Gene-Environment interactions in asthma: what we know today?

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INTRODUCTION

Asthma is a complex respiratory disease characterized by episodes of variable airflow obstruction with clinical symptoms such as wheezing, coughing and shortness of breath. The classic definition of asthma as a single disease has begun to wane and the emerging concept is that asthma is an entity or a syndrome consisting of various different phenotypes, each involving different pathogenetic mechanisms¹². This idea has resulted from the large number of asthmatic phenotypes described and the numerous risk factors and immunological mechanisms that have been identified³⁵.

Asthma, with its various phenotypes, is a complex disease, resulting from the action of multiple environmental and genetic susceptibility factors. The development of the complex disease or syndrome is a process that depends on the interaction of these factors, in gene-gene, environment-environment and gene-environment manners, within a complex biological system⁶. Through years of research and effort, hundreds of genetic and environmental factors have been shown to be associated with asthma⁷⁸. Despite these efforts, however, only few of these factors have become widely accepted. The two main reasons for this difficulty are the heterogeneity of asthma phenotypes and the inherent complexity of the disease.

Asthma and environment

The rising prevalence of asthma over the past 50 years has emphasized the importance of environmental exposure in susceptibility to asthma, as genetic susceptibility factors within the population cannot change over such a short time frame. In a study conducted by the World Health Organization (WHO), it was estimated that 44% of the asthma burden worldwide is due to the environment⁹. Pollution, both outdoor and indoor, is thought to affect the development, persistence and exacerbation of asthma¹⁰. The most abundant compounds among outdoor pollutants are inhalable particulate matter, ozone, nitrogen oxides (NOx), sulphur dioxide (SO2) and diesel exhaust particles¹¹. Many studies have documented the role of these pollutants in asthma exacerbations¹¹,¹² and, to a lesser extent, in the development of asthma¹²,¹³. Exposure to indoor air pollution has been associated with asthma in a pattern similar to that of outdoor air pollution. The most thoroughly evaluated indoor pollutants are indoor combustion for heating
and cooking, environmental tobacco smoke (ETS), mould and dampness\textsuperscript{14}. The effect of ETS on the development and severity of asthma is strong and consistent in both in vitro and in vivo studies\textsuperscript{13,16-18}.

Early life sensitization to common Aeroallergens has been also associated with the risk of asthma development\textsuperscript{19-21}. Sensitization to house dust mite (HDM) is the most frequently evaluated allergen\textsuperscript{22-24}. Other allergenic factors associated with asthma are sensitization to pet allergens\textsuperscript{19,25,26}, pollen allergens\textsuperscript{13,27,28} and IgE specific to fungal species present in mould\textsuperscript{29-31}. Respiratory viral infections, mainly respiratory syncytial virus (RSV) and rhinovirus (RV), have been associated with the inception and exacerbation of asthma in both children and adults\textsuperscript{32-34}. Other environmental factors associated with asthma phenotypes are cigarette smoking\textsuperscript{35,36}, obesity\textsuperscript{37} and diet\textsuperscript{38,39}. In addition, occupational exposures are important environmental factors for a significant minority of patients with asthma and are reported to account for around 9-15\% of asthma in adults of working age\textsuperscript{40}. Agents that cause occupational asthma can be divided into compounds of high molecular weight (e.g., proteins of animal or plant origin and latex) and low molecular weight (mainly isocyanates, acid anhydrides, some metallic compounds such as platinum salts, cleaning agents and wood dusts)\textsuperscript{40,41}.

**Asthma and genes**

The role of genetic factors in asthma and atopy is unquestionable. It was initially postulated from the observation of familial clustering, and twin studies have subsequently shown that there is a genetic element to asthma susceptibility, with heritability of the condition estimated at between 0.36 and 0.77\textsuperscript{42-44}. The first study to link a specific genetic locus, namely chromosome 11q13, to asthma was published in 1999\textsuperscript{45}, since when polymorphisms in more than 200 candidate genes, described in more than 1,000 publications, have been reported to be associated with asthma or a related phenotype. In general, three main approaches have been used to identify genetic susceptibility locus in complex diseases such as asthma: linkage analysis, positional cloning and genetic association studies.

Linkage analysis tests co-segregation of genetic markers and a trait of interest in the absence of previous hypotheses\textsuperscript{46}, using short tandem repeat polymorphisms (STRPs) and single-nucleotide polymorphisms (SNPs). Linkage studies in asthma have resulted in more than 20 possible regions\textsuperscript{47}, the most promising of which are those that are well replicated in multiple populations, namely: 2p, 4q, 5q31-33, 6p21-24, 11q13-21, 12q21-24, 13q12-14, 16q21-23 and 19q17. In a recent meta-analysis of 20 genome-wide linkage studies, linkage was detected for asthma in two regions (2p21-p14 and 6p21) in European families\textsuperscript{48}.

Once a set of markers close to a disease susceptibility locus has been identified using techniques such as genome-wide linkage analysis, fine mapping analysis and positional cloning are then used for the identification of susceptibility genes and mutations\textsuperscript{49}. To date, several genes have been identified as a result of this technique, although their specific functions have not been clarified. ADAM metallopeptidase domain 33 (ADAM33) on chromosome 20p was the first identified as an asthma and bronchial hyperresponsiveness (BHR) susceptibility gene\textsuperscript{50}. Other genes identified with positional cloning for allergic disease phenotypes include DPP10 and IL1RN on chromosome 2q\textsuperscript{51,52}, PCDH1, CYFIP2 and SPINK5 on chromosome 5q\textsuperscript{53-55}, HLAG on chromosome 6p\textsuperscript{56}, GPRA on chromosome 7p\textsuperscript{57}, PHF11 on chromosome 13q\textsuperscript{58}, PTGDR on chromosome 14q\textsuperscript{59} and PLUAR on chromosome 18\textsuperscript{60}.

