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REVIEW

Asthma from a pharmacogenomic point of view

C Szalai[1,2], I Ungvári[3], L Pelyhe[4], G Tölgyesi[3] and A Falus[2,3]

[1]*Laboratory of Molecular Biology, Heim Pál Pediatric Hospital, Budapest, Hungary;* [2]*Inflammation Biology and Immunogenomics Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary;* [3]*Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary and* [4]*Faculty of Biology, Eötvös Lóránd University, Budapest, Hungary*

Pharmacogenomics, a fascinating, emerging area of biomedical research is strongly influenced by growing availability of genomic databases, high-throughput genomic technologies, bioinformatic tools and artificial computational modelling approaches. One main area of pharmacogenomics is the discovery of new drugs and drug targets with molecular genetic, genomic or even bioinformatic methods; the other is the study of how genomic differences influence the variability in patients' responses to drugs. From a genetic point of view, asthma is multifactorial, which means that the susceptibility to the disease is determined by interactions between multiple genes, and involves important non-genetic factors such as the environment for their expression. In this review, we summarize collective evidence from linkage and association studies that have consistently reported suggestive linkage or association of asthma or its associated phenotypes to polymorphic markers and single nucleotide polymorphisms in selected chromosomes. Genes that have been found implicated in the disease are potential new drug targets and several pharmacological investigations are underway to utilize these new discoveries. Next, we will focus on the inter-individual variability in anti-asthmatic drug responses and review the recent results in this topic.
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Abbreviations: AHR, airway hyper-responsiveness; BHR, bronchial hyper-responsiveness; GC, glucocorticoid; SNP, single nucleotide polymorphism

Introduction

Pharmacogenomics offers a new tool for the discovery of new targets for drug development purposes, and for the individual variation in drug response. In this review, we will delineate some of the genomic and genetic methods for identifying genome regions, genes and genetic variations that might play a role in the pathomechanism of asthma, and then we will discuss some of the most important results. Genes that have been found implicated in the disease are potential new drug targets and several pharmacological investigations are underway to utilize these new discoveries. Next, we will focus on the inter-individual variability in anti-asthmatic drug responses and review the recent results in this topic.

Correspondence: Professor A Falus, Department of Genetics, Cell and Immunobiology, Semmelweis University, Nagyvárad tér 4, Budapest H-1089, Hungary.
E-mails: faland@dgci.sote.hu (or) afalus@gmail.com
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Investigation of the genomic background of asthma

Asthma is a pulmonary disease characterized by intermittent narrowing of the small airways of the lung with subsequent airflow obstruction, increased bronchial responsiveness to a variety of stimuli and symptoms of wheeze, cough and breathlessness. The majority of asthmatics are also atopic, with manifestation of allergic diathesis including clinical allergy to aeroallergens and foods, or subclinical allergy manifest by skin test reactivity to allergen or elevated serum immunoglobulin (Ig) E. Allergic asthma can present for the first time at any age, but the incidence is highest in children (Dodge and Burrows, 1980). It is the most common chronic disease of childhood and the most frequent reason for paediatric hospital admission, and its incidence is on the rise (Mannino *et al*., 2002).

Previous studies suggest that asthma is a multifactorial disease influenced by genetic and environmental factors (Kelley *et al*., 2005). Studies of twins have shown generally that concordance rates for asthma are significantly higher in monozygotic twins than dizygotic twins, and that the heritability of asthma varies between 36 and 79% (Weiss and Raby, 2004). Importantly, there is evidence that genetic

liability for asthma, airway responsiveness and allergic traits are regulated through distinct loci, although there is likely some shared overlap as well (Barnes, 2000).

Given the likely presence of genes of strong effect, it is reasonable expectation that understanding the genetics of asthma will lead to improvements in its diagnosis, prevention and treatment. As a result, programmes aimed at the discovery of genes that predispose individuals to this illness are being carried out worldwide. Studies on the genetics of asthma are hampered by the fact that there is no standard definition of asthma (Tattersfield *et al.*, 2002). Attempts to define asthma have generally resulted in descriptive statements invoking notions of variable airflow obstruction over short periods of time, sometimes in association with markers of airway or bronchial hyper-responsiveness (AHR or BHR) and cellular pathology of the airway; they have not, however, provided validated quantitative criteria for these characteristics to enable diagnosis of asthma to be standardized for clinical, epidemiological or genetic purpose. For this reason, investigators have defined and commonly used objective quantitative traits, such as total and specific IgE levels, AHR and skin prick test, as surrogate markers of asthma. The danger in using these intermediate phenotypes is the assumption that their genetic basis is the same as that of the disease and that they represent the full range of disease states. For example, although atopy is one of the strongest risk factors for asthma, it alone is not sufficient to induce asthma, as many atopic individuals do not have asthmatic symptoms.

The other difficulty that hampers the efforts to identify the specific genes involved in asthma is the multigenic nature of the disease. It means that more than one gene in each individual might interact to produce the disease phenotype (polygenic inheritance), different disease alleles might exist in different individuals (genetic heterogeneity) and interaction with the environment might lead to incomplete penetrance. An individual might also develop disease owing to environmental factors alone (phenocopy). By contrast with single-gene disorders, genes that predispose to asthma will not usually contain mutations that lead to a gross aberration in function. Most often they will be variants of normal genes, the evolutionary advantages of which have become obscure (Ropers, 2007).

Results of the association studies and genome-wide screens in humans

Until now more than 600 gene association studies for asthma were published and more than 120 genes have been found associated with an asthma- or atopy-related phenotype, 54 genes that have been replicated in 2–5 independent samples, 15 genes in 6–10 independent samples and 10 genes in >10 independent samples (Ober and Hoffjan, 2006). In the last few years, positional cloning strategies revealed seven novel genes *ADAM33* (Van Eerdewegh *et al.*, 2002), *DPP10* (Allen *et al.*, 2003), G-protein-coupled receptor for asthma susceptibility (*GPRA*) (Laitinen *et al.*, 2004), *HLA-G* (Nicolae *et al.*, 2005), cytoplasmic fragile X mental retardation protein-interacting protein 2 (*CYFIP2*) (Noguchi *et al.*, 2005), arginine/serine-rich 8 (*SFRS8*) (Brasch-Andersen *et al.*, 2006) and *ORMDL3* (Moffatt *et al.*, 2007). Some genes were associated with asthma phenotypes rather consistently across studies and populations. In particular, variation in 10 genes has been associated with asthma phenotypes in 10 or more studies: interleukin-4 (*IL4*), *IL13*, β2 adrenergic receptor (*ADRB2*), major histocompatibility complex, class II, DQ β1 (*HLA-DQB1*), tumour necrosis factor-α (*TNFα*), lymphotoxin-α (*LTA*), high-affinity IgE receptor (*MS4A2*), IL-4 receptor (*IL4R*), *CD14* and *ADAM33*. It is notable that the first positionally cloned asthma gene *ADAM33* (Van Eerdewegh *et al.*, 2002) has now been associated with asthma or a related phenotype in >10 independent samples. These loci likely represent true asthma or atopy susceptibility loci or genes important for disease modification. However, no one gene will be an 'asthma' gene in all populations. This likely reflects the complex aetiology of these conditions, the modest effects of these genes on risk and the important roles of gene–gene and gene–environment interactions in determining susceptibility. Chromosomal localization and possible function of some candidate genes in asthma, and related diseases are presented in Table 1.

