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

Eleftherios Zervas[1], John W. Holloway[2-3], Valia Kehagia[2]

[1]7th Pneumonology Dept. and Asthma Centre, SOTIRIA" Hospital for Chest Diseases, Athens, Greece
[2]Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, United Kingdom
[3]Human Development and Health, Faculty of Medicine University of Southampton, Southampton General Hospital, Southampton, United Kingdom

Key words:
- asthma
- genes
- environment
- interaction
- review

Correspondence:
Eleftherios Zervas,
Pneumonologist, 7th Pneumonology Dept. and Asthma Centre, "SOTIRIA" Hospital for Chest Diseases, Mesogion Ave. 152, 11527 Athens, Greece
Tel: 2107763306, Fax: 2107781911,
E-mail: lefzervas@ath.forthnet.gr lefzervas@yahoo.gr

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 mechanisms[1,2]. This idea has resulted from the large number of asthmatic phenotypes described and the numerous risk factors and immunological mechanisms that have been identified[3-5].

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 system[6]. Through years of research and effort, hundreds of genetic and environmental factors have been shown to be associated with asthma[7,8]. 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 environment[9]. Pollution, both outdoor and indoor, is thought to affect the development, persistence and exacerbation of asthma[10]. The most abundant compounds among outdoor pollutants are inhalable particulate matter, ozone, nitrogen oxides (NOx), sulphur dioxide (SO_2) and diesel exhaust particles[11]. Many studies have documented the role of these pollutants in asthma exacerbations[11,12] and, to a lesser extent, in the development of asthma[13,14]. 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[15]. The effect of ETS on the development and severity of asthma is strong and consistent in both *in vitro* and *in vivo* studies[13,16-18].

Early life sensitization to common aeroallergens has been also associated with the risk of asthma development[19-21]. Sensitization to house dust mite (HDM) is the most frequently evaluated allergen[22-24]. Other allergenic factors associated with asthma are sensitization to pet allergens[19,25,26], pollen allergens[13,27,28] and IgE specific to fungal species present in mould[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[32-34]. Other environmental factors associated with asthma phenotypes are cigarette smoking[35,36], obesity[37] and diet[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[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)[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[42-44]. The first study to link a specific genetic locus, namely chromosome 11q13, to asthma was published in 1999[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[46], using short tandem repeat polymorphisms (STRPs) and single-nucleotide polymorphisms (SNPs). Linkage studies in asthma have resulted in more than 20 possible regions[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 19q[47]. 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[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[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[50]. Other genes identified with positional cloning for allergic disease phenotypes include DPP10 and IL1RN on chromosome 2q[51,52], PCDH1, CYFIP2 and SPINK5 on chromosome 5q[53-55], HLAG on chromosome 6p[56], GPRA on chromosome 7p[57], PHF11 on chromosome 13q[58], PTGDR on chromosome 14q[59] and PLUAR on chromosome 18[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[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[62]. Genes that have been extensively replicated include the β2 adrenergic receptor gene ADRB2[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[66-69]; and genes involved in the cellular responses that characterize atopic disease, such as IL13 and its receptor and the FCER1B gene[72-75].

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[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[77]. The first GWAS for asthma discovered a novel associated locus on chromosome 17q21 encompassing the genes ORMDL3, GSDMB and ZPBP2[78]. 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[79-84]. Other genes identified in more than one GWAS are IL1RL1 on chromosome 2[81,85-88], RAD50 on chromosome 5q31[89,90], PDE4D gene on chromosome 5q12[91,92], HLA- DR/DQ on chromosome 6[89,93] and IL33 on chromosome 9[85,86,92]. Additional novel susceptibility genes identified in a single study include DENND1BI[94], SMAD3[86], CHI3L1[95], IL2RB[86] and ATPAF1[96]. 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[97]. 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[8]. 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[98]. The literature on gene-environment interactions in asthma has grown considerably during the last few years[99-102], butdespite 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[99]. 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*[103] 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)[103]. 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*[104]. 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)[104]. 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*[105], 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 NO_2 for asthma prevalence ($p = 0.02$) and new-onset asthma ($p = 0.04$).