Association studies using genetic factors look for statistical correlation between one or more genetic polymorphisms and a trait. Two types of approach have been developed according to prior hypotheses by which a number of polymorphisms have been evaluated, namely candidate gene and, more recently, whole genome association studies. In a candidate gene association study, a particular gene (or set of genes) is selected for study based on its biological plausibility or suspected role in the phenotype of interest\textsuperscript{61}. Most of the genes known to modify asthma have been identified through candidate gene studies, although surprisingly few of these candidate gene discoveries have been rigorously replicated, and many have been examined and failed replication in subsequent studies\textsuperscript{62}. Genes that have been extensively replicated include the β2 adrenergic receptor gene ADRB2\textsuperscript{63-65}; the cytokines, receptors, signalling proteins, and transcription factors involved in Th1 and Th2 differentiation of T cells, such as the interleukins (IL) IL4, IL4RA, IFNG, IFNGR1, STAT6, GATA3, and TBX21\textsuperscript{66-69}; and genes involved in the cellular responses that characterize atopic disease, such as IL13 and its receptor and the FCER1B gene\textsuperscript{70-75}.

In the most recent development in genetic studies, the genome-wide association study (GWAS), a dense set of SNPs across the genome is genotyped to survey the most common genetic variation for a trait\textsuperscript{76}. Like the candidate gene association study, this design facilitates
the collection of a large number of cases and controls for analysis, increasing the statistical power. In contrast, however, it permits a hypothesis-free search for gene variants associated with disease, revealing new targets for researchers. To date, 12 GWAS have been conducted to look for susceptibility loci for asthma and related traits. The first GWAS for asthma discovered a novel associated locus on chromosome 17q21 encompassing the genes ORMDL3, GSDMB and ZPBP2. None of these genes would have been selected in a candidate association study based on current knowledge of the functions of these genes, but the findings have been consistently replicated in multiple populations from diverse ethnic backgrounds. Other genes identified in more than one GWAS are IL1RL1 on chromosome 29, RAD50 on chromosome 5q31, PDE4D gene on chromosome 5q12, HLA-DR/DQ on chromosome 6, IL33 on chromosome 9, and DENND1B on chromosome 17q21. Additional novel susceptibility genes identified in a single study include DENND1B, SMAD3, CHI3L1, IL2RB, and ATPAF1. A recent GWA study on two independent populations of African descent yielded 3 SNPs in genes of potential biological relevance to asthma and allergic disease, but none of the associations observed in the African groups were replicated in European studies or in 4 additional case-control studies of African Americans. This illustrates the complexity of identifying associations for a complex disease such as asthma, in admixed and heterogeneous populations with different genetic backgrounds and patterns of environmental exposure.

As summarized above, both genetic and environmental factors are involved in the aetiology of complex diseases such as asthma, acting through a complex biological system that involves interactions between them. In genetically predisposed subjects, exposure to a specific environmental factor can lead to the development of disease. Studies on gene-environment interaction may help in the elucidation of the mechanisms of the disease process and the identification of genetic risk factors with small marginal effects. This information could also help in the design of novel strategies of intervention at both the preventive and therapeutic level for the population at risk. The literature on gene-environment interactions in asthma has grown considerably during the last few years, but despite a relatively high number of studies, only modest advances have been achieved in the understanding of the relevance of genetic background in the causation of asthma in relation to environmental exposures. This article reviews studies that have examined interactions between genes, environmental and occupational exposures, and asthma and its related phenotypes.

**Gene-environment interactions in asthma**

This review is based on a bibliographic search conducted online through PubMed (http://www.ncbi.nlm.nih.gov/entrez/) in March 2011, using the keywords “asthma AND (gene OR genes OR polymorphism) AND (environment OR environmental OR occupational) AND (association OR interaction) NOT review”. Of 283 papers initially identified with some reference to gene–environment interactions in asthma and related phenotypes, 134 studies had assessed true gene–environment interactions in asthma and their results are summarized and commented upon here.

**Outdoor air pollution - gene interaction in asthma**

Acute and chronic exposure to outdoor air pollutants has been associated with asthma development and increased asthma morbidity. The main outdoor air pollutants evaluated in clinical studies are inhalable particulate matter (PM2.5, PM10), ozone, nitrogen oxides, sulfur oxides and diesel exhaust particles. The first study on gene-outdoor air pollutants interaction was published in 2001. In this study, Winterton et al. explored whether genes with polymorphisms shown to be associated with asthma (i.e., ADRB2, IL4RA, CC16, TNFα, LTA and NQO1) are associated with the BHR to SO2 observed in some patients with asthma. In this population of 62 patients with asthma, only tumour necrosis factor-α (TNFα) promoter polymorphism appeared to interact with the response to SO2 (OR = 16.25; 95% CI 1.5 to infinite). The relationship between functionally significant polymorphisms in NQO1 (Pro187Ser) and GSTM1 (homozygous deletion) and asthma risk in children with a high lifetime exposure to ozone was tested in the study of David et al. They examined 218 children with asthma from Mexico City and found a protective effect of the NQO1 position 187 polymorphism in the population of GSTM1-null children with high ozone exposure (RR = 0.4; 95% CI 0.2 to 0.8). In the same study, dietary supplementation with antioxidants was shown to be more beneficial for GSTM1 null genotype carriers. In contrast, in the study of Castro-Giner et al., genetic polymorphism in the NQO1 gene was related to asthma susceptibility among persons exposed to local traffic-related air pollution. In this cohort of 2,920 adults from the second European Community Respiratory Health Survey (ECRHS II), a significant interaction was found between NQO1 rs2917666 and NO2 for asthma prevalence (p = 0.02) and new-onset asthma (p = 0.04).

