Genomic Strategies in Pharmacology of Asthma and Autoimmunity

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Abstract: Pharmacogenomics, a fascinating, emerging area of biomedical research is strongly influenced by growing availability of genomic databases, high-through-put genomic technologies, bioinformatic tools and artificial computational modeling approaches. Multinational clusters, such as the regional and internet-driven pharmaco-grids generated an entirely new environment for research and development in pharmacology. Although the field of pharmacogenomics is in its infancy, the promise of pharmacogenomics lies in its potential to predict genomic sources of interindividual variability in drug response (both efficacy and toxicity), thus allowing individualization of therapy to maximize effectiveness and minimize risk. Thus, pharmacogenomics holds the promise for individualized medicine adapted to each person's own genetic makeup. Environmental factors including diet, age, and lifestyle as well as infection can influence a person's response to medicines, but understanding an individual's genetic background is thought to be the key to creating personalized drugs with greater efficacy and safety. Similar to other biomedical fields, in allergic and autoimmune diseases pharmacogenomics combines traditional pharmaceutical sciences such as biochemistry with annotated knowledge of genes, proteins, and single nucleotide polymorphisms (SNP). One of the major challenges now are developing and applying the statistical and computational capacity to store, manage, analyze and interpret the wealth of data being generated. This review summarizes the recent pharmacogenomic trends in inflammatory diseases with particular attention to autoimmune conditions and asthma.

Keywords: Pharmacogenomics, asthma, autoimmunity, single nucleotide polymorphism.

INTRODUCTION

Pharmacogenomics offers a new tool for the discovery of new targets for drug development purposes, and for the determination/prediciton of individual variations in drug response (efficacy, toxicity). Studies focusing on the genomical background of the differential responsiveness of patients to therapies, may allow the development of individualized or more efficient therapies. In this review, we will highlight some genes and genetic variations that might play a role in the pathomechanism of asthma, as well as some of the most important data. Genes that have been found implicated in the disease are potential new drug targets and several parmacological investigations are underway to utilize these new discoveries. Next, we will focus on the inter-individual variability in antiasthmatic drug responses and review the recent results in this topic.

INVESTIGATION OF THE GENOMIC BACKGROUND OF ASTHMA

Asthma is a pulmonary disease characterised 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 E (IgE). Allergic asthma can emerge for the first time at any age, but the incidence is highest in children [1]. It is the most common chronic disease of childhood and the most frequent reason for pediatric hospital admission, and its incidence is on the rise [2].

Previous studies suggest that asthma is a multifactorial disease influenced by genetic and environmental factors [3]. 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 vary between 36% and 79% [4]. 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 [5].

Given the likely presence of genes of strong effect, it is a 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 [6]. 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 hyperresponsiveness (AHR) and cellular pathology of the airway; they have not, however, provided validated quantitative cri-

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teria for these characteristics to enable diagnosis of asthma to be standardised 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.

Results of the Association Studies and Genome-Wide Screens in Humans

Till the beginning of 2006 more than 500 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 410 independent samples. [7]. In the last few years positional cloning strategies revealed six novel genes. Some genes were associated with asthma-phenotypes rather consistently across studies and populations. In particular, variation in ten genes have been associated with asthma-phenotypes in ten or more studies: interleukin-4 (IL)-4, IL-13, β_2 adrenergic receptor (ADRB2), human leukocyte antigen (HLA) DQB1, tumor necrosis factor-α (TNF-α), lymphotoxin-α (LTA), the β chain of the high affinity receptor for IgE (FcεRI-β), IL-4 receptor (IL-4R), CD14 and a disintegrin and metalloprotease 33 (ADAM33). It is notable that the first positionally cloned asthma gene, *ADAM33* [8] has now been associated with asthma or a related phenotype in more than 10 independent samples. These loci likely represent true asthma or atopy susceptibility loci or genes important for disease modification. However, no single gene will be an 'asthma' gene in all populations. This likely reflects the complex etiology 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**.

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 single nucleotide polymorphisms (SNPs) in selected chromosomes.

Chromosome 2

Evidence for linkage of asthma and related phenotypes to chromosome 2q arm has been reported in several studies [9, 10]. Mouse genome screens have also linked AHR to the region homologous to 2q in the human [11]. This 2q14 region includes the IL-1 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 [12].

The 2q33 region harbors the candidate gene cytotoxic T-lymphocyte antigen 4 (CTLA-4), an important regulator of T-cell activation and differentiation. Transmission disequilibrium test analysis showed that several SNPs in the CTLA-4 gene were significantly associated with serum IgE levels, allergy, asthma, and forced expiratory volume in 1 second (FEV1) predicted below 80%, but not with AHR, and CTLA-4 polymorphisms of potentially direct pathogenic significance in atopic disorders were identified.

Allen and colleagues [9] positionally cloned a novel asthma gene through an effort that was aimed at mining the candidate linkage region on 2q. 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. Based on 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.

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 [13]. It contains the cytokine gene cluster that plays an important role in the pathomechanism of asthma and atopic disorders.

IL-4 is important in IgE isotype switching and the regulation of allergic inflammation. The 3017 G/T variant of IL-4 or the haplotype it identifies was found to be influencing significantly the ability of IL-4 to modulate total serum IgE levels. Large-scale association studies in 1120 German schoolchildren were conducted to determine the effect of all polymorphisms present in the IL-4 gene on the phenotypic expression of atopic diseases. A total of 16 polymorphisms were identified in the IL-4 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 [14].

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 IL-13 gene. The most significant associations were observed to asthma, AHR, and skin-test responsiveness with the -1111 promoter polymorphism. The Q110 IL-13 variant displayed significantly increased binding capacity to its receptor compared with R110 IL-13 and was associated with elevated IgE level and asthma [15].