Lee and colleagues[106] 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 NO_x and SO_2 (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[106]. In a subsequent study by Lee *et al* in the same cohort of children with asthma[107], 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*[108] on a birth cohort of 4,089 children. They found that children with GSTP1 Ile105Val/Val105Val genotypes were at increased risk of sensitization to any allergen when exposed to elevated levels of traffic NOx (OD = 2.4; 95% CI, 1.0-5.3). In accordance also with Winterton's results[103], in children with TNF-308 GA/AA genotypes, the GSTP1-NOx interaction effect was even more pronounced. The study of Islam *et al*[109] 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[110] 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)[110]. 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[111]. 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 been 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*[112] 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[113] 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*[114], 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[115] 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*[116] 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*[117] 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[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[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[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[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[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[123], and similarly for PCDH1[55] and the 17q21 susceptibility locus containing ORMDL3 and GSDML[80]. The results of linkage studies stratified by ETS exposure suggest that this may be a common phenomenon[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*[130], 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[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 gene and HDE exposure in relation to the development of an allergic phenotype was also observed in the study of Simpson *et al*[132], who found increasing HDE exposure to be associated with reduced risk ofallergic 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*[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[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[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 *et al*[136] and one in a transgenic mouse model of airway inflammation conducted by Rajagopalan *et al*[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 *et al*[138], Torres-Galván *et al*[139], Moffatt *et al*[140] and one in a population of Greek children conducted by Parapanissiou and colleagues[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 *et al*[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[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 *et al*[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 *et al*[145], the C-509T polymorphism of the TGFB1 gene was associated with total IgE levels and specific IgE to *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[146], allele 122 of the IL-9 gene[147], CA-repeat SNP in the NOS1 exon 29[148], IL10 SNPs[149] and Butyrophilin-like 2 (BTNL2) gene polymorphisms[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 *et al*[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[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 *et al*[153] and Kalpaklioğlu *et al*[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[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 *et al*[156] reported a decreased frequency of HLA-DQB1*03 allele in children with *Alternaria*-sensitive moderate-severe asthma, suggesting that HLA-DQB1*03 may be protective against the development of mold-sensitive severe asthma. Wu and colleagues[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[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[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, Schuttelaar *et al*[160] 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[161]. Variations in TLRs may therefore affect innate immune defences and contribute to asthma susceptibility[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[163], and later studies have confirmed this lack of association of TLR4 polymorphisms with asthma[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[167-170], TLR6[171-173], TLR7[174], TLR8[174], TLR9[175] and TLR10 genes[176,177] with asthma or atopy, although the number of studies is relatively small and the results not widely replicated. Custovic and colleagues[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[178]. These results are in line with the findings of Hoffjan and colleagues[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[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[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[181], who, examining lung function, showed that the haplotype carrying MUC7*5 was associated with higher FEV_1, reduced age-related decline of FEV_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[182]. NOD1 is a cytosolic protein mediating innate and acquired immunity by recognizing bacterial molecules and it also synergistically increases TLR-induced responses[183]. Weidinger *et al*[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[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[185]. 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[40]. 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[186] 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*[187], Mapp *et al*[188] and Kim *et al*[93] confirmed the hypothesis that HLA-DQB1*0503 has a role in conferring susceptibility to IAA, while the studies of Rihs *et al*[189], Bernstein *et al*[190] and Beghé *et al*[191] 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*[192] 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*[193] 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*[194], 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*[195] 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*[196]. 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*[197] 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[198] 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 atopy[199,200]. 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 homes[201]. 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 al*[202] 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 al*[168] 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 al*[203] 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 al*[170] 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 al*[204] 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 al[205] 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 findings[93,186-188]. 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 pollution[104,106,119,120,126-128,206].

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 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 heterogeneity[99,207,208]. 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[99,100]. 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 gene-environment interaction studies, 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[209].

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[210]. 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[211].

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

1. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention: NHLBI/WHO workshop Report: National Institutes of Health, National Heart, Lung and Blood Institute. NIH publication No. 02-3659; updated 2009. Available on www.ginasthma.org.
2. Borish L, Culp JA. Asthma: a syndrome composed of heterogeneous diseases. Ann Allergy Asthma Immunol 2008;101:1-8.
3. Wenzel SE, Schwartz LB, Langmack EL et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001-1008.
4. Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet 2006;368:804-813.
5. Samitas K, Vittorakis S, Chorianopoulos D, Oikonomidou E, Gaga M. Immunological Mechanisms in the Lung. Pneumon 2007; 20:274-278
6. Wu X, Jin L, Xiong M. Mutual information for testing gene-environment interaction. PLo S One 2009;4:e4578.
7. Ho SM. Environmental epigenetics of asthma: an update. J Allergy Clin Immunol 2010;126:453-465.
8. Holloway JW, Yang IA, Holgate ST. Genetics of allergic disease. J Allergy Clin Immunol 2010;125:S81-S94.
9. Pruss-Ustun A, Corvalan C. How much disease burden can be prevented by environmental interventions? Epidemiology 2007;18:167-178.
10. D'Amato G, Liccardi G, D'Amato M, Holgate S. Environmental risk factors and allergic bronchial asthma. Clin Exp Allergy 2005;35:1113-1124.
11. Umstead T, Phelps D, Floros J. Ground level ozone is both a regional and global problem. Pneumon 2011, 24:20-23
12. Kunzli N, Kaiser R, Medina S et al. Public-health impact of outdoor and traffic-related air pollution: a European assessment. Lancet 2000;356:795-801.
13. Gilmour MI, Jaakkola MS, London SJ, Nel AE, Rogers CA. How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. Environ Health Perspect 2006;114:627-633.
14. Jacquemin B, Sunyer J, Forsberg B et al. Association between modelled traffic-related air pollution and asthma score in the ECRHS. Eur Respir J 2009;34:834-842.
15. Bernstein JA, Alexis N, Bacchus H et al. The health effects of non-industrial indoor air pollution. J Allergy Clin Immunol 2008;121:585-591.
16. Baena-Cagnani CE, Gomez RM, Baena-Cagnani R, Canonica GW. Impact of environmental tobacco smoke and active tobacco smoking on the development and outcomes of asthma and rhinitis. Curr Opin Allergy Clin Immunol 2009;9:136-140.
17. Sturm JJ, Yeatts K, Loomis D. Effects of tobacco smoke exposure on asthma prevalence and medical care use in North Carolina middle school children. Am J Public Health 2004;94:308-313.
18. Yu M, Zheng X, Peake J, Joad JP, Pinkerton KE. Perinatal environmental tobacco smoke exposure alters the immune response and airway innervation in infant primates. J Allergy