Lee and colleagues investigated whether GSTP1...
genotypes and outdoor air pollution were interactive risk factors for childhood asthma. They examined 436 schoolchildren from 3 districts with different air pollution levels in southern Taiwan and observed that GSTP1 Ile105 homozygote carriers have a higher risk of asthma produced by outdoor air pollution, defined by levels of NOx and SO2 (OR = 5.5; 95% CI 1.6 to 21.3). They also found that the risk of asthma revealed a clear dose-response relationship with outdoor air pollution only in children who were Ile-105 homozygotes. In a subsequent study by Lee et al in the same cohort of children with asthma, those with TNF-308 GG genotype had a significantly reduced risk of bronchitic symptoms with low-ozone exposure (OR= 0.53; 95% CI: 0.31-0.91), but this risk was not reduced in children living in high-ozone communities. These interactions were replicated in the study of Melén et al on a birth cohort of 4,089 children. They found that children with GSTP1 Val105Val/Val105Val genotypes were at increased risk of sensitization to any allergen when exposed to elevated levels of traffic NOx (OR = 2.4; 95% CI, 1.0-5.3). In accordance also with Winterton’s results, in children with TNF-308 GA/AA genotypes, the GSTP1-Nox interaction effect was even more pronounced. The study of Islam et al produced similar results, where the risk for asthma in a cohort of 1,610 school children was highest among GSTP1 Ile105 homozygotes who participated in 3 or more sports in the high ozone communities (HR = 6.15, 95% CI 2.2 to 7.4).

Another study by the same author examined the role of ozone and functional polymorphisms of antioxidant genes HMOX-1, CAT and MNSOD in asthma pathogenesis. Using a population-based cohort of 1,125 non-Hispanic and 586 Hispanic white children from California who were followed annually for 8 years to ascertain new-onset asthma, they found that HMOX-1 “short” alleles (<23 repeats) were associated with a reduced risk for new-onset asthma among non-Hispanic whites (HR = 0.64; 95% CI 0.41-0.99), with this protective effect being largest in children residing in low-ozone communities (HR = 0.48; 95% CI, 0.25-0.91) (interaction p value = 0.003). In 2009, Wenten and colleagues reported an epistasis between functional polymorphisms in the CAT/MPO loci, which differed according to the levels of oxidant-stress-producing air pollutants. In a population of 1,136 US elementary schoolchildren, risk of respiratory-related school absences was elevated for children with the CAT (G/G) and MPO (G/A or A/A) genes (RR = 1.35, 95% CI: 1.03 - 1.77), with this epistatic effect being most evident in communities exhibiting high ambient ozone levels (p-interaction = 0.03).

Outdoor aeroallergens - gene interaction in asthma
Airborne allergens are known to be among the major causes of allergic diseases and asthma. Almost 20 years ago, Blumenthal et al found that MHC-linked gene or genes (HLA-B7, SC31, DR2) controlled the IgE immune response to ragweed allergens and demonstrated an association with asthma in ragweed pollen-sensitive subjects. Human leukocyte antigen (HLA) class II genes were also shown to be associated with outbreaks of asthma caused by the inhalation of soybean dust. Soriano and colleagues studied the distribution of both HLA-DR and HLA-DQ in 78 soybean epidemic asthma patients and found that the risk of epidemic asthma was particularly associated with the DRB1*13 gene.

Pollen sensitivity appears to be a major contributor to the asthma phenotype. Blumenthal et al, on behalf of the Collaborative Study on the Genetics of Asthma, reported a genome-wide search for quantitative trait loci contributing to variation in seasonal pollen reactivity. Chromosomal regions that exhibited suggestive linkage (logarithm of the odds >1.18; P < .01) to seasonal pollen reactivity were identified on chromosomes 13q34, 20p12, and 21q21, but with extensive heterogeneity between African American, European American and Hispanic subjects. Especially for olive pollen allergy, Llanes and colleagues genotyped 7 polymorphisms of the IL13, IL4RA, IL5 and ADRB2 genes in 146 patients allergic to olive pollen with seasonal rhinitis/asthma and 50 control subjects, and found that the interaction between IL4RA SNPs I50V/Q551R was strongly associated with the asthma phenotype (OR = 2.48, p = 0.007). Di Somma et al reported an association of a TNF-308 allele (TNF2/DRB1*1104) with asthma, in a population recruited on the basis of allergy to the pollen of Parietaria.

Indoor air pollution - gene interaction in asthma
It is well known that poor indoor air quality increases the severity and frequency of respiratory symptoms experienced by people with asthma. The main indoor irritant associated with asthma is ETS. Although smoking and ETS may not be the primary factors in the changing prevalence of asthma, they are important contributors, with significant potential for interaction with genetic factors to influence disease propensity. In 2001 Wang et al conducted a study in 128 patients with asthma and 136 control individuals, investigating the interaction between the β(2)AR gene polymorphisms and cigarette smoking on risk of asthma. They found that compared with never-smoking Gly-16 homozygotes, those ever-smokers who...
are Arg-16 homozygotes had a significantly increased risk of asthma (OR = 7.81; 95% CI: 2.07 to 29.5). This association showed a clear dose-response relationship with the number of cigarettes smoked.

