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SERIES "CONTROVERSIES IN OCCUPATIONAL ASTHMA"
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The role of genetic factors in occupational asthma

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The role of genetic factors in occupational asthma. C.E. Mapp. ©ERS Journals Ltd 2003.
ABSTRACT: This article explores the influence of genetic factors on the development of sensitisation and occupational asthma (OA).

First, several types of studies aimed at examining the role of genes, as well as the role of gene-environment interactions in asthma, including the available data for OA specifically, were reviewed. Genetic approaches include linkage and allele-sharing analysis and segregation analysis. Secondly, deoxyribonucleic acid banking for epidemiological studies was focused upon, highlighting the factors to be considered in choosing the appropriate specimens for genotyping.

OA, like asthma, is a multifactorial condition and, to date, no ideal genetic study has been described to examine complex gene-environment interactions. Most studies in OA have examined human leukocyte antigen-associated polymorphisms with some nonreproducible results.

The search for genes in occupational asthma is still in progress, and much of the information obtained has been based on small sample sizes, using different strategies for the recruitment of subjects. The best methodological approach still needs to be determined and the results of genetic identification need to be confirmed in different samples.
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Bronchial asthma is a multifactorial disease and appears to be genetically heterogeneous. Indeed, it involves several genes, as well as environmental factors that influence the expression of the disease. Moreover, there are the problems of incomplete penetrance (*i.e.* an individual bearing a predisposing polymorphism does not necessarily develop asthma), of a high phenocopy rate (*i.e.* asthma may develop in the absence of a genetic predisposition, when the environmental stimulus is strong enough) and finally of intermediate phenotypes complicating the picture.

Previously, it has been reported that as much as 40% of the genetic liability for allergic diseases is due to environmental factors [1]. Environment is defined as the complex of factors that represent the external components of an individual's life, including the following: place of upbringing, diet, types of illnesses, educational background, work history, and exposure to various materials or psychological events. The influence of the environment is mainly evident in the determination of multifactorial characters.

Asthma and allergies are good models for studying complex diseases resulting from the interaction of genetic and environmental factors, and occupational asthma (OA) is a good model for studying the natural history of adult-onset asthma and investigating gene-workplace interactions [2, 3]. The amount of time spent in the workplace is an important component of an individual's environment.

Many steps are involved in the current approach to mapping common and genetically complex traits; the step that defines interactions between genes (gene-gene), and between genes and environment (gene-environment), is important for complex diseases (*i.e.* asthma and allergic diseases), but unfortunately, it is the least developed step [4].

A given environmental exposure may influence the risk of disease in people with different genotypes, or alternatively, a particular genotype may influence the risk of disease in people exposed to different environmental factors [5]. The most powerful means of detecting a gene-environment interaction is to measure both the genotype and the environmental risk factor [6]. Moreover, larger sample sizes are needed for the studies to detect interactions. A software program that computes sample size for studies of gene-gene interactions and for studies of gene-environment interactions is freely available [7, 8]. Understanding the relationships between genes and environment has implications for society and public health, since some environmental and workplace factors that influence genetic risk are modifiable.

Several books and reviews on the genetics of allergy and asthma and on approaches to gene-mapping in human diseases have been published in the past decade [9]. This article will focus on controversial issues (table 1), on the current approaches to the analysis of gene-environment interactions and on the strategies for studying the aetiology of complex traits.

Previous articles in this series: No. 1: Vandenplas O, Malo J-L. Definitions and types of work-related asthma: a nosological approach. *Eur Respir J* 2003; 21: 706–712. **No. 2:** Moscato G, Malo J-L, Bernstein D. Diagnosing occupational asthma: how, how much, how far? *Eur Respir J* 2003; 21: 879–885.

Table 1. – Difficulties and controversial issues in genetic studies of occupational asthma

Adequacy of models of gene-environment interaction
Complex genetic diseases require genetic and epidemiological approaches
Are traditional linkage studies relevant? Inconsistent results found in the case of asthma induced by diisocyanates
Inconsistent results of whole genome-screening studies
Difficulty in determining the genetic localisation of susceptibility loci in asthma
Small sizes of samples and lack of statistical power to detect genes of modest effect, with a consequent lack of consistency of results
Small number of single nucleotide polymorphisms typed in a given gene
Susceptibility genes for quantitative traits (levels of total IgE, airway responsiveness) not necessarily equivalent to the susceptibility genes for the disease (asthma)
Modest level of association, if any, which does not help in surveillance programmes of high-risk workforces
Comparability of various possibilities of collecting DNA (blood spots, whole blood, buccal smears, *etc.*)
Adequacy of the HLA-system polymorphism studies

Ig: immunoglobulin; DNA: deoxyribonucleic acid; HLA: human leukocyte antigen.

