

A review of asthma genetics: gene expression studies and recent candidates

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Abstract. Recent evidence indicates an important role of inflammation pathways, airways remodeling and epithelium activation in asthma genetics. In particular, transcriptome studies have detected differentially expressed genes involved in eosinophil apoptosis, the arginase pathway, response to allergens or interleukins, and to inhaled corticosteroids. Candidate gene and genome wide studies have localized genetic regions involved in the disease, such as the *AIAR* and *CLCA1* genes (chromosome 1), *IL-1RN* and *DPP10* (2q14), *HLA-G* and *TNF- α* (6p21), *GPR4* (7p14), *Fc ϵ RI* and *GSTP1* (11q13), *NOS1*, *IFNG*, *STAT6*, *VDR*, and other genes (12q13-26), *PHF11* and flanking genes (13q14), *AACT* and *PTGDR* (14q), and *ADAM33* (20p13). The role of these and other genetic determinants has to be confirmed in future, preferably longitudinal, studies.

Key words: association, asthma, atopy, expression, gene, genome, haplotype.

Introduction

Asthma is a common and complex condition, with considerable heterogeneity both in its phenotype and in the underlying pathophysiology. The major symptoms of asthma are paroxysms of dyspnoea, wheezing and cough, although symptoms may vary from one patient to another, and response to treatment may differ markedly even in patients with similar symptoms (Holgate 1999). An important goal of asthma research is to understand the genetic and environmental triggers for asthma and the factors that lead to variations in its natural history. It is known that inflammation is a key element in the diathesis (Wills-Karp and Ewart 2004).

Cells and mediators

Recent studies have demonstrated that airway inflammation is a principal feature in the patho-

physiology of asthma (Gern et al. 1999). The disorder is multifactorial (in both initiation and progression) because of the involvement of numerous resident and recruited inflammatory cells. T cells and IgE-mediated responses are known to be a key factor in the allergic response (Elias et al. 2003). The dendritic cells are the bridge between allergens and T cells through antigen-processing events. In response to allergens the T lymphocytes produce a restricted array of cytokines. In particular the pro-inflammatory cytokines are synthesized by the Th2 subtype of T helper cells. The other subtype, the Th1 cells are involved in virus defense and antagonize the allergic response. An imbalance in the expression of T cell phenotype is thought to play an important role in the pathophysiology of asthma (Yazdanbakhsh et al. 2002; Martinez 2001). Atopic patients show increased levels of interleukin 4 (IL-4), which is responsible for the induction of the Th2 cell response that in turn provides the signals for IgE

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production. T helper cells are not the only source of proallergic cytokines: mast cells, basophils, eosinophils, CD8⁺ T cells and bronchial, fibroblast and smooth muscle cells can all produce inflammatory molecules as those encoded by the 5q cytokine cluster, including IL-4, IL-5, IL-9 and IL-13 (Key et al. 2002; Renauld 2001). Cross-linking to the IgE on mast cells β subunit of the high affinity IgE receptor (Fc ϵ RI) triggers the release of preformed vasoactive mediators, synthesis of prostaglandins and leukotrienes, and transcription of cytokines (Wenzel et al. 2003). The allergen-specific IgE are also induced by the interaction of the IgE with the Fc ϵ RII receptor (CD23) on B cells. IgE production is associated with allergic hypersensitivity responses to inhaled allergens. The release of neutral protease from mast cells, through the IgE receptor, can activate the receptors of endothelial and epithelial cells that in turn leads to the production of cytokines and adhesion molecules that selectively recruit eosinophils and neutrophils, cells relevant to the inflammatory manifestations (Cohn et al. 2004). Eosinophils play a key role in the pathogenesis of allergic asthma (Bandeira-Melo and Weller 2003). They secrete a number of inflammatory mediators including cytokines, tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β), prostaglandin E2 (PGE2), cysteinyl leukotriens and platelet activating factor (PAF), as well a number of reactive oxygen intermediates, cytotoxic peptides and degradation enzymes such as elastase and collagenase (Carey et al. 2003; Chanez et al. 1990; Behm and Ovington 2000). Thus the inflammatory response is also involved in the damage and repair of the host tissue.