A number of studies have investigated possible interaction between genetic variability and in utero smoke (IUS) exposure on the development of asthma. Rouse and colleagues\textsuperscript{118}, in an experimental animal model of asthma, showed that IUS exposure alters the expression of genes in the lungs of adult mice and that this differential expression is reflected in differential respiratory and immune responses to nontobacco allergens. A clear interaction between the genetic background of children and maternal smoking in human asthma was first documented by Gilliland and colleagues\textsuperscript{119}. In a study of GSTM1 genotype and maternal smoking during pregnancy on 2,950 children, an increased risk of asthma and asthma-related phenotypes was conferred by GSTM1-null homozygosity in children only in those with a history of IUS exposure. These results were replicated in the study of Rogers and colleagues\textsuperscript{120}, in a different family-based cohort of children with asthma. Interaction between IUS exposure and a number of cytokine polymorphisms has also been reported in determining the risk of asthma and related phenotypes. Ramadas and colleagues\textsuperscript{121} found that in the first decade of life, interaction between a polymorphism of the IL-1 receptor antagonist (IL1RN) gene, rs2234678, and maternal smoking during pregnancy increased the risk for childhood asthma. Similar results were reported by Sadeghnejad and colleagues\textsuperscript{122} for the interactive effect of the IL-13 and tobacco smoke on persistent childhood wheezing and asthma, with the effect of maternal smoking during pregnancy on asthma being stronger in children with the common IL13 haplotype pair than in those without it (OR 5.58 and OR 1.29, respectively; p-interaction = 0.014).

As well as interaction between candidate asthma genes and tobacco smoke exposure, a number of groups have examined interaction between smoke exposure and novel asthma susceptibility genes identified through a hypothesis independent approach, such as by genome-wide linkage and association studies. One example of this was a report of an interaction between IUS and ADAM33 polymorphisms in the development of asthma and BHR\textsuperscript{123}, and similarly for PCDH1\textsuperscript{55} and the 17q21 susceptibility locus containing ORMDL3 and GSDML\textsuperscript{60}. The results of linkage studies stratified by ETS exposure suggest that this may be a common phenomenon\textsuperscript{124-129}.

A few studies have examined the interaction of indoor air pollutants other than ETS with genes in asthma susceptibility. The first such study, performed within the German follow-up of the European Community Respiratory Health Survey conducted by Werner et al\textsuperscript{126}, examined whether the effect of endotoxin concentration in settled house dust on asthma is modified by the presence of variation in the TLR4 gene. They found that in the non-carrier group of G299/I399 polymorphism in the TLR4 gene, the prevalence of asthma was significantly increased in the presence of elevated endotoxin levels in house dust (OR=6.24; 95% CI, 1.33-29.17 in the second tertile, compared with the lowest endotoxin tertile). The carriers of the polymorphisms (n = 55) showed a non-significant trend towards a lower risk of asthma. Zambelli-Weiner and colleagues\textsuperscript{131} examined endotoxin load, evaluated the role of the CD14 C-260T polymorphism and tested for interaction between this genotype and house dust endotoxin (HDE) exposure on atopic phenotypes. They found that the CD14-260TT genotype might protect against asthma for individuals with low HDE exposure (OR= 0.09; 95% CI, 0.03-0.24), but may be a risk factor for individuals with high HDE exposure (OR= 11.66; 95% CI, 1.03-131.7), suggesting a gene-environment interaction. A relationship between the CD14 gen and HDE exposure in relation to the development of an allergic phenotype was also observed in the study of Simpson et al\textsuperscript{132}, who found increasing HDE exposure to be associated with reduced risk of allergic sensitization (OR= 0.70; 95% CI, 0.55-0.89) but with increased risk of non-atopic wheeze (OR= 1.42; 95% CI, 1.01-1.99) in children with the CC genotype at -159 on the CD14 gene. This interaction between C-T polymorphism at position 159 in the promoter of CD14 and endotoxin exposure may be modulated through the effect of this genetic variant on the cytokine response of the inflammatory cells, as implicated in the study by Keskin et al\textsuperscript{133}. Lastly, in a very recent study from Taiwan the association between home dampness and genetic polymorphisms in childhood asthma were investigated. Tsai and colleagues\textsuperscript{134} investigated 3,810 schoolchildren in the Taiwan Children Health Study and they found that home dampness was a risk factor for asthma and wheeze among children, especially for those with the TNF-308 A allele.

**Indoor allergens - gene interaction in asthma**

Common indoor allergens also include house dust mite (HDM), cockroach, animal dander, and certain molds. A strong and consistent association is demonstrated between asthma and exposure to these allergens, and there is evidence to suggest that children sensitized to common...
indoor allergens are at a several-fold higher risk of asthma and allergy\textsuperscript{135}. This effect appears to depend both on the dose and type of allergen and on the underlying genetic susceptibility of the child.