Genetic and epidemiological concepts

Susceptibility

A susceptible individual is one who presents the phenotype (disease) if the relevant exposure is present and does not present the phenotype (disease) if the exposure is absent. The phenotype represents a genotype-environment interaction. Genetic susceptibility may influence the disease in several ways. First, it can influence the risk of disease itself; secondly, it may augment the expression of an environmental risk factor; and thirdly, the risk factor may augment the genetic effect [10].

Three models of gene-environment interaction have been described [11]. In the first model, disease risk is increased only in the presence of the susceptibility genotype and the environmental risk factor; in the second one, disease risk is increased by the environmental risk factor alone but not by the genotype alone; and finally, in the third model, disease risk is increased by the genotype in the absence of the environmental risk factor but not by the risk factor alone.

The association of genetic and epidemiological methods in the study of complex genetic interactions has a greater chance of success in investigating the multifactorial nature of asthma and allergic diseases. Traditional linkage methods alone may not be powerful enough for investigating the genetics of asthma and allergies.

Linkage studies

Linkage analysis is one way of identifying genetic regions likely to harbour a disease gene. Asthma is related to both airway hyperresponsiveness and atopy. The most convincing regions of linkage to asthma or atopy phenotypes are the regions 5q31–33, 6p21, 11q13, 12q, 13q and 16p12 [12]. Some of these regions harbour important candidate genes such as interleukin (IL)-4, IL-13 (5q31), tumour necrosis factor (TNF)-α (6p21), IL-4R-α (16p12) and FceRI-β (11q13).

Since asthma is difficult to define, the search for intermediate phenotypes has been the focus of many genetic studies [13]. The use of intermediate phenotypes, such as serum immunoglobulin (Ig)E levels or airway responsiveness, permits selection of subjects for the extremes of distribution, increasing the power to detect linkage [14]. More recently, it has been suggested that testing for linkage is enhanced by the use of quantitative traits compared with a categorical asthma phenotype [15].

Whole genome screens undertaken in humans have resulted in many inconsistencies between the location of linkages, and have failed to discover a functional mutation affecting asthma or atopy susceptibility [13]. However, a new putative asthma susceptibility gene (ADAM33) was recently identified by positional cloning in an outbred population [16]. ADAM proteins are membrane-anchored metalloproteases with diverse functions, including the shedding of cell-surface proteins, such as cytokines and cytokine receptors [17].

Despite much progress in defining asthma and atopy, accompanied by progress in the technology for detecting single nucleotide polymorphisms (SNPs), the genetic localisation of susceptibility loci in asthma is not always precise enough to allow positional cloning of new genes in the disease. Candidate loci linkage-disequilibrium-mapping techniques, using SNPs, may be helpful to understand the mechanisms operating in asthma [18].

A new methodology, such as the meta-analysis of linkage studies between 5q31–33 markers and total serum IgE levels, suggested that these markers have a modest effect on total serum IgE levels, confirming many limitations in the genome screens for asthma and atopy phenotypes [19]. The most common limitations are the small sample sizes and the lack of statistical power to detect genes of modest effect, with a consequent lack of consistency in results [18]. Other limitations include the small number of SNPs typed in a given gene, with the consequent possibility of generating misleading results [20]. Other authors [21] have emphasised the fact that there is insufficient agreement about definitions, genetic models and statistics to apply meta-analysis to asthma and atopy. The same authors have suggested that more experience is needed to force agreement on genetic models, and that whatever the model, statistics should be presented as a log score or as Chi-squared, or as other large-sample statistics in order to give both a pooled estimate and test of heterogeneity among samples. Finally, the assumption that the susceptibility genes for quantitative traits such as total IgE or airway responsiveness associated with asthma and allergic diseases are equivalent to the susceptibility genes for disease risk may not necessarily be valid [13].

Strategy in the study of the aetiology of complex traits

Complex genetic diseases require genetic and epidemiological approaches.

Genetic approaches. Genetic approaches include linkage and allele-sharing analysis, and segregation analysis.

Linkage and allele-sharing analysis. These methods are useful in studying gene-gene interactions that may occur in a multiplicative or additive manner. The use of linkage analysis requires a narrow and precise definition of the disease, or the restriction of the patient population, to improve the study of the genetic aetiology of complex diseases.

Nonparametric allele-sharing methods, such as affect sib-pair analysis, are preferred to traditional linkage analysis [22].