Genes

The activation of many different cellular types (B and T cells, eosinophils, dendritic cells, etc) and molecules (cytokines, intracellular mediators) in space and time (tissue remodeling, early phase, late phase) show how complex and heterogeneous the ethiopathogenesis of asthma is. So it should not be surprising that many genes have been reported and that the linkage results and association studies have been replicated with difficulty among the studies. Many genes might contribute to the variability of the phenotypes according to environmental exposure and genetic background of the population. Environmental factors may uncover pre-existing susceptibility genes within popula-

tions. Moreover, methodological issues (estimation of significance, power of the study, definition of the phenotype, selection criteria, sample structure and size, DNA markers used) that affect the reliability of the results, as discussed in many reports, must be taken into account (Hoh and Ott 2003; Zondervan and Cardon 2004). There is strong evidence that genes confer susceptibility rather than necessarily the disease itself as in Mendelian disease (Clarke et al. 2000; Skadhauge et al. 1999; Chen et al. 2001; Sampogna et al. 2000; Holberg et al. 1996; Xu et al. 1995). The early genetic studies confirmed the biophysiological observations by reporting linkage or association with the expected candidate MHC II region (Blumenthal et al. 1992), the IL-4 gene cluster (Meyers et al. 1994; Levitt et al. 1995) and the high affinity receptor for IgE with a proposed maternal inheritance (Shirakawa et al. 1994; Trabetti et al. 1998; Doull et al. 1996; Cui et al. 2003). Other studies followed, reporting several genes or chromosomal regions such as chromosome 12 (*IFGN*, *ITG β -7*, *STAT6*, *NOS1*, *STAT6*), 5 (*IL-4* cluster genes, *ADRB2*, *CD14*, *SPINK5*), 7 (*TCRG*), 11 (*Fc ϵ RI*), 13, 14 (*AACT*), 19 (*Fc ϵ RII*), and many others (Hakonarson and Wjst 2001; Venanzi et al. 2001; Trabetti et al. 1998; Hoffjan et al. 2003; Gao et al. 2004).

Recently, positional cloning and fine mapping studies indicated novel genes, often with unclear functions, to be linked to asthma in several populations.

In this paper we review some recent results from fine mapping and gene expression studies, and we describe insights into genetic programs of disease pathogenesis from experimental models of asthma induced by different allergens and protocols (exposure to cytokines, drugs, etc.). Table 1 reports a summary of asthma related genes mentioned in this review.

Gene expression studies

DNA microarray techniques can be used to study gene expression of a large number of loci. The expression can be monitored simultaneously and the expression profiles may be compared in different samples. The difference in the sample may be due to the time interval at which the gene expression is observed, exposure to a treatment, cell types studied and other causes. The heterogeneity of disease phenotype may be studied with many different methods in order to collect information that may contribute to the overall understanding of the disease. This technology has been used in

asthma with different intents and reveals to be a reliable tool to confirm previous observations or to provide new clues for metabolic pathways involved in the pathophysiology of the disease.

Eosinophil apoptosis

Gene expression of the eosinophils and their role in the pathophysiology of asthma have been extensively studied (Temple et al. 2001). The study reported changes in messenger RNA expression profile after treatment with IL-5. Eighty genes were observed to show a difference in regulation between eosinophils treated or non-treated with IL-5. The majority of these gene (73) were up-regulated, whereas only seven genes were down-regulated. The up-regulated genes are supposed to be involved in adhesion (*ICAM-1*, *CD24*), migration, activation (*IL-8*, *ERK-3*, *CCR-1*, *CD69*), or survival of eosinophils or hematopoietic cells (*Pim-1*, *EGR-1*). The expression of four genes (*Pim-1*, *SLP-76*, *DSP-5*, *CD24*) that play a role in the survival of eosinophils in response to IL-5 was reported, 2 of which (*Pim-1* and *SLP-76*) were restricted to eosinophils. These two genes may be members of important pathways that regulate inflammatory events by controlling the survival of eosinophils.