The genetic complexity of asthma is also underlined by the results of family-based genome-wide screens (also known as linkage studies), carried out by microsatellite markers, which have highlighted 20 genomic regions as being likely to contain asthma susceptibility genes. The size of these linked regions (10–20 million bp) means that they typically harbour hundreds of candidate genes. For the most part, each study has indicated that several loci are linked to asthma or related traits, supporting the multigenic model for the disease. However, few of the reported linkages have met the accepted criteria for significant genome-wide linkage. This lack of clear frontrunners has made it difficult to set priorities for narrowing gene regions and gene discovery. Despite this, many of these linkages have been replicated in multiple screens. The consistency with which these regions have been detected in asthma scans indicates that they might indeed contain asthma susceptibility genes.

In recent years, several companies offered methods for high-throughput single nucleotide polymorphism (SNP) screenings at a relatively cheap price. The most powerful and promising methods are the genome-wide association (GWA) studies. GWA involves scanning thousands of samples, either as case–control cohorts or in family trios, utilizing hundreds of thousands of SNP markers located throughout the human genome. Algorithms are applied that compare the frequencies of either single SNP alleles, genotypes or multimarker haplotypes between disease and control cohorts. In comparison to family linkage-based approaches, association studies have two key advantages. First, they are able to capitalize on all meiotic recombination events in a population, rather than only those in the families studied. Because of this, association signals are localized to small regions of the chromosome containing only a single to a few genes, enabling rapid detection of the actual disease susceptibility gene. Second, GWA allows the identification of disease genes with only modest increases in risk, a severe limitation in linkage studies and the very type of genes one expects for common disorders. Until now one GWA has been

Table 1 Chromosomal localization and possible function of candidate genes in asthma, and related phenotypes

Chromosomal region	*Population example*	*Candidate gene*	*Function*	*Phenotype*
2q14	German, Italian	*IL1* gene family (Gohlke *et al*., 2004)	Influencing inflammatory response	Asthma, atopy
	Australian, British	*DPP10* (Allen *et al*., 2003)	Regulation of chemokines and cytokines	Asthma, high IgE
2q33	English, Dutch, Norwegian	*CTLA4* (Munthe-Kaas *et al*., 2004)	Regulator of T-cell activation and differentiation	Asthma, high IgE
5q31–q33	Amish, German, USA white, USA Hispanic, Chinese, Koreans	*IL4*, *IL13*, *CSF2* (Kabesch *et al*., 2003; Chen *et al*., 2004)	IgE isotype switching, induction of Th2 response	High IgE, asthma, AHR
		IL5 (Namkung *et al*., 2007)	Eosinophil activation, maturation	Asthma
		IL9 (Temann *et al*., 2007)	Role in T-, B- and mast cell functions	Asthma
		SPINK5 (Walley *et al*., 2001)	Possible epithelial differentiation	Atopy, asthma
		CD14 (Vercelli *et al*., 2001; Leung *et al*., 2003)	Bacterial LPS binding receptor	High IgE, atopy
		HAVCR1, *HAVCR2* (earlier TIM1, TIM3) (McIntire *et al*., 2004)	Th1, Th2 differentiation	Asthma
		ADRB2 (D'amato *et al*., 1998; Liggett, 2003)	Influencing the effect of β2-agonists and smoking	Asthma
		Leukotriene C_4 synthase (Kedda *et al*., 2004)	Enzyme for leukotriene synthesis	Aspirin-intolerant asthma, asthma
		CYFIP2 (Noguchi *et al*., 2005)	Increases adhesion properties of CD4+ cells	Asthma
5p13	German, Hutterites	*ZFR*, *NPR3*, *ADAMTS12 PRLR*, *IL7R*, *LIFR*, *PTGER4* (Kurz *et al*., 2006)	Diverse functions	Asthma, BHR
6p21.3	USA white Dutch, Chinese, Japanese	*HLA-D* (Marsh *et al*., 1989; Cookson, 1999)	Antigen presentation	Specific IgE
		HLA-G (Nicolae *et al*., 2005	Immunoregulation	Asthma, BHR
		TNF (Tölgyesi *et al*., 2006)	Proinflammatory cytokine	Asthma
		LTA (Migita *et al*., 2005)	Induces the expression of cell adhesion molecules and cytokines	Asthma
7p	Finnish, Canadian, Australian	*GPRA* (Laitinen *et al*., 2004)	Unknown	Asthma, atopy
11q13	Australian whites and aborigine, Chinese	*MS4A2* (Shirakawa *et al*., 1994; Hill *et al*., 1995; Hill and Cookson, 1996)	High-affinity IgE receptor	Atopy, asthma
		SCGB1A1 (CC16) (Laing *et al*., 2000)	Regulation of airway inflammation	Asthma
	Japanese	*CHRM1* (Maeda *et al*. 2006)	Airway constriction and inflammation, epithelial cell proliferation	Asthma
11p		*ETS2*, *ETS3* (Brooks-Wilson, 1999)	Transcription factors	Asthma
12q14.3–q24.31	Caucasian, Japanese, Indian, Taiwanian	*INFG* (Nagarkatti *et al*., 2002; Wang *et al*., 2006)	Inhibition of IL-4 activity	Asthma, atopy, high IgE
		KIT ligand (Sutcliffe *et al*., 2006)	IL-4 production, mast cell maturation	
		STAT6 (Gao *et al*., 2004; Schedel *et al*., 2004)	Cytokine-regulated transcription factor	
		NOS1 (Grasemann *et al*., 2000; Shao *et al*., 2004)	NO: vasodilation, inflammatory regulation	Asthma
12q24.21–q24.33	Danish	*SFRS8* (Brasch-Andersen *et al*., 2006)	Regulation of the splicing of CD45	Asthma
13q	Australian, British	*PHF11*, *(SETDB2, RCBTB1*(?)) (Zhang *et al*., 2003)	Transcriptional regulation (?)	High IgE
14q22.1–24	USA white and black	*PTGDR* (Oguma *et al*., 2004)	T-cell chemotaxis	Asthma
	Mexican	Arginase 2 (Li *et al*., 2006)	Inhibition of NO generation	Asthma
16p21	Chinese, German, Spanish	*IL4R* (Hytonen *et al*., 2004)	α subunit is part of the receptor for IL-4 and IL-13	Atopy, asthma