Segregation analysis. This method is very sensitive to sample size and to ascertainment bias, but it requires computer resources. The use of regression models has been successful in the study of both qualitative and quantitative traits [23] and in the exploration of the interactions between loci [24]. The combination of linkage and segregation analyses increases the power to determine genetic interactions. However, it also generates some disadvantages. For example, combining the two methods works better only for the analysis of quantitative traits.

Epidemiological approaches. The epidemiological approach makes it possible to test models for investigating the relationship between genetic susceptibility and environmental risk factors. The case-control method appears to be powerful enough to study this relationship [25]. At variance with family studies, this approach does not incorporate the inheritance pattern of the trait.

Case-control method. In this method, cases are defined as those affected with the studied disease, whereas controls, although at risk for developing the disease (*i.e.* exposed for many years to a sensitiser, which causes OA in the cases), are unaffected by the disease. Controls should be selected using the same criteria applied to the cases, they must be at risk for developing the disease of interest and their selection must be performed during the same time period as the cases.

Association studies have often used the case-control method to detect disease-susceptibility genes. In the classical candidate gene approach, the frequency of the genetic marker of interest or the frequency of the disease-susceptibility gene in the cases is compared with the frequency in the group of controls. Using two controls in the same family may increase the power of such an analysis [26].

Cohort study. Study subjects are selected on the basis of their exposure status (*i.e.* family history of disease, or a susceptibility genotype), are disease-free at the beginning of the follow-up period and are followed until onset of disease. The cohort design makes it possible to study rare exposures, multiple effects of a single exposure, and to determine a temporal relationship between exposure and disease. However, these studies are costly and time-consuming and are not effective for studying diseases with late onset.

Epidemiological measures. Associations between the risk factor and the disease may be quantified with measures such as the relative risk (RR), the odds ratio (OR) and the attributable risk (AR). The latter represents a measure of impact or the excess risk of disease among individuals who are genetically susceptible compared with those who are not. The genetic AR is dependent on the proportion of disease due to the susceptibility gene and the magnitude of the RR among gene carriers and noncarriers.

Confounding bias. To control for confounding factors in assessing the association between a risk factor and a disease, stratification and logistic regression are common methods employed for case-control studies, whereas in cohort studies, survival analyses are used.

Sensitivity, specificity and predictive value of a positive genetic test. The value of a genetic test for predicting a disease can be characterised by measures such as sensitivity and specificity. When the sensitivity of the test is high, the number of false-negatives decreases, whereas high specificity results in a low number of false-positives (individuals with the disease who are classified as test-negative).

Predictive values. The predictive values are a function of the sensitivity, specificity and prevalence of disease. The positive-predictive value is an estimate of the accuracy of the test in predicting the presence of the disease, whereas the negative-predictive value is an estimate of the accuracy of the test in predicting the absence of disease.

Deoxyribonucleic acid banking for epidemiological studies. Factors to be considered in choosing the appropriate specimens include quality and quantity of deoxyribonucleic acid (DNA), convenience of collection and storage, and cost and ability to accommodate further needs for genotyping [27].

Blood spots. Blood spots are a stable, inexpensive source of DNA for genotyping polymorphisms for association studies [28]. Hundreds of genotypes can be obtained from one blood spot. These samples can be collected without a phlebotomist and safely transported by regular mail. However, specimens may not have been collected without obtaining informed consent to perform genetic studies [29].

Whole-blood specimens. The National Heart, Lung and Blood Institute has published guidelines for obtaining these specimens [30] and, recently, a review has also been published [28]. This technique provides high-quality DNA in amounts sufficient for current applications. Blood is collected into vials containing, usually, ethylene diamine tetraacetic acid. Cells can be stored in whole blood, noncoagulated or as a clot, or in buffy coats. Getting consistent results with buffy coats is often problematic, since the technique is time-consuming and the multiple-step procedure requires great care. Clotted blood offers the advantage of obtaining DNA from clots and serum for other analyses.

Transformed fresh or cryopreserved lymphocytes. The viability of lymphocytes was studied in a multicentre study [31]. The indications are that cell separation and storage must be performed within 6 h of collection, using a controlled-rate freezer to cryopreserve the cells. The advantage of using transformed cell lines is that it provides an unlimited supply of DNA; the disadvantages are high costs, complicated methods and the possibility of obtaining DNA or ribonucleic acid (RNA) only, with no possibility of measuring other compounds in serum or whole blood.