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	IL-1 gene family	Influencing inflammatory response	Asthma, atopy
	Australian, U.K.	DPP10	Regulation of chemokines and cytokines	Asthma, high IgE
2q33	English, Dutch, Norwegian	CTLA-4	Regulator of T-cell activation and differentiation	Asthma, high IgE
5q31-q33	Amish, German, U.S.A. white, U.S.A. Hispanic	IL-4, IL-13, GM-CSF	IgE isotype switching, induction of Th2 response	High IgE, asthma, AHR
		IL-5	Eosinophil activation, maturation	Asthma
		IL-9	Role in T, B and mast cell functions	Asthma
		SPINK5	Possible epithelial differentition	Atopy, asthma
		CD14	Bacterial LPS binding receptor	High IgE, atopy
		TIM1, TIM3	Th1, Th2 differentiation	Asthma
		ADRB2	Influencing the effect of β2-agonists and smoking	Asthma
		LTC_4 synthase	Enzyme for leukotriene synthesis	Aspirin intolerant asthma, asthma
		CYFIP2	Increases adhesion properties of $CD4^+$ cells	Asthma
6p21.3	U.S.A white Dutch, Chinese	HLA-D	Antigen presentation	Specific IgE
		HLA-G	Immunoregulation	Asthma, BHR
		TNFα	Proinflammatory cytokine	Asthma
		LTA	Induces the expression of cell adhesion molecules and cytokines	Asthma
7p	Finnish, Canadian, Australian	GPRA	Unknown	Asthma, atpoy
11q13	Australian whites and aborigine, Chinese	FcεRI-β	High affinity IgE receptor	Atopy, asthma
		CC16	Regulation of airway inflammation	Asthma
11p		ETS-2, ETS-3	Transcription factors	Asthma
12q14.3-q24.31	Caucasian, Japanese	INF-γ	Inhibition of IL-4 activity	Asthma, atopy, high IgE
		SCF	IL-4 production, mast cell maturation	
		STAT6	Cytokine regulated transcriptin factor	
		NFY-β	Elevation of IL-4 and HLA-D gene transcription	
		NNOS	NO: vasodilation, inflammatory regulation	Asthma

(Table 4) contd…..

Chromosomal region	Population example	Candidate gene	Function	Phenotype
13q	Australian, British	PHF11, (SETDB2, RCBTB1(?))	Transcriptional regulation (?)	High IgE
14q22.1	U.S.A. white and black	DP2R	T cell chemotaxis	Asthma
16p21	Chinese, German, Spanish	IL-4R	α subunit is part of the receptor for IL-4 and IL-13	Atopy, asthma
17q11.2	Hungarian, Korean	RANTES, MCP-1, eotaxin	Attracting and stimulating of leukocytes	Asthma
20p13	U.S.A. white, English, German, Japanese	ADAM33	Possible role in bronchial contractility or remodelling	Asthma, AHR

ADAM33: a disintegrin and metalloprotease 33; ADRB2: β_2 adrenergic receptor; AHR: airway hyperresponsiveness; BHR: bronchial hyperresponsiveness; CC16: clara cell protein 16; CTLA-4: cytotoxic T lymphocyte antigen 4; CYFIP2: cytoplasmic fragile X mental retardation protein-interacting protein 2; DP2R: prostanoid D2 receptor; DPP10: dipeptidyl-peptidase 10; ETS: epithelium specific transcription factor; FcεRI-β: high affinity IgE receptor β subunit; GM-CSF: granulocyte-macrophage colony-stimulating factor; GPRA: G protein-coupled receptor for asthma susceptibility; HLA: human leukocyte antigen; IgE: immunoglobulin E; IL: interleukin; INF: interferon; LPS: lipopolysaccharide; LTA: lymphotoxin-α; LTC_4: leukotriene C_4; MCP-1: monocyte chemoattractant protein-1; MHC: major histocompatibility complex; NFY-β: β-subunit of nuclear factor Y; NNOS: neuronal nitric oxide synthase; NO: nitric oxide; PHF11: plant homeodomain finger protein-11; RANTES: regulated on activation normal T cell expressed and secreted; RCBTB1: regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1; SCF: stem cell factor; SETDB2: SET domain bifurcated 2; SPINK5: serine peptidase inhibitor, Kazal type 5; Th: T helper lymphocyte; STAT6: signal tranducer and activator of transcription 6; TIM: T-cell integrin mucin-like receptor; TNF: tumor necrosis factor.

CD14 is located on chromosome 5q31 and it is a receptor that has specificity for lipopolysaccharides (LPS) 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 helper T lymphocytes (Th)1. 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.

The gene underlying Netherton disease (serine peptidase inhibitor, Kazal type 5; SPINK5) encodes a 15-domain serine proteinase inhibitor (lymphoepithelial Kazal-type-related 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 Glu420→Lys variant was found to be significantly associated with atopic dermatitis and atopy with weaker correlation with asthma in two independent panels of families [16].

Noguchi and co-workers [17] 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 cytoplasmic fragile X mental retardation protein-interacting protein 2 (*CYFIP2*) gene were associated significantly with the development of asthma ($p = 0.000075$; odds ratio, OR: 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.

Chromosome 6

The major histocompatibility gene complex (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 [10]. This region contains many molecules involved in innate and specific immunity. The class II genes of the MHC have recognized influences on the ability to respond to particular allergens. The strongest and most consistent association is between the minor component of ragweed antigen (Amb a V) and HLA-DR2. It was demonstrated that all but 2 of 80 white IgE responders to Amb a V carried HLA-DR2 and Dw2 (DR2.2). This was significantly higher than the frequency of this haplotype among nonresponders (approximately 22%) [18]. 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, and sensitivity to inhaled acid anhydrides and HLA-DR3 [19].

Both class I and class III genes of the MHC as well as nonclassic MHC genes may also affect asthma through allergic or nonallergic pathways, respectively. The TNF-α gene is located on chromosome 6 between the class I and III clusters of the human MHC. It is a potent proinflammatory cytokine, which is found in excess in asthmatic airways. The -308A allele in the promoter region of the TNF-α gene is transcribed *in vitro* at seven times the rate of the -308G allele. Several reports found associations between the –308A allele and asthma [20].

The LTA gene is located next to the TNF-α gene, and induces the expression of cell adhesion molecules and proinflammatory cytokines including E selectin, TNF-α and IL-1A and B. An intronic SNP (+252), a promoter polymorphism (-753 G/A) and a haplotype were found to be associated with increased susceptibility to asthma in different

populations. These latter results emphasise the inflammatory nature of the asthmatic response, as distinct from its allergic basis [21].

Nicolae and co-workers conducted a genome wide screen of families who participated in the Collaborative Study on the Genetics of Asthma [3]. 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 microsatellita 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 bronchial hyperresponsiveness (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 was highly significant. Similar analyses stratified by father's asthma status did not show as significant a trend. Furthermore, the prevalences of BHR were 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 [22].

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 [23, 24].

Using strategies of genetic mapping and positional cloning Laitinen and co-workers [25] identified new molecular players in asthma and allergy on chromosome 7p. They adopted a hierarchical genotyping design, leading to the identification of a 133-kilobase risk-conferring segment containing two genes. One of these coded for an orphan G protein-coupled receptor (GPCR) named G protein-coupled receptor for asthma susceptibility (GPRA), which showed distinct distribution of protein isoforms between bronchial biopsies from healthy and asthmatic individuals. In three cohorts from Finland and Canada, single nucleotide polymorphism-tagged haplotypes associated with high serum IgE or asthma. The putative ligand, isoforms of GPRA, and their putative downstream signaling molecules may define a new pathway critically altered in asthma.