Table 1 *Continued*

Chromosomal region	*Population example*	*Candidate gene*	*Function*	*Phenotype*
17q11.2–q21	Hungarian, Korean	RANTES (*CCL5*) (Yao *et al*., 2003), MCP-1 (*CCL2*) (Szalai *et al*., 2001), eotaxins (*CCL11* and *CCL24*) (Shin *et al*., 2003; Chang *et al*., 2005)	Attracting and stimulating of leukocytes	Asthma
	British, German	*ORMDL3* (Moffatt *et al*., 2007)	Unknown	Asthma
20p13	USA white, English, German, Japanese	*ADAM33* (Van Eerdewegh *et al*., 2002)	Possible role in bronchial contractility or remodelling	Asthma, AHR

Abbreviations: *ADAM33*, A disintegrin and metalloproteinase domain 33; *ADAMTS12*, A disintegrin and metalloproteinase domain with thrombospondin type 1 motif 12; *ADRB2*, β2 adrenergic receptor; AHR, airway hyper-responsiveness; BHR, bronchial hyper-responsiveness; *CC16*, Clara cell protein 16 or uteroglobin; *CHRM1*, cholinergic receptor muscarinic-1; *CSF2*, colony-stimulating factor 2 (granulocyte-macrophage); *CTLA4*, cytotoxic T-lymphocyte antigen 4; *CYFIP2*, cytoplasmic fragile X mental retardation protein-interacting protein 2; *DPP10*, dipeptidyl peptidase 10; *ETS*, epithelium-specific transcription factor; *GPRA*, G-protein-coupled receptor for asthma susceptibility; *HAVCR*, hepatitis A virus cellular receptor; HLA, human leukocyte antigen; Ig, immunoglobulin; IL, interleukin; *IL7R*, IL-7 receptor; *INF*, interferon; *LIFR*, leukemia inhibitory factor receptor; LPS, lipopolysaccharide; *LTA*, lymphotoxin-α; *MCP-1*, monocyte chemoattractant protein-1; MHC, major histocompatibility complex; *MS4A2*, membrane-spanning 4 domains, subfamily A, member 2 (high-affinity IgE receptor β subunit); *NOS1*, neuronal nitric oxid synthase; *NPR3*, natriuretic peptide receptor C; *PHF11*, plant homeodomain finger protein-11; *PRLR*, prolactin receptor; *PTGDR*, prostaglandin D2 receptor; *PTGER4*, prostaglandin E4 receptor; *RANTES*, regulated on activation normal T-cell expressed and secreted; SCF, stem cell factor; *SFRS8*, splicing factor, arginine/serine-rich 8; *STAT6*, signal transducer and activator of transcription 6; *TIM*, T-cell integrin mucin-like receptor; *TNF*, tumor necrosis factor-α; *ZFR*, zinc-finger RNA-binding protein.

published in asthma with promising results (Moffatt *et al*., 2007).

Next, we will systematically summarize collective evidence from linkage and association studies that have consistently reported suggestive linkage or association of asthma or its associated phenotypes to polymorphic markers and SNPs in selected chromosomes.

Chromosome 2

Evidence for linkage of asthma and related phenotypes to chromosome 2q arm has been reported in several studies (Allen *et al*., 2003, Hersh *et al*., 2007) (Table 1). Mouse genome screens have also linked AHR to the region homologous to 2q in the human (De Sanctis *et al*., 1995).

This 2q14 region includes the *IL1* gene family. Single-marker, two-locus and three-locus haplotype analysis of SNPs yielded several significant results for asthma ($P<0.05$–0.0021) for the human *IL1RN* gene encoding the IL-1 receptor antagonist protein, an anti-inflammatory cytokine that plays an important role in maintaining the balance between inflammatory and anti-inflammatory cytokines (Gohlke *et al*., 2004). The study was carried out in German population and was replicated and confirmed in an independent Italian family sample. Furthermore, a single G/T base exchange at +4845 in exon 5 of the *IL1A* gene results in an amino-acid substitution of alanine for serine.

The 2q33 region harbours the candidate gene cytotoxic T-lymphocyte antigen 4 (*CTLA4*), an important regulator of T-cell activation and differentiation. Transmission disequilibrium test analysis showed that several SNPs in the *CTLA4* gene were significantly associated with serum IgE levels, allergy, asthma and FEV1 % (forced expiratory volume 1 s) predicted below 80%, but not with AHR, and *CTLA4* polymorphisms of potentially direct pathogenic significance in atopic disorders were identified (Munthe-Kaas *et al*., 2004). The particular SNP alleles found positively associated with these phenotypes were previously shown to be associated negatively with autoimmune disorders. As autoimmune disorders are TH1-skewed diseases and asthma and atopic diseases are TH2 diseases, these data suggest a role for *CTLA4* polymorphisms in determining the TH1/TH2 balance.

Allen *et al*. (2003) positionally cloned a novel asthma gene through an effort that was aimed at mining the candidate linkage region on 2q. They found and replicated association between asthma and the D2S308 microsatellite, 800 kb distal to the *IL1* cluster on 2q14. The strongest associations were with SNP WTC122, in close proximity to D2S308. After an extensive search for the gene that contains the associated SNPs, they identified *DPP10*. This gene encodes a homologue of dipeptidyl peptidases (DPPs), which are thought to cleave terminal dipeptides from various proteins. On the basis of homology of this gene to other members of this family, the authors speculate that *DPP10* regulates the activity of various chemokine and cytokine genes by removing N-terminal dipeptides from them in a proline-specific manner. They suggest that DPP10 might cleave various pro-inflammatory and regulatory chemokines and cytokines. If this is the case, DPP10 might modulate inflammatory processes in the airways. As comparisons between *DPP10* expression in asthmatic and normal tissues are yet to be carried out, it is still unclear whether differences in the quantity or pattern of expression of *DPP10* will be associated with asthma.

Chromosome 5

After an original observation of genetic linkage of total IgE levels to the 5q31 region in extended Amish pedigrees and confirmation of linkage to the same region, chromosome 5q31–33 has become one of the most studied candidate asthma regions (Marsh *et al*., 1994). It contains the cytokine

gene cluster that plays an important role in the pathomechanism of asthma and atopic disorders (Table 1).

IL-4 is important in IgE isotype switching and the regulation of allergic inflammation. The 3017 G/T variant of the *IL4* gene or the haplotype it identifies was found to be influencing significantly IL-4's ability to modulate total serum IgE levels (Kabesch *et al*., 2003). Large-scale association studies in 1120 German schoolchildren were conducted to determine the effect of all polymorphisms present in the *IL4* gene on the phenotypic expression of atopic diseases. A total of 16 polymorphisms were identified in the *IL4* gene. A significant association between a cluster of polymorphisms in strong linkage disequilibrium with each other and a physician's diagnosis of asthma and total serum IgE levels was found.