Buccal cells. Buccal cells can be obtained for DNA isolation using cytobrushes, swabs or oral lavage. They have been used in epidemiological studies either as primary specimens or in combination with whole-blood specimens. There is growing evidence that the use of mouthwash (excluding bacterial contamination) gives a greater yield and a higher quality of DNA [32–36]. The use of alcohol-containing mouthwash has been recommended, as this is the optimal collection medium to prevent bacterial growth on swabs. Mouthwash specimens seem to be superior to cytobrushes for obtaining high molecular weight DNA [35]. A recent study showed that cells isolated from either mouthwash or cytobrush samples collected by mail from adults are adequate sources of DNA, although a single mouthwash sample provides larger amounts and higher molecular weight DNA than two cytobrush samples [35]. The same authors showed that storage at -80°C for up to 1 yr did not significantly deplete the amount of hybrid (h)DNA in the samples. Another study assessed the feasibility of obtaining buccal-cell DNA by mail

and using mouthwash collection kits [37]. It was concluded that under field conditions, this method yields adequate genomic DNA [37]. A pilot study, to determine the optimal conditions for the collection of buccal cells by using a mouthwash collection protocol, established that in order to maximise hDNA yield, buccal cells should be collected before brushing teeth (higher yield by 40%) and processed within 5 days of collection [36].

Self-collection of oral epithelial DNA by oral rinses appears satisfactory and efficient for epidemiological studies [32]. Self-collection can be carried out in the subject's home or workplace, under instructions from a nonmedically trained interviewer. Short-term storage, up to 3 days, at room temperature did not affect the specimens. Although larger quantities of DNA were obtained from males than from females, polymerase chain reaction assay amplification did not differ by sex. The self-collection method achieved high participation rates, one of the goals in epidemiological studies.

Recently, another method of collecting buccal-cell DNA, the toothbrush-rinse method, combined with a modified Gentra Puregene (Minneapolis, MN, USA) DNA extraction protocol, was recommended for large-scale epidemiological studies [38]. This method requires only inexpensive and commonly available materials, produces samples that are stable at room temperature and yields a large amount of high-quality DNA.

The advantages of obtaining DNA from buccal cells are that the method is noninvasive and that participants can collect and mail the specimen themselves [32]. The drawbacks are the low DNA content and high variable yield (factors: method of collection, method of DNA extraction, unsupervised collection of specimens, transport, mouthwash methods require individuals to expectorate), the inability to control bacterial contamination, and the lack of other compounds to study besides DNA and RNA. By using primer extension pre-amplification, mouthwash DNA can be increased 500–1,000-fold, but a portion of that amplification could be due to bacterial DNA and the method is not routinely performed [39].

In conclusion, DNA extracted from whole blood yields large quantities of high-quality genomic DNA, the cost of storage is low and the method allows for many immediate or future applications. Despite the drawbacks of yielding limited amounts of DNA and wide interindividual variation, buccal cells should be recommended when noninvasive, self-administered or mailed collection protocols are required. Transformed lymphocytes should be considered when sophisticated techniques and funds are available, and when future and collaborative genetic studies are anticipated.

Studies of occupational asthma with a latency period

In the past decade, studies of OA have shown exposure relationships for both sensitisation (*i.e.* IgE production) and disease (*i.e.* asthma). The risk of developing asthma grows with increasing exposure to its cause, but the time course has the pattern of an epidemic curve, with a median period of ~1 yr, consistent with the development of sensitisation and asthma in a susceptible population [40]. However, the latent period between the onset of exposure and the onset of symptoms is highly variable, with some subjects developing asthma only after many years of exposure [41].

Human leukocyte antigen system

Most reported genetic studies of OA have investigated the importance of human leukocyte antigen (HLA) class-II polymorphisms in increasing or decreasing the risk of developing sensitisation and OA. HLA class-II molecules are highly polymorphic and are therefore plausible candidate genes that influence the development of a specific immunological response. HLA associations with OA have been shown in workers exposed to low molecular weight chemicals, including acid anhydrides, isocyanates, platinum salts and western red cedar [42–45]. However, in isocyanate asthma, one study did not find significant associations with HLA-DR or HLA-DQ alleles [46]. HLA associations provide evidence for a specific immunological response in asthma caused by low molecular weight sensitisers. All of these studies were performed on small sample sizes and the level of association, often modest, does not help in the identification of susceptible individuals before exposure. The strength of association is usually expressed as an OR with appropriate confidence intervals. An OR >100 indicates a major genetic determinant, but in OA, ORs of this magnitude have never been found, suggesting that other genes and/or environmental factors are more relevant. Indeed, the level of exposure and tobacco smoking are more important in determining the risk of developing sensitisation to platinum salts than the HLA phenotype, emphasising the role of environmental factors in OA [40]. It is also important to take into account the social, geographical and ethnic factors, and to test the association in different populations of similar ethnicity (*i.e.* replication of the results). It is also possible that by chance, two genes would occur together more frequently than expected, with the result that a disease associated with an HLA molecule may be a marker for another gene with which it is in linkage disequilibrium.