HLA class II genetic polymorphism has been variably implicated in susceptibility to immune reaction to HDM allergens. The findings of two studies, one in vivo study reported by Kim \textit{et al}\textsuperscript{136} and one in a transgenic mouse model of airway inflammation conducted by Rajagopalan \textit{et al}\textsuperscript{137}, suggested that HLA-DRB1 alleles may play an important role in increasing a Th2-predominant immune response to HDM. Other studies, including those from Holloway \textit{et al}\textsuperscript{138}, Torres-Galván \textit{et al}\textsuperscript{139}, Moffatt \textit{et al}\textsuperscript{140} and one in a population of Greek children conducted by Parapanissiou and colleagues\textsuperscript{141}, failed to demonstrate any significant association between any HLA haplotype and house mite allergic bronchial asthma. Plasminogen activator inhibitor (PAI)-1 polymorphisms have also been studied in relation to HDM sensitive asthma. Pampuch \textit{et al}\textsuperscript{142} found in patients with newly diagnosed HDM-sensitive allergic asthma that the 4G/4G PAI-1 genotype was associated with an increased risk of allergic asthma (OR= 3.48; 95% CI, 1.54-7.89), BHR and raised levels of IgE. These results were replicated in the study of Kowal and colleagues\textsuperscript{143}, in a cohort of 372 patients with HDM-allergic asthma. Another gene implicated with HDM exposure in more than one study is transforming growth factor-β1 (TGFB1). In the study by Sharma \textit{et al}\textsuperscript{144}, HDM exposure modified the effect of TGFB1 SNPs on airway responsiveness and asthma exacerbations in children with asthma. In addition, in the study by Acevedo \textit{et al}\textsuperscript{145}, the C-509T polymorphism of the TGFB1 gene was associated with total IgE levels and specific IgE to \textit{D. pteronyssinus} in patients with asthma. Many other gene epitopes have been reported to be associated with HDM sensitivity and asthma related phenotypes in a single study, such as 148C and 13925C SNPs in the IL18 gene\textsuperscript{146}, allele 122 of the IL-9 gene\textsuperscript{147}, CA-repeat SNP in the NOS1 exon 29\textsuperscript{148}, IL10 SNPs\textsuperscript{149} and Butyrophilin-like 2 (BTNL2) gene polymorphisms\textsuperscript{150}, but replication and confirmation of these findings are still awaited.

Another aeroallergen source often implicated in severe asthma pathogenesis is the cockroach. The first genome-wide study screening for genes influencing, among other, cockroach-specific IgE responsiveness was published by Hizawa \textit{et al}\textsuperscript{151}, and was a part of the Collaborative Study on the Genetics of Asthma (CSGA). They found that specific IgE response toward cockroach showed evidence of linkage to chromosomes 5q31-q33 (P=0.0050) and 11q13 (P=0.017). The linkage revealed between the 5q31 region and the specific response to cockroach antigen, was attributed by Gao and colleagues\textsuperscript{152} to polymorphisms in the CD14 gene located in this region. The results of their study suggest that differentially expressed genes, particularly CD14 and genes in the IFN signalling pathway, may play an important role in the immune response to cockroach allergen. HLA class II genetic polymorphism has also been associated with sensitization to cockroach allergens. Both Donfack \textit{et al}\textsuperscript{153} and Kalpaklioğlu \textit{et al}\textsuperscript{154} reported an association between HLA class II allele of DRB1 and the expression of atopy in cockroach-sensitive patients.

Documentation on genetic risk factors underlying other indoor allergens, such as mold sensitivity and pet dander is limited. Two gene epitopes have been linked with protection from allergic sensitization to mold allergens; Weiss and colleagues\textsuperscript{155} found that variation in the integrin-β3 gene (ITGB3), an integrin gene within an asthma linkage peak on chromosome 17, was strongly associated with asthma susceptibility and protection from mold allergen sensitization. Knutsen \textit{et al}\textsuperscript{156} reported a decreased frequency of HLA-DQB1*03 allele in children with \textit{Alternaria}-sensitive moderate-severe asthma, suggesting that HLA-DQB1*03 may be protective against the development of mold-sensitive severe asthma. Wu and colleagues\textsuperscript{157} explored whether interactions between high fungal exposure and common genetic variants in genes for the two chitinases (CHIT1 and CHIA, enzymes that cleave chitin which is present in fungal cells), and the chitinase 3-like 1 gene, CHI3L1, are associated with asthma-related outcomes. They genotyped 395 subjects and their parents, as part of the Childhood Asthma Management Program, and found that high mold exposure significantly modified the effect of CHIT1 SNPs on severe asthma exacerbations. Vicencio and colleagues\textsuperscript{158} confirmed these findings in a study of 6 children who carried the diagnosis of severe asthma with fungal sensitization. All 6 patients were heterozygous for a 24-base pair duplication in the CHIT1 gene, which has been associated with decreased levels of circulating chitinases and susceptibility to fungal infection. Regarding exposure to pet dander, Bottema and colleagues\textsuperscript{159} analyzed polymorphisms in the IL13 and CD14 genes in 3,062 children from three Dutch cohort studies: Prevention and Incidence of Asthma and Mite Allergy (PIAMA), the Prevention of Asthma in Children (PREVASC), and the Child, Parent, Health, Focus on Lifestyle and Predisposition (KOALA), and tested for interaction with tobacco smoke and pet exposure at ages 1, 2, 4 and 8 years. They found that in CD14, the rs2569190-TT and
rs2569191-CC genotypes were associated with lower IgE and decreased risk of atopy at 4 and 8 years in children exposed to pets, with an opposite effect in non-exposed children. Based on the PIAMA cohort, Schutteelaar et al.\textsuperscript{60} reported their findings on the contribution of the filaggrin gene (FLG) mutations to the development of eczema and asthma. In this population, cat exposure enhanced the effect of a FLG mutation on the development of eczema, with a later development of asthma and hay fever.