IL-13 is one of the major cytokine in asthma. It enhances mucus production, AHR, and the production of the main eosinophil chemoattractant eotaxin. The receptors for IL-13 and IL-4 share a common α chain and the functions of the two cytokines overlap. Several polymorphisms were found in the *IL13* gene. The most significant associations were observed to asthma, AHR and skin test responsiveness with the -1111 promoter polymorphism (Chen *et al*., 2004). The Gln110 IL-13 variant displayed significantly increased binding capacity to its receptor compared with Arg110 IL-13 and was associated with elevated IgE level and asthma.

CD14 is located on chromosome 5q31 and it is a receptor that has specificity for lipopolysaccharides and other bacterial wall-derived components. Engagement of CD14 by these bacterial components is associated with strong IL-12 responses by antigen-presenting cells, and IL-12 is regarded as an obligatory signal for the maturation of naive T cells into Th1 cells (Verhasselt *et al*., 1997). A C/T SNP at position –159 in the promoter of the gene encoding CD14 was found to be associated with increased levels of soluble CD14 and decreased total serum IgE (Vercelli *et al*., 2001; Leung *et al*., 2003).

The gene underlying Netherton disease (*SPINK5*) encodes a 15-domain serine proteinase inhibitor (LEKTI), which is expressed in epithelial and mucosal surfaces and in the thymus.

SPINK5 is at the distal end of the cytokine cluster on 5q31. A Glu420Lys variant was found to be significantly associated with atopic dermatitis and atopy with weaker correlation with asthma in two independent panels of families (Walley *et al*., 2001).

Noguchi *et al*. (2005) performed a mutation screening and association analyses of genes in 5q33 in 9.4-Mb-long human linkage region. Transmission disequilibrium test analysis of 105 polymorphisms in 155 families with asthma revealed that six polymorphisms in *CYFIP2* gene were associated significantly with the development of asthma ($P = 0.000075$; odds ratio $= 5.9$). These six polymorphisms were in complete linkage disequilibrium. Subjects homozygous for the haplotype overtransmitted to asthma-affected offspring showed significantly increased level of *CYFIP2* gene expression in lymphocytes compared with ones heterozygous for the haplotype. CYFIP2 is member of a widely expressed, highly conserved protein family, highly abundant in CD4+ cells from multiple sclerosis patients and it is suggested that overabundance of CYFIP2 protein facilitates increased adhesion properties of T cells.

A broad region on the short arm of chromosome 5 (5p13) flanking the D5S1470 microsatellite marker separated by >9 Mb was identified as an asthma susceptibility locus in several populations. Fine mapping and positional candidate studies of this region in the Hutterites and an outbred case–control sample from Germany by genotyping 89 SNPs in 22 genes revealed that this region contains at least two and possibly five asthma or BHR susceptibility loci (Kurz *et al*., 2006).

Chromosome 6

The MHC region on chromosome 6p21.3 has shown consistent linkage to asthma-associated phenotypes in several studies and is considered to be a major locus influencing allergic diseases (Szalai *et al*., 2002). This region contains many molecules involved in innate and specific immunity (Table 1). The class II genes of the MHC have recognized influences on the ability to respond particular allergens. The strongest and most consistent association is between the minor component of ragweed antigen (Amb a 5) and *HLA-DR2*. It was demonstrated that all but 2 of 80 white IgE responders to Amb a 5 carried *HLA-DRB2* (DW2.2/DR2.2). This was significantly higher than the frequency of this haplotype among non-responders (approximately 22%) (Marsh *et al*., 1989). Many other possible positive and negative associations of the MHC with allergen reactivity have been described. Stronger HLA effects may be seen when the antigen is small and contains a single or very few antigenic determinants. This may be the case with aspirin-induced asthma and DPB1*0301 allele of the *HLA-DPB1* gene (Cookson, 1999).

Both class I and III genes of the MHC as well as non-classic MHC genes may also affect asthma through allergic or non-allergic pathways, respectively. The *TNF* gene is located on chromosome 6 between the class I and III clusters of the human MHC. It is a potent pro-inflammatory cytokine, which is found in excess in asthmatic airways. The -308A allele in the promoter region of the *TNF* gene is transcribed *in vitro* at seven times the rate of the -308G allele (Wilson *et al*., 1997). Several reports found associations between the –308A allele and asthma. An example for the gene–environmental interaction in asthma is, that children positive for *Chlamydophila pneumoniae*-specific IgG carrying the *TNF* -308A allele have considerably higher risk of developing asthma than children with similar infection status carrying wild-type genotypes (Tölgyesi *et al*., 2006).

Nicolae *et al*. (2005) conducted a genome-wide screen of families who participated in the Collaborative Study on the Genetics of Asthma. The strongest linkage signal in white families was on chromosome 6p21 at marker D6S1281, which is 2.5 cM telomeric to the HLA complex. To further narrow the linked region, they genotyped Chicago families and trios for additional microsatellite markers and SNPs. Analysis of the individual variants revealed that polymorphisms in *HLA-G* were associated with asthma in both families and trios. The strongest association was found between BHR and -964 G/A polymorphism. These results were confirmed altogether in four independent samples.

The −964A allele was overtransmitted to children with BHR if the mother was unaffected, whereas the −964G allele was overtransmitted to children with BHR if the mother was affected. The differences in transmission patterns of alleles to children with BHR from mothers with and without BHR were highly significant. Similar analyses stratified by father's asthma status did not show as significant a trend. Furthermore, the prevalence of BHR was not different if maternal status was ignored, but the prevalence of BHR among children with the GG genotype was significantly influenced by maternal status. Among children whose mothers have BHR, 56% of GG children have BHR; among children of mothers without BHR, 26% of GG children have BHR. No such relationship was observed for AA or AG children. In Dutch families, GG children were less likely to be atopic but were more likely to have BHR if their mother also had BHR. None of the other five markers typed in the Dutch families showed associations with either BHR or atopy.

Chromosome 7

The first published genome-wide scan in asthma suggested six tentative genetic loci, among them chromosome 7p, which was then implicated in a study of Finnish and Canadian families and confirmed in West Australian families (Daniels *et al*., 1996; Laitinen *et al*., 2001).

Using strategies of genetic mapping and positional cloning, Laitinen *et al*. (2004) identified new molecular players in asthma and allergy on chromosome 7p. They adopted a hierarchical genotyping design, leading to the identification of a 133-kb risk-conferring segment containing two genes. One of these coded for an orphan G-protein-coupled receptor named GPRA, which showed distinct distribution of protein isoforms between bronchial biopsies from healthy and asthmatic individuals. In three cohorts from Finland and Canada, SNP-tagged haplotypes were associated with high-serum immunoglobulin E or asthma. The murine orthologue of GPRA was upregulated in a mouse model of ovalbumin-induced inflammation. The properties of GPRA make it a strong candidate for involvement in the pathogenesis of asthma. GPRA might act as a receptor for an unidentified ligand. The putative ligand, isoforms of GPRA and their putative downstream signalling molecules may define a new pathway critically altered in asthma (Table 1).