Chromosome 11

Linkage of atopy to a genetic marker on chromosome 11q13 was first reported in 1989 [26]. The β chain of FcεRI was subsequently localized to the region [27]. Affected sib-pair analysis showed that linkage of atopy to chromosome 11 markers was to maternal alleles in many families [28]. Several coding and non-coding polymorphisms have been identified in the gene that encodes FcεRI-β. Ile/Leu181 and Val/Leu183 have been found in several populations. Maternal inheritance of both these variants was found to be associated with severe atopy. Ile/Leu181 has also been associated with levels of IgE in heavily parasitized Australian aborigines, implying a protective role for the gene in helminthiasis. Another polymorphism, E237G, 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. No such associations were found in some other populations.

Chromosome 12

This chromosome has been linked to both atopy and asthma. Several asthma associated genes are located on chromosome 12q21-24 including stem cell factor (SCF), interferon-γ (INF-α), signal transducer and activator of transcription 6 (STAT6). According to gene association studies IFN-γ does not seem to be responsible for the linkage.

STAT6 is a critical signalling molecule in the Th2 signalling pathway, and mice lacking STAT6 are protected from allergic pulmonary manifestations. 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. A number of common polymorphisms have been identified, including a GT repeat in exon 1 and three common SNPs (G4219A, A4491G, and A4671G) 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 [29, 30].

Neurally derived nitric oxide (NO), produced by neuronal NO synthase (NOS1; nNOS), is physiologically linked to asthma as it is a neurotransmitter for bronchodilator nonadrenergic noncholinergic nerves. Mice lacking a functional NOS1 gene were shown to be hyporesponsive to methacholine challenge compared with wild type mice [31]. The frequencies of the number of a CA repeat in exon 29 were significantly different between Caucasian asthmatic and nonasthmatic population [32]. Recently, the *NOS1* intron 2 GT repeat and *STAT6* exon 1 GT repeat were associated with childhood asthma in a Japanese population [33].

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. Recently, Zhang and co-workers [34] progressed from broad linkage to gene identification in this region. First, they confirmed linkage of atopy and total serum IgE concentrations to this region using standard linkage approaches. Next, they made a saturation map that indicated that the locus associated with atopy was within 7.5 cM of the linkage peak. Confining their analysis to those polymorphisms with a minor allele frequency > 0.15, they identified 49 SNPs, 4 deletion-insertion polymorphisms and a GGGC repeat. To determine whether IgE levels were associated with any of these SNPs, they did linkage

disequilibrium analyses in Australian families and found significant associations of the natural log of IgE concentrations (LnIgE) within a 100-kb region on chromosome 13. They confirmed their findings using transmission tests of association. Subsequent haplotype analysis indicated that the region of association to LnIgE centered on one gene, plant homeodomain (PHD) finger protein-11 (*PHF11*), and extended to two flanking genes, SET domain bifurcated 2 (*SETDB2*) and regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1 (*RCBTB1*). The precise function of PHF11 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 and colleagues [34] 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 [34].

Chromosome 14

Using 175 extended Icelandic families that included 596 patients with asthma, Hakonarson and co-workers [35] performed a genome-wide scan with 976 microsatellite markers. Linkage of asthma was detected to chromosome 14q24, with an allele-sharing a highest logarithm of 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 [35].

Prostanoid DP receptor2 (DP2R) is located on chromosome 14q22.1 and was found to be required for the expression of the asthma phenotype in mice [36]. Prostanoid DP mediates the chemotaxis of T cells that follows the degranulation of mast cells. Six SNPs in DP2R and its vicinity have been found. These define four common three-SNP haplotypes, which vary in their ability to support transcription of DP2R and have distinct DNA-binding-protein affinity profiles. Individual DP2R SNPs were significantly associated with asthma in white and black population in the U.S.A.. 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 DP2 as an asthma-susceptibility gene [37].

Chromosome 16

Several studies have shown linkage between region on chromosome 16p21 and atopic phenotypes of specific IgE. The strongest candidate gene in this region is the IL-4R, which also serves as the α-chain of the IL-13R. At least three of the eight reported SNPs that result in amino acid substitutions in the IL-4R gene have been associated with the atopic phenotypes and less commonly with asthma [17]. Although the alleles or haplotypes showing the strongest evidence differed between the populations.

The Chemokine System and Chromosome 17

Linkage between asthma and chromosome 17 was detected in several ethnic groups, although no such linkage was shown to other atopic diseases [5, 38]. There are several candidate genes for asthma in this region, but the most important of them are genes in the chemokine gene cluster. Chemotactic cytokines, or chemokines, are small signalling proteins which are deeply involved in the physiology and pathophysiology of acute and chronic inflammatory processes, by attracting and stimulating specific subsets of leukocytes. Chemotaxis and chemokine gene clusters represent gene sets serving a basic cell-physiological activity and by targeting these genes or proteome by novel strategies of drug delivery may provide a new tool in pharmacological treatment. Polymorphisms of the key signaling molecules are detected frequently among pathological conditions. Consequently, evaluation of the most significant molecular families of chemotaxis assures prognostic values as well as these data could support development of new drug-targeting agents and techniques as well.

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. Interestingly, in the group of chemotactic ligands relatively low SNP frequencies were found in the two classes of chemokines (CCL and CXCL) representing several members of this family (clustered on 17q12 and 4q21 respectively). However, characterization based on the number of SNPs can provide only a guide to pharmacogenetical research, its prognostic value of whether the alterations of the chemotaxis related sequences are expressed in the proteomic level, is rather limited. Collecting information about genomic sequences, whether the polymorphisms are located in coding regions of the protein or in other sequences (e.g. in regulatory regions), provides more information concerning the biological significance of these alterations of the genome.

Monocyte chemoattractant protein-1 (MCP-1) may play a significant role in the allergic responses because of its ability to induce mast cell activation and leukotriene $(LT)C_4$ release into the airway, which directly induces AHR. A biallelic A/G polymorphism in the MCP-1 distal gene regulatory region at position –2518 has been found that affects the level of MCP-1 expression in response to an inflammatory stimulus. Associations were found between carrying G at –2518 of the MCP-1 gene regulatory region and the presence of childhood asthma and between asthma severity and homozygosity for the G allele. In asthmatic children the MCP-1 –2518G also correlated with increased eosinophil level [38].

RANTES (regulated on activation normal T cell expressed and secreted) is one of the most extensively studied chemokines in allergic and infectious diseases. Two polymorphisms in the *RANTES* promoter region (-28 C/G and –403 G/A) have been found affecting the transcription of the RANTES 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 [38-40].

Eotaxin is 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 123 G/A polymorphism was related to total serum IgE in asthmatics, although the effects of the different SNPs in the eotaxin gene were quite inconclusive.

