# Genes and severe asthma

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**SUMMARY. Asthma is a chronic inflammatory disease of the airways, affecting approximately 155 million people worldwide. The causes are multifactorial and are attributed to several interactions between various different genes and environmental exposures. These interactions can affect either the susceptibility of a person to develop of asthma or the severity of the disease. This review article presents recent approaches to the understanding of the genetic influences on the severity of asthma expression. Specifically, genetic studies conducted in adults with severe asthma, and the spectrum of the genetics of lung function are reviewed, with special focus on pharmacogenetics and the genetic factors that affect the response to asthma treatment. Finally, environmental interactions and epigenetic mechanisms are discussed. *Pneumon 2011, 24(3):321-329.***

## INTRODUCTION

Asthma is a chronic inflammatory disease of the airways, characterized by recurrent episodes of symptomatic airflow obstruction and various degrees of bronchial hyperreactivity, that affects approximately 155 million people worldwide[1,2]. The causes of asthma are multifactorial and are attributed to several interactions between various different genes and environmental exposures *(Figure 1)*. Despite reports that heritability in asthma varies between 36% and 79%[3-5], no clear-cut pattern of inheritance has been established. For example, it is known that when one of a child's parents suffers from asthma that child's risk of developing asthma is doubled. Additionally, a greater concordance of asthma in monozygotic twins than in dizygotic twins has been demonstrated in heritability studies[6,7]. The interaction between genetic factors and environmental exposures are responsible not only for the development but also for the expression and progression of the disorder[8]. The severity of asthma can be influenced either by specific genes that may lead to a decline in pulmonary function[9], or by exogenous interactions in which an individual may have reduced responsiveness or be resistant to a specific form of asthma therapy, such as corticosteroids[8]. Genetic causes of susceptibility and severity in asthma are attributed to a number of gene variants or single nucleotide polymorphisms (SNPs), each of which affect asthma susceptibility and/or disease severity. Recent papers

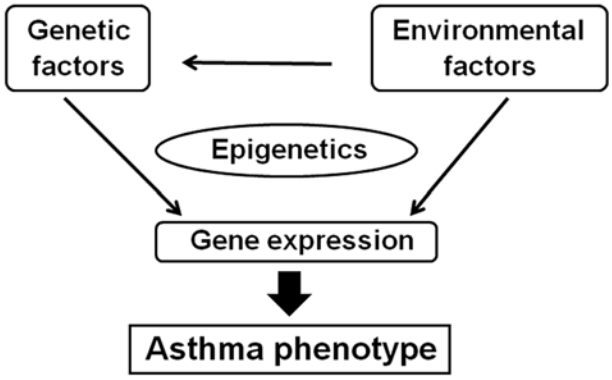


**FIGURE 1.** The pathogenesis of asthma

have reviewed the latest developments in the genetic mechanisms of asthma susceptibility and severity[10-12].

The aim of this review is to present the recent genetic approaches to the understanding of the severity in asthma expression. Specifically, a review is made of studies conducted on severe adult asthma[13] and the spectrum of the genetics of lung function, with focus on pharmacogenetics and genetic factors that affect the response to asthma treatment. Finally, environmental interactions and epigenetic mechanisms are discussed.

## THE ASTHMA GENETICS APPROACH

Genome-wide association studies (GWAS) have become the most effective approach to the study of the genetics of human diseases. Regarding asthma, GWAS analyze genetic variants, namely SNPs, in order to determine which variants are associated with either asthma susceptibility or asthma severity. GWAS have been applied among family members or between unrelated subjects comparing the frequency of each genetic variant between subjects with asthma (asthma cases) and asthma-free control subjects. In this approach, a statistically significant increase in the frequency of a SNP in subjects with asthma compared to control subjects provides evidence that the genetic variant is related to the disease susceptibility. In addition to GWAS, replication studies and meta-analyses are also required. To date, five genes/regions have been consistently found to be associated with asthma: ORMDL3-GSDMB, IL-33, IL-1RL1, RAD50-IL13 and HLA-DR/DQ.

Linkages have been found in specific ethnic groups, using different phenotypes. The regions of the human chromosomes that have been studied more frequently are 2q33, 5q23-31, 6p24-21, 11q21-13, 12q24-12, and 13q14-12, because they contain a large number of candidate genes[14]. With techniques such as high-throughput sequencing, SNP genotyping and linkage disequilibrium (LD) mapping, investigators have managed to identify various different susceptibility genes. In addition to linkage studies, association studies can be useful. Another type of genetic variation, copy number variants (CNV) has also been studied, as structural variations may have more functional impact on phenotypic variation than SNPs[15].

All these studies aimed to identify a group of genetic variants that can predict either the susceptibility or the severity of asthma. In addition, it is possible that specific pathways may serve as a guide to therapeutic intervention[8]. For this reason, current emphasis has been placed on genomic asthma research to identify genes that not only are involved in disease susceptibility but also separately affect disease progression and asthma severity *(Table 1)*. The major studies that are analyzed in this review are displayed in *Table 2*.

## GWAS OF SEVERE ASTHMA

Initially, genetic studies evaluated only asthma susceptibility, often using limited phenotypes and older GWAS, and aimed to identify genes associated with childhood-onset asthma or asthma susceptibility. There is also a need to understand whether the same or other genes contribute to the predisposition to more severe or difficult-to-treat asthma.

Li et al[16] performed a GWAS for asthma susceptibility and severity in a longitudinal cohort of 473 cases of asthma in non-Hispanic white subjects in the The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) population and 1,892 control subjects in the general population[17,18]. In this analysis, several variants in the RAD50/IL13 region of chromosome 5q31.1 were found to be associated with asthma. Additional studies have begun to identify various different genes associated with asthma severity[19-21]. For instance, one study showed that IL-13 appears to be a major regulator of allergen-induced asthma in mice[19,20], and identified variants in the HLA-DR/DQ region on chromosome 6p21.3

**TABLE 1.** The asthma genetics approach

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| 1. Candidate gene approach |
| 2. Genome-wide association studies |
| 3. Linkage studies |

**TABLE 2.** Genes associated with severe asthma

| Authors | Subject |
|---|---|
| Li X et al (2010)[15] | Several variants of **RAD50/IL 13** of **chromosome 5q31.1** are associated with asthma |
| Booth M et al (2006)[21], Munthe-Kaas MC et al (2007)[22], Moffatt MF et al (2010)[23] | **HLA DR/DQ** region of **chromosome 6p21.3** is associated with asthma |
| Jongepier H et al (2004)[9], Van Eerdewegh P et al (2002)[27], Howard TD et al (2003)[28] | **ADAM 33 gene,** located in **chromosome 20p13** is associated with asthma susceptibility and severity |
| Hancock DB et al (2010)[31], Repapi E et al (2010)[32] | **HHIP gene** is associated with $FEV_1$ or the $FEV_1$/FVC ratio |
| Li X et al (2011)[33] | **HHIP/rs1512288** variant on **chromosome 4q31** is a predictor of $FEV_1$ and FVC in asthma |
| Gudbjartsson DF et al (2009)[34] | An **IL-1RL1 variant** is associated with eosinophil counts in asthma |
| Wenzel SE et al (2007)[35] | **Minor alleles IL-4R, E375A, Q551R** are predictors of severe asthma exacerbations |
| **RESPONSE TO THERAPY** | |
| Vercelli D et al (2008)[40], Pascual RM et al (2010)[41], Lima JJ et al (2009)[42] | Polymorphisms in **ADRB2 gene** are responsible for the variability of bronchodilator response |
| Green SA et al (1994)[43], Green SA et al (1995)[44], Martinez FD et al (1997)[45], Cho SH et al (2005)[46], Kotani Y et al (1999)[47], Tan S et al (1997)[48] | **Gly16Arg** and **Gln27Glu** mutations in **ADRB2 gene** alter the bronchodilator response |
| Tantisira KG et al (2004)[53] | Haplotype in **CRHR1 gene** is a predictor of corticosteroid response |
| Hawkins GA et al (2009)[54] | Variation in **STIP1 gene** is a predictor of changes in $FEV_1$ in response to inhaled corticosteroid treatment |
| Huizenga NA et al (1998)[57] | **Asn363Ser minor allele** in **NR3C1 gene** increases sensitivity to exogenous corticosteroids |
| Drazen JM et al (1999)[58] | Promoter variation in **ALOX5 gene** is associated with no response in 5-LO pathway inhibitors |
| Pillai SG et al (2004)[65] | Mutated form of **CYSLTR2 gene** is associated with reduced response to anti-leukotriene treatment |
| Meyers DA (2008)[67] | **Genetic variations** in **TNF pathway** are associated with different responsiveness to anti-TNF therapy |
| Slager RE et al (2010)[68], Slager RE et al (2011)[70] | **Genetic variations** in **IL-4/13 pathway** are associated with different responsiveness to anti-TNF therapy and reduced asthma exacerbations |
| **GENES AND ENVIRONMENT** | |
| Ober C et al (2011)[75], Koppelman GH et al (2006)[76] | Environmental exposure and the presence of **-159T allele** of **CD14 gene** increases the risk of asthma. The presence of -**159C allele** is associated with asthma in non- exposed subjects |
| Meyers DA et al (2005)[77], Colilla S et al (2003)[78] | Linkage between BHR and **chromosome 5q** in children exposed to passive smoking |
| **EPIGENETICS** | |
| Hew M et al (2006)[82] | Decreased activity of **histone deacetylase** in severe asthma |
| Ito K et al (2002)[84], Cosio BG et al (2004)[85] | Increased activity of **histone acetyltransferase** in asthma |
| Breton CV et al (2009)[87] | Different states of methylation in **8 genes** of **CpG** in children, according to their exposure to maternal smoking during pregnancy |

associated with asthma, a genomic region which has been replicated in many candidate genes studies[22,23] and GWAS[24]. Since this genomic region was also identified in a GWAS of serum immunoglobulin E (IgE) levels[25] and a majority of the TENOR subjects had allergic asthma, RAD50/IL13 variation may be particularly critical to the progression of disease in patients with severe, persistent allergic asthma.

## GENETICS OF LUNG FUNCTION AND ASTHMA SEVERITY

In order to assess the genetic influences on asthma severity, earlier studies focussed on the genetic determinants of pulmonary function, which is an indicator of asthma severity[26,27]. The A disintegrin and metalloprotease 33 (ADAM33) gene, located on chromosome 20p13 is a member of a gene family that plays a role in cell fusion, signalling and adhesion. It was first identified as an asthma susceptibility gene using positional cloning in non-Hispanic white families[28], and this was subsequently confirmed in several candidate gene studies. SNPs in ADAM33 were significantly associated with asthma risk in each ethnic group, but no single polymorphism or haplotype was significant across all populations, probably due to ancestral differences in haplotype structure[29].

ADAM33 variants were also tested in 200 non-Hispanic white asthma cases with data collected over a period of more than 25 years. The minor allele of the ADAM33 S_2 polymorphism was a significant predictor of increased decline in forced expiratory volume in 1 second ($FEV_1$), suggesting that this gene may be related to disease progression in asthma[9]. Additionally, SNPs of ADAM33 have also been associated with risk of chronic obstructive pulmonary disease (COPD) in a Dutch cohort[30] and cross-sectional pulmonary function measurements in a population of 880 long-term tobacco smokers[31].

Two large meta-analyses of GWAS for normal lung function have been carried out in the European general population. The CHARGE consortium and the SpiroMeta consortium both identified the hedgehog interacting protein (HHIP) gene to be associated with either $FEV_1$ or the $FEV_1$/FVC ratio[32,33]. Eleven other genomic regions for normal pulmonary function were also discovered, including: TNS1, GPR126, ADAM19, AGER-PPT2, FAM13A, PTCH1, PID1, HTR4, the INTS12-GSTCD-NPNT region, and THSD4. To determine whether or not these genetic variants also modulate lung function or the severity in asthma populations, Li et al[34] examined 14 SNPs in the 11 candidate genes identified for association with pulmonary function measures, in 1,441 subjects with asthma (non-Hispanic whites and African-Americans) from the Severe Asthma Research Program (SARP), CSGA and TENOR cohorts. The meta-analysis of these populations showed that the HHIP/rs1512288 variant on chromosome 4q31 was a significant predictor of $FEV_1$ and FVC in patients with asthma. HHIP variants were also associated with reversibility but not bronchial hyperresponsiveness (BHR). An increasing number of risk variants in normal lung function genes were found to be strongly associated with lower $FEV_1$ and increased asthma severity based on American Thoracic Society (ATS) criteria or SARP phenotypic cluster assignment[34].

The blood eosinophil count, a biomarker related to the inflammatory response and possibly asthma pathogenesis, was assessed in a large population-based GWAS of 9,392 subjects in Iceland that was designed to identify genetic variants associated with the eosinophil count. An IL-1RL1 variant was found to be associated with both the eosinophil count and asthma. SNPs in WDR36, IL-33 and MYB that were associated with the eosinophil count were also related to atopic asthma. One other study also implicated IL-1R1 in the modulation of asthma-related phenotypes[35].

Despite the advances in identification of asthma susceptibility genes, the pathogenesis of severe asthma is still poorly understood and little is known about the genetic and environmental factors that contribute to asthma severity, due to the small proportion of subjects with severe asthma. The SARP cohort, sponsored by the National Heart, Lung and Blood Institute, is one of the few studies with a sufficient number of patients with severe asthma for research purposes[26].

Five primary phenotypes of increasing asthma severity were generated in order to define asthma heterogeneity in SARP, using a hypothesis-free clustering analysis[27]: 1) mild allergic asthma, 2) mild-moderate allergic asthma, 3) late-onset less allergic asthma, 4) severe allergic asthma, and 5) severe asthma with fixed airflow obstruction. Clusters 1, 2 and 4 represent allergic asthma of increasing severity and also have the largest percentages of individuals who report a family history of asthma, compared with clusters 3 and 5.

The initial SARP genetic studies tested candidate genes that had primarily been identified in susceptibility studies, in order to determine if these genes were also associated with disease progression. For example, non-synonymous polymorphisms in the IL-4 receptor α gene (IL-4R) were tested for association with severe

asthma phenotypes (i.e. exacerbations, reduced lung function and increased inflammation) in SARP patients with asthma and in patients with severe asthma from a similar cohort. The IL-4R, E375A and Q551R non-Hispanic white minor alleles were predictors of severe exacerbations and lower performance on lung function tests. The E375A C allele was also associated with increased mast cell related inflammatory response[36].

Novel genotyping technologies and comprehensive phenotyping are expected to facilitate investigation of the genetics of asthma severity in the future. Sequencing can provide genomic coverage better than existing genotyping platforms and help to uncover biologically relevant causal variants.

## PHARMACOGENETICS - RESPONSE TO THERAPY

Another genetic mechanism that has been investigated is related to pharmacogenetics, where responsiveness to asthma therapy is altered. For example, reduced pharmacological response to the regular use of short-acting β2-agonists (SABAs), but not to long-acting β2-agonists (LABAs), has been reported in ADBR2 gene Arg16 homozygotes[37-40].

ADRB2 is a small, intronless gene that encodes the β2-adrenergic receptor, and it has been extensively studied in asthma[41]. Many studies explored whether polymorphisms in ADRB2 are responsible for the variability in bronchodilator response among asthma patients, and if they can identify a subgroup of patients with worsening symptoms despite β-agonist treatment[42,43].

Most of the ADRB2 genetic studies have focussed on the Gly16Arg and Gln27Glu mutations, which have been shown *in vitro* to alter receptor activity[44,45]. The initial pharmacogenetic studies suggested that children with asthma who were homozygous for both the Arg16 and Gln27 variants were more likely to have improved response to albuterol, compared with children who were heterozygous for Arg/Gly16 or homozygous for Gly16[46]. Subsequent studies showed a similar positive relationship between the Arg16 variant and response to SABA therapy[47-49], but others demonstrated an adverse effect with the same genotype[37,50,51]. Several studies have indicated that the long-term use of LABAs without the use of inhaled corticosteroids (ICS) was related to adverse outcome[52,53]. A very large analysis with a second replicate study of asthma patients treated with LABAs in combination with ICS showed no effects of the ADBR2 genotype on the therapeutic response, strongly suggesting that there was no adverse effect in patients with moderate to severe asthma receiving combination LABA/ICS treatment[40].

Two prospective, genotype-stratified trials showed no effect of the Gly16Arg genotype in response to LABA therapy; one evaluated LABA (salmeterol) administered both alone and in LABA/ICS combination therapy, and did not demonstrate any effect of the Gly16Arg genotype on response to therapy[38,39].

Variability to the corticosteroid response is documented in both childhood and adult asthma[38,54,55].

As previous studies have shown, steroid treatment is not equally effective in all patients[56,57]. Several studies have focussed on NR3C1, the glucocorticoid receptor gene. For example, elderly subjects from a chronic disease study who had the NR3C1 Asn363Ser minor allele were more sensitive to exogenous glucocorticoid[58]. Another target in this pathway is the STIP1 gene, which encodes a protein that is part of the heterocomplex that activates the glucocorticoid receptor. STIP1 variation was shown to be a predictor of changes in $FEV_1$ in response to treatment with ICS[55]. This shows heterogeneity in corticosteroid responsiveness, with half of the STIP1 haplotypes producing lower corticosteroid response compared with the haplotypes that show greater sensitivity to ICS.

An analysis performed by Tantisira et al[54], evaluating polymorphisms in 14 genes in a primary clinical trial for association with improved lung function in patients on corticosteroids, indicated that a common haplotype in the corticotrophin-releasing hormone receptor (CRHR1) gene was a predictor of corticosteroid response. CRHR1 was associated with response independently of the type of steroid administered, and appears to contribute to modulation of this pathway in both adults and children with asthma[54].

Variability is also observed in the response to anti-leukotriene treatment, i.e.: cysteinyl leukotriene receptor 1 antagonists (montelukast and zafirlukast); and inhibitors of the 5-lipoxygenase (5-LO) enzyme (zileuton).

An initial pharmacogenetic study showed that promoter variation in the ALOX5 gene identified a subset of asthma subjects with reduced enzyme activity and no response to treatment with 5-LO pathway inhibitors[59]. Leukotriene signalling occurs through G protein-coupled receptors called cys-LTRs that are encoded by two genes, CYSLTR1 and CYSLTR2[60,61]. Analysis of the leukotriene C4 synthase gene showed an effect of genotype on the synthesis of leukotriene C4 by blood eosinophils, and genotype-specific responses to zafirlukast treatment in patients with severe asthma[62]. Genetic studies of CYSLTR1

and CYSLTR2 and of the genes that encode intermediates in leukotriene biosynthesis have predominantly been limited to investigation of their association with asthma and atopy susceptibility[63-65]. Two pharmacogenetic studies tested genetic variation in CYSLTR1 and CYSLTR2. An analysis of the common form of CYSLT2 in comparison to a mutated form of CYSLT2 (with Val instead of Met in the amino acid position 201) demonstrated that altered CYSLT2 receptors containing Val did not respond as well to cys-leukotriene D4, which may affect asthma patients with this mutation[66]. A study that evaluated four CYSLT1 genetic variants in patients with asthma treated with montelukast did not demonstrate any relationship between these polymorphisms and improvement in lung function[67].

Finally, two pharmacogenetic studies of the tumor necrosis factor (TNF)[68] and IL-4/13 pathway[69] provided promising results by identifying by genotype a subset of patients with asthma who had improved responsiveness to an anti-TNF antibody (inhibitor of the Th2 pathway)[69,70]. An IL-4R genetic variation was also found to be associated with reduced asthma exacerbations in a larger mechanistic trial[71].

## GENE - ENVIRONMENT INTERACTIONS

Despite the significance of environmental factors in the development of asthma, only a few gene/environment interactions have been identified for asthma severity. One of the interactions most consistently replicated in asthma studies has been for the -159 promoter variant of the CD14 gene, which encodes a subunit of the endotoxin receptor found on mononuclear cells. Several types of exposure have been studied for interaction with this gene, including levels of endotoxin in house dust, pets, laboratory animals and farm animals[72-75]. Increased risk for asthma has been demonstrated in exposed subjects with the -159T allele while the -159C allele is associated with asthma in non-exposed subjects. This pattern of association may explain the varying allele associations observed for the CD14-159C/T polymorphism in asthma susceptibility studies that do not include environmental exposure[76,77].

A genetic linkage analysis in 200 families evaluated the interaction between gene and passive smoke exposure interaction, contributing to asthma risk and BHR. Linkage signals for asthma and BHR were observed on chromosomes 3p and 5q; however, childhood exposed to passive smoking accounted for BHR linkage to 5q[78,79].

## EPIGENETICS

The findings of genetic studies are sometimes lacking in consistency between different locations and populations, which suggests that an additional factor could be involved in pathogenesis of asthma. Epigenetics is the term used to describe heritable changes in gene expression. These are not coded in the DNA sequence itself but caused by post-translational modifications in DNA and histone proteins, or modified by microRNA expression[80]. Specifically, in histone, such modifications include phosphorylation, acetylation, methylation, sumoylation, oxidation, nitrosylation and ubiquitination. Epigenetic regulation is not only critical for generating diversity of cell types during mammalian development, but it is also important for maintaining the stability and integrity of the expression profiles of different cell types. Thus, the non-coding event is of importance as well as the genetic event; for example, disruption of epigenetic processes can cause cancer, syndromes involving chromosomal instabilities, defects of learning and memory, and diabetes mellitus. In addition, there is emerging evidence for the role of epigenetics in the pathology of respiratory disorders such as asthma and COPD.

Histone deacetylase (HDAC) is an enzyme which deacetylates histone, thus controlling proinflammatory gene expression. Recently, HDAC2 has been shown to be a cofactor of glucocorticoid receptors and a prerequisite molecule for steroid action[81,82].

HDAC is reported to be decreased in severe asthma[83] and COPD[84]. Oxidative stress, such as produced by cigarette smoking, causes reduction of HDAC activity, and leads to enhancement of steroid-insensitive inflammation. In addition, histone acetylatransferase activity, which acetylates histone, has been found increased in bronchial biopsy samples and alveolar macrophages in asthma, where it will enhance inflammation[85,86]. There is also evidence that Th2 cytokine production is epigenetically controlled in asthma[87]. Several studies have focussed on the interaction between environmental exposures and epigenetic mechanisms such as methylation of DNA, and have investigated how these epigenetic modifications alter gene expression, and finally affect the course of the disease.

Children whose mothers smoked during pregnancy showed decreased methylation of the AluYb8 repeat sequence, a marker of overall DNA hypomethylation. In an analysis of global CpG methylation, 8 genes were identified with methylation states that differed between exposed and non-exposed children. The AXL and PTPRO

genes were hypermethylated, a finding that replicated previous studies, but it is not known whether these genes contribute to asthma risk. Perhaps of greater relevance to their asthma susceptibility, smoking-induced effects on the methylation of long interspersed repetitive elements-1 (LINE1) were noted in children with the glutathione- S-transferase mu 1 (GSTM1) null genotype only. This finding suggests a possible mechanism for interaction between epigenetic modulation and a gene identified in asthma studies, which could result in increased risk for children exposed to maternal smoking during pregnancy[88]. Future studies may provide additional insight into the mechanism of exposure-induced epigenetic changes that lead to increased risk of chronic lung disease, including asthma.

Lastly, another non-coding epigenetic mechanism has been identified, namely microRNAs (miRNAs), which are small non-coding RNAs consisting of 18 to 25 nucleotides. These have been reported to regulate inflammatory gene expression via an RNA interference mechanism, and the expression of some of the miRNAs are associated with the development of asthma or its severity[89,90].

## CONCLUSIONS

A number of genes are variously associated with susceptibility to development of asthma, disease severity and treatment response. Current evidence suggests that gene polymorphism can cause abnormalities in the quality and quantity of proteins, and that epigenetic control and environmental factors can also affect gene expression. The interactions between gene induction, gene variants and epigenetic mechanisms should be considered in the future, in order to understand better the mechanisms of asthma development.

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