Chromosome 11

Linkage of atopy to a genetic marker on chromosome 11q13 was first reported in 1989 (Cookson *et al*., 1989) (Table 1). The gene for the β chain of the high-affinity receptor for IgE (FcεRI-β) *MS4A2* was subsequently localized to the region (Hill and Cookson, 1996). Affected sib-pair analysis showed that linkage of atopy to chromosome 11 markers was to maternal alleles in many families (Cookson *et al*. (1992). Several coding and non-coding polymorphisms have been identified in the gene that encodes FcεRI-β. Ile181Leu and Val183Leu have been found in several populations. Maternal inheritance of both these variants was found to be associated with severe atopy (Shirakawa *et al*., 1994). Ile181Leu has also been associated with levels of IgE in heavily parasitized Australian aborigines, implying a protective role for the gene in helminthiasis (Hill *et al*., 1995). Another polymorphism, Glu237Gly, was found to be associated with various measures of atopy, as well as, bronchial reactivity to methacholine in Australian population and with asthma in Chinese population (Hill and Cookson, 1996). No such associations were found in some other populations.

The human cholinergic receptor muscarinic-1 (CHRM1) is widely distributed in the lungs. In patients with asthma, CHRM1 may be involved in airway constriction, airway epithelial cell proliferation and airway inflammation. The *CHRM1* gene is located on chromosome 11q13. A case–control study carried out in Japanese patients identified two common SNPs (−9697C/T and −4953A/G) and a haplotype associated with asthma (Maeda *et al*., 2006).

Another candidate in this region is the uteroglobin (also known as Clara cell secretory protein or CC16, whose official gene name is now *SCGB1A1*), a 16-kDa protein, which is also located on 11q13 and primarily expressed in the respiratory tract by non-ciliated bronchiolar secretory cells, accounting for 7% of the total protein content in the bronchoalveolar lavage fluid of healthy non-smokers. The immunomodulatory activity of uteroglobin has been well documented and its mRNA levels have been proposed as markers of lung maturation and epithelial differentiation (Oshika *et al*., 1998). The CC16 (now it is *SCGB1A1*) gene was screened for mutations and a polymorphism (38A/G) was identified and associated with an increased risk of physician-diagnosed asthma in a population of Australian children, and increased AHR in a population of Australian infants. The 38A sequence was associated with reduced plasma CC16 levels and individuals with lower plasma CC16 levels were more likely to have asthma (Laing *et al*., 2000). However, studies on populations of Japanese and British adults and North American children did not replicate these associations (Gao *et al*., 1998).

A collaboration among Sequana Therapeutics, Boehringer-Ingelheim and the University of Toronto identified *ETS2* and *ETS3* genes, which are adjacent to each other on chromosome 11p and code for epithelium-specific transcription factors, as being asthma-associated genes in a genome-wide screen of the population of Tristan da Cunha, a volcanic island in the South Atlantic Ocean (Brooks-Wilson, 1999). This small population of 290 inbred individuals, derived from a small set of founders who settled on the island in the nineteenth century, has a high incidence of asthma (∼30%) and shares a relatively homogeneous living environment. This association was replicated in an outbred population from Toronto and also in three population samples from the USA and Denmark; however, no such association was reported in a study of an outbred European-American population. *ETS2* and *ETS3* are expressed in airway epithelial cells and may function as transcriptional activators or repressors of genes expressed in these cells.

Chromosome 12

This chromosome has been linked to both atopy and asthma. Several asthma-associated genes are located on chromosome 12q21–24, including KIT ligand (also known

as stem cell factor), interferon-γ (*IFNG*), signal transducer and activator of transcription-6 (*STAT6*) (Table 1).

According to gene association studies, *IFNG* does not seem to be responsible for the linkage in several populations, but a CA repeat polymorphism in the *IFNG* gene was significantly associated with total serum IgE levels in an Indian population, and with the risk of asthma for children in Taiwan (Nagarkatti *et al*., 2002; Wang *et al*., 2006).

STAT6 is a critical signalling molecule in the Th2 signalling pathway, and mice lacking STAT6 are protected from allergic pulmonary manifestations (Kuperman *et al*., 1998). The importance of STAT6 in asthma is also evident from studies showing that *STAT6* gene expression is markedly upregulated in airway epithelial cells in asthma (Matsukura *et al*., 1999). A number of common polymorphisms have been identified, including a GT repeat in exon 1 and three common SNPs (4219G/A, 4491A/G and 4671A/G) in the human *STAT6* gene. All four of these polymorphisms and a haplotype have been shown to be associated with allergic phenotypes in various populations (Gao *et al*., 2004; Schedel *et al*., 2004).

Neurally derived nitric oxide (NO), produced by neuronal NO synthase (NOS1), is physiologically linked to asthma as it is a neurotransmitter for bronchodilator non-adrenergic non-cholinergic nerves. Mice lacking a functional *NOS1* gene were shown to be hyporesponsive to methacholine challenge compared with wild-type mice (De Sanctis *et al*., 1999). The frequencies of the number of a CA repeat in exon 29 were significantly different between Caucasian asthmatic and non-asthmatic population (Grasemann *et al*., 2000). Recently, the *NOS1* intron 2 GT repeat and *STAT6* exon 1 GT repeat were associated with childhood asthma in a Japanese population (Shao *et al*., 2004).

A genome-wide scan in Danish families identified 12q24.21–q24.33 as an asthma susceptibility region, and then a fine-scale mapping was carried out in 167 families (Brasch-Andersen *et al*., 2006). In this sample set, three SNPs in the splicing factor, *SFRS8* gene showed the most significant association with asthma. The *SFRS8* gene codes for a splice factor which not only regulates its own splicing but also the splicing of CD45, an important molecule in the activation process of T cells. The activation of T cells depends on different splice variants of CD45. T cells are known to be involved in the pathogenesis of atopic diseases such as asthma, so *SFRS8* is a candidate gene in the region.

Chromosome 13

Linkage of chromosome 13q to atopy, asthma and allergy to house dust mites in children with asthma was found in different studies and genome-wide scans (Table 1).

Recently, Zhang *et al*. (2003) progressed from broad linkage to gene identification in this region. Haplotype analysis indicated that the region of association to LnIgE centred on one gene, *PHF11* (plant homeodomain finger protein-11), and extended to two flanking genes, *SETDB2* and *RCBTB1*. By conducting an analysis in which they held each SNP in *PHF11* constant in a serial manner, they identified three SNPs in introns 5 and 9 and in the 3′-untranslated region as having independent effects. They then showed that the intron 5 and 3′-untranslated region variants were also associated with severe clinical asthma: an association that was confirmed in an unrelated British population.

The precise function of *PHF11* gene has not been determined, but the presence of two zinc-finger motifs in the translated protein suggests a role in transcriptional regulation. The gene is expressed in most tissues, but Zhang *et al*. observed consistent expression in many immune-related tissues. Moreover, they identified multiple transcript isoforms, including variants expressed exclusively in the lung and in peripheral blood leukocytes. Because variation in this gene was strongly associated with serum IgE levels and, as described by Zhang, with circulating IgM, and because the gene is expressed heavily in B cells, the authors suggest that this locus may be an important regulator in immunoglobulin synthesis.