Clin Immunol 2008;122:640-647.
19. Bush RK. Indoor allergens, environmental avoidance, and allergic respiratory disease. Allergy Asthma Proc 2008;29:575-579.
20. Illi S, von ME, Lau S et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet 2006;368:763-770.
21. Sporik R, Squillace SP, Ingram JM et al. Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools. Thorax 1999;54:675-680.
22. Custovic A, Taggart SC, Francis HC, Chapman MD, Woodcock A. Exposure to house dust mite allergens and the clinical activity of asthma. J Allergy Clin Immunol 1996;98:64-72.
23. Ohshima Y, Yamada A, Hiraoka M et al. Early sensitization to house dust mite is a major risk factor for subsequent development of bronchial asthma in Japanese infants with atopic dermatitis: results of a 4-year followup study. Ann Allergy Asthma Immunol 2002;89:265-270.
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.
25. Bertelsen RJ, Carlsen KC, Carlsen KH et al. Childhood asthma and early life exposure to indoor allergens, endotoxin and beta(1,3)-glucans. Clin Exp Allergy 2010;40:307-316.
26. Brussee JE, Smit HA, van Strien RT et al. Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. J Allergy Clin Immunol 2005;115:946-952.
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.
28. Kihlstrom A, Lilja G, Pershagen G, Hedlin G. Exposure to birch pollen in infancy and development of atopic disease in childhood. J Allergy Clin Immunol 2002;110:78-84.
29. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. Eur Respir J 2006;27:615-626.
30. Zureik M, Neukirch C, Leynaert B et al. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. BMJ 2002;325:411-414.
31. Perzanowski MS, Sporik R, Squillace SP et al. Association of sensitization to Alternaria allergens with asthma among school-age children. J Allergy Clin Immunol 1998;101:626-632.
32. Rosenthal LA, Avila PC, Heymann PW et al. Viral respiratory tract infections and asthma: the course ahead. J Allergy Clin Immunol 2010;125:1212-1217.
33. Jackson DJ, Gangnon RE, Evans MD et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am.J.Respir.Crit Care Med 2008;178:667-672.
34. Kusel MM, de Klerk NH, Kebadze T et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007;119:1105-1110.
35. Hylkema MN, Sterk PJ, de Boer WI, Postma DS. Tobacco use in relation to COPD and asthma. Eur Respir J 2007;29:438-445.
36. Rovina N, Gratziou C. Asthma and smoking. Pneumon 2006; 19:202-215.
37. Lugogo NL, Kraft M, Dixon AE. Does obesity produce a distinct asthma phenotype? J Appl Physiol 2010;108:729-734.
38. Arvaniti F, Priftis KN, Panagiotakos DB. Dietary habits and asthma: a review. Allergy Asthma Proc 2010;31:e1-10.
39. Allen S, Britton JR, Leonardi-Bee JA. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. Thorax 2009;64:610-619.
40. Zervas E, Gaga M. Work-related and occupational asthma. In: Palange P., Simonds A., eds. ERS Handbook, Respiratory Medicine.: ERS; 2010:268-272.
41. Tarlo SM, Balmes J, Balkissoon R et al. Diagnosis and management of work-related asthma: American College Of Chest Physicians Consensus Statement. Chest 2008;134:1S-41S.
42. Koppelman GH, Los H, Postma DS. Genetic and environment in asthma: the answer of twin studies. Eur Respir J 1999;13:2-4.
43. Nieminen MM, Kaprio J, Koskenvuo M. A population-based study of bronchial asthma in adult twin pairs. Chest 1991;100:70-75.
44. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. Am Rev Respir Dis 1990;142:1351-1358.
45. Deichmann KA, Starke B, Schlenther S et al. Linkage and association studies of atopy and the chromosome 11q13 region. J Med Genet 1999;36:379-382.
46. Dawn TM, Barrett JH. Genetic linkage studies. Lancet 2005; 366:1036-1044.
47. Wills-Karp M, Ewart SL. Time to draw breath: asthma-susceptibility genes are identified. Nat Rev Genet 2004;5:376-387.
48. Bouzigon E, Forabosco P, Koppelman GH et al. Meta-analysis of 20 genome-wide linkage studies evidenced new regions linked to asthma and atopy. Eur J Hum Genet 2010;18:700-706.
49. Howard TD, Meyers DA, Bleecker ER. Mapping susceptibility genes for allergic diseases. Chest 2003;123:363S-368S.
50. Van EP, Little RD, Dupuis J et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 2002;418:426-430.
51. Allen M, Heinzmann A, Noguchi E et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nat Genet 2003;35:258-263.
52. Gohlke H, Illig T, Bahnweg M et al. Association of the interleukin-1 receptor antagonist gene with asthma. Am J Respir Crit Care Med 2004;169:1217-1223.
53. Noguchi E, Yokouchi Y, Zhang J et al. Positional identification of an asthma susceptibility gene on human chromosome 5q33. Am J Respir Crit Care Med 2005;172:183-188.
54. Walley AJ, Chavanas S, Moffatt MF et al. Gene polymorphism in Netherton and common atopic disease. Nat Genet 2001; 29:175-178.
55. Koppelman GH, Meyers DA, Howard TD et al. Identification of PCDH1 as a novel susceptibility gene for bronchial hyperresponsiveness. Am J Respir Crit Care Med 2009;180:929-935.
56. Nicolae D, Cox NJ, Lester LA et al. Fine mapping and positional candidate studies identify HLA-G as an asthma susceptibility gene on chromosome 6p21. Am J Hum Genet 2005;76:349-357.