Although the CC chemokine receptor (CCR) gene cluster is located on 3p21.3, regarding its importance in asthma as possible drug targets and its functionally connection with the CC (C-C motif) chemokines, results about the genetic variations of the CCRs will be discussed here. The chemokine receptor CCR5, which is expressed on monocytes, macrophages, Th1 (but not Th2) cells, is responsible for transducing chemotaxis response to RANTES, macrophage inflammatory protein- (MIP)-1α and MIP-1β. A common 32-base pair (bp) deletion mutation in the CCR5 gene (CCR5Δ32), which causes truncation and loss of CCR5 receptors on lymphoid cell surfaces has been described. The CCR5Δ32 allele was found to be common in the Caucasian population but was not found in people of African or Asian ancestry. Some data indicate an association of the CCR5Δ32 allele with reduced risk of asthma in some populations (e.g. in Scottish children), while in families from Western Australia, Southern England and Hungary there was no significant association for atopy or asthma/wheeze.

In the receptor of eotaxin CCR3 three polymorphisms were identified in Japanese (Asian) and British (Caucasian) subjects. Multiple logistic regression analysis showed that CCR3 T51C was associated with asthma in the British population (OR: 2.83, $p < 0.02$), but not in the Japanese population independent of atopic phenotypes such as high levels of total or house dust mite-specific IgE in serum.

Chromosome 20

The first report of positional cloning of an asthma gene in a human population was published in 2002 [41]. In this study, a multi-point linkage analysis for asthma was done in 460 affected sibling-pair Caucasian families from the U.S. and the U.K.. 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 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 Antiasthmatic Drug Responses

There are four major classes of asthma pharmacotherapy currently in widespread use: (1) β_2-agonists used by inhalation for the relief of airway obstruction (e.g. albuterol, salmeterol, fenoterol), (2) glucocorticosteroids for both inhaled and systemic use (e.g. beclomethasone, triamcinolone, 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 LT (cysLT) pathway (e.g., montelukast, pranlukast, zafirlukast, 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 are reasons to believe that genetic factors underlie much of the observed treatment variance. A study of treatment response to a glucocorticosteroids, a β_2 agonist, and an experimental LT inhibitor has found that up to 60–80% of the variance in drug response may be due differences between individuals. 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 [42].

To date, investigations in the field of asthma pharmacogenomics have focused on three classes of asthma therapies: β_2-agonists, LT antagonists and glucocorticosteroids. The data summarized below provide evolving evidence that response to asthma therapy is highly variable between individuals with asthma, and genetic differences can help to predict the response to treatment in asthma.

The 5q31-33 is an important pharmacogenomic region for asthma [60]. β_2-agonists are used widely by inhalation for the relief of airway obstruction. These drugs act *via* binding to the β_2 adrenergic receptor (ADRB2), a cell surface GPCR 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 [43]. The Gly-16 receptor exhibits enhanced downregulation *in vitro* after agonist exposure. In contrast, Arg-16 receptors are more resistant to downregulation. Because of linkage disequilibrium, individuals who are homozygous for arginine (Arg/Arg) at position 16 are much more likely to be homozygous for glutamate (Glu/Glu) at position 27, individuals who are homozygous for glycine (Gly/Gly) at position 16 are much more likely to be homozygous for glutamine (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 Arg/Arg patients, rather than Gly/Gly individuals, at position 16. Bronchodilator treatments avoiding β_2-agonist may be appropriate for patients with the Arg/Arg genotype [44]. Additionally, smoking subjects homozygous for Arg16 had an almost 8-fold risk for developing asthma, than non-smoking subjects with Gly/Gly genotype at position 16.

LTs, 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 [45]. Of the three enzymes exclusively involved in the formation of the LTs (ALOX5, 5-lipoxygenase; LTC_4 synthase, and LTA_4 epoxide hydrolase), ALOX5 is the enzyme required for the production of both the cysLTs (LTC_4 , LTD_4 , and LTE_4) and LTB_4. 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 cysLTs at their receptor is associated with an amelioration of asthma. A polymorphism located in the promoter of the ALOX-5 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 5.

In a study in the United States approximately 6% of asthma patients did not carry a wild-type allele at the *ALOX5* core promoter locus [46]. 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 antileukotriene 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% to 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.

LTC_4 synthase is a membrane-bound glutathione transferase expressed only by cells of hematopoietic origin and is a key enzyme in the synthesis of cysLTs, converting LTA_4 to LTC_4. The gene encoding LTC_4 synthase is located on 5q35. An adenine to cytosine transversion has been found 444 bp upstream (-444) of the translation start site of the LTC_4 synthase gene and reported that the polymorphic C -444 allele occurred more commonly in patients with aspirin intolerant asthma (AIA) [47, 48]. A 5-fold greater expression of LTC_4 synthase has been demonstrated in individuals with AIA when compared with patients with aspirin-tolerant asthma, furthermore, the expression of LTC_4 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 AIA. In addition, it was found that, among subjects with asthma treated with zafirlukast (a LT 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 [49].

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. Since 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 [50].

One study evaluating the role of IL-4 in glucocorticoid (GC)-resistant asthma performed genotyping of the IL-4 C589T SNP in a case-controlled manner comparing 24 patients with GC-resistant asthma and 682 GC-sensitive asthmatics. The IL-4 589T allele was found to be associated with increased IL-4 gene transcription and GC-resistant asthma [51, 52].

The association of longitudinal change in lung function and SNPs from candidate genes crucial to the biologic actions of corticosteroids were 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 [53]. 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 (CAMP). 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 [53].

In the T-bet gene, one common nonsynonymous SNP has been described to date, rs2240017, which codes for a replacement of histidine 33 with glutamine (H33Q). In a study of 701 children from the CAMP, 4.5% were found to be heterozygous for this variant [54]. After limiting the analysis to Caucasian children, each of the 33HQ heterozygous individuals on inhaled corticosteroids demonstrated a marked improvement in airway hyperresponsiveness compared with either 33HH homozygotes or any individual not taking inhaled steroids. According to these results the T-bet and the CRHR1 may be important determinants for the pharmacogenetic response to the therapy of asthma with inhaled corticosteroids.

For searching for genes influencing GC sensitivity an alternative strategy was used by Hakonarson and co-workers [55] 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 (PBMC) freshly isolated from GC-sensitive and GC-resistant asthma patients. They have found

15 genes that most accurately separated GC responders from the nonresponders. 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.

Histamine is a bronchoconstrictor involved in the pathogenesis of asthma, and histamine *N*-methyltransferase plays a dominant role in histamine metabolism in human bronchial epithelium. A common polymorphism in the methyltransferase coding gene decreases the level of the enzyme, and is therefore expected to result in increased bronchoconstriction. According to this hypothesis, a study on 192 asthmatic patients and 237 unaffected controls indicated a significantly increased allele frequency of the T 314 allele, associated with low enzyme activity, in asthmatics, and it was suggested to consider in these patients the use of H_1 antihistamines which do not inhibit the enzyme [56].