Chromosome 14

Using 175 extended Icelandic families that included 596 patients with asthma, Hakonarson *et al*. (2002) performed a genome-wide scan with 976 microsatellite markers. Linkage of asthma was detected to chromosome 14q24, with an allele-sharing logarithm of the odds (LOD) score of 2.66. After the marker density was increased within the locus to an average of one microsatellite every 0.2 cM, the LOD score rose to 4.00.

Prostaglandin D2 receptor (*PTGDR*) is located on chromosome 14q22.1 and was found to be required for the expression of the asthma phenotype in mice (Matsuoka *et al*., 2000). Prostaglandin mediates the chemotaxis of T cells that follows the degranulation of mast cells. Six SNPs in *PTGDR* and its vicinity have been found (Oguma *et al*., 2004). These define four common three SNP haplotypes, which vary in their ability to support transcription of *PTGDR* and have distinct DNA-binding-protein affinity profiles. Individual *PTGDR* SNPs were significantly associated with asthma in white and black population in the USA. Multivariate analysis of the haplotype combinations (diplotypes) demonstrated that both whites and blacks who had at least one copy of the haplotype with a low transcriptional efficiency had a lower risk of asthma than subjects with no copies of the haplotype. These functional and genetic findings identify *PTGDR* as an asthma susceptibility gene.

Another potential candidate, arginase 2 (*ARG2*), lies close to this region on 14q24. Arginases may contribute to asthma pathogenesis through various mechanisms, including inhibition of nitric oxide generation, reduced arginine bioavailability and increased ornithine production, which could lead to airway remodelling caused by altered polyamine and proline synthesis. Using a case–parent triad design in Mexico City, two SNPs in the *ARG2* gene were found to be associating with increased relative risk of childhood asthma (Li *et al*., 2006) (Table 1).

Chromosome 16

Several studies have shown linkage between region on chromosome 16p21 and atopic phenotypes of specific IgE (Table 1). The strongest candidate gene in this region is the *IL4R*, which also serves as the α-chain of the IL-13 receptor.

At least three of the eight reported SNPs that result in amino-acid substitutions in the *IL4R* gene have been associated with the atopic phenotypes and less commonly with asthma. However, the alleles or haplotypes showing the strongest evidence differed among the populations (Hytonen *et al*., 2004).

Chromosome 17

Linkage between asthma and chromosome 17 was detected in several ethnic groups, although no such linkage was shown to other atopic diseases (Barnes, 2000; Moffatt *et al*., 2007). There are several candidate genes for asthma in this region, but the most important of them are genes in the chemokine gene cluster (Table 1). Chemotactic cytokines, or chemokines, are small signalling proteins that are deeply involved in the physiology and pathophysiology of acute and chronic inflammatory processes, by attracting and stimulating specific subsets of leukocytes. A number of chemokines have been identified in human asthma whose production appears to be related to the severity of asthmatic inflammation and reactive airway responses. A good summary about chemokine and their receptors as potential targets in asthma can be read in the review by Palmqvist *et al*. (2007).

Monocyte chemoattractant protein-1 (MCP-1, official name is now CCL2) may play a significant role in the allergic responses because of its ability to induce mast cell activation and leukotriene C_4 release into the airway, which directly induces AHR. Neutralization of CCL2 drastically reduces bronchial hyper-reactivity, lymphocyte-derived inflammatory mediators, and T cell and eosinophil recruitment to the lung (Gonzalo *et al*., 1998). A biallelic A/G polymorphism in the *CCL2* distal gene regulatory region at position –2518 has been found that affects the level of CCL2 expression in response to an inflammatory stimulus (Rovin *et al*., 1999). Associations were found between carrying G at –2518 of the *CCL2* gene regulatory region and the presence of childhood asthma, and between asthma severity and homozygosity for the G allele. In asthmatic children, the *CCL2* –2518G also correlated with increased eosinophil levels (Szalai *et al*., 2001).

RANTES (regulated on activation normal T-cell expressed and secreted) or CCL5 is one of the most extensively studied chemokines in allergic and infectious diseases. CCL5 is likely to be important in airway inflammation because blocking antibodies to CCL5 inhibits airway inflammation in a murine model of allergic airway disease. Furthermore, eosinophil chemotactic activity that appears in the bronchoalveolar lavage fluid of asthma patients following allergen challenge was found to be due to CCL5. Two polymorphisms in the *CCL5* promoter region (–28 C/G and –403 G/A) have been found affecting the transcription of the *CCL5* gene. Both polymorphisms have been found associated with asthma, or a phenotypic variant of asthma (–28G: near fatal asthma) in some populations, but not in others (Szalai *et al*., 2001; Yao *et al*., 2003; Al-Abdulhadi *et al*., 2005).

The chemokine receptor CCR5 expressed on monocytes, macrophages and Th1 cells (but not on Th2 cells) is responsible for transducing chemotaxis response to CCL5. A common 32-bp deletion mutation in the *CCR5* gene (*CCR5Δ32*), which causes truncation and loss of CCR5 receptors on lymphoid cell surfaces, has been described. Some data indicate an association of the *CCR5Δ32* allele with reduced risk of asthma in some populations, while in other studies there was no such association for atopy or asthma/wheeze (Szalai *et al*., 2000). A good example of the role of the gene–environmental interaction in multifactorial diseases is the association between *CCR5Δ32* and the chronic *Mycoplasma pneumoniae* infection. Furthermore, carrying the *CCR5Δ32* polymorphism seems to reduce the susceptibility to asthma in the infected children (Ungvári *et al*., 2007).

Eotaxins are the main chemoattractant for eosinophils, the most important cellular mediator of AHR. The expression of eotaxin mRNA and protein was found to be increased in the bronchial epithelium and submucosal layer of the airways of chronic asthmatics. In a Korean population, the 123G/A polymorphism in the eotaxin1 (*CCL11*) gene was related to total serum IgE in asthmatics, the –384A/G polymorphisms with asthma, while the 1265A/G SNP in the eotaxin2 (*CCL24*) gene was associated with asthma (Shin *et al*., 2003; Chang *et al*., 2005).

In a GWM study genotyping more than 317 000 SNPs in British and German subjects using family- and case-referent panels, multiple markers on chromosome 17q21 were strongly and reproducibly associated with childhood onset asthma (Moffatt *et al*., 2007). The findings were confirmed in independent studies on subjects from the same Caucasian populations. The SNPs associated with childhood asthma were consistently and strongly associated ($P<10^{-22}$) in cis with transcript levels of *ORMDL3*, a member of a gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum. In this study, genomic regions other than the 17q21 locus did not contain multiple markers with significant evidence of association at the stringent 1% false discovery rate threshold in either of the individual collections or the combined samples. Presently, the function of the *ORMDL3* gene is unknown and its possible role in asthma requires further studies. ORMDL3 was found to be expressed in many tissues, particularly liver and peripheral blood lymphocytes. The SNPs showing the strongest association to asthma and *ORMDL3* transcript abundances are contained within an island of linkage disequilibrium between 35.2 and 35.4 Mb on chromosome 17q21. The one LOD support unit for SNPs showing maximum association to ORMDL3 levels lies within the first intron of the neighbouring *GSDML* gene. This non-coding sequence shows significant homology among species, and contains an element with high homology to the pro-inflammatory transcription factor C/EBPb.

