (4.7%)

Abbreviation: HLA, human leukocyte antigen.
Source: From Ref. 42.

In the best studied, and only replicated, example of HLA-DPB1 Glu 69 and CBD, although 25% to 30% of the beryllium-exposed population were Glu 69 positive and the disease virtually limited to Glu 69 positive individuals, only 12% of Glu 69 positive individuals developed disease. More than 85% (36/41) of the exposed individuals who were Glu 69 positive did not develop CBD, suggesting that while HLA type has an influence on the development of the disease, other genetic and environmental factors are at least as important. One of the factors described above was intensity of exposure to beryllium, but even among machinists (the high exposure group) only 10% overall and 25% who were Glu 69 positive developed CBD. Accurate prediction of individuals at risk of developing allergic lung disease, including OA, will have to await the identification of other relevant genetic polymorphisms, knowledge of which will need to be integrated into an understanding of exposure–response relationships, their modification by environmental (e.g., tobacco smoking) factors, and by the possibly varying influence of genetic susceptibility at different levels of exposure.

Association Studies in Asthma

Although MHC variants are strongly associated with asthma, genes which regulate inflammatory and allergic components are also involved. Many of these genes are found on chromosomes 5q31-q33, 6p21, 11q13, and 12q (22,43) including cytokines IL-4, -5, -9, -13, and TNFα, which are either determining or modifying factors in immunologic responses to asthma. Tumor growth factor (TGF)β1, IFNγ, IL-1β, IL-4 receptor α (IL-4Rα), IL-10, -12B, TNFα, and IL-13 are some of the candidate cytokine genes with known polymorphic variants involved in asthma and related phenotypes (44–50). Of these, TGFβ1, IL-4, -4Rα, -10, and -13 are more likely involved in the development of immune-mediated asthma while IFNγ, TNFα, IL-1β, and -12β are involved in inflammation. Recently, variations in other chromosomal regions have been associated with asthma phenotypes including toll-like receptor (TLR)–10, ADAM 33, CD14, monocyte chemoattractant protein–1 (MCP-1), and angiotensin-converting enzyme genes (51–54). Examples of single nucleotide polymorphisms (SNPs) associated with asthma or its partial phenotypes are given in Table 7.

The potential for gene–gene interactions in disease initiation and severity also exists for polygenic diseases such as asthma. For example, a significant gene–gene interaction exists between IL-4Rα (+478) and IL-13 (–1111), such that individuals homozygous for both the IL-4Rα variant and heterozygous or homozygous for the IL-13 variant are almost five times more likely to develop asthma than those without the genotype (55). In addition, individuals with the IL-4 590C and the IL-4Rα Arg551 genotype have been reported to have a higher risk of developing asthma (56). However, the role of these SNPs, and others with similar activities, has not been comprehensively examined in OA. As in other forms of asthma, inflammatory changes and allergen-specific T lymphocytes are found in the airways of many patients with OA, along with eosinophils, cytokines, and serum IgE antibodies (57–59). Thus, similar genetic associations as in immune-mediated asthma might be expected to occur in OA.

A role for low-penetrance genes in disease modification has been identified for OA. Oxidative stress is a major component of inflammation, and impaired ability to detoxify reactive oxygen species (ROS) may perpetuate the inflammatory processes and precipitate asthma symptoms (60). In this respect, genes coding for antioxidant enzymes such as glutathione S-transferases (GSTs) and *N*-acetyl transferase (NAT)

Table 7 Examples of Associations Between Polymorphisms and Asthma or Related Phenotypes in Different Populations

Gene	SNP position	Summary	References
IL-10	–627	–627A allele reported to be a risk factor of developing atopic asthma	(47)
IL-12B	–4237, –6402	–4237 and –6402 polymorphisms were associated with asthma severity-phenotype	(48)
IL-13	–1055	–1055TT genotype was found to be associated with the development allergic asthma	(50)
TLR-10	+1031, +2322	+1031 and +2322 SNPs were associated with physician-diagnosed asthma	(51)
ADAM 33	ST+7	ST+7 SNP was associated with asthma	(53)
IL-4Rα	S478P	Significant association was found between S478P and high IgE levels	(55)
TNFα	–308	Homozygosity for –308 allele was associated with increased risk of physician-diagnosed asthma	(79)
TGFβ	–509	–509T variant was associated with asthma severity	(80)
IL-4	–589	–589T variant was associated with the development of asthma and the regulation of total serum IgE	(81)
IL-3	Ser27Pro	27 Pro allele showed protective effect on development of asthma in nonatopic subjects	(82)
GSTP1	Ile-105	Gene–environment interaction was reported between Ile-105 homozygotes and outdoor air pollution for childhood asthma	(83)
CD14	–159	–159T variant was associated with expression of a more severe asthma	(84)

Abbreviations: IL, interleukin; TLR, toll-like receptor; TNF, tumor necrosis factor; GST, glutathione S-transferases; TGF, tumor growth factor; CD, cytotoxic drug.