**Viral and bacterial infections - gene interaction in asthma**

Viral and bacterial infections, especially in early infancy, have long been thought to be involved in asthma pathogenesis and morbidity, but direct evidence for a gene-environment interaction in this area are surprisingly few. Most studies on the role of respiratory infection in asthma inception provide indirect evidence on alteration of genetic factors controlling host immune responses to respiratory infection and the subsequent influence these may exert on asthma pathogenesis. The toll-like receptors (TLRs) are the principal receptors for microbial ligands and play a critical role in the innate immune response to infectious, both bacterial and viral\textsuperscript{161}. Variations in TLRs may therefore affect innate immune defences and contribute to asthma susceptibility\textsuperscript{162}. The first study to examine this possibility, published in 2002, focussed on genetic variation at the TLR4 locus. In this study Raby and colleagues, using a family-based approach, found no evidence to support a significant association between TLR4 polymorphisms and a diagnosis of asthma\textsuperscript{163}, and later studies have confirmed this lack of association of TLR4 polymorphisms with asthma\textsuperscript{164-166}. In contrast, the majority of genetic association studies published in the last decade have found positive associations of single nucleotide polymorphisms in the TLR2\textsuperscript{167-170}, TLR6\textsuperscript{171-173}, TLR7\textsuperscript{174}, TLR8\textsuperscript{174}, TLR9\textsuperscript{175} and TLR10 genes\textsuperscript{176,177} with asthma or atopy, although the number of studies is relatively small and the results not widely replicated. Custovic and colleagues\textsuperscript{178} recently tested the hypothesis that the same genetic variation in TLR2/-16934 is associated with reduced risk of developing allergic phenotypes among urban children attending day care in early life. Day care is consistently associated with increased prevalence of infections in early life, through which an effect of day-care attendance on the development of asthma has been suggested. In two population-based birth cohorts, from Manchester, UK and Tucson, USA, approximately 1,000 participants were recruited prenatally and followed prospectively for from 5 to 11 years. A significant interaction between day care attendance and TLR2/-16934 on the development of sensitization and atopic wheezing was demonstrated, providing indirect evidence that the protective effects of day care on the development of allergic disease may be mediated through microbial exposure\textsuperscript{178}. These results are in line with the findings of Hoffjan and colleagues\textsuperscript{179} in their study in the Childhood Onset of Asthma (COAST) cohort of children. They investigated the interactions between day care exposure in the first 6 months of life and genotypes for 72 polymorphisms at 45 candidate loci and their effects on cytokine response profiles and on the development of atopic phenotypes in the first year of life. They found 6 interactions (in 4 polymorphisms at 3 loci) with day care attendance that had an effect on early-life immune phenotypes. Furthermore, the interactive effects of day care and the FCER1B Glu237Gly genotype on IL-5 response profiles and the IL4RA Ile50Val genotype on IFN-γ responses was related to the increased number of viral infections in the children attending day care\textsuperscript{179}.

A number of studies have examined the associations between asthma and polymorphisms in the genes regulating innate and mucosal immune responses to bacterial microorganisms. Kirkbride and colleagues\textsuperscript{180} examined the role of mucin MUC7, a glycoprotein that plays a role in bacterial clearance, and reported a significantly lower frequency of the MUC7*5 allele in individuals with atopic asthma than in other groups. These results were replicated in the study of Rousseau and colleagues\textsuperscript{181}, who, examining lung function, showed that the haplotype carrying MUC7*5 was associated with higher FEV\textsubscript{1}, reduced age-related decline of FEV\textsubscript{1}, and also a reduced incidence of wheeze, suggesting a protective role of MUC7*5 on respiratory function and asthma. Another gene locus implicated in interactions with microbial pathogens is the nucleotide-binding oligomerization domain protein 1 (NOD1) gene, located on chromosome 7p14-p15, a region that has been linked with asthma-related traits\textsuperscript{182}. NOD1 is a cytosolic protein mediating innate and acquired immunity by recognizing bacterial molecules and it also synergistically increases TLR-induced responses\textsuperscript{183}. Weidinger et al.\textsuperscript{184} evaluated 11 NOD1 polymorphisms for associations with atopic phenotypes within a large German cohort (≈ 2,500), demonstrating significant associations for polymorphisms rs2907748, rs2907749, and rs2075822 with atopic eczema and asthma, which indicated a role of these NOD1 genetic variants in atopic susceptibility. Similar findings were reported by Reijmerink and colleagues\textsuperscript{87} in their study of 3,062 Dutch children from the birth cohorts PIAMA, PREVASC and KOALA, which also provided evidence of
interaction between several TLR-related pathway genes important in atopy and/or asthma development.

Endotoxins, part of the outer membrane of Gram negative (-) bacteria, are a potent inducer of airway inflammation. In the domestic environment, significant concentrations may be generated from pets kept indoors, carpeting and air conditioning. In the agricultural setting, endotoxins may be transported from the animal quarters into the home environment of farmers. Genetic variations, especially polymorphisms in innate-immune genes such as CD14, NOD1 or TLRs, are thought to be responsible for variations in the individual susceptibility to the effects of Gram (-) endotoxins. Gene-endotoxin interactions with respect to asthma, are discussed in other chapters (indoor air pollution - gene interaction and living conditions - gene interaction) of this review.