Chromosome 20

The first report of positional cloning of an asthma gene in a human population was published in 2002 (Van Eerdewegh *et al*., 2002) (Table 1). In this study, a multi-point linkage analysis for asthma was carried out in 460 affected sibling-pair Caucasian families from the USA and the UK. The strongest linkage signal was to 20p13 (LOD score 2.94). The investigators identified a cluster of SNPs in the *ADAM33* gene in this region that demonstrated significant associations

with asthma. The *ADAM33* gene is expressed ubiquitously in muscle of every type, including the smooth muscle of bronchioles. It is also expressed in fibroblasts, lymph nodes, thymus and liver, but not in leukocytes or bone marrow. ADAM proteins are zinc-dependent metalloproteinases that belong to a disintegrin- and metalloproteinase-containing family. The exact function of ADAM33 is unknown but its expression profile and the functions of related proteins suggest a role for ADAM33 in bronchial contractility. Alternatively, it has been suggested that its position in these tissues might allow it to modify the process of bronchial remodelling (scarring) that follows chronic asthmatic airway inflammation. A further possibility is that ADAM33 might activate other as-yet-unknown cytokines.

Investigations of the inter-individual variability in anti-asthmatic drug responses

There are four major classes of asthma pharmacotherapy currently in widespread use (Barnes, 2006): (1) β2-agonists used by inhalation for the relief of airway obstruction (for example, albuterol, salmeterol and fenoterol); (2) glucocorticosteroids for both inhaled and systemic use (for example, beclomethasone, triamcinolone and prednisone); (3) theophylline and its derivatives, used for both the relief of bronchospasm and the control of inflammation and (4) inhibitors and receptor antagonists of the cysteinyl-leukotriene pathway (for example, montelukast, pranlukast, zafirlukast and zileuton).

Variability in individual asthma treatment response may be due to many factors, including the severity and type of disease, treatment compliance, intercurrent illness, other medication taken (drug–drug interaction), environmental exposures and age. However, there is reason to believe that genetic factors underlie much of the observed treatment variance. A study of treatment response to glucocorticosteroids, a β2 adrenergic agonist and an experimental leukotriene inhibitor has found that up to 60–80% of the variance in drug response may be due to differences among individuals (Drazen *et al*., 2000). This value corresponds to the maximum limit of genetic variance, and indicates that a clinically relevant part of the response to the main classes of asthma drugs may be due to genetic determinants.

To date, investigations in the field of asthma pharmacogenomics have focused on three classes of asthma therapies: β2-agonists, leukotriene antagonists and glucocorticosteroids. The data summarized below provide evolving evidence that response to asthma therapy is highly variable among individuals with asthma, and genetic differences can help to predict the response to treatment in asthma.

Interaction between genetic variations and β2-agonists

The 5q31–33 is an important pharmacogenomic region for asthma (Liggett, 2000). β2-agonists are used widely by inhalation for the relief of airway obstruction. These drugs act via binding to the β2 adrenergic receptor (ADRB2), a cell surface G-protein-coupled receptor located on 5q32. Responses to this drug are currently the most investigated pharmacogenomic pathway in asthma. Two coding variants (at positions 16 and 27) within the *ADRB2* gene have been shown *in vitro* to be functionally important (Liggett, 2003). The Gly16 receptor exhibits enhanced downregulation *in vitro* after agonist exposure. In contrast, Arg16 receptors are more resistant to downregulation. Because of linkage disequilibrium, individuals who are Arg/Arg at position 16 are much more likely to be Glu/Glu at position 27; individuals who are Gly/Gly at position 16 are much more likely to be Gln/Gln at position 27. The position 27 genotypes influence but do not abolish the effect of the position 16 polymorphisms with regard to downregulation of phenotypes *in vitro*. Retrospective studies and prospective clinical trials have suggested that adverse effects occur in patients homozygous for arginine (Arg/Arg), rather than glycine (Gly/Gly), at position 16. Bronchodilator treatments avoiding β2-agonist may be appropriate for patients with the Arg/Arg genotype (Israel *et al*., 2004; Wechsler *et al*., 2006).

Interaction between genetic variations and leukotriene antagonists

Leukotrienes, released by eosinophils, mast cells and alveolar macrophages, are among the main mediators in asthma, inducing airway obstruction, migration of eosinophils and proliferation of smooth muscle (Barnes, 2006). Of the three enzymes exclusively involved in the formation of the leukotrienes (5-lipoxygenase (ALOX5), leudotriene C4 (LTC4) synthase and LTA4 epoxide hydrolase), ALOX5 is the enzyme required for the production of both the cysteinyl-leukotrienes (LTC4, LTD4 and LTE4) and LTB4. ALOX5 activity in part determines the level of bronchoconstrictor leukotrienes present in the airways, and pharmacological inhibition of the action of ALOX5 or antagonism of the action of the cysteinyl-leukotrienes at their receptor is associated with an amelioration of asthma. A polymorphism located in the promoter of the *ALOX5* gene decreases gene transcription, and less enzyme is produced when the number of repeats of an Sp1-binding motif GGGCGG, which acts as a transcription modulating site, is different from the usual number of five (Kalayci *et al*., 2006). In a study in the USA, approximately 6% of asthma patients did not carry a wild-type allele at the *ALOX5* core promoter locus (Drazen *et al*. (1999). It was hypothesized that patients possessing the altered promoter might be less responsive to a leukotriene modifier. In randomized, double-blind, placebo-controlled trials of ABT-761, an ALOX5 inhibitor, which is a derivative of the anti-leukotriene drug zileuton, this hypothesis was investigated. The primary outcome of the clinical study was improvement in FEV1. In the unstratified population, the inhibitor produced a 12–14% improvement in FEV1. Patients homozygous for the wild-type promoter had a 15% improvement in FEV1. In contrast, those patients homozygous for the mutant version of the promoter had a significantly decreased FEV1 response. Otherwise the *ALOX5* core promoter locus does not account for all patients who did not respond to ALOX5 inhibition, which suggests that there may be other gene defects in the pathway leading to a lack of response to this form of treatment. It was suggested that patients who fail to respond to ALOX5 inhibition are

those in whom other mechanisms are responsible for asthmatic airway obstruction.