AUTOIMMUNE DISEASES

Autoimmune diseases are characterized by pathological immune responses to self antigens. Even though individual autoimmune diseases are not frequent, collectively they affect approximately 5% of the adult population. Autoimmune diseases have complex genetic background and they are characterized by multifactorial inheritance, in which numerous disease susceptibility genes and environmental factors contribute to the development of the disease.

Autoimmune diseases are associated with a number of shared disease susceptibility loci. This is further confirmed by the observation that they are often clustered in affected families. These observations are suggestive of the existence of common disease pathways. Gene products of shared disease susceptibility alleles may be primary targets in drug development. Disease susceptibility alleles are often organized in gene clusters shown both in humans and in experimental autoimmune animal models. Polymorphisms that dictate susceptibility to autoimmune diseases are also found in healthy individuals, and the specific constellation/coexistence of several such susceptibility alleles in a given genome contributes to the development of the pathological phenotype.

Recently, a group of rare monogenic autoimmune diseases has received significant attention. In these monogenic diseases the mutations point to key factors that play a major role in the development of autoimmunity. The autosomal-recessive autoimmune polyglandular syndrome type I (APS1; APECED, automimmune polyendocrinopathy candidiasis ectodermal dystrophy; Online mendelian inheritance in man - OMIM 240300) is caused by mutations in the autoimmune regulator (AIRE) gene that encodes for the transcription factor AIRE [57]. AIRE is considered to be a "master regulator" of promiscuous gene expression of the thymus that results in central tolerance induction to those tissue-specific self antigens that are recognized by high affinity T cell receptor (TCR) binding [58, 59]. Autoimmune lymphoproliferative syndrome (ALPS; OMIM 601859) is caused by the mutations in the TNF receptor superfamily, member 6 (TNFRFS6; Fas) gene that lead to apoptosis defect and an increased number of double negative ($CD4^-/CD8^-$) T cells [60]. Immundysregulation, polyendocrinopathy, enteropathy, X-linked inheritance (IPEX) is caused by the mutations of the forkhead box P3 (FOXP3) gene on Xp11.23 encoding for the FOXP3 transcription factor, the regulator of the regulatory T (Treg) cells. IL-2Rα deficiency (OMIM 606367) is caused by the deletion of the IL-2R α chain (CD25) that leads to decreased peripheral immune tolerance associated with Treg cells [61].

By mapping disease susceptibility genes, pharmacogenomics of the autoimmune diseases may help to identify disease pathways and novel drug targets. Furthermore, it may provide information about drug metabolizing enzymes and drug transporters of an individual.

Selected Genes Associated with Autoimmune Diseases

Chromosome 1

1. Complement Component C1q (C1q; 1p36.3-p34.1)

The first subcomponent of the C1 complex, C1q binds to immunocomplexes as a first step of the classic complement activation. While the activation of complement in systemic lupus erythematosus (SLE) contributes to tissue injury, inherited deficiency of the classic pathway components, particularly C1q, is associated with the development of SLE. It is hypothesized that a physiologic event early in the classic pathway protects against the development of SLE and C1q may play a key role in this respect [62].

2. Interleukin-10 (IL-10; 1q31-q32)

IL-10 is a Th2 cytokine that has been shown to be secreted by Treg cells as well. High IL-10 production is associated with autoimmune diseases such as rheumatoid arthritis (RA) and SLE. The IL-10 promoter is polymorphic and may account for different levels of cytokine production [63]. The promoter -2849A/G polymorphism of the IL-10 gene was not associated with the incidence of RA, but instead, it was correlated with the progression of the disease. RA patients with the G allele, which is associated with high IL-10 production, also had higher autoantibody titers at baseline [64].

IL-10 is thought to play a key role in psoriasis. Its highly polymorphic promoter contains two informative microsatellites, IL-10.G and IL-10.R. A clear differential distribution was revealed at the IL-10.G locus when patients were stratified according to whether they had a positive family history of psoriasis ($p = 0.04$) due to an overrepresentation of the IL-10.G13 allele in those patients with familial disease (40.4% *vs* 19.6%, chi square = 7.292, $p = 0.007$). The positive association of allele IL-10.G13 with familial psoriasis was especially strong when patients with early onset were compared with those with early onset against a nonfamilial background (39.6% *vs* 14.5%, chi square = 8.959, $p = 0.003$). Patients with age of onset of less than 40 were 4-fold more likely to have a psoriatic family background if they carried the IL-10.G13 allele. These data suggested that the IL-10 locus contributes to the heritability of psoriasis susceptibility. In a case-control study of Crohn's disease [65] the authors found a significant association of the higher-producing IL-10 -1082G and TNF-α -857C alleles with structuring disease.

3. Fas Ligand (FasL; 1q23)

This cell surface receptor is implicated in apoptosis induction. FasL triggers apoptosis by binding to Fas on the surface of cells. The apoptosis genes FAS and FASL are candidate contributory genes in SLE, as mutations of these genes result in autoimmunity in several murine models of SLE. In humans, FAS mutations result in ALPS [66].

4. Fc Gamma Receptor II b (FCGR2B; 1q22)

An autoimmune disorder that may result from the disruption of inhibitory receptors, particularly in their conserved intracellular immunoreceptor tyrosine-based inhibitory motifs (ITIMs), the FCGR2B being an example. In SLE the -343G-C polymorphism has been associated with decreased transcription of the *FCGR2B* promoter and resulted in decreased binding of the activator protein-1 (AP-1). It was found that homozygosity for a -343C promoter polymorphism was increased in SLE patients compared to controls [67]. Homozygosity for an Ile232→Thr (I232T) polymorphism in the transmembrane region of the FCGR2B gene was significantly increased in SLE patients compared to control subjects [68], while an expression variant of FCGR2B was suggested to be a risk factor for SLE [69]. The loss of function polymorphism (-232T) in homozygotes was shown to lead to reduced FCGR2B-mediated inhibition of B cell receptor-triggered proliferation [70].

5. Protein Tyrosine Phosphatase, Nonreceptor-Type, 22 (PTPN22; 1p13)

It is one of the most important loci that appear to exert a general effect on the autoimmune disease process. The PTPN22 gene encodes for a lymphoid tyrosine phosphatase (LYP), involved in T-cell activation, as a negative regulator [71].

A 1858C-T transition in the PTPN22 gene, resulting in an Arg620→Trp (R620W) substitution was shown to be associated with insulin-dependent diabetes mellitus (IDDM) [72]. Moreover, RA was shown to be associated with the minor allele, 1858T, of the R620W SNP in *PTPN22* [73], which was also associated with type 1 diabetes mellitus and Graves thyroiditis [74], while a functional R620W variant has now been conclusively shown to confer approximately 2-fold risk for seropositive RA [75]. These findings imply that *PTPN22* may be a general autoimmune disease susceptibility locus.