**Occupational exposure - gene interaction in asthma**

Occupational asthma is defined as asthma induced by exposure in the working environment to airborne dusts, vapours or fumes, in individuals with or without pre-existing asthma, and account for 9-15% of cases of asthma in adults of working age. Genetic variants that determine susceptibility to environmental exposures may contribute greatly to the development of occupational asthma in the setting of specific exposures occurring in the workplace. Isocyanates are one of the main causes of occupational asthma and there is growing evidence of interaction between exposure to this irritant and genetic factors. In 1994 by Bignon and colleagues reported on their study of whether HLA Class II genetic markers contribute to susceptibility or resistance to isocyanate-induced asthma (IAA) in exposed workers. They found that allele DQB1*0503 and the allelic combination DQB1*0201/0301 were associated with susceptibility to the disease, while allele DQB1*0501 and the DQA1*0101-DQB1*0501-DR1 haplotype conferred significant protection on exposed healthy control subjects. Since then, HLA II interactions with IAA have been evaluated by several studies. Balboni et al., Mapp et al. and Kim et al. confirmed the hypothesis that HLA-DQB1*0503 has a role in conferring susceptibility to IAA, while the studies of Rihs et al., Bernstein et al. and Beghé et al. did not demonstrate any significant interaction with HLA II. It has been also suggested that genetic susceptibility to IAA may be related to differences in anti-oxidant metabolism and several studies have focussed on the relevant gene markers. Piirilä et al. examined whether polymorphisms in the glutathione S-transferase (GST) genes modify allergic responses to diisocyanate exposure in 182 diisocyanate exposed workers. Lack of the GSTM1 gene (null genotype) was associated with a 1.89-fold risk of diisocyanate-induced asthma, while the combination of GSTM1 null and the GSTM3 AA genotype was strongly associated with lack of diisocyanate-specific IgE antibodies (OR=0.09, 95% CI 0.01 - 0.73). Wikman et al. extended the latter study to examine the possible role of N-acetyltransferase (NAT) genotypes in the development of diisocyanate-induced ill effects, and found an increase in asthma risk for the concurrent presence of the GSTM1 null genotype and either NAT1 (OR=4.53; 95% CI 1.76 to 11.6) or NAT2 (OR=3.12, 95% CI 1.11 to 8.78) slow acetylator genotypes, suggesting that the NAT genes play an important role in the inception of asthmatic reactions to occupational exposure to diisocyanates. The role of GST polymorphisms in IAA was supported by the study of Mapp et al., in which the homozygosity for the GSTP1 *Val allele was shown to confer protection against asthma and BHR. A recent genome-wide association study was conducted by Kim et al. in 84 patients with toluene diisocyanate (TDI)-induced asthma compared with 263 unexposed healthy control subjects. In this study a genetic polymorphism of catenin α 3, α-T catenin (CTNNA3) (rs1786929) was found significantly associated with the TDI-induced asthma phenotype (p=0.015), suggesting that polymorphisms of CTNNA3 may be determinants of susceptibility to TDI-induced asthma.

Gene-environment interactions in asthma produced by the western red cedar among workers in sawmill industries, were evaluated in the study of Horne et al. They assessed the distribution of DRB1 and DQB1 HLA class II alleles and DRB1-DQB1 haplotypes in 56 Caucasian patients with proven red cedar asthma and 63 healthy Caucasian control subjects exposed to red cedar dust. An increased risk for asthma was observed in HLA DQB1*0603 (OR= 2.9, p=0.05) and DQB1*0302 (OR= 4.9, p=0.02) genotypes, while a lower risk was found for DQB1*0501 allele (OR= 0.3, p=0.01). HLA Class II genetic markers were also found to be associated with sensitization to organic acid anhydrides, a low molecular weight industrial chemical. Young et al. found a significant excess of HLAII-DR3 in the cases with specific IgE to acid anhydrides compared with control subjects (50% versus 14%, OR = 6, p = 0.05), while Jones et al. reported that another allele, HLAII-DQB1 (*)0501, confers susceptibility to development of IgE-associated allergy to acid anhydrides (OR= 3.0; 95% CI 1.2 - 7.4). These findings suggest MHC II proteins may be an important determinant of the specificity of the IgE response to inhaled irritants such as acid anhydrides.
Living conditions - gene interaction in asthma

Another field of interest for addressing interactions between genes and the environment is study of farming populations. Growing up and/or working on a farm appears to confer a protection against the development of asthma and atopy199,200. A possible explanation for this protective effect may be the exposure to bacterial products of animal origin such as endotoxins and lipopolysaccharide (LPS) in stables, dairies and homes201. Findings from genetic studies in farming and non-farming populations indicate that genetic variations in innate-immune genes such as those for CD14, NOD1 or TLRs may modulate this effect, depending on environmental conditions. Lauener et al202 from the Allergy and Endotoxin (ALEX) study team, a multicentre study conducted in Austria, Switzerland and Germany, showed that blood cells from farmers’ children express significantly higher amounts of CD14 (0.96 vs 0.43, p=0.0013), and TLR 2 (0.11 vs 0.04, p<0.0001) than those from children of non-farmers. Eder et al203 reported that those farmers’ children carrying a T allele in TLR2/-16934 were significantly less likely than children with genotype AA to have a diagnosis of asthma (3% vs 13%, p = .012), current asthma symptoms (3% vs 16%, p = .004), atopic sensitization (14% vs 27%, p = .023), and current hay fever symptoms (3% vs 14%, p = .01). This association between TLR2/-16934 and asthma among children of farmers was independent of atopy. In the same cohort, Eder et al203 also explored whether this previously observed inverse association between exposure to microbial products and asthma and allergies in childhood is modified by genetic variation in Caspase recruitment domain protein (CARD) 4, an intracellular pattern recognition receptor containing NOD1 protein. They found a strong protective effect of a farming environment on allergies in children homozygous for the T allele in CARD4/-21596, but not in children carrying the minor allele (C), giving indications of a modifying role of polymorphisms in CARD4/NOD1 to the protective effect of exposure to a farming environment.

In a study by Smit et al204 in a cohort of 1,901 young Danish farmers, the potential associations between SNPs in CD14 and atopy and new-onset asthma were tested. They found that the CD14/-260T allele was significantly associated with less atopy (OR = 0.39; 95% CI 0.21-0.72), while the CD14/-651T allele was positively associated with atopy (OR = 2.53; 95% CI 1.33-4.80). Bieli et al205 from the Prevention of allergy – Risk factors for Sensitization in children related to Farming and Anthroposophic Lifestyle (PARSIFAL) group, extended these results, reporting a significant interaction between genetic variation in CD14/-1721 and farm milk consumption. The protective effect of farm milk consumption on allergic diseases is stronger in children carrying the A allele in CD14/-1721 than in children homozygous for the G allele and this might be mediated through farm milk-induced up-regulated CD14 gene expression, but a recent genome-wide interaction analysis for asthma and atopy, reported by Ege et al206 from the GABRIELA study group, failed to replicate these results. The GABRIELA researchers investigated 500,000 genotyped SNPs and farm-related exposures in 1,708 children from 4 rural regions of Central Europe, and tested selectively for interactions between farm exposures and 7 SNPs that had emerged in a large meta-analysis of childhood asthma as genome-wide significant in interacting with farm exposures for asthma or atopy. They found that neither the asthma-associated SNPs nor SNPs previously reported as having interactions with asthma showed significant interactions in the GABRIELA cohort.