LTC4 synthase is a membrane-bound glutathione transferase expressed only by cells of haematopoietic origin and is a key enzyme in the synthesis of cys-LTs, converting LTA4 to LTC4. The gene encoding LTC4 synthase is located on 5q35. An adenine to cytosine transversion has been found 444 bp upstream (−444) of the translation start site of the LTC4 synthase gene and reported that the polymorphic C −444 allele occurred more commonly in patients with aspirin-intolerant asthma (Sanak *et al*., 1997, 2000). A fivefold greater expression of LTC4 synthase has been demonstrated in individuals with aspirin-intolerant asthma when compared with patients with aspirin-tolerant asthma; furthermore, the expression of LTC4 synthase mRNA has also been shown to be higher in blood eosinophils from asthmatic subjects compared with control subjects and was particularly increased in eosinophils from patients with aspirin-intolerant asthma. In addition, it was found that, among subjects with asthma treated with zafirlukast (a leukotriene receptor antagonist), those homozygous for the A allele at the −444 locus had a lower FEV1 response than those with the C/C or C/A genotype (Palmer *et al*., 2002).

Interaction between genetic variations and corticosteroids

Corticosteroids taken by the inhalational route are the most effective and commonly used drugs for the treatment of asthma but may also be associated with serious adverse effects. Large inter-individual variation, including a significant number of non-responders, exists in the treatment response to these drugs. In one study of asthmatics, 22% of individuals taking inhaled beclomethasone had decrements in their FEV1 after 12 weeks of therapy, while in a second study 38% of patients randomized to either budesonide or fluticasone demonstrated FEV1 improvements of 5% over the course of 24 weeks. As the intra-individual response to inhaled corticosteroid treatment is highly repeatable, it is reasonable to postulate a genetic difference for the response to inhaled corticosteroids in asthma (Pignatti, 2004).

One study evaluating the role of IL-4 in glucocorticoid (GC)-resistant asthma performed genotyping of the *IL4* 589C/T SNP in a case-controlled manner comparing 24 patients with GC-resistant asthma and 682 GC-sensitive asthmatics. The *IL4* 589 T allele was found to be associated with increased IL-4 gene transcription and GC-resistant asthma (Leung and Bloom, 2003; Tantisira and Weiss, 2005).

The association of longitudinal change in lung function and SNPs from candidate genes crucial to the biologic actions of corticosteroids was evaluated in three independent asthmatic clinical trial populations utilizing inhaled corticosteroids as the primary therapy in at least one treatment arm. Variations in corticotropin-releasing hormone receptor 1 (*CRHR1*) gene were consistently associated with enhanced response to therapy in the three populations (Tantisira *et al*., 2004b). Individuals homozygous for the SNP rs242941 manifested a doubling to quadrupling of the lung function response to corticosteroids compared with lack of the variants. In addition, a common haplotype (frequency 27%), termed GAT, was associated with a significantly enhanced response to inhaled corticosteroids in two populations. The estimated 8-week improvement in FEV1 for those subjects imputed to have the homozygous GAT/GAT haplotype was more than twice that for those homozygous for non-GAT haplotypes in the Adult Study, and nearly three times that in Childhood Asthma Management Program. Improvement in those heterozygous for the GAT haplotype was intermediate between the two groups, suggesting an additive effect. As the primary receptor mediating the release of adrenocorticotropic hormone, which regulates endogenous cortisol levels, CRHR1 plays a pivotal, pleiotropic role in steroid biology. These data indicate that genetic variants in *CRHR1* have pharmacogenetic effects influencing asthmatic response to corticosteroids, provide a rationale for predicting therapeutic response in asthma and other corticosteroid-treated diseases, and suggest this gene pathway as a potential novel therapeutic target.

In the *TBX21* (also known as *T-bet*) gene, one common non-synonymous SNP has been described to date, rs2240017, which codes for a replacement of histidine 33 with glutamine (His33Gln). In a study of 701 children, 4.5% were found to be heterozygous for this variant (Tantisira *et al*., 2004a). After limiting the analysis to Caucasian children, each of the 33 His/Gln heterozygous individuals on inhaled corticosteroids demonstrated a marked improvement in airway hyper-responsiveness compared with either 33 His/His homozygotes or any individual not taking inhaled steroids. According to these results, the *TBX21* and the *CRHR1* may be important determinants for the pharmacogenetic response to the therapy of asthma with inhaled corticosteroids.

Combination inhalers with a corticosteroid and a long-acting β2-agonist are the most effective treatment so far available in asthma. Tantisira *et al*. (2005) investigated a possible interaction between β2-agonist and corticosteroid pathways. β2 adrenergic receptor carries out signal transduction by activating the heterotrimeric stimulatory guanine nucleotide-binding protein, whose α subunit activates the effector adenylyl cyclase. A non-synonymous polymorphism of adenylate cyclase type 9 (*ADCY9*) gene (Ile772Met) influenced the catalytic activity of the enzyme in cell cultures, and cells cultured in the presence of GC-expressing Met772 had a significantly increased albuterol-stimulated adenylyl cyclase response. The hypothesis that Met772 *ADCY9* is associated with an improved albuterol bronchodilator response in asthmatics was investigated in 436 asthmatic children who were followed for 4 years and randomized to receive placebo or the inhaled corticosteroid budesonide. Met772 carriers on budesonide showed a significant improvement in FEV1. Moreover, a highly significant interaction was found for budesonide treatment and the *ADCY9* polymorphism. The Met772 *ADCY9* polymorphism represents one of most likely several multi-gene polymorphisms along the receptor-relaxation axis, which together may provide for a composite pharmacogenetic index for asthma therapy.

For searching for genes influencing GC sensitivity, an alternative strategy was used by Hakonarson *et al*. (2005) in an Icelandic population. A total of 11 812 genes were examined with high-density oligonucleotide microarrays

to search for differences in mRNA expression in peripheral blood mononuclear cells freshly isolated from GC-sensitive and GC-resistant asthma patients. They have found 15 genes that most accurately separated GC responders from the non-responders. They suggested that this method could predict clinical response to inhaled GC therapy with meaningful accuracy. Upon validation in an independent study, these results can support the development of a diagnostic test to guide GC therapy in asthma patients.

Concluding remarks

In the last few years, our knowledge about the structure and function of the human genome improved considerably. Still, we are very far from the perfect understanding of the genomic background of complex diseases such as allergy or asthma. Regarding the multiple gene–gene and gene–environmental interactions, it is very likely that we will never forecast whether a newborn will have asthma in the future, or not, but with the available sequence information (including non-coding genome and the increasingly recognized series of regulatory microRNA profiles), the completion of a high-quality physical map of the human and mouse genome, the different animal models (KO and transgenic animals) and the advance of bioinformatics and different methods (microarray, DNA sequencing, high-throughput screenings and GWA) will make it possible that several additional asthma genes and gene regulatory networks will be identified in the next years. And hopefully, this knowledge will be translated into improved diagnosis, prevention and therapeutic strategies for this chronic disease.

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Conflict of interest

The authors state no conflict of interest.

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