are strong candidates for OA association studies. GSTs play an important role in the protection of cells from ROS damage because they detoxify a wide variety of electrophiles such as lipid and DNA peroxides. GSTP, located on chromosome 11q13, provides more than 90% of the GST activity in the lung and contains two genetic polymorphisms: an A→G transition at nucleotide +313 that leads to the 105 *Ile/Val* substitution, and a C→T transition at nucleotide +314. It has been demonstrated that the *val* variant alters specific activity and affinity for electrophilic substrates. For example, sevenfold greater catalytic activity for polycyclic aromatic hydrocarbon diol epoxides and a threefold lower activity for 1-chloro-2,4-dinitrobenzene were reported in individuals with the rare allele (61,62). An association was also found between the 105 *val* variant and lung cancer and COPD (63–65). Earlier, the

GSTP1 *val/val* genotype was reported to confer a sixfold lower risk of asthma (OR = 0.16; 95% CI: 0.05–0.55) and 10-fold lower risk (OR = 0.11, 95% CI: 0.02–0.50) for high IgE levels (60). Although it did not achieve statistical significance, a protective effect of *val/val* genotype was observed in TDI-induced asthma in subjects exposed to TDI for 10 or more years (OR = 0.23, 95% CI: 0.05–1.13) (66). In these studies the homozygosity for the *val* variant was lower in subjects with asthma (5.1%) after 10 years of TDI exposure than in those without asthma (18.8%). Although the sample size was low and statistical significance was not attained, this finding points to the importance of exposure intensity and other genetic factors in the development of asthma after short-term exposure (67). In another study, 182 diisocyanate-exposed workers were examined for GST polymorphisms (68). GSTM1 null and GSTM3 AA genotypes were related to late reactions in the specific bronchial provocation test, individually (OR = 2.82, 95% CI: 1.15–6.88 and OR = 3.75, 95% CI: 1.26–11.2, respectively) or in combination (OR = 11.0, 95% CI: 2.19–55.3). GSTM1 catalyzes the reaction of glutathione for a wide variety of organic compounds to form thioethers. The homozygosity for the GSTM1 null allele results in loss of gene function. Although relatively little is known about the role of the GSTM3 in enzyme metabolism, it is known to have overlapping substrate specificities with GSTM1 (69). GSTM1 null and the combination of GSTM1 null and GTM3AA genotypes were also reported to be related with lack of diisocyanate-specific IgE antibodies. Although the GSTP1 *val/val* genotype was associated with higher total serum IgE levels (OR = 5.46, 95% CI: 1.15–26.0), no statistically significant association was observed between GSTP1 genotypes and risk of diisocyanate-induced asthma. The difference in total serum IgE levels with respect to GSTP1 *val/val* genotype, compared to the data of Fryer et al. (60), may be explained by the small sample size, differences in the study populations, and the level and nature of exposure.

Like GSTs, there are large inter-individual variations in enzyme responses of NATs. NATs are involved in the deactivation of aromatic amines that can be formed from aromatic diisocyanates (70) and also play a role in the inactivation of proinflammatory cysteinyl leukotrienes which are potent mediators of airway narrowing (71). Polymorphisms in the NAT genes have been associated with lung cancer (72,73). The NAT1 variant, responsible for slow acetylation, was found to be associated with a 2.5-fold higher risk for developing diisocyanate-induced asthma (95% CI: 1.32–4.91) and 7.7-fold risk for developing TDI-induced asthma (95% CI: 1.18–51.6) (74). The presence of GSTM1 null and either NAT1 or NAT2 genotypes also conferred an increased risk (OR = 4.53; 95% CI: 1.76–11.6 and OR = 3.12; 95% CI: 1.11–8.78, respectively). Similar associations were also reported for the NAT1 and NAT2 slow acetylator genotypes (OR = 4.20; 95% CI: 1.51–11.6).

FUTURE DIRECTIONS

Meta-analyses of genetic association studies are currently underway to help identify susceptibility loci in candidate genes. Reports from multiple studies may help to establish genes with modest effects for multifactorial diseases such as asthma (75,76). Advance high-throughput technologies available for data generation and the amount and complexity of the data that can be obtained require the creation of new algorithms and methodologies for use in the computational interpretation and analysis of biological data. These advances could lead to better predictive models to incorporate genetic variability for human risk assessment (77).

The conduct of genetic studies raises the ethical, legal, and social implications of such research. The scientific profit from genetic research requires full integration of ethics components into the structure and functioning of genetic studies (78). The formulation and adoption of societal issues and widespread education are required to protect individuals against genetic-based discrimination or inappropriate use of the results (80). Despite the increasing availability of such data, there has been little effort to incorporate genetic information into the risk assessment process. Molecular epidemiology studies in workplace would provide more accurate information on the inter-individual variability that could be used in risk assessment and for improving the regulation and redefinition of acceptable exposure levels in the workplace. This information would also be useful in the development of more appropriate disease models that help to investigate disease risk, gene–environment interactions, and new therapeutic or treatment regimens.

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And Related Conditions

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