CONCLUSIONS

Increasing evidence is being accumulated in support of the concept that gene-environment interactions play a critical role in the pathogenesis of asthma. Combinations of genetic and environmental factors may explain a large part of the differences in the prevalence of asthma and related phenotypes between populations, although gene studies have provided little replicable evidence on such interactions. The interaction of HLAII-DQB1 alleles and the occupational exposure to isocyanates is among the most consistent of study findings93,186-188. Documentation is also consistent on the role of the genes involved in oxidative stress protection, mainly GSTs, in the susceptibility to asthma induced by tobacco related exposures and by air pollution104,106,119,120,126-128,206.

The study of gene-environment interactions is a relatively new field, with the number of studies increasing every year. As all study in its initial steps, that of interactions is not free from problems and limitations. The main problem in evaluating gene-environment interactions is derived from the highly complicated models that characterize complex diseases such as asthma and allergy. This involves an enormous number of genetic loci associated with the disease (genetic heterogeneity), the large number of environmental factors, the moderate effects of genetic variants, and aetiological heterogeneity99,207,208. For this reason, one of the main limitations of the studies conducted to date is the statistical power needed to
evaluate interaction, which can be achieved only in large studies. Most of the studies evaluated in this review had been conducted in small populations and did not have the power either to detect interactions conclusively or to reasonably exclude false positive results. Limited sample size is also a cause of non-identification of true correlations (false negatives or type II error). This is a particular problem for the replication of positive results. Another problem is the publication bias. Publication bias occurs when negative results tend not to be considered for publication by researchers and publishers. In a new field, the publication bias is even a greater problem because the prior probability to detect interactions is generally low and also because of the increased interest for publishing positive reports in a relatively new field.

Another essential issue in studies of gene-environment relations in asthma is how to define interactions. The term interaction has a different interpretation for biologists and statisticians. Biological interpretation of interaction describes the co-participation of two factors in the same causal mechanism. In the statistical sense, interaction between two factors represents the deviation from a mathematical model for joint effects. Therefore, although the statistical interaction is commonly used to describe a relationship between two factors, it must not be confused with biological interaction. For this reason, it is considered important to limit the assessment of gene-environment interactions to those interactions that have biological plausibility. In addition, the accurate assessment of environmental exposure in terms of dose and timing across the life course constitutes a further limitation.

Despite these problems and limitations, some advances have been achieved in the understanding of gene-environment interactions in asthma in recent years. These advances have already helped to unravel the biology of certain exposures, such as cigarette smoke, air pollution, endotoxin and occupational hazards, in relation to specific genotypes. A better understanding of gene-environment interactions in asthma is inseparable from a better understanding of the mechanisms by which environmental factors increase the risk for asthma or protect against it. The large number of potential interactions between genes and environmental exposures and their complexity clearly demonstrates the need for larger, well designed multi-centre studies to be conducted, in order to obtain better and more reliable results.

REFERENCES


24. Tovey ER, Almqvist C, Li Q, Crisafulli D, Marks GB. Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort. J Allergy Clin Immunol 2008;122:114-118.


27. D’Amato G. Environmental urban factors (air pollution and allergens) and the rising trends in allergic respiratory diseases. Allergy 2002;57(Suppl)72:30-33.


82. Leung TF, Sy HY, Ng MC et al. Asthma and atopy are associated with chromosome 17q21 markers in Chinese children. Allergy 2009;64:621-628.


84. Wu H, Romieu I, Sierra-Monge J et al. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. Allergy 2009;64:629-635.


91. Himes BE, Hunninghake GM, Baurley JW et al. Genome-wide


96


135. Kim YK, Oh HB, Oh SY et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of asthma and allergy. Clin Exp Allergy 2001;31:110-115.


146. Wang TN, Chen WY, Huang YF et al. The synergistic effects of the IL-9 gene and environmental exposures on asthmatic Taiwanese families as determined by the transmission/disequilibrium test. Int J Immunogenet 2006;33:105-110.


154. Kim YK, Oh HB, Oh SY et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite Dermatophagoides pteronyssinus. Clin Exp Allergy 2001;31:110-115.


159. Kim YK, Oh HB, Oh SY et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite Dermatophagoides pteronyssinus. Clin Exp Allergy 2001;31:110-115.


161. Kim YK, Oh HB, Oh SY et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite Dermatophagoides pteronyssinus. Clin Exp Allergy 2001;31:110-115.


163. Kim YK, Oh HB, Oh SY et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite Dermatophagoides pteronyssinus. Clin Exp Allergy 2001;31:110-115.


165. Kim YK, Oh HB, Oh SY et al. HLA-DRB1*07 may have a susceptibility and DRB1*04 a protective effect upon the development of a sensitization to house dust mite Dermatophagoides pteronyssinus. Clin Exp Allergy 2001;31:110-115.
single nucleotide polymorphisms, HLA-DR and HLA-DQ in children with Alternaria-sensitive moderate-severe asthma. Clin Mol Allergy 2010;8:5.


189. Rihs HP, Barbalho-Krolls T, Huber H, Baur X. No evidence for the influence of HLA class II in alleles in isocyanate-induced...