The arginase pathway

Zimmerman and co-workers performed a gene expression study of 12422 genes, and they found that 291 genes were commonly involved in disease pathogenesis after exposure to several different allergens. They concluded that 6.5% of the tested transcriptome was altered in an asthmatic lung (Zimmerman et al. 2003). In particular, they found a concerted overexpression of the genes encoding molecules involved in the metabolism of arginine.

The enzyme arginase I is involved in the production of polyamines (e.g. putrescine, spermidine) and proline which controls cell proliferation and collagen production. Thus, it may be involved in the regulation of apoptosis. The authors propose that arginine is metabolized not only by nitric oxide synthase (NOS) enzymes (producing NO and L-citrulline), but also by arginase (producing polyamines and proline). In 2004 Kocyigit et al. reported that arginase activity correlates with manganese (Mn), an element required for arginase activity and stability. Lower Mn concentration could cause lower arginase activity favouring the upregulation of NO in asthma (Kocyigit et al. 2004a; 2004b).

The response to allergen or IL-4

A microarray study (40,000 genes) has been performed in a monkey model of allergic asthma. Gene expression was evaluated after the inhalation of the *Ascaris suum* antigen (bronchoconstriction) or IL-4 (allergic response). Gene expression of the lung tissue was measured 4, 18 and 24 h after antigen challenge. One hundred and forty nine genes were differentially expressed 4 hours after the challenge. The authors described 5 gene clusters that comprise pulmonary and activation-related chemokines, vascular cell adhesion molecule 1 (VCAM1), IL-4 inducible genes (eotaxin, VCAM1, MCP-1, MCP-3), tissue remodeling factors (tissue inhibitor of metalloproteinase I TIMPI, plasminogen activator inhibitor-1 PAI-1), chitinase protection from damage by proteolytic enzymes, and several antioxidants (SOD1, SOD2, GPX). This confirms the evidence that oxidative stress and reactive oxygen species contribute to inflammation in asthma (Kinnula and Crapo 2003). It is worth noting that among the differentially expressed genes there is alpha1-antichymotrypsin (*AACT*), a proteinase inhibitor that was associated with elevated IgE in a sample of Italian families (Malerba et al. 2001). The largest change in expression level was observed in the cluster of downregulated genes (CCAAT binding transcription factor β (*NF-Y\beta*), *SYBII*, aminopeptidase A, SLAM inducing Th2 responses), showing that they do not respond to IL-4 treatment, but to allergen challenge. Moreover, other genes showed a differential expression 24 h after IL-4 inhalation, but not by antigen challenge.

Profiling atopy and asthma

Atopy and asthma are strongly correlated. Brutsche and co-workers searched for a cluster of genes, which may help in assessing disease status, evaluating the expression of 609 genes in atopic asthmatics, non-atopic asthmatics and healthy individuals (Brutsche et al. 2002). They developed a composite atopy gene expression (CAGE) score including 10 genes dysregulated in atopic individuals. The CAGE score was reported to be better than IgE in differentiating atopic from non-atopic individuals. The score correlated with IgE levels and showed a trend of correlation with asthma severity. It is interesting to note that IL-1R is one of the ten genes that were chosen to compute the CAGE score and it maps in the IL-1 gene

Table 1. Asthma related genes and their location

Description	Gene	Chromosome	Genomic location from p-ter
1	2	3	4
Early growth response protein 1	<i>EGR-1</i>	1p34	45.5
Prostaglandin E receptor 3	<i>PTGER3</i>	1p31	71
Chloride channel calcium activated family member 1	<i>CLCA1</i>	1p31	86
Vascular cell adhesion protein 1 precursor	<i>V-CAM 1</i>	1p21	100.9
Glutathione-S-transferase	<i>GSTM1</i>	1p13	109.9
Adenosine A3 receptor	<i>A3AR;ADORA3</i>	1p13	111.7
Adenosine A1 receptor	<i>A1AR;ADORA1</i>	1q32	199.8
Transforming growth factor beta 2 precursor	<i>TGF-β-2</i>	1q41	214.9
Interleukin-1 receptor	<i>IL-1R1</i>	2q11	102.2
High affinity interleukin-8 receptor A	<i>IL-8RA; CXCR1</i>	2q35	218.9
Interleukin-1 receptor antagonist protein precursor	<i>IL-1RN</i>	2q13	113.6
Interleukin-1 alpha precursor	<i>IL-1α</i>	2q13	113.2
Interleukin-1 beta precursor	<i>IL-1β</i>	2q13	113.3
Dipeptidylpeptidase 10 isoform 1	<i>DPP10</i>	2q14	114.9
C-C chemokine receptor type 1	<i>CCR1</i>	3p21	46.2
Interleukin-8 precursor	<i>IL-8</i>	4q13	75.0
Aminopeptidase A	<i>APA</i>	4q25	111.8
Interleukin-5 precursor	<i>IL-5</i>	5q23	131.9
Interleukin-4 precursor	<i>IL-4</i>	5q23	132
Interleukin-13 precursor	<i>IL-13</i>	5q23	132
Interleukin-9 precursor	<i>IL-9</i>	5q31	135
Monocyte differentiation antigen CD14 precursor	<i>CD14</i>	5q31	140
Serine protease inhibitor Kazal-type 5 precursor	<i>SPINK5</i>	5q32	147.4
Beta-2 adrenergic receptor	<i>ADRB2</i>	5q32	148.2
Plasma glutathione peroxidase precursor	<i>GPX3</i>	5q33	150.4
SH2 domain-containing leucocyte protein	<i>SLP-2 LCP2</i>	5q35	169.6
Lymphocyte cytosolic protein 2	<i>SLP-76</i>	5q35	169.6
HLA class I histocompatibility antigen – alpha chain G precursor	<i>HLA G</i>	6p21	30
Major histocompatibility complex – class II – DR beta 1	<i>HLA-DRB1</i>	6p21	32.7
Tumor necrosis factor precursor	<i>TNF-α</i>	6p21	37.1
Pim-1 oncogene	<i>PIM1</i>	6p21	37.2
Peroxisome assembly factor-2	<i>PAF-2</i>	6p21	43
Arginase I	<i>ARG1</i>	6p23	131.9
Superoxide dismutase 2 mitochondrial	<i>SOD2</i>	6q25	160.1
Interleukin-6	<i>IL-6</i>	7p15	22.5
G-protein-coupled receptor for asthma susceptibility	<i>GPRA</i>	7p14	34.3
T cell receptor gamma	<i>TCRG</i>	7p14	38
Epidermal growth factor receptor precursor	<i>EGFR</i>	7p11	59.9
Plasminogen activator inhibitor-1 precursor	<i>PAI-1 o SEPRINE1</i>	7q22	100.4
Nitric-oxide synthase – endothelial	<i>eNOS; NOS3</i>	7q36	150.1
Peroxisome assembly factor-1	<i>PAF-1</i>	8q21	78.1

1	2	3	4
Prostaglandin E synthase	<i>PTGES</i>	9q34	129.6
Mucin 2	<i>MUC2</i>	11p15	
Prostaglandin D2 receptor DP	<i>PTGDR</i>	11q	51.8
High affinity immunoglobulin epsilon receptor beta-subunit	<i>FcεRI</i>	11q12.1	59.6
Glutathione-S-transferase	<i>GSTP1</i>	11q	67.1
Early activation antigen CD69	<i>CD69</i>	12p13	9.8
Vitamin D3 receptor	<i>VDR</i>	12q13	46.5
Signal transducer and activator of transcription 6	<i>STAT6</i>	12q13	55.8
Interleukin-1 receptor-associated kinase 3	<i>IRAK3</i>	12q14	64.9
Interleukin-22 precursor	<i>IL-22</i>	12q15	66.9
Interferon gamma precursor	<i>IFNG</i>	12q15	68.8
Kit ligand precursor	<i>KITLG</i>	12q21	87.4
Nuclear transcription factor Y subunit beta	<i>NF-YB</i> ; <i>CCAAT</i> -binding transcription factor subunit A	12q23	103
Nitric-oxide synthase type I	<i>nNOS</i> ; <i>NOS1</i>	12q24	116.9
SET domain bifurcated 2	<i>SETDB2</i>	13q14	48.9
PHD finger protein 11	<i>PHF11</i>	13q14	49
Regulator of chromosome condensation	<i>RCBTB1</i>	13q14	49
Regulator of chromosome condensation	<i>RCC1</i> ; <i>RCBTB1</i>	13q14	49
Prostaglandin E receptor 2	<i>PTGER2</i>	14q22	51.9
Arginase II	<i>ARG2</i>	14q24	67.2
Alpha-1-antichymotrypsin precursor	<i>AACT</i>	14q32	94.1
Extracellular signal-regulated kinase 3	<i>ERK-3</i>	15q21	50.1
Arachidonate 15-lipoxygenase	<i>ALOX15</i>	17p13	4.5
Nitric oxide synthase - inducible	<i>iNOS</i> ; <i>NOS2</i>	17q11	23.1
Small inducible cytokine A2 precursor	<i>CCL2</i> ; <i>MCP-1</i>	17q12	29.6
Small inducible cytokine A7 precursor	<i>CCL7</i> ; <i>MCP-3</i>	17q12	29.6
Squamous cell carcinoma antigen 1	<i>SCCA-1</i> <i>SerpinB4</i>	18q21	59.5
Low affinity immunoglobulin epsilon Fc receptor	<i>Fc-ε-RII</i> ; <i>CD23</i>	19p13	7.7
Intercellular adhesion molecule-1 precursor	<i>ICAM-1</i>	19p13	10.2
Prostaglandin E receptor 1	<i>PTGER1</i>	19q13	14.4
Transforming growth factor beta 1 precursor	<i>TGF-β-1</i>	19q13	46.5
Disintegrin and metalloproteinase domain 33	<i>ADAM33</i>	20p13	3.6
Superoxide dismutase [Cu-Zn]	<i>SOD1</i>	21q22	32.0
Prostaglandin-E(2) 9-reductase	<i>CBR1</i>	21q22	36.4
Glutathione-S-transferase	<i>GSTT1</i>	22q11	22.7
Tissue inhibitor of metalloproteinase 1	<i>TIMP1</i>	Xq11	47.2
Synaptobrevin-like protein 1	<i>SYBL1</i>	Xq28	154.7
Signal transducer CD24 precursor	<i>CD24</i>	Yq11	19.5
Synaptobrevin-like protein 1	<i>SYBL1</i>	Yq12	57.6

Chromosome and genomic locations have been extracted from the Ensembl Genome Browser – Ensembl v27 (<http://www.ensembl.org/>). Genes were arbitrarily ordered by location from chromosome 1 to the sex chromosomes.

cluster on chromosome 2, that recently has been shown to be linked to asthma (see chromosome 2).

Inhaled corticosteroid therapy

Laprise et al. reported a microarray study (12000 oligonucleotide probes) to detect differentially expressed genes in bronchial biopsies of asthmatic patients after inhaled corticosteroid therapy (ICS). They identified 79 differentially expressed genes in patients versus controls. A comparison of the patients before and after ICS treatment revealed a difference of expression for 128 genes, suggesting that the treatment triggers new metabolic pathways. Expression levels of 26 of the 79 genes were partially or totally corrected after inhaled corticosteroid therapy, including the expression of the proteolytic enzymes.

Candidates, fine-mapping and genome studies

Chromosome 1: The *A1AR* and *CLCA1* genes

Adenosine is a regulatory nucleoside that is generated in response to cellular stress and injury. Distinct pro- and anti-inflammatory activities are likely to be mediated by the expression profile of specific adenosine receptors (ARs) on different cells. Some studies have shown that A1 adenosine receptor (A1AR) signaling can produce neutrophil and monocyte activation. Adenosine is a signaling molecule that has been associated with the regulation of asthma and other pulmonary diseases (Banerjee et al. 2002; Tilley and Boucher 2005). Sun et al. studied the contribution of the A1AR signaling to the lung inflammation and damage observed in adenosine deaminase deficient (ADA deficient) mice that exhibit elevated adenosine levels (Sun et al. 2005). The removal of the *A1AR* gene resulted in enhanced pulmonary inflammation, along with increased mucus metaplasia and alveolar damage associated with excessive expression of IL-4 and IL-13 in the lungs, with an increased expression of chemokines and matrix metalloproteinases. A1AR signaling may serve to regulate the severity of pulmonary inflammation and remodeling in chronic lung disease by controlling the levels of important mediators of inflammation and damage. Adenosine contributes to mucus hypersecretion by airway epithelial cells and upregulates mucin (MUC2) expression. The pathway is initiated at the A1AR that transduces signals through a Ca²⁺ activated Cl⁻ channel and the epidermal growth factor receptor (EGFR). In 2004, Kamada et al. reported the association of *CLCA1* gene polymorphisms and

haplotypes with childhood and adult asthma in the Japanese population (Kamada et al. 2004). In 2002, Toda and co-workers reported the up-regulation of *CLCA1* by IL-9, and supported the hypothesis that the *CLCA1* channel may be responsible, in part, for the overproduction of mucus in asthmatic patients (Toda et al. 2002).

This shows the value of information that could be derived from partial or complete knowledge of pathways for the formulation of new candidate genes. A1AR plays a role in the regulation of inflammation by promoting IL-4 and IL-13 production, and stimulates mucus production by the regulation of *CLCA1* (McNamara et al. 2004). The combinations of several approaches (experimental models, gene expression, frequency of gene variation) has led to new insight into a pathway that is likely to be involved in mucus production in inflammation.

Chromosome 2: One or more genes?

Two independent groups have recently reported the results of their positional cloning study on chromosome 2. The two groups described 2 different candidate genes, *IL-R1N* and *DPP10*, both mapping in the region of the IL-1 cluster on chromosome 2q14. Gohlke and al. reported an association with SNPs in the *IL-1RN* gene in the German and Italian asthmatic populations (Gohlke et al. 2004). They did not study SNPs in the *DPP10* gene. Allen and al. reported a lack of association in the *IL-1RN* gene in British and German populations, but found an association with SNPs of the *DPP10* gene, which is positioned close to the *IL-1RN* gene (Allen et al. 2003). Neither study has resulted in a definitive conclusion.

IL-1RN is a 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. The region was identified after a genome scan, and a subsequent fine mapping approach using 219 SNPs. A mouse model for ovalbumin-induced airway hyperresponsiveness and one for metacholine-induced airway responsiveness described linkage with the human syntenic region (Heinzmann et al. 2002). A stronger association was found in the *IL-1RN* gene where sequencing showed 28 additional DNA variants. The region showed a strong (LD) and 6 SNPs were found to be sufficient for tagging all haplotypes with a prevalence greater than 1%. This means that the analysis of only 6 SNPs would capture the haplotype variability in the *IL-1RN* gene. Three SNPs (rs2234678, rs878972,

rs454078) were associated with asthma in the German sample and 2 SNPs (rs2234678, rs878972) in the Italian families. However, the authors were not able to find a single causative mutation. The authors stated that it is unlikely that a gene flanking the *IL-1RN* gene is responsible for the association results, because all significantly associated SNPs or haplotypes are located in, or close to, *IL-1RN* in the German families. *DPP10* is 800Kb distal to the IL-1 gene cluster. The linkage and fine mapping on 244 UK families identified a haplotype located in a LD island which was associated with asthma. The same haplotype was also associated with asthma in a sample of 1047 German asthmatic children. Moreover, a haplotype (WTC122P*1 – D2S308*3) was more frequent among steroid-dependent asthmatics. The only gene expressed from the region was *MEX4FB-1*, coding for the DPP10 protein. No coding polymorphism in the *DPP10* gene was found, suggesting that the effect on asthma susceptibility may reside in regulatory elements leading to alternative splicing.

Chromosome 6: One more susceptibility gene and maternal effect

The human major histocompatibility (MHC) genes and many other genes that play an important role in the regulation of the immune system map on chromosome 6p21 (Shiina et al. 2004). The 6p21 region has shown strong linkage to atopic phenotype and asthma in many studies (Cookson 2002; Hakonarson and Wjst 2001; Moffat et al. 2003), and it is considered a major locus influencing allergic diseases. Other researchers reported an association with the *TNF- α* gene (Trabetti et al. 1999; Shin et al. 2004). Recently, Nicolae and co-workers described the association of HLA-G in 3 populations (Nicolae et al. 2005). They reported evidence from 4 independent samples in support of the *HLA-G* gene as a novel asthma and bronchial hyperresponsiveness susceptibility gene in the human leukocyte antigen region. By the positional cloning approach they identified a gene polymorphism (–944 A/G) associated with asthma in two samples. They also reported a differential association of alleles with childhood disease on the basis of the maternal affection status. The –964 GG genotype was associated with asthma among children of mothers with bronchial hyperresponsiveness (BHR), whereas the –964 AA genotype was associated with asthma among children of mothers with a negative BHR affection status. Thus, the susceptibility for asthma linked to

6p21 is complex and may be influenced by maternal factors.

Chromosome 7: The *GPRA* gene

Populations isolated for a long time or originating from a relative small number of individuals are likely to exhibit greater genetic homogeneity than outbred populations and are potentially important in multigenic disease mapping due to a lower number of risk genes for a given disease. In Finnish and French-Canadian families in 2001, a genome scan for asthma and IgE showed linkage of a 20cM region on chromosome 7p14-p15, and in 2004 the orphan G protein-coupled receptor gene (*GPRA*, G-protein-coupled receptor for asthma susceptibility) has been associated with elevated IgE (Laitinen et al. 2001; 2004). Some SNPs and several haplotypes in the region of 70 Kb were found to be associated with elevated IgE levels, clinical asthma, and atopy. At least one haplotype was observed to be associated with elevated IgE levels in each population. However, the risk haplotypes were different in the different populations.

Chromosome 11: Not only the *Fc ϵ RI* gene

The glutathione-S-transferase (*GST*) genes and other genes involved in the oxidation stress have been described to be involved in asthma and atopy (Barnes 1990; Fryer et al. 2000; Aynacioglu et al. 2004; Tamer et al. 2004; Gilliland et al. 2002; Hemmingsen et al. 2001). Gene polymorphisms and differential expression levels of the *GST* genes have been associated with asthma, atopy and lung function (Brasch-Andersen et al. 2004). It is remarkable that the *GSTP1* maps on the same region of the frequently mentioned *Fc ϵ RI* gene, and it has been proposed as an alternative or complementary explanation for the linkage of chromosome 11q13. In 2003 Child and co-workers reported a maternal association of genetic variants of the *GSTP1* with asthma in children, supporting the hypothesis of maternal inheritance and providing an alternative candidate to *Fc ϵ RI* (Child et al. 2003).

Chromosome 12: Oxidative stress and the *VDR* gene

Several studies have shown linkage of chromosome 12q13-26 to asthma or related phenotypes (Malerba et al. 2000; Raby et al. 2003; Xu et al. 2002; Barnes et al. 1999; Wjst et al. 1999) in different populations. The linkage shown spans a wide region, suggesting that more than 1 asthma susceptibility gene may be located on chromosome 12 (Cookson and Moffatt 2004; Hoffjan and

Ober 2002; Gao and Huang 2004). The hunt for gene identification is still on.

Oxidative stress, with the formation of reactive oxygen species is a key component of inflammation (Barnes 1990). The NOS enzymes are involved in the synthesis of nitric oxide (NO) from arginine. The role of NO, a molecule having a number of important biological functions, in case of asthma is still unknown. It has been proposed to be a marker for airways inflammation and NO concentration, closely correlated with the percentage of eosinophils in BAL fluids (Warke 2002; Warke 2004). NO is produced by a group of enzymes referred to as nitric oxide synthase: endothelial (eNOS, chromosome 7), neuronal (nNOS, chromosome 12q32) and inducible NOS (iNOS, chromosome 17). The association of some nNOS marker with asthma or related phenotypes has been reported (Gao et al. 2000; De Sanctis et al. 1999), and recently confirmed in the Japanese population (Shao et al. 2004).

Recently Vollmert et al. studied the region containing the integrin beta-7 (*ITG β -7* with 7 SNP) and the vitamin receptor D (with the sole protein-modifying SNP *FokI* 46). They concluded that neither the *ITG- β 7* gene nor the *FokI* SNP in the *VDR* gene seem to be associated with asthma or related phenotypes in 172 German asthmatic families (Vollmert et al. 2004). At the same time Raby et al. reported the association of *VDR* gene polymorphisms in child and adult asthma after studying 7 candidate genes (*IFNG*, *STAT6*, *CPM*, *KITLG*, *IL-22*, *IRAK3*, *VDR*) with 28 DNA markers in 582 family trios (Raby et al. 2004b). Of the 7 SNPs in the *VDR* gene the *FokI* 46 did not show any association with asthma and was not in linkage disequilibrium with any of the other 6 SNPs. The association with the *VDR* SNPs was replicated in a case-control study (1034 individuals), but the allele associated was different from that observed in the trios (Poon et al. 2004). A recent study of experimental models showed the importance of the vitamin D endocrine system in the generation of the Th2- driven inflammation in the lung (Wittke et al. 2004).

Chromosome 13: Genes influencing IgE levels

Several studies confirmed linkage of atopy and the related phenotype to chromosome 13q14 (Hakonarson and Wjst 2001). Zhang et al. reported a region linked to IgE levels in asthma (Zhang et al. 2003). The region centered on the *PHF11* gene and extended to two flanking

genes, *SETDB2* and *RCBTB1*. They identified 3 SNPs having independent effects and a 3 marker haplotype showing consistent association in other 3 sample sets. The recent genome scan in the Italian population showed a multipoint linkage of D13S156 and the elevated IgE, supporting the linkage of 13q14 with the IgE levels (Malerba, in preparation).

Chromosome 14: The *PTGDR* gene

Linkage of chromosome 14 markers with asthma or related phenotypes has been illustrated in several reports and some gene associations have been described, such as *AATC* (Malerba et al. 2001; Hakonarson et al. 2002).

Recently Mansur et al. reported the linkage and association of the D14S63 marker with total serum IgE levels in asthmatic families (Mansur et al. 2004). Marker D14S63 is physically located 16 Mb from the prostaglandin D2 receptor (DP) gene (*PTGDR*). *PTGDR* is present on mast cells and eosinophils, which generate the effector molecules of the asthmatic diathesis (Kabashima and Narumiya 2003). Oguma and co-workers have described a specific haplotype of the gene to be associated with asthma in both white and black individuals, suggesting that the gene polymorphisms play a common role in different populations (Oguma et al. 2004). The authors observed that the haplotype with the lower risk of asthma presented a low transcription efficiency, providing a functional role to the haplotype.

Chromosome 20: Airways remodeling

In 2002, Van Eerdewegh et al. reported a new gene associated with asthma, referred to as *ADAM33* (chromosome 20p), the function of which remains unclear. The ADAM proteins are implicated in proteolysis during extracellular signaling (Bridges et al. 2004). It has been suggested that *ADAM33* may be involved in cellular adhesion and airway wall remodeling (Cakelbread et al. 2004; Holloway et al. 2004). Several studies have confirmed the association with asthma (Lee et al. 2004; Jongepier et al. 2004; Powell et al. 2004; Werner et al. 2004), whereas others did not observe any association (Lind et al. 2003). Howard reported the association of SNPs in the *ADAM33* gene with asthma in 4 populations (US Caucasian, Dutch, US Hispanic and Afro-American) (Howard et al. 2003). However, no single SNP was associated across all the populations tested and no single haplotype accounted for asthma susceptibility risk. These results validate the new paradigm for asthma pathogenesis, in which exaggerated

inflammation and remodeling are the consequence of abnormal injury and repair responses (Davies et al. 2002).

Conclusions

Gene identification analyses have indicated several chromosomal regions and genes, involved in the causation and progression of asthma symptoms.

On the other hand, the role of environmental and lifestyle factors has to be determined, in order to unravel the complex interactions of genetic and environmental determinants of the disease and the response to therapy in individual patients (Pignatti 2004a, b).

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