Oxidative stress

Some important chemical sensitisers, such as isocyanates, may cause oxidative stress at the epithelial surface when they form conjugates with human proteins. Isocyanate exposure induces intracellular hydrogen peroxide production and intercellular adhesion molecule (ICAM)-1 expression on cultured mononuclear cells, suggesting that the production of reactive oxygen species by monocytes at the site of exposure of an isocyanate may contribute to tissue damage [47]. The production of hydrogen peroxide upregulates ICAM-1 expression, which may potentiate the infiltration and adhesion of cells at the site of exposure [3, 47]. Therefore, defects in antioxidant defences could contribute to the susceptibility of isocyanate-induced asthma.

Recently, a relationship has been shown between asthma induced by exposure to toluene diisocyanate (TDI) and allelic variants of the glutathione-*S*-transferase (GST) enzyme, which serves as an important protector of cells from oxidative stress products [48]. The π-class GST locus gene product represents >90% of the GST activity in the lung [49] and is located on chromosome 11q13 close to other markers associated with asthma. A similar association between the protective Val^{105}/Val^{105} phenotype and a reduced risk of being atopic has also been shown, suggesting that a defect in antioxidant defences may enhance the risk of becoming sensitised as well as the risk of asthma [50].

PIIRILA *et al.* [51] found that in asthma induced by exposure to isocyanates, the polymorphic GSTs, especially the mu-class GSTs, play an important role in occupational exposure to isocyanates. The same authors also showed that, in addition to GSTs, the *N*-acetyltransferase slow acetylator genotype posed a 7.77-fold risk of asthma among workers exposed to TDI [52].

Cytokines

In a mouse model of OA induced by isocyanates, the cytokine TNF-α plays a central role, since its deficiency abrogated TDI-induced T-helper cell-2 cytokines in airway response and resulted in a significant reduction in the migration of airway dendritic cells to the draining lymph nodes [53]. This cytokine could influence both inflammatory processes and specific immune events.

Peripheral blood mononuclear cells of subjects exposed to isocyanates showed significantly enhanced secretion of monocyte chemoattractant protein-1; an increased production of IL-8 and TNF-α was also present in supernatants, suggesting a role for both chemokines and cytokines in isocyanate-induced OA [54].

Rhinitis

Allergic rhinitis and allergic asthma may be linked in the natural history of asthma. Allergic rhinitis precedes or develops concurrently with the development of allergic asthma [55] and it has been suggested that both are manifestations of the same disease entity [55, 56].

Rhinitis precedes OA induced by high molecular weight agents, and it may be considered a risk factor for this type of OA [57]. It has also been shown that the determinants for developing specific skin sensitisation, symptoms and disease were different for atopic and nonatopic subjects exposed to laboratory animal-derived allergens; baseline rhinitis on contact with pets and perannual rhinitis symptoms were important in atopics, and airway hyperresponsiveness was important in nonatopics [58].

To date, there have been no conclusive studies on determining susceptibility genes in the case of irritant-induced asthma. However, one study has suggested that an interaction between host and environmental factors may also occur in this type of OA [59].

Conclusions

Genetics has a significant influence on asthma and allergic diseases, but the "weight" of genetic susceptibility and gene-environment interactions have not yet been established. Rapid advances in several fields, especially in molecular biology and statistical analysis, have allowed the increased understanding of the development of these diseases, but a clear picture of the mechanisms that lead to asthma onset and persistence is still lacking. To fully understand the genetics of these complex diseases, it is essential to establish a team of investigators with backgrounds in genetics, immunology, epidemiology and statistics, not to mention the important contribution of the clinician in defining an accurate phenotype of the disease. Once the mechanisms and how the genes interact with environmental factors are understood, subjects who are predisposed to develop asthma and allergic diseases should be identifiable. OA, an important and frequent clinical condition, remains a challenge for investigators. As a recent editorial emphasised [3], more use of this natural model should be made in order to assess many aspects of asthma that are difficult to address in childhood.

This article attempted to address relevant questions regarding the validity of current genetic designs and the establishment of the "ideal" genetic study to examine gene-environment interactions. These questions remain open, since, to date, the search for genes in asthma and allergies is still in progress, and much of the information has been fragmentary and unconfirmed. Moreover, even in the natural model of occupational asthma, and even at the time of diagnosis, patients are heterogeneous. The next step should be to design a genetic study with the contribution of investigators with expertise in the above-mentioned fields, and to remember that findings need to be replicated.

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