6. Peptidylarginine Deiminases (PADs; 1p36)

PADs are posttranslational modification enzymes that convert arginine to citrulline in the presence of calcium ions. Four PADI genes (*PADI1 - 4*) are located in the region 1p36. *PADI4* has been identified as a susceptibility locus for RA (P = 0.000008) [76] and a functional *PADI4* haplotype affected the stability of transcripts and was associated with levels of antibody to citrullinated peptides in RA sera, acting as autoantigens [76].

Chromosome 2

CTLA-4 (2q33) is a member of the immunoglobulin superfamily, expressed by activated T cells and currently considered to be one of the common autoimmune disease susceptibility genes implicated in IDDM, Graves disease, Hashimoto thyroiditis, celiac disease, and SLE. The first reports on the association of CTLA-4 with IDDM [77, 78] were confirmed by meta-analysis [79].

An association of the 49A/G polymorphism with Graves disease was reported [80, 81], albeit another study could not confirm it [82]. From the pharmacogenomic aspect, the study of Kinjo and co-workers [83] is of particular interest. When investigating the 49A/G polymorphism of the CTLA-4 gene, the patients were divided according to the time of disappearance of the thyroid-stimulating hormone (TSH) receptor antibody after initiation of antithyroid drug treatment. Patients with positive TSH receptor antibody for over 5 years did not have the AA genotype and had higher frequencies of the GG genotype and the G allele compared to those showing positive TSH receptor antibody for 1 and 2 years. The periods of time until remission were significantly shorter in the AA genotype. The conclusion driven from these results was those patients suffering from Graves disease with the G allele need to continue antithyroid drug treatment for longer periods [83].

A significantly increased frequency of the 49A allele was found in patients with celiac disease compared to controls [84], and recently, a common CTLA-4 haplotype was shown to have strong association with the disease [85]. Van Belzen and co-workers [86] found no significant difference between patients and controls in the frequency of the 49G allele, but did find an increase in the frequency of the CT60 G allele in celiac disease patients. Finally, association has been documented between SLE and SNPs of the CTLA-4 gene. The -1722(T/C) polymorphism was shown to be significantly associated with SLE [87], this result being confirmed later by a meta-analysis [88].

Chromosome 5

Tokuhiro and co-workers [89] found that an intronic SNP in the runt-related transcription factor 1 (RUNX1; acute myeloid leukemia 1, aml1) binding site of solute carrier family 22 (organic cation transporter), member 4 (SLC22A4; 5q31). *SLC22A4*, designated slc2F2, is associated with RA in the Japanese population. A 2-allele risk haplotype indicating risk to Crohn's disease was found to be in strong linkage disequilibrium: a 1672C-T substitution in the SLC22A4 gene causing a leu503→phe (L503F) missense change, and a -207G-C transversion in the SLC22A5 gene [90]. Because of some conflicting findings, other authors suggested that certain haplotypes in defined populations may confer susceptibility or protection to Crohn's disease [91].

Chromosome 6

The MHC (6p21) has been shown to be associated with most, if not all common autoimmune diseases [92]. MHC is considered to play a role in determining genetic susceptibility to a given autoimmune disease by shaping the pool of presented peptides to T cells. While some diseases are associated with a single HLA-allele (like HLA-B27 in ankylosing spondylitis), other diseases including IDDM and multiple sclerosis (MS) are associated with groups of alleles [93, 94]. Besides MHC-encoded molecules involved in the cell surface antigen presentation, other MHC-encoded alleles also have been documented to be associated with autoimmune

diseases (including the gene encoding for TNF-α; 6p21.3). Recently, a relatively common Caucasian haplotype including the C4A null genotype, a short single C4B gene and the TNF2 allele of the TNF-α gene have been shown to be associated with smoking [95]. The significance of this finding is that tobacco smoking has been proposed to be a significant environmental factor in autoimmune diseases [96, 97].

In RA an association between the disease and the HLA complex has been observed in many different populations and most studies have focused on the role of HLA-DRB1 in disease susceptibility. Using 54 markers distributed across the entire HLA complex, HLA associations with RA were found to be complex and not completely explained by the class II, DR beta 1 (*DRB1*) locus [98].

A diallelic polymorphism in the promoter region of the nuclear factor of kappa light chain gene enhancer in B cells inhibitor-like 1 (NFKBIL1; 6p21.3) gene was shown to represent the second RA susceptibility locus within the HLA region, the first being HLA-DRB1 [99].

Chromosome 10

Several mutations of the FAS (10q24.1) gene have been identified in ALPS. The disease is characterized by massive nonmalignant lymphadenopathy, autoimmune phenomena, and expanded populations of TCR-$CD3^+CD4^-CD8^-$ lymphocytes, and shows defective FAS-mediated T-lymphocyte apoptosis *in vitro* [100-102].

Chromosome 16

A -168A-G polymorphism in the type III promoter of the MHC, class II, transactivator (MHC2TA; 16p13) gene was associated with increased susceptibility to RA, MS as well as with lower expression of MHC2TA after stimulation of leukocytes with INF-γ [103].

Chromosome 21

AIRE (21q22.3)was identified as the gene responsible for APECED. The AIRE protein contains motifs suggestive of a transcription factor, including 2 zinc finger (PHD-finger) motifs. Mutations of the AIRE gene have been associated with autoimmune polyendocrinopathy syndrome [104-110].

The transcription factor originally identified as a polyomavirus enhancer-binding protein (PEBP2) is composed of an alpha subunit, which binds to DNA *via* a Runt domain, and a beta subunit, which increases the affinity of the alpha subunit for DNA, but shows no DNA binding by itself. Runt-related transcription factor 1 (21q22.3) is one of several genes that encode the alpha subunit**.** Tokuhiro and co-workers [111] identified a SNP of RUNX1, designated runx1, in intron 6 (24658C-G) that was strongly associated with RA. Recently, RUNX1 has been also been implicated in the pathomechanism of psoriasis and SLE [112].

Chromosome X

FOXP3 (Xp11.23-q13.3), is a member of the forkhead/winged-helix family of transcriptional regulators, highly conserved in humans, and its protein product, scurfin, is essential for normal immune homeostasis. FOXP3 is expressed primarily in the $CD4^+CD25^+$ Treg cells. Immune dysregulation, polyendocrinopathy, enteropathy, and IPEX is one of a group of clinical multisystem autoimmune diseases [113]. Clinically, IPEX manifests most commonly with diarrhea, insulin-dependent diabetes mellitus, thyroid disorders, and eczema. Several mutations of the FOXP3 gene were found to be associated with IPEX [114-117].

Pharmacogenomic Approaches to Study Responsiveness to Anti-TNF-α Therapy in Crohn's Disease

TNF-α plays a central role in the pathophysiology of Crohn's disease including in the development of the mucosal lesions. The incidence, progression and the effective pharmacotherapy of Crohn's disease may depend on the genetic variations of TNFα gene, TNF receptor (TNFR) genes, TNFR1 and TNFR2, and genetics of postreceptor signaling molecules like the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and the nuclear factor kappa B (NF-κB) [118, 119]. The genetic variation may be also responsible for the adverse effects of pharmacotherapy.

The relatively recent development of genetically engineered agents has the potential to alter the treatment of Crohn's disease radically, and drugs that inhibit TNF-α, have been introduced as a new therapeutic class with high efficacy, rapid onset of action, prolonged effect, and improved tolerance. However, these agents are expensive and at least one-third of the eligible patients fail to show any beneficial response [120]. Finding a means to predict those who will respond, and to anticipate relapses, are therefore of obvious importance.

The anti-TNF-α monoclonal antibody, infliximab, induced remission in 30-40% of Crohn's disease patients. TNFR2 (exon 6, 196Arg; and 3'-UTR 1663, 1690) and TNF-α promoter polymorphisms [-238, -308, -376, -857, -1031, TNFR1 -609, +36 (exon 1)] were not associated with responsiveness to treatment in two independent cohorts (n= 444 and n=90), or with refractory Crohn's disease itself, and, therefore, they were suggested to be excluded as pharmacogenetic markers for a treatment response to infliximab and as etiologic factors for Crohn's disease [121]. Similarly, the three mutations in the caspase recruitment domain 15 (CARD15; NOD2) gene, which are independently associated with susceptibility to Crohn's disease, were not found to be associated with responsiveness to treatment [122].

In another study, the role of the TNFR functional mutations, TNFR2T587G and TNFR1A36G, in the pathogenesis of Crohn's disease and ulcerative colitis was investigated in 344 and 152 patients, respectively; with respect to disease phenotypes [119]. The TNFR2 587G allele was more frequent in ulcerative colitis patients and both single nucleotide polymorphisms were negatively associated with smoking in Crohn's disease. The complex and multifactorial etiology of Crohn's disease was shown by this association with smoking behavior. A relation between TNFR1A36G and pancolitis was found in ulcerative colitis. In the evaluation of the association of these mutations with the response to infliximab in 166 Crohn's disease patients, there was no clear effect of the polymorphisms on infliximab response, although the rare

TNFR1 36G allele was associated with a lower response to the drug (OR: 0.47) [119].

Although the studied TNFR polymorphisms did not alter significantly the response to infliximab in Crohn's disease patients, the expression of cytokines and cytokine receptors were modified by several factors, including steroid hormones. The outcome of therapy with drugs such as steroids may be influenced by a wide range of genetic factors including polymorphisms in the multi-drug resistance 1 (MDR1, ABCB1, PGP) gene, polymorphisms in glucocorticoid receptor genes, variations of transport proteins in the peripheral blood and other not defined factors. The association of TNF-α gene polymorphism and steroid responsiveness was studied in 193 patients *vs* 98 ethnically matched controls [123], while the aforementioned G-308A variation was the first published biallelic polymorphism of the TNF-α promoter [124]. Althought the frequencies of –308A allele, TNF2, were reported to be slightly, but not significantly lower in Crohn's disease patients than in control subjects, a more prominent difference in TNF2 frequencies and in the ratio of TNF2 carriers was found when comparing subgroups of patients. The frequency of the TNF2 allele was significantly higher in steroid-dependent (28.1%) than in non-steroid-dependent disease (19.3%), and tended to be higher in colonic than in small bowel disease and in fistulizing than in stricturing disease. TNF2 carriers tended to be more frequent in patients with steroid-dependent than non-steroid-dependent disease in patients with fistulizing and with colonic disease, while patients carrying at least one copy of the allele were reported to produce slightly more TNF-α at the colonic level [123]. Thus, it was concluded that TNF2 may have an influence on the behaviour of Crohn's disease. Carriage of the allele may favor steroid-dependent disease and to a lesser extent fistulizing and colonic disease, possibly secondary to a TNF-α-driven inflammatory reaction at the mucosal level [123].

Pharmacogenomic Approaches to Investigate Drug Metabolizing Enzymes and/or Drug Transporters in Autoimmune Diseases

Pharmacogenomics of autoimmune diseases links the genomic background of individual patients suffering from diseases with complex genetic background with efficiency or tolerability of the therapeutic interventions in order to develop optimal individualized therapy [125]. 6-Mercaptopurine, 6-thioguanine, and azathioprine are thiopurine drugs that are used in the therapy of autoimmune diseases. Thiopurines are metabolized by the enzyme thiopurine methyltransferase (TPMT; EC 2.1.1.67), the activity of which is controlled by a common polymorphism [126]. Patients with low or absent TPMT activity, when treated with standard thiopurine doses, are at life-threatening risk of thiopurine toxicity [127]. On the contrary, patients with genetically determined high TPMT activity may experience a decreased therapeutic effect when treated by thipurine [128].

These observations have opened new vistas towards individualized drug therapy. As an example, in RA, azathioprine doses can be optimized by reducing the doses for patients homozygous for mutant TPMT alleles, thus leading to reduced drug-induced morbidity due to severe myelosuppression and leucopenic events and to cost savings. In thiopurine toxicity and/or efficacy one important risk factor is the thiopurine S-methylation pathway catalyzed by TPMT. This is probably the best known example, as yet, for the clinical application of the pharmacogenomic knowledge.

Methotrexate (MTX) is the most widely used disease modifying antirheumatic drug (DMARD) in the treatment of RA, its use being limited by its variable efficacy and toxicity [129]. While in oncology an increasing body of evidence is currently available about the genomic background of MTX resistance, in RA limited evidence is available in the field. Two SNPs of the methylenetetrahydropholate reductase (MTHFR) gene have been investigated: C677T and A1298C. It was shown that RA patients, treated by MTX, are characterized by elevated plasma homocysteine levels that are even further increased by the C677T polymorphism. Elevated homocysteine levels were suggested to be associated with the GI toxicity of MTX [130]. In a separate study, it was shown that the effects of MTX on homocysteine metabolism (leading to elevated transaminase activities) were associated with the C677T polymorphism [131]. The presence of the A1298C polymorphism was shown to be associated with the improvement of several laboratory, but not clinical, parameters in patients with RA undergoing MTX treatment, while there was no effect of this SNP on MTX toxicity. On the contrary, the C677T polymorphism rendered RA patients more sensitive to MTX toxicity [132].

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 ideal understanding of the genomic background of complex diseases like allergy, asthma or autoimmunity. Regarding the multiple gene-gene and gene-environmental interactions, it is likely that we will never forecast whether a new-born will have asthma in the future. However, the available sequence information (including non-coding genome and the increasingly recognized series of regulatory microRNA profile); the completion of a high-quality physical map of the human and mouse genome; the different animal models [knock-out (KO) and transgenic animals]; and the advance of bioinformatics and different techniques (e.g. microarray, DNA sequencing, high throughput screenings) will make it possible that several additional asthma or autoimmune susceptibility genes and gene regulatory networks will be identified in the next years.

The limitations of the pharmacogenomical approaches are typical: while focusing on the genomic background, such studies lack a way to take in account very important factors, like the multitude of epistatic interactions, stochastic environmental effects and drug interactions. A major advantage of pharmacogenomical approaches is, however, that these days such studies can directly benefit from the recent developments of microarray-based methodologies and our currently growing understanding of systems biology, as well as of the wide use of bioinformatics in data analysis. Hopefully, this extremely rapidly knowledge will be soon translated into improved diagnosis, prevention and therapeutic strategies for these chronic diseases.

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LIST OF ABBREVIATIONS

ADAM	=	A disintegrin and metalloprotease domain
ADRB2	=	β_2 Adrenergic receptor
AHR	=	Airway hyperresponsiveness
AIA	=	Aspirin intolerant asthma
AIRE	=	Autoimmune Regulator
ALOX5	=	5-Lipoxygenase
ALPS	=	Autoimmune lymphoproliferative syndrome
Amb a V	=	Ragweed antigen
AP-1	=	Activator protein-1
APECED	=	Automimmune polyendocrinopathy candidiasis ectodermal dystrophy
APS1	=	Autoimmune polyglandular syndrome type I
BHR	=	Bronchial hyperresponsiveness
bp	=	Base pair
C1q	=	Complement Component C1q
CAMP	=	Childhood asthma management program
CC	=	C-C motif chemokines
CC16	=	Clara cell protein 16
CCL	=	Chemokine (C-C motif) ligand
CCR	=	CC chemokine receptor
CCR5Δ32	=	Deletion mutation in the CCR5
CRHR1	=	Corticotropin-releasing hormone receptor 1
CTLA-4	=	Cytotoxic T lymphocyte-associated antigen 4
CXCL	=	Chemokine (C-X-C motif) ligand
CYFIP2	=	Cytoplasmic fragile X mental retardation protein-interacting protein 2
CYFIP2	=	Cytoplasmic fragile X mental retardation protein-interacting protein 2
cysLT	=	Cysteinyl LT ()
DMARD	=	Disease modifying antirheumatic drug
DP2R	=	Prostanoid DP receptor 2
DPP	=	Dipeptidyl peptidase
DPP10	=	Dipeptidyl-peptidase 10
ETS	=	Epithelium specific transcription factor
FAS	=	TNF receptor superfamily, member 6
FASL	=	Fas ligand (; 1q23)
FCGR2B	=	Fc gamma receptor II b
FcεRI-γ	=	High affinity IgE receptor β subunit
FEV1	=	Forced expiratory volume in 1 second
FOXP3	=	Forkhead box P3
GC	=	Glucocorticoid
GM-CSF	=	Granulocyte-macrophage colony-stimulating factor
GPCR	=	G protein-coupled receptor
GPRA	=	G protein-coupled receptor for asthma susceptibility
HLA	=	Human leukocyte antigen
IDDM	=	Insulin-dependent diabetes mellitus
IgE	=	Immunoglobulin E
IL	=	Interleukin
INF	=	Interferon
IPEX	=	Immundysregulation, polyendocrinopathy, enteropathy, X-linked inheritance
ITIM	=	Intracellular immunoreceptor tyrosine-based inhibitory motif
JNK	=	c-Jun N-terminal kinase
KO	=	Knock-out
LEKTI	=	Lymphoepithelial Kazal-type-related inhibitor; SPINK5
LnIgE	=	Natural log of IgE concentrations
LOD	=	Logarithm of odds
LPS	=	Lipopolysaccharide
LPS	=	Lipopolysaccharide
LT	=	Leukotriene
LTA	=	Lymphotoxin-α
LYP	=	Lymphoid tyrosine phosphatase
MCP-1	=	Monocyte chemoattractant protein-1
MDR1	=	Multi-drug resistance 1 (ABCB1, PGP)
MHC	=	Major histocompatibility gene complex
MHC2TA	=	Major histocompatibility complex, class II, Transactivator
MIP	=	Macrophage inflammatory protein
MS	=	Multiple sclerosis
MTHFR	=	Methylenetetrahydropholate reductase
MTX	=	Methotrexate
NF-κB	=	Nuclear factor kappa B
NFKBIL1	=	Nuclear factor of kappa light chain gene enhancer in B cells inhibitor-like 1
NFY-β	=	β-Subunit of nuclear factor Y

NNOS	=	Neuronal nitric oxide synthase (NOS1; nNOS)
NO	=	Nitric oxide
OMIM	=	Online mendelian inheritance in man
OR	=	Odds ratio
PAD	=	Peptidylarginine deiminase
PBMC	=	Peripheral blood mononuclear cells
PEBP2	=	Polyomavirus enhancer-binding protein
PHD	=	Plant homeodomain
PHD 2	=	Zinc finger motif
PHF11	=	Plant homeodomain finger protein-11
PTPN22	=	Protein tyrosine phosphatase, nonreceptor-type, 22
RA	=	Rheumatoid arthritis
RANTES	=	Regulated on activation normal T cell expressed and secreted
RCBTB1	=	RCC1 and BTB (POZ) domain containing protein 1
RCC1	=	Regulator of chromosome condensation
RUNX1	=	Runt-related transcription factor 1 (aml1: acute myeloid leukemia 1)
SAPK	=	Sstress-activated protein kinase
SCF	=	Stem cell factor
SETDB2	=	SET domain bifurcated 2
SLC22A4	=	Solute Carrier Family 22 (Organic Cation Transporter), Member 4
SLE	=	Systemic lupus erythematosus
SNP	=	Single nucleotide polymorphism
SPINK5	=	Serine peptidase inhibitor, Kazal type 5; LEKTI
STAT6	=	Signal transducer and activator of transcription 6
T-bet	=	T-box expressed in T cells
TCR	=	T cell receptor
Th	=	T helper lymphocyte
TIM	=	T-cell integrin mucin-like receptor
TNF	=	Tumor necrosis factor
TNF2	=	–308A allele of the TNF-α
TNFR	=	TNF receptor
TNFRFS6	=	TNF receptor superfamily, member 6; Fas
TPMT	=	Thiopurine methyltransferase
Treg	=	Regulatory T cells
TSH	=	Thyroid-stimulating hormone

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