57. Laitinen T, Polvi A, Rydman P et al. Characterization of a common susceptibility locus for asthma-related traits. Science 2004;304:300-304.
58. Zhang Y, Leaves NI, Anderson GG et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. Nat Genet 2003;34:181-186.
59. Oguma T, Palmer LJ, Birben E et al. Role of prostanoid DP receptor variants in susceptibility to asthma. N Engl J Med 2004;351:1752-1763.
60. Barton SJ, Koppelman GH, Vonk JM et al. PLAUR polymorphisms are associated with asthma, PLAUR levels, and lung function decline. J Allergy Clin Immunol 2009;123:1391-1400.
61. Tabor HK, Risch NJ, Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev Genet 2002;3:391-397.
62. March ME, Sleiman PM, Hakonarson H. The genetics of asthma and allergic disorders. Discov Med 2011;11:35-45.
63. Zhang G, Hayden CM, Khoo SK et al. Beta2-Adrenoceptor polymorphisms and asthma phenotypes: interactions with passive smoking. Eur Respir J 2007;30:48-55.
64. Hawkins GA, Tantisira K, Meyers DA et al. Sequence, haplotype, and association analysis of ADR beta2 in a multiethnic asthma case-control study. Am J Respir Crit Care Med 2006;174:1101-1109.
65. Guerra S, Graves PE, Morgan WJ et al. Relation of beta2-adrenoceptor polymorphisms at codons 16 and 27 to persistence of asthma symptoms after the onset of puberty. Chest 2005;128:609-617.
66. Amirzargar AA, Movahedi M, Rezaei N et al. Polymorphisms in IL4 and iLARA confer susceptibility to asthma. J Investig Allergol Clin Immunol 2009;19:433-438.
67. Pykalainen M, Kinos R, Valkonen S et al. Association analysis of common variants of STAT6, GATA3, and STAT4 to asthma and high serum IgE phenotypes. J Allergy Clin Immunol 2005;115:80-87.
68. Beghe B, Barton S, Rorke S et al. Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a Caucasian population. Clin Exp Allergy 2003;33:1111-1117.
69. Nakao F, Ihara K, Kusuhara K et al. Association of IFN-gamma and IFN regulatory factor 1 polymorphisms with childhood atopic asthma. J Allergy Clin Immunol 2001;107:499-504.
70. Sandford AJ, Chagani T, Zhu S et al. Polymorphisms in the IL4, IL4RA, and FCERIB genes and asthma severity. J Allergy Clin Immunol 2000;106:135-140.
71. Zhu S, Chan-Yeung M, Becker AB et al. Polymorphisms of the IL-4, TNF-alpha, and Fcepsilon RIbeta genes and the risk of allergic disorders in at-risk infants. Am J Respir Crit Care Med 2000;161:1655-1659.
72. Beghe B, Hall IP, Parker SG et al. Polymorphisms in IL13 pathway genes in asthma and chronic obstructive pulmonary disease. Allergy 2010;65:474-481.
73. Black S, Teixeira AS, Loh AX et al. Contribution of functional variation in the IL13 gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort. Allergy 2009;64:1172-1178.
74. Hunninghake GM, Soto-Quiros ME, Avila L et al. Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood. J Allergy Clin Immunol 2007;120:84-90.
75. Hizawa N, Maeda Y, Konno S et al. Genetic polymorphisms at FCER1B and PAI-1 and asthma susceptibility. Clin Exp Allergy 2006;36:872-876.
76. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. Nat Rev Genet 2005;6:95-108.
77. Akhabir L, Sandford AJ. Genome-wide Association Studies for Discovery of Genes Involved in Asthma. Respirology 2011; 16:396-406.
78. Moffatt MF, Kabesch M, Liang L et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature 2007;448:470-473.
79. Bisgaard H, Bonnelykke K, Sleiman PM et al. Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. Am J Respir Crit Care Med 2009;179:179-185.
80. Bouzigon E, Corda E, Aschard H et al. Effect of 17q21 variants and smoking exposure in early-onset asthma. N Engl J Med 2008;359:1985-1994.
81. Ferreira MA, McRae AF, Medland SE et al. Association between ORMDL3, IL1RL1 and a deletion on chromosome 17q21 with asthma risk in Australia. Eur J Hum Genet 2011;19:458-464.
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.
83. Madore AM, Tremblay K, Hudson TJ, Laprise C. Replication of an association between 17q21 SNPs and asthma in a French-Canadian familial collection. Hum Genet 2008;123:93-95.
84. Wu H, Romieu I, Sienra-Monge JJ et al. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. Allergy 2009;64:629-635.
85. Gudbjartsson DF, Bjornsdottir US, Halapi E et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009;41:342-347.
86. Moffatt MF, Gut IG, Demenais F et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-1221.
87. Reijmerink NE, Bottema RW, Kerkhof M et al. TLR-related pathway analysis: novel gene-gene interactions in the development of asthma and atopy. Allergy 2010;65:199-207.
88. Wu H, Romieu I, Shi M et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. J Allergy Clin Immunol 2010;125:321-327.
89. Li X, Howard TD, Zheng SL et al. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. J Allergy Clin Immunol 2010;125:328-335.
90. Weidinger S, Gieger C, Rodriguez E et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS Genet 2008;4:e1000166.
91. Himes BE, Hunninghake GM, Baurley JW et al. Genome-wide

association analysis identifies PDE4D as an asthma-susceptibility gene. Am J Hum Genet 2009;84:581-593.
92. Melen E, Himes BE, Brehm JM et al. Analyses of shared genetic factors between asthma and obesity in children. J Allergy Clin Immunol 2010;126:631-637.
93. Kim SH, Oh HB, Lee KW et al. HLA DRB1*15-DPB1*05 haplotype: a susceptible gene marker for isocyanate-induced occupational asthma? Allergy 2006;61:891-894.
94. Sleiman PM, Flory J, Imielinski M et al. Variants of DENND1B associated with asthma in children. N Engl J Med 2010;362:36-44.
95. Ober C, Tan Z, Sun Y et al. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med 2008;358:1682-1691.
96. Schauberger EM, Ewart SL, Arshad SH et al. Identification of ATPAF1 as a novel candidate gene for asthma in children. J Allergy Clin Immunol 2011; 128:753-760.
97. Mathias RA, Grant AV, Rafaels N et al. A genome-wide association study on African-ancestry populations for asthma. J Allergy Clin Immunol 2010;125:336-346.
98. Simpson A, Custovic A. Prevention of allergic sensitization by environmental control. Curr Allergy Asthma Rep 2009;9:363-369.
99. Castro-Giner F, Kauffmann F, de CR, Kogevinas M. Gene-environment interactions in asthma. Occup Environ Med 2006; 63:776-786.
100. London SJ, Romieu I. Gene by environment interaction in asthma. Annu Rev Public Health 2009;30:55-80.
101. von ME. Gene-environment interactions in asthma. J Allergy Clin Immunol 2009;123:3-11.
102. Steiropoulos P, Ito K. Genes and severe asthma. Pneumon 2011;24:321-329.
103. Winterton DL, Kaufman J, Keener CV et al. Genetic polymorphisms as biomarkers of sensitivity to inhaled sulfur dioxide in subjects with asthma. Ann Allergy Asthma Immunol 2001;86:232-238.
104. David GL, Romieu I, Sienra-Monge JJ et al. Nicotinamide adenine dinucleotide (phosphate) reduced:quinone oxidoreductase and glutathione S-transferase M1 polymorphisms and childhood asthma. Am J Respir Crit Care Med 2003;168:1199-1204.
105. Castro-Giner F, Kunzli N, Jacquemin B et al. Traffic-related air pollution, oxidative stress genes, and asthma (ECHRS). Environ Health Perspect 2009;117:1919-1924.
106. Lee YL, Lin YC, Lee YC et al. Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. Clin Exp Allergy 2004;34:1707-1713.
107. Lee YL, McConnell R, Berhane K, Gilliland FD. Ambient ozone modifies the effect of tumor necrosis factor G-308A on bronchitic symptoms among children with asthma. Allergy 2009;64:1342-1348.
108. Melen E, Nyberg F, Lindgren CM et al. Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. Environ Health Perspect 2008;116:1077-1084.
109. Islam T, Berhane K, McConnell R et al. Glutathione-S-transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. Thorax 2009;64:197-202.
110. Islam T, McConnell R, Gauderman WJ et al. Ozone, oxidant defense genes, and risk of asthma during adolescence. Am J Respir Crit Care Med 2008;177:388-395.
111. Wenten M, Gauderman WJ, Berhane K et al. Functional variants in the catalase and myeloperoxidase genes, ambient air pollution, and respiratory-related school absences: an example of epistasis in gene-environment interactions. Am J Epidemiol 2009;170:1494-1501.
112. Blumenthal M, Marcus-Bagley D, Awdeh Z et al. HLA-DR2, [HLA-B7, SC31, DR2], and [HLA-B8, SC01, DR3] haplotypes distinguish subjects with asthma from those with rhinitis only in ragweed pollen allergy. J Immunol 1992;148:411-416.
113. Soriano JB, Ercilla G, Sunyer J et al. HLA class II genes in soybean epidemic asthma patients. Am J Respir Crit Care Med 1997;156:1394-1398.
114. Blumenthal MN, Langefeld CD, Barnes KC et al. A genome-wide search for quantitative trait loci contributing to variation in seasonal pollen reactivity. J Allergy Clin Immunol 2006;117:79-85.
115. Llanes E, Quiralte J, Lopez E et al. Analysis of polymorphisms in olive pollen allergy: IL13, IL4RA, IL5 and ADRB2 genes. Int Arch Allergy Immunol 2009;148:228-238.
116. Di SC, Charron D, Deichmann K, Buono C, Ruffilli A. Atopic asthma and TNF-308 alleles: linkage disequilibrium and association analyses. Hum Immunol 2003;64:359-365.
117. Wang Z, Chen C, Niu T et al. Association of asthma with beta(2)-adrenergic receptor gene polymorphism and cigarette smoking. Am J Respir Crit Care Med 2001;163:1404-1409.
118. Rouse RL, Boudreaux MJ, Penn AL. In utero environmental tobacco smoke exposure alters gene expression in lungs of adult BALB/c mice. Environ Health Perspect 2007;115:1757-1766.
119. Gilliland FD, Li YF, Dubeau L et al. Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. Am J Respir Crit Care Med 2002;166:457-463.
120. Rogers AJ, Brasch-Andersen C, Ionita-Laza I et al. The interaction of glutathione S-transferase M1-null variants with tobacco smoke exposure and the development of childhood asthma. Clin Exp Allergy 2009;39:1721-1729.
121. Ramadas RA, Sadeghnejad A, Karmaus W et al. Interleukin-1R antagonist gene and pre-natal smoke exposure are associated with childhood asthma. Eur Respir J 2007;29:502-508.
122. Sadeghnejad A, Karmaus W, Arshad SH et al. IL13 gene polymorphisms modify the effect of exposure to tobacco smoke on persistent wheeze and asthma in childhood, a longitudinal study. Respir Res 2008;9:2.
123. Reijmerink NE, Kerkhof M, Koppelman GH et al. Smoke exposure interacts with ADAM33 polymorphisms in the development of lung function and hyperresponsiveness. Allergy 2009;64:898-904.
124. Jaakkola JJ, Nafstad P, Magnus P. Environmental tobacco smoke, parental atopy, and childhood asthma. Environ Health Perspect

2001;109:579-582.
125. Colilla S, Nicolae D, Pluzhnikov A et al. Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. J Allergy Clin Immunol 2003;111:840-846.
126. Palmer CN, Doney AS, Lee SP et al. Glutathione S-transferase M1 and P1 genotype, passive smoking, and peak expiratory flow in asthma. Pediatrics 2006;118:710-716.
127. Panasevich S, Lindgren C, Kere J et al. Interaction between early maternal smoking and variants in TNF and GSTP1 in childhood wheezing. Clin Exp Allergy 2010;40:458-467.
128. Li YF, Tseng PJ, Lin CC et al. NAD(P)H: Quinone oxidoreductase 1, glutathione S-transferase M1, environmental tobacco smoke exposure, and childhood asthma. Mutat Res 2009;678:53-58.
129. Meyers DA, Postma DS, Stine OC et al. Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. J Allergy Clin Immunol 2005;115:1169-1175.
130. Werner M, Topp R, Wimmer K et al. TLR4 gene variants modify endotoxin effects on asthma. J Allergy Clin Immunol 2003;112:323-330.
131. Zambelli-Weiner A, Ehrlich E, Stockton ML et al. Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study. J Allergy Clin Immunol 2005;115:1203-1209.
132. Simpson A, John SL, Jury F et al. Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. Am J Respir Crit Care Med 2006;174:386-392.
133. Keskin O, Birben E, Sackesen C et al. The effect of CD14-c159T genotypes on the cytokine response to endotoxin by peripheral blood mononuclear cells from asthmatic children. Ann Allergy Asthma Immunol 2006;97:321-328.
134. Tsai CH, Tung KY, Chen CH, Lee YL. Tumour necrosis factor G-308A polymorphism modifies the effect of home dampness on childhood asthma. Occup Environ Med 2011; 68:771-776.
135. Arshad SH. Does exposure to indoor allergens contribute to the development of asthma and allergy? Curr Allergy Asthma Rep 2010;10:49-55.
136. 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.
137. Rajagopalan G, Tilahun AY, Iijima K et al. HLA-DR polymorphism modulates response to house dust mites in a transgenic mouse model of airway inflammation. Tissue Antigens 2011; 77:589-592.
138. Holloway JW, Doull I, Begishvili B et al. Lack of evidence of a significant association between HLA-DR, DQ and DP genotypes and atopy in families with HDM allergy. Clin Exp Allergy 1996;26:1142-1149.
139. Torres-Galvan MJ, Quiralte J, Blanco C et al. Linkage of house dust mite allergy with the HLA region. Ann Allergy Asthma Immunol 1999;82:198-203.
140. Moffatt MF, Schou C, Faux JA et al. Association between quantitative traits underlying asthma and the HLA-DRB1 locus in a family-based population sample. Eur J Hum Genet 2001;9:341-346.
141. Parapanissiou E, Papastavrou T, Deligiannidis A et al. HLA antigens in Greek children with allergic bronchial asthma. Tissue Antigens 2005;65:481-484.
142. Pampuch A, Kowal K, Bodzenta-Lukaszyk A et al. The -675 4G/5G plasminogen activator inhibitor-1 promoter polymorphism in house dust mite-sensitive allergic asthma patients. Allergy 2006;61:234-238.
143. Kowal K, Bodzenta-Lukaszyk A, Pampuch A et al. Analysis of -675 4 g/5 G SERPINE1 and C-159T CD14 polymorphisms in house dust mite-allergic asthma patients. J Investig Allergol Clin Immunol 2008;18:284-292.
144. Sharma S, Raby BA, Hunninghake GM et al. Variants in TGFB1, dust mite exposure, and disease severity in children with asthma. Am J Respir Crit Care Med 2009;179:356-362.
145. Acevedo N, Vergara C, Gusmao L et al. The C-509T promoter polymorphism of the transforming growth factor beta-1 gene is associated with levels of total and specific IgE in a Colombian population. Int Arch Allergy Immunol 2010;151:237-246.
146. Shin HD, Kim LH, Park BL et al. Association of interleukin 18 (IL18) polymorphisms with specific IgE levels to mite allergens among asthmatic patients. Allergy 2005;60:900-906.
147. 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.
148. Martinez B, Barrios K, Vergara C et al. A NOS1 gene polymorphism associated with asthma and specific immunoglobulin E response to mite allergens in a Colombian population. Int Arch Allergy Immunol 2007;144:105-113.
149. Hunninghake GM, Soto-Quiros ME, Lasky-Su J et al. Dust mite exposure modifies the effect of functional IL10 polymorphisms on allergy and asthma exacerbations. J Allergy Clin Immunol 2008;122:93-98.
150. Konno S, Takahashi D, Hizawa N et al. Genetic impact of a butyrophilin-like 2 (BTNL2) gene variation on specific IgE responsiveness to Dermatophagoides farinae (Der f) in Japanese. Allergol Int 2009;58:29-35.
151. Hizawa N, Freidhoff LR, Ehrlich E et al. Genetic influences of chromosomes 5q31-q33 and 11q13 on specific IgE responsiveness to common inhaled allergens among African American families. Collaborative Study on the Genetics of Asthma (CSGA). J Allergy Clin Immunol 1998;102:449-453.
152. Gao P, Grigoryev DN, Rafaels NM et al. CD14, a key candidate gene associated with a specific immune response to cockroach. Clin Exp Allergy 2010;40:1353-1364.
153. Donfack J, Tsalenko A, Hoki DM et al. HLA-DRB1*01 alleles are associated with sensitization to cockroach allergens. J Allergy Clin Immunol 2000;105:960-966.
154. Kalpaklioglu AF, Turan M. Possible association between cockroach allergy and HLA class II antigens. Ann Allergy Asthma Immunol 2002;89:155-158.
155. Weiss LA, Lester LA, Gern JE et al. Variation in ITGB3 is associated with asthma and sensitization to mold allergen in four populations. Am J Respir Crit Care Med 2005;172:67-73.
156. Knutsen AP, Vijay HM, Kariuki B et al. Association of IL-4RA

single nucleotide polymorphisms, HLA-DR and HLA-DQ in children with Alternaria-sensitive moderate-severe asthma. Clin Mol Allergy 2010;8:5.
157. Wu AC, Lasky-Su J, Rogers CA, Klanderman BJ, Litonjua AA. Fungal exposure modulates the effect of polymorphisms of chitinases on emergency department visits and hospitalizations. Am J Respir Crit Care Med 2010;182:884-889.
158. Vicencio AG, Chupp GL, Tsirilakis K et al. CHIT1 mutations: genetic risk factor for severe asthma with fungal sensitization? Pediatrics 2010;126:e982-e985.
159. Bottema RW, Reijmerink NE, Kerkhof M et al. Interleukin 13, CD14, pet and tobacco smoke influence atopy in three Dutch cohorts: the allergenic study. Eur Respir J 2008;32:593-602.
160. Schuttelaar ML, Kerkhof M, Jonkman MF et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. Allergy 2009;64:1758-1765.
161. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000;406:782-787.
162. Yang IA, Fong KM, Holgate ST, Holloway JW. The role of Toll-like receptors and related receptors of the innate immune system in asthma. Curr Opin Allergy Clin Immunol 2006;6:23-28.
163. Raby BA, Klimecki WT, Laprise C et al. Polymorphisms in toll-like receptor 4 are not associated with asthma or atopy-related phenotypes. Am J Respir Crit Care Med 2002;166:1449-1456.
164. Smit LA, Bongers SI, Ruven HJ et al. Atopy and new-onset asthma in young Danish farmers and CD14, TLR2, and TLR4 genetic polymorphisms: a nested case-control study. Clin Exp Allergy 2007;37:1602-1608.
165. Yang IA, Barton SJ, Rorke S et al. Toll-like receptor 4 polymorphism and severity of atopy in asthmatics. Genes Immun 2004;5:41-45.
166. Bjornvold M, Munthe-Kaas MC, Egeland T et al. A TLR2 polymorphism is associated with type 1 diabetes and allergic asthma. Genes Immun 2009;10:181-187.
167. Bottema RW, Kerkhof M, Reijmerink NE et al. Gene-gene interaction in regulatory T-cell function in atopy and asthma development in childhood. J Allergy Clin Immunol 2010;126:338-346.
168. Eder W, Klimecki W, Yu L et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. J Allergy Clin Immunol 2004;113:482-488.
169. Qian FH, Zhang Q, Zhou LF et al. Polymorphisms in the toll-like receptor 2 subfamily and risk of asthma: a case-control analysis in a Chinese population. J Investig Allergol Clin Immunol 2010;20:340-346.
170. Smit LA, Siroux V, Bouzigon E et al. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. Am J Respir Crit Care Med 2009;179:363-368.
171. Hoffjan S, Stemmler S, Parwez Q et al. Evaluation of the toll-like receptor 6 Ser249Pro polymorphism in patients with asthma, atopic dermatitis and chronic obstructive pulmonary disease. BMC Med Genet 2005;6:34.
172. Kormann MS, Depner M, Hartl D et al. Toll-like receptor heterodimer variants protect from childhood asthma. J Allergy Clin Immunol 2008;122:86-92.
173. Tantisira K, Klimecki WT, Lazarus R et al. Toll-like receptor 6 gene (TLR6): single-nucleotide polymorphism frequencies and preliminary association with the diagnosis of asthma. Genes Immun 2004;5:343-346.
174. Moller-Larsen S, Nyegaard M, Haagerup A et al. Association analysis identifies TLR7 and TLR8 as novel risk genes in asthma and related disorders. Thorax 2008;63:1064-1069.
175. Lazarus R, Klimecki WT, Raby BA et al. Single-nucleotide polymorphisms in the Toll-like receptor 9 gene (TLR9): frequencies, pairwise linkage disequilibrium, and haplotypes in three U.S. ethnic groups and exploratory case-control disease association studies. Genomics 2003;81:85-91.
176. Heinzmann A, Brugger M, Bierbaum S et al. Joint influences of Acidic-Mammalian-Chitinase with Interleukin-4 and Toll-like receptor-10 with Interleukin-13 in the genetics of asthma. Pediatr Allergy Immunol 2010;21:e679-e686.
177. Lazarus R, Raby BA, Lange C et al. TOLL-like receptor 10 genetic variation is associated with asthma in two independent samples. Am J Respir Crit Care Med 2004;170:594-600.
178. Custovic A, Rothers J, Stern D et al. Effect of day care attendance on sensitization and atopic wheezing differs by Toll-like receptor 2 genotype in 2 population-based birth cohort studies. J Allergy Clin Immunol 2011;127:390-397.
179. Hoffjan S, Nicolae D, Ostrovnaya I et al. Gene-environment interaction effects on the development of immune responses in the 1st year of life. Am J Hum Genet 2005;76:696-704.
180. Kirkbride HJ, Bolscher JG, Nazmi K et al. Genetic polymorphism of MUC7: allele frequencies and association with asthma. Eur J Hum Genet 2001;9:347-354.
181. Rousseau K, Vinall LE, Butterworth SL et al. MUC7 haplotype analysis: results from a longitudinal birth cohort support protective effect of the MUC7*5 allele on respiratory function. Ann Hum Genet 2006;70:417-427.
182. Laitinen T, Daly MJ, Rioux JD et al. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. Nat Genet 2001;28:87-91.
183. van Heel DA, Ghosh S, Butler M et al. Synergistic enhancement of Toll-like receptor responses by NOD1 activation. Eur J Immunol 2005;35:2471-2476.
184. Weidinger S, Klopp N, Rummler L et al. Association of NOD1 polymorphisms with atopic eczema and related phenotypes. J Allergy Clin Immunol 2005;116:177-184.
185. Radon K. The two sides of the "endotoxin coin". Occup Environ Med 2006;63:73-78.
186. Bignon JS, Aron Y, Ju LY et al. HLA class II alleles in isocyanate-induced asthma. Am J Respir Crit Care Med 1994;149:71-75.
187. Balboni A, Baricordi OR, Fabbri LM et al. Association between toluene diisocyanate-induced asthma and DQB1 markers: a possible role for aspartic acid at position 57. Eur Respir J 1996;9:207-210.
188. Mapp CE, Beghe B, Balboni A et al. Association between HLA genes and susceptibility to toluene diisocyanate-induced asthma. Clin Exp Allergy 2000;30:651-656.
189. Rihs HP, Barbalho-Krolls T, Huber H, Baur X. No evidence for the influence of HLA class II in alleles in isocyanate-induced

asthma. Am J Ind Med 1997;32:522-527.
190. Bernstein JA, Munson J, Lummus ZL, Balakrishnan K, Leikauf G. T-cell receptor V beta gene segment expression in diisocyanate-induced occupational asthma. J Allergy Clin Immunol 1997;99:245-250.
191. Beghe B, Padoan M, Moss CT et al. Lack of association of HLA class I genes and TNF alpha-308 polymorphism in toluene diisocyanate-induced asthma. Allergy 2004;59:61-64.
192. Piirila P, Wikman H, Luukkonen R et al. Glutathione S-transferase genotypes and allergic responses to diisocyanate exposure. Pharmacogenetics 2001;11:437-445.
193. Wikman H, Piirila P, Rosenberg C et al. N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk. Pharmacogenetics 2002;12:227-233.
194. Mapp CE, Fryer AA, De MN et al. Glutathione S-transferase GSTP1 is a susceptibility gene for occupational asthma induced by isocyanates. J Allergy Clin Immunol 2002;109:867-872.
195. Kim SH, Cho BY, Park CS et al. Alpha-T-catenin (CTNNA3) gene was identified as a risk variant for toluene diisocyanate-induced asthma by genome-wide association analysis. Clin Exp Allergy 2009;39:203-212.
196. Horne C, Quintana PJ, Keown PA, Dimich-Ward H, Chan-Yeung M. Distribution of DRB1 and DQB1 HLA class II alleles in occupational asthma due to western red cedar. Eur Respir J 2000;15:911-914.
197. Young RP, Barker RD, Pile KD, Cookson WO, Taylor AJ. The association of HLA-DR3 with specific IgE to inhaled acid anhydrides. Am J Respir Crit Care Med 1995;151:219-221.
198. Jones MG, Nielsen J, Welch J et al. Association of HLA-DQ5 and HLA-DR1 with sensitization to organic acid anhydrides. Clin Exp Allergy 2004;34:812-816.
199. Riedler J, Braun-Fahrlander C, Eder W et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. Lancet 2001;358:1129-1133.
200. Von Ehrenstein OS, Von ME, Illi S et al. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 2000;30:187-193.
201. Braun-Fahrlander C, Riedler J, Herz U et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-877.
202. Lauener RP, Birchler T, Adamski J et al. Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. Lancet 2002;360:465-466.
203. Eder W, Klimecki W, Yu L et al. Association between exposure to farming, allergies and genetic variation in CARD4/NOD1. Allergy 2006;61:1117-1124.
204. Bieli C, Eder W, Frei R et al. A polymorphism in CD14 modifies the effect of farm milk consumption on allergic diseases and CD14 gene expression. J Allergy Clin Immunol 2007;120:1308-1315.
205. Ege MJ, Strachan DP, Cookson WO et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. J Allergy Clin Immunol 2011;127:138-144.
206. Kabesch M, Hoefler C, Carr D et al. Glutathione S transferase deficiency and passive smoking increase childhood asthma. Thorax 2004;59:569-573.
207. Yang Q, Khoury MJ. Evolving methods in genetic epidemiology. III. Gene-environment interaction in epidemiologic research. Epidemiol Rev 1997;19:33-43.
208. Ottman R. An epidemiologic approach to gene-environment interaction. Genet Epidemiol 1990;7:177-185.
209. Colhoun HM, McKeigue PM, Davey SG. Problems of reporting genetic associations with complex outcomes. Lancet 2003;361:865-872.
210. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. Lancet 2001;358:1356-1360.
211. Yang IA, Holloway JW. Asthma: advancing gene-environment studies. Clin Exp Allergy 2007;37:1264-1266.