The multifactorial nature of food allergy
van Ginkel, Cornelia Doriene

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
CHAPTER 2

THE GENETICS OF ALLERGIC DISORDERS

C. DORIENE VAN GINKEL\textsuperscript{1,2}, ANTHONY E.J. DUBOIS\textsuperscript{1,2}, GERARD H. KOPPELMAN\textsuperscript{1,2}

\textsuperscript{1}University of Groningen, University Medical Center Groningen, Department of Paediatric Pulmonology and Paediatric Allergy, Groningen, the Netherlands, \textsuperscript{2}University of Groningen, University Medical Center Groningen, GRIAC Research Institute, Groningen, the Netherlands

MANUAL OF ALLERGY AND CLINICAL IMMUNOLOGY FOR OTOLARYNGOLOGISTS. EDITORS: DAVID L. ROENSTREICH, MARVIN P. FRIED, GABRIELE S. DE VOS AND ALEXIS H. JACKMAN. COPYRIGHT © 2016 PLURAL PUBLISHING, INC. ALL RIGHTS RESERVED. USED WITH PERMISSION.
INTRODUCTION TO THE HERITABILITY OF ALLERGIC DISEASES

MULTIFACTORIAL ETIOLOGY
With an estimated prevalence ranging between 16% and 57% in the United States, Europe, Australia, New Zealand, and Taiwan, atopy is the most common disorder of the immune system and is defined as sensitization to atopic allergens. Sensitization is defined as the production of specific immunoglobulin E (sIgE), and atopic allergens are defined as allergens that may show epidemiologically significant cosensitization with known “reference” atopic allergens such as house dust mite. Despite the high prevalence of atopic allergic diseases such as asthma, rhinitis, food allergy, and atopic dermatitis, the pathogenesis of these disorders remains poorly understood.

Atopic diseases have a multifactorial etiology, with a complex interaction of genetic risk factors and environmental triggers. A well-known purported environmental influence is described in the hygiene hypothesis, which states that exposure to microbes and microbial products early in life is associated with a decrease in risk of developing allergic disease. This is supported by multiple epidemiological studies which have shown that there is an inverse relationship between markers of early life exposure to microbes and microbial products (such as number of siblings, day care attendance or growing up on a farm) and allergic disease.

HERITABILITY
The genetic basis of atopy is widely established, and in the past, atopy was even defined as “a personal or familial tendency to produce IgE antibodies in response to low doses of allergens.” Atopic diseases coexist in individuals and are clustered in families and populations. As early as 1916, positive family histories were reported in half of asthmatic patients. Since then, numerous epidemiologic studies have reported familial clustering of atopic diseases as well as higher concordance rates among monozygotic twins than dizygotic twins. For example, one study reported a 5-fold increase for the risk of peanut allergy for a child with a peanut allergic sibling or parents. For atopy in general, current heritability estimations vary between 34% and 84%, which means that this proportion of variance is due to heritable factors. Heritability estimates of allergic diseases vary between 35% and 95% for asthma, 33% and 91% for allergic rhinitis, and 71% and 84% for atopic dermatitis.

Furthermore, many patients suffer from multiple atopic diseases. For example, 90% of all children referred to a tertiary clinic for food allergy have been diagnosed with atopic diseases other than food allergy, most commonly atopic dermatitis. Is the co-occurrence of atopic diseases due to a shared etiology, or they can be regarded as different clinical manifestations of atopy? The answer to this question is as yet unknown although a recent report on 12 European birth cohorts with over 27 000 children showed that the population attributable risk of IgE sensitization was only 38% for having an atopic comorbidity (asthma, atopic dermatitis, and rhinitis). This suggests that IgE sensitization can no longer be considered the only causal mechanism for comorbidity in allergic diseases.

In this chapter, we will provide an overview of the genetics of allergic disease, and start with an introduction into genetic and epigenetic research that has revolutionized our
knowledge about atopy and atopic diseases over the past two decades. In addition, we will
explain the pathway leading to IgE sensitization and genetic markers associated with the
different atopic diseases. Emphasis will be on the causal mechanism behind the genetic
markers, and different causal theories will be discussed.

Atopic diseases have a multifactorial etiology, with a complex interaction of genetic risk
factors and environmental triggers. For atopy in general, current heritability estimations
vary between 34% and 84%. Genetic studies may provide an answer to the question
whether the co-occurrence of atopic diseases is due to a shared etiology or if they can be
regarded as different clinical manifestations of atopy.

RESEARCH ON THE GENETICS OF ATOPY AND ALLERGY
In the last two decades, approaches to studying the genetics of allergy have evolved
enormously. Since the first study reporting an association between a genetic locus and atopic
disease in 1989, over 1000 candidate gene studies have been published. These candidate
gene studies are hypothesis driven and therefore focus on preidentified genetic variations,
which have been selected based on their function in known pathways leading to allergy. The
genetic markers used are single nucleotide polymorphisms (SNPs), which are changes of one
base pair in the genome that account for over 90% of all genetic variation. Alternatively, other
sources of genetic variation, such as insertions, deletions, or copy number variants can be
studied. The study design is usually a comparison of the frequency of a gene variant in a
population of cases versus a population of healthy controls. Replicating findings from these
candidate gene studies has proved to be challenging due to a variety of reasons, such as lack
of standardized phenotyping and lack of power. An important aspect of genetic studies is that
the identified genetic variants can have a direct, functional effect in a gene, and thereby
increase disease risk. Alternatively, a SNP can be only indirectly involved by being in linkage
disequilibrium (LD) with the pathogenic variant. The LD is the association of two genetic
variants, in which both are inherited together, mostly because they are close to each other on
the genome.

To generate information on new, as yet undiscovered pathways, genome-wide
approaches were then developed which analyze all regions of the genome and are therefore
hypothesis free. The first approach that was used is called positional cloning, which combines
linkage studies in families with subsequent fine mapping studies using a case-control
approach. In linkage studies, genetic markers evenly spaced across the genome are studied
for cosegregation in affected relatives. This method is based on the hypothesis that the
“disease variant” is inherited and therefore similar in affected relatives. Combining data in
different families provides information on large chromosomal regions associated with allergy
of several millions of base pairs, which generally contain dozens to hundreds of genes.
Therefore, with linkage studies it is difficult to pinpoint one or more genes, and this method
is then followed up by positional candidate gene studies, where genes in the linked regions are investigated for association with the disease using a case-control design.

Although several interesting genes have been identified with this method, the current method of choice is called the Genome Wide Association study (GWAS). In a GWAS, between three hundred thousand and one million SNPs are analyzed using DNA chips. The number of SNPs tested requires statistical correction for multiple testing errors and therefore requires large sample sizes. The GWAS select for prevalent genetic variations (ie, present in >5% in the general population). A recent development is the use of resequencing studies. Because GWAS are only sensitive to common variants, this method aims for rare variants that may have a relatively strong effect on the risk of developing the disease. These rare variants are studied for enrichment in cases compared to controls.

All approaches have advantages and disadvantages (Table 4–1) and provide relevant information on the genetic origins of atopy. Study outcomes are discussed below.
The genetics of allergic disorders - Chapter 2  PART I

is then followed up by positional candidate gene studies, where genes in the linked regions are investigated for association with the disease using a case-control design. Although several interesting genes have been identified with this method, the current method of choice is called the Genome Wide Association study (GWAS). In a GWAS, between three hundred thousand and one million SNPs are analyzed using DNA chips. The number of SNPs tested requires statistical correction for multiple testing errors and therefore requires large sample sizes. The GWAS select for prevalent genetic variations (ie, present in >5% in the general population). A recent development is the use of resequencing studies. Because GWAS are only sensitive to common variants, this method aims for rare variants that may have a relatively strong effect on the risk of developing the disease. These rare variants are studied for enrichment in cases compared to controls.

All approaches have advantages and disadvantages (Table 4–1) and provide relevant information on the genetic origins of atopy. Study outcomes are discussed below.

**TABLE 4–1. APPROACHES FOR GENE DISCOVERY**

In the last two decades, approaches to studying the genetics of allergy have evolved from the hypothesis-driven candidate gene studies toward hypothesis-free genome-wide association studies. Identified genetic variants can have a direct, functional effect in a gene, and thereby increase disease risk, or can be only indirectly involved by being associated with the pathogenic variant.

**Gene-Environment Interactions and Epigenetics**

Although results from candidate gene and genome-wide analyses are promising, the SNPs currently identified can only explain a few percent of the total estimated heritability of atopy. Therefore, current research is directed at the cause of this “missing heritability.” This can be due to as yet unidentified prevalent variants with very modest effects, rare variants with strong effects, or other sources of genetic variation such as copy number variation. Furthermore, interactions between genes and environment and gene-gene interactions have been proposed.

Currently, epigenetics as a cause of missing heritability is an emerging field of research. Epigenetics refers to the regulation of DNA expression by methylation, chromatin modifications, or regulatory RNA molecules, which do not affect the DNA sequence itself, yet are heritable. It has been shown that environmental factors can influence epigenetic mechanisms, such as the expression of DNA by influencing the degree of DNA compaction and accessibility for gene transcription, regulating gene expression in a temporal and tissue-specific manner. The epigenetic regulation of DNA may be transmitted through multiple generations. Technology to study epigenetic regulation on a genome-wide scale has recently emerged. The best described epigenetic mechanisms are microRNAs, DNA methylation, and histone modifications, which will be explained briefly below.

DNA is transcribed into messenger RNA (mRNA) and undergoes splicing, a RNA processing event in which the introns are removed and exons joined together. After splicing, mRNA is transcribed into proteins. Specific parts of DNA can also be transcribed into regulatory RNAs, which include long noncoding RNAs and microRNAs (miRNA). The latter are small RNAs that do not code for a protein but can bind to mRNA and thereby degrade or modify the mRNA and inhibit the protein transcription. One miRNA can modify multiple mRNAs, and one mRNA can be modified by multiple miRNAs, which indicates the complexity of this regulation.⁹

DNA methylation is the best studied epigenetic modification of DNA, and it occurs predominantly on CpG dinucleotides. Methylation describes the addition of a methyl group to a cytosine to form a 5’-methylcytosine. Approximately 75% of all CpG dinucleotides in the genome are methylated, and methylation is more prevalent in gene bodies and exons. CpG islands are generally less methylated regions where CpG dinucleotides cluster, and these are frequently located in regulatory regions such as promotor regions which are the starting point of DNA transcription. When methylated, the CpG islands in promotor regions inhibit DNA
transcription by blocking the binding of transcription factors. Methylation in gene bodies might stimulate transcription or influence splicing or chromosomal stability.

DNA is wrapped around histones, which are proteins that interact with the long DNA chain to organize it into tightly wound nucleosomes. The amino acid tails of histones can be methylated, acetylated, phosphorylated, or otherwise modified causing changes in the structure of the DNA wrapping. These changes influence the accessibility of DNA for gene transcription. Histone acetylation has been most extensively studied and is associated with transcriptional activation. Histone acetyltransferase promotes transcription by attaching an acetyl group to lysine residues, which indirectly recruits activator proteins to the DNA. The acetyl group can be removed from histones by histone deacetylase enzyme, which leads to decreased gene expression. Levels of histone acetyltransferase and histone deacetylase enzyme are therefore indicators for gene expression, and their role in atopy and atopic diseases is currently under investigation.

Genetic studies conducted so far are not able to account for all the estimated heritability. Epigenetic influences and gene-environment interaction are therefore proposed to partly explain this “missing heritability.” Epigenetics refers to the regulation of DNA expression by methylation, chromatin modifications, or regulatory RNA molecules, which do not affect the DNA sequence itself, yet are heritable.

DIFFERENCE BETWEEN SENSITIZATION AND CLINICAL DISEASE

DEFINING SENSITIZATION
Atopic individuals produce specific immunoglobulin E (sIgE) after exposure to atopic allergens, which is termed sensitization. The sIgE may then bind to mast cells and/or basophils which may then, in turn, initiate an allergic reaction after re-encountering the allergen.

The prevalence of IgE sensitization differs between populations, with reported prevalences varying between 16% and 57% in the United States, Europe, Australia, New Zealand, and Taiwan. Sensitization is a strong risk factor for allergic disease although it is well established that sensitization does not always lead to symptoms, or clinical disease. The exact prevalence of asymptomatic sensitization is not really known. However, in the case of food allergy, only approximately 50% of children highly suspected of being food allergic and commonly sensitized to that suspect food are found to be clinically reactive to that food, as ascertained by a positive reaction to a double-blind placebo-controlled food challenge. The prevalence of sensitization among asthma patients is about 60% to 80%, and the ratio of rhinitis associated with and without sensitization is 3:1. Approximately 20% of patients with atopic dermatitis are not sensitized to any allergen. These data indicate that not all patients with atopic diseases are sensitized.

The mechanisms that result in atopy and the pathway driving sensitized individuals to become clinically allergic remain unknown. Since the genetic makeup of a child influences
these differences it is likely that the genetic studies on these phenotypes may lead to knowledge on the pathogenetic mechanisms of allergy.

**IMPORTANCE OF DISEASE DEFINITION**

The definition of the phenotype used is of great importance when interpreting data on genetics of atopy and allergic diseases. Moreover, there is an important difference between atopic sensitization and atopic disease. Sensitization is usually defined as either a specific IgE level above 0.35 kU/l, a positive allergen skin prick test, a high total serum IgE, or a combination of these tests. High total serum IgE is not always associated with atopy, since it can also be associated with helminthic infections or atopic dermatitis without evidence of atopy. Moreover, no proper cut off for total serum IgE has been defined above which the diagnosis of atopy may be reliably made and the upper limit of “normal” is unknown.

There are several diagnostic methods used to diagnose each atopic disease. When comparing studies, different definitions have been used, which complicates the replication of genetic findings. Asthma is difficult to define, especially in young children with symptoms of wheeze that may also be due to viral respiratory infections. Furthermore, there are several subgroups, including allergic asthma, exercise-induced asthma, and occupational asthma. These diseases are highly likely to have different pathophysiologies, which may be difficult to distinguish in genetic studies where the phenotypes have not been specifically defined. Allergic rhinitis is diagnosed based on symptoms, temporal pattern (seasonal or chronic), skin prick tests and response to medications. There are nonallergic rhinitis syndromes such as idiopathic rhinitis, infectious rhinitis and hormonal, alcohol, food or drug-induced rhinitis which are sometimes difficult to differentiate from allergic rhinitis. For food allergy, one can use open food challenges or the double-blind placebo controlled food challenge, the latter being the gold standard. Open food challenges have a higher false-positive rate than double-blind placebo controlled challenges, and studies using the former for case definition of food allergy may therefore fail to differentiate between asymptomatic sensitization and clinical reactivity to foods. This difference is clinically relevant since asymptomatic sensitization does not require therapy. The diagnosis of atopic dermatitis is not based on a specific test but is instead based on physical examination, history, and reported symptoms. The prevalence based on skin examination is approximately two-thirds of the prevalence of self-reported or questionnaire-based atopic dermatitis.

To differentiate between the specific atopic diseases, the control group selected is also important. Are patients with one atopic disease compared to nonatopic healthy controls or are different groups of patients with a specific atopic disease compared? To differentiate between sensitization and atopic disease, a sufficient number of asymptomatically sensitized patients should be included in the study. For example, when comparing food allergic patients to the general population, genetic markers thought to be associated with food allergy may in reality be associated with sensitization to foods since both of these traits are often seen together in the cases but are generally both lacking in (nonatopic) controls.
Sensitization does not always lead to symptoms or atopic disease. It is therefore highly important to distinguish these phenotypes from one another. Genetic studies may give insight in the pathway(s) driving sensitized individuals to become clinically allergic.

GENES IMPORTANT IN THE PATHWAY LEADING TO ATOPY AND ALLERGY
In this section, we briefly describe the pathway leading to sensitization. We then describe current knowledge on the mechanisms of atopy, and how genetic and epigenetic (candidate) gene studies have provided a model to understand the relationship between (epi)genetic variation and disease development. We cannot provide a complete overview of all candidate gene studies, but have selected well-replicated genes to illustrate novel insights that such studies have provided.

PATHWAY LEADING TO SENSITIZATION
Individuals may become sensitized by skin contact, inhalation, or ingestion of (glyco-)proteins. Dendritic cells are specialized in antigen presentation and capture these proteins in the gut or other sites of entry, after which they home to secondary lymphoid organs such as lymph nodes. The dendritic cell processes the protein and presents an allergen peptide on a major histocompatibility complex (MHC) class II molecule to naïve CD4⁺ T lymphocytes. In immune responses to atopic allergens, dendritic cells concurrently secrete the cytokine interleukin-4 (IL-4). Both the peptide containing MHC receptor and IL-4 bind to CD4⁺ T cells, which induces expression of signal transducer and activator of transcription 6 molecule (STAT6). This in turn stimulates the CD4⁺ T cells to differentiate into T helper 2 cells (Th2). The Th2 cells then activate B cells by producing CD40 ligand, IL-4, IL-5, and IL-13. Naïve mature B cells also present antigens in the lymph nodes and are activated by Th2 cells to undergo IL-4 and IL-13 induced immunoglobulin class-switching. This results in the production of allergen-specific IgE antibodies by the activated B cells. The allergen-specific IgE (sIgE) antibodies may then bind to resident tissue mast cells as can be demonstrated by immediate skin testing, or the sIgE may also be measured directly in many body fluids including blood.
PART I  Chapter 2 - Prevalence of EAs in food-allergic adolescents

EPITHELIAL BARRIER
The epithelia of the skin, lung, and gastrointestinal tract are the first sites to encounter allergens. An impaired barrier function may therefore permit intact proteins to pass the barrier and to elicit an immune response. The filaggrin (FLG) gene on chromosome 1q21 lies within the epidermal differentiation complex. These genes play an important role in skin barrier function since the filaggrin protein helps aggregate the epidermal cytoskeleton to form a protein-lipid barrier. Loss of function (LOF) variants of the FLG gene result in a defective form of the filaggrin protein and have a prevalence of about 10% in Western populations. FLG LOF variants are associated with ichthyosis vulgaris, characterized by palmar hyperlinearity, keratosis pilaris, and a fine white scale on the extremities. Ichthyosis vulgaris is strongly associated with atopy; 37% to 50% of people with ichthyosis vulgaris have atopic diseases and about 8% of atopic dermatitis patients have ichthyosis vulgaris. Furthermore, the FLG LOF variants are strong risk factors for atopic dermatitis and sensitization. In children with atopic dermatitis, they are also associated with asthma. Other studies suggest a role of FLG in sensitization to foods and clinical food allergy. The genetic findings of FLG being an important gene for food allergy have led to the dual-allergen exposure hypothesis for the pathogenesis of food allergy. This proposes that low-dose cutaneous exposure to food allergens triggers Th2 responses and IgE production by B cells while early oral exposure induces tolerance by stimulating regulatory T cells and Th1 cells. This hypothesis is supported by a study showing that epicutaneous exposure to peanut protein causes Th2-type immunity with high levels of peanut-specific IgE and prevents the development of oral tolerance to peanut. A recent study showed that early life environmental exposure to peanuts, as measured by peanut in household dust, is indeed associated with peanut sensitization and allergy as confirmed by open food challenges, but only in children carrying FLG mutations. This percutaneous priming is also proposed for the role of FLG in asthma and rhinitis. However, future studies are needed to define the effect of FLG loss of function variants on sensitization and atopic diseases since it is yet unclear whether FLG is important only in the pathway leading to sensitization or also in the pathway leading from sensitization to specific allergic diseases. The GWAS on asthma have identified other SNPs important in the epithelial barrier (see below under Asthma).

Recent studies have identified genetic markers important in epithelial barrier function, such as the filaggrin gene, to be associated with atopic diseases. It is therefore hypothesized that cutaneous exposure to allergens in individuals with an impaired barrier function may trigger Th2 responses and allergy.
T AND B CELL DIFFERENTIATION

Genes encoding STAT6, IL-4, IL-13, and the IL-4 receptor alpha chain are among the best-replicated candidate genes in allergic disease. As discussed above, the IL-4 cytokine stimulates T cells to differentiate into Th2 cells, and IL-4 and IL-13 stimulate B cells to produce IgE. Several chromosome 5q SNPs located in the gene coding for IL-4, IL-13, and another chromosome 16 (IL-4RA) gene that codes for their receptor have been reproducibly associated with sensitization and asthma. Variants in the IL-13 gene have been associated with sensitization to both allergenic foods and inhalants. Interestingly, epigenetic mechanisms may also be important, since recent data have shown that early farm exposure is associated with hypomethylation of the IL-13 gene. Studies have also shown that Th2 polarization occurs through epigenetic regulation of the IL-4 gene by demethylation and active histone modifications, leading to greater IL-4 expression. The RHS7 gene, one of the 4 RAD50 DNase I hypersensitivity sites (RHS4-7) is located closely to Th2 associated cytokines. It was reported to affect methylation of the IL-13 promoter and expression of IL-4. Variants of the RHS7 gene were also associated with total serum IgE levels. Because of these findings, DNA methylation of the IL-13 promotor has been suggested to mediate this genetic effect on IgE levels.

Variants in the STAT6 gene located on chromosome 12q are associated with total serum IgE levels, allergic sensitization, as well as a tree nut allergy. In another study, early exposure to a farm environment was associated with hypomethylation of this gene. The CD14 gene encodes a lipopolysaccharide receptor. Lipopolysaccharides are the main component of endotoxin, which is present in house dust. CD14 gene variants are reported to be associated with atopy, and thought to influence the balance between Th1 and Th2 responses to antigens. The -159T promoter variant of the CD14 gene is associated with asthma in patients highly exposed to microbes and endotoxin and the -159C variant is associated with this phenotype among patients with low exposure. Thus, gene variants in this gene may interact with the environment, in particular microbial exposures. Figure 4–1 illustrates the association between the -159C/T genotype and IgE sensitization among exposed and nonexposed patients in the Manchester Asthma and Allergy study.
PART I  Chapter 2 - Prevalence of EAs in food-allergic adolescents

FIGURE 4–1.

Genetic variation in genes coding for cytokines such as IL-4 and IL-13, important in B and T cell differentiation, is associated with sensitization. An individual’s response to environmental factors such as house dust may be influenced by their genetic makeup, such as shown for the CD14 gene.

ANTIGEN PRESENTATION
Major histocompatibility class II (MHC II) antigens are cell surface proteins expressed on antigen-presenting cells such as dendritic cells. In humans, these are termed human leukocyte antigens (HLA). As explained above, antigens captured by antigen-presenting cells are degraded to peptides and the resulting peptide fragments are inserted into the cleft of an appropriate MHC class II molecule (Figure 4–2). The resulting complex is transported to the surface of the antigen-presenting cell where it is presented to naïve CD4+ T cells with receptors recognizing the peptide MHC II complex. In the case of responses to allergens, these CD4+ cells are activated and then differentiate into Th2 cells. Genetic variation in the HLA region (HLA-DQ and HLA-DR) is strongly associated with (adult) onset asthma and IgE levels.
The genetics of allergic disorders - Chapter 2  PART I

FIGURE 4–1.

ANTIGEN PRESENTATION
Major histocompatibility class II (MHC II) antigens are cell surface proteins expressed on antigen-presenting cells such as dendritic cells. In humans, these are termed human leukocyte antigens (HLA). As explained above, antigens captured by antigen-presenting cells are degraded to peptides and the resulting peptide fragments are inserted into the cleft of an appropriate MHC class II molecule (Figure 4–2). The resulting complex is transported to the surface of the antigen-presenting cell where it is presented to naïve CD4+ T cells with receptors recognizing the peptide-MHC II complex. In the case of responses to allergens, these CD4+ cells are activated and then differentiate into Th2 cells. Genetic variation in the HLA region (HLA-DQ and HLA-DR) is strongly associated with (adult) onset asthma and IgE levels, respectively.40,41 Since the structure of the MHC II molecule determines which antigen peptides are presented to CD4+ cells and which are not, they may be important in explaining why certain antigenic proteins have a tendency to elicit production of IgE antibodies (ie, allergens) and others do not (ie, nonallergens). One hypothesis is that allergens are distinguished from other antigens by the fact that they do not trigger danger signals that result in Th1 or Th17 cytokine production. However, studies showing associations between MHC II and sensitization suggest that multiple disease mechanisms may be important. Furthermore, due to the strong linkage disequilibrium on chromosome 6 (the HLA gene repertoire) it has been difficult to pinpoint which genes are important.

FIGURE 4–2.
Basic molecules and cells contributing to the IgE network, IgE function and IgE regulation. Gene products identified by GWA studies on total serum IgE are depicted in red (with chromosomal loci provided). IL-4 denotes interleukin-4; MHC II, major histocompatibility complex, class II; APC, antigen-presenting cell; IL-13, interleukin-13; CD40L, CD40 ligand; mlgE, membrane IgE; FceRI, the high-affinity IgE receptor; CSR, class-switch recombination; (m/s) CD233, (membrane/soluble) low-affinity IgE receptor, trimeric form. Reprinted with permission from John Wiley and Sons. Potaczek DP, Kabesch M. Current concepts of IgE regulation and impact of genetic determinants. Clin Exp Allergy. 2012;42:852–871.

FCERI RECEPTOR
Activated B cells produce antigen-specific IgE antibodies which bind to the high-affinity FcεRI receptor on mast cells, basophils, and eosinophils. When IgE binds to the high-affinity FcεRI receptor and subsequently binds the allergen, these resulting complexes cross-link and
activate the mast cell or basophil, which will then release proinflammatory substances such as

- Biogenic amines such as histamine which increase vascular permeability, vascular smooth muscle cell relaxation, bronchial smooth muscle contraction, and peristalsis
- Granule enzymes and proteoglycans such as tryptase and chymase which induce tissue damage and mucus secretion
- Lipid mediators such as prostaglandin D2 which act as a vasodilator and bronchoconstrictor and promote neutrophil accumulation at inflammatory sites
- Cytokines such as IL-4, IL-5 (the latter induces eosinophil activation), IL-6, IL-13 (the latter induces mucus production) which all induce inflammation. TNF-α enhances the expression of adhesion molecules on endothelial cells, allowing influx of inflammatory cells at sites of inflammation.

Candidate gene studies of the beta chain of the FcɛRI (published in 1993) that showed an association with asthma, were among the first genetic studies in asthma. However, subsequent replication studies have produced inconsistent results, with some reports confirming, and other refuting this association.

REGULATORY T CELLS

Allergy can also be defined as the failure to tolerate allergens and regulatory T cells are important in the induction of tolerance. There are several types of regulatory T cells, including CD4+CD25 regulatory T cells, which express the transcription factor, forkhead/winged helix transcription factor box protein 3 (FOXP3). The levels of FOXP3 gene expression are considered the most reliable marker for the functionality of regulatory T cells and correlate with their suppressive activity. SNPs in the X chromosomal FOXP3 gene were reported to have opposite effects among different sexes since they were associated with sensitization to egg and inhalant allergens in girls, and remission of sensitization in boys.43 Secondhand smoking and air pollution exposure in children are reported to be associated with increased methylation levels of the promoter of the FOXP3 gene.44,45 This increased methylation is associated with a decreased expression of FOXP3 and has been reported to be associated with a higher risk of asthma and/or persistent wheezing.44 Furthermore, FOXP3 methylation was significantly decreased in peanut-allergic patients undergoing peanut oral immunotherapy compared to patients undergoing regular care. Interestingly, in patients who remained tolerant to peanut 6 months after oral immunotherapy (as shown by open food challenges), the FOXP3 DNA methylation level was lower compared to those patients who lost their peanut tolerance.46 A similar phenomenon occurs in allergic rhinitis patients receiving sublingual immunotherapy for grass and dust mites who also show decreased methylation of FOXP3 in regulatory T cells compared to those on placebo.47 These findings suggest that FOXP3
methylation is influenced by environmental factors and may be associated with the development of clinical tolerance to allergens.

**Specific Atopic Disease Genes Identified by GWAS**

Aside from genes that have been associated with atopic sensitization and genes that are shared between atopic diseases, there are also disease-specific genes (as discussed above and in Figure 4–3 and Table 4–2). GWA studies that systematically compare sensitization, asthma, allergic rhinitis, and atopic dermatitis have provided more insight into the genetic overlap of these diseases (shown in Figure 4–3). Importantly, some of the genetic markers found to be associated with a specific atopic disease are not associated with specific or total serum IgE levels, indicating that mechanisms unrelated to IgE may also be important in asthma and atopic dermatitis.

**Figure 4–3.** Venn diagram illustrating genes identified through genome-wide association studies as associated with the allergic diseases asthma, atopic dermatitis, and allergic rhinitis. Genes highlighted in black identify those discovered in Caucasian populations, with italics defining promising genes that did not quite achieve genome-wide significance. Genes highlighted in blue identify those genes discovered in non-Caucasian populations, while those in red identify
The specific symptoms that occur following a particular allergen encounter are determined by several factors that currently remain largely unknown. Tissue-specific expression of atopy may relate to the route of allergen exposure or the local presence and activation of inflammatory cells, including mast cells, or relate to the homing of active T cells to the target tissue. Genetic factors related to the microstructure of the target tissues may thus be important in determining the organ specificity of allergic disease. In addition to factors that relate to increased risk, it is tempting to speculate that local protective factors exist, that may be either genetic or epigenetic. The next section provides an overview how genetic studies have increased our understanding of the unique and shared origin of these diseases.
### The Genetics of Allergic Disorders

**Chapter 2**

**Part I**

The specific symptoms that occur following a particular allergen encounter are determined by several factors that currently remain largely unknown. Tissue-specific expression of atopy may relate to the route of allergen exposure or the local presence and activation of inflammatory cells, including mast cells, or relate to the homing of active T cells to the target tissue. Genetic factors related to the microstructure of the target tissues may thus be important in determining the organ specificity of allergic disease. In addition to factors that relate to increased risk, it is tempting to speculate that local protective factors exist, that may be either genetic or epigenetic. The next section provides an overview how genetic studies have increased our understanding of the unique and shared origin of these diseases.

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Chr</th>
<th>Association</th>
<th>Potential Function</th>
<th>GWAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6R</td>
<td>1q21</td>
<td>Asthma</td>
<td>Regulatory T cell function, T cell differentiation</td>
<td>58</td>
</tr>
<tr>
<td>DENND1B</td>
<td>1q31</td>
<td></td>
<td>Memory T cell functions.</td>
<td>59B</td>
</tr>
<tr>
<td>IL1RL1</td>
<td>2q11</td>
<td>IL-33 receptor-recruitment of inflammatory cells</td>
<td>41, 60, 61A, 62B, 63</td>
<td></td>
</tr>
<tr>
<td>PDE4D</td>
<td>5q12</td>
<td>Cell signaling, inflammation, ASM function</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>TSLP</td>
<td>5q22</td>
<td></td>
<td>Activates dendritic cells, Th2 immune responses</td>
<td>41</td>
</tr>
<tr>
<td>SLC22A4/RAD50/IL13</td>
<td>5q31</td>
<td>Organic cationic transporter/DNA repair/Th2 cytokine</td>
<td>62B, 65A, 41, 63</td>
<td></td>
</tr>
<tr>
<td>HLA-DRA/DRQ</td>
<td>6p21*</td>
<td>T cell responses/many additional genes in region</td>
<td>62B</td>
<td></td>
</tr>
<tr>
<td>CDHR3</td>
<td>7q22</td>
<td>Epithelial polarity, cell-cell contact and differentiation</td>
<td>41, 60, 62B</td>
<td></td>
</tr>
<tr>
<td>IL33</td>
<td>9p24</td>
<td>Recruitment/activation of inflammatory cells</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>C11orf30/LRRC32</td>
<td>11q13</td>
<td>Regulates gene expression, epithelial barrier/regulatory T cell function</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>SMAD3</td>
<td>15q22</td>
<td>TGF-β signaling intermediate, fibrosis</td>
<td>41, 61A, 62B</td>
<td></td>
</tr>
<tr>
<td>ORMDL3/GSDMB</td>
<td>17q21</td>
<td>Sphingolipid synthesis/cell apoptosis</td>
<td>52C</td>
<td></td>
</tr>
<tr>
<td>IL2RB</td>
<td>22q12</td>
<td>Binds IL-2/IL-15, lymphoid cell differentiation</td>
<td>59, 41</td>
<td></td>
</tr>
<tr>
<td>C11orf30/LRRC32</td>
<td>11q13</td>
<td>Allergic rhinitis Regulates gene expression, epithelial barrier/regulatory T cell function</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>FLG, LCE3A</td>
<td>1q21*</td>
<td>Atopic dermatitis Epidermal differentiation and structure</td>
<td>67, 68, 69, 70</td>
<td></td>
</tr>
<tr>
<td>IL1RL1, SLC9A4</td>
<td>2q12*</td>
<td>IL-33 receptor/sodium-hydrogen exchanger</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>IL2-IL21</td>
<td>4q27</td>
<td>T cell survival/B cell proliferation and IgE production</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>SLC22A4/RAD50/IL13/KIF3A</td>
<td>5q31</td>
<td>Organic cationic transporter/DNA repair/Th2 cytokine/cilia protein</td>
<td>68, 69, 70</td>
<td></td>
</tr>
<tr>
<td>HLA-B (BAT1/TNXB/CREBL1)</td>
<td>6p21</td>
<td>T cell responses/many additional genes in region</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>PRR5L</td>
<td>11p13</td>
<td>Cellular apoptosis</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>
Susceptibility Genes for Asthma, Allergic Rhinitis, and Atopic Dermatitis Recently Identified by GWAS


Note. Genes focused to those meeting conventional genome-wide significance (P<5 × 10−8) and/or independent replication in Caucasian studies only. ^Severe Asthma, #Childhood severe asthma with exacerbation, and $Childhood onset asthma. *Using Immunochip array.

*Association also observed in the Asian population GWAS.

II, interleukin; IL6R, IL-6 receptor; DENND1B, Denn/madd domain-containing 1b; IL1RL1, IL-1 receptor like 1; PDE4D, phosphodiesterase 4d, cAMP specific; ASM, airway smooth muscle; TSLP, thymic stromal lymphopoietin; Th2, T helper 2; SLC22A4, solute carrier family 22 (organic cation/zwitterion transporter), member 4; RAD50, S. cerevisiae, homolog of (DNA repair); IL13. IL-13; HLA-, major histocompatibility complex, class II; CDHR3, cadherin-related family member 3; IL33, IL-33; C11orf30, chromosome 11 open reading frame 30; LRRC32, leucine-rich repeat containing 32; SMAD3, mothers against decapentaplegic drosophila homolog3; TGF-β, transforming growth factor beta; ORMDL3, orm1-like protein 3; GSDMB, gasdermin b; IL2RB, IL-2 receptor beta; Ig, immunoglobulin; FLG, filaggrin; LCE3A, late cornified envelope 3A; IL1RL1, interleukin receptor-like 1; SLC9A4, solute carrier family 9, subfamily A (NHE4, cation proton antiporter 4), member 4; IL2-IL21, interleukin 2/21; KIF3A, kinesin family member 3A; PRRSL, proline rich 5-like; OVOL1, ovo-like 1(Drosophila); CLEC16A, C-type lectin domain family 16, member A; ZNF652, zinc finger protein 652; ADAMTS10, ADAM metallopeptidase with thrombospondin type 1 motif, 10; ACTL9, actin-like 9; TNFRSF6B, tumor necrosis factor receptor superfamily, member 6b, decoy.
ASTHMA

Asthma is a chronic inflammation of the airways with a prevalence of 8% in the US population that is characterized by reversible bronchial airway obstruction, chronic bronchial inflammation, and associated airway remodelling. The airway obstruction is partly caused by bronchial smooth muscle cell contraction and hypertrophy, mucosal edema, and by the production of mucus. The inflammation is characterized by mast cell, basophil, and eosinophil activation. These produce mediators of inflammation and airway remodelling.\textsuperscript{40,48–50}

Family-based linkage studies were the first approach that could be used to identify novel genes that were previously not implicated in asthma. The first gene discovered by this approach was ADAM33 (a disintegrin and matrix metalloproteinase 33).\textsuperscript{51} ADAM33 was associated with asthma and bronchial hyperresponsiveness, but not sensitization, and subsequent functional studies showed a role in smooth muscle and vascular remodeling.\textsuperscript{1} Several other asthma genes found by positional cloning were mainly expressed in structural cells of the airways. This suggests that these cells, in particular the airway epithelial cells, are important in asthma susceptibility.\textsuperscript{50}

The GWA studies have now provided a replicable set of genes that are associated with asthma (Table 4–2). The first GWA study in asthma in 2007 revealed that SNPs on chromosome 17q12-21 were associated with childhood onset asthma.\textsuperscript{52} These SNPs regulate ORMDL3 and GSDMB expression, genes of unknown function but potentially of importance to eosinophil trafficking, endoplasmic reticulum stress, and epithelial cell remodelling.\textsuperscript{1,48} The 17q12-21 locus is strongly associated with childhood-onset asthma, and to a lesser extent with allergic rhinitis, but not with atopic dermatitis or sensitization.\textsuperscript{1}

The GWA studies of asthma have revealed three genes that are in one single functional pathway: The IL33 gene encodes the IL-33 cytokine released by damaged cells, whereas IL1RL1 encodes the IL-33 receptor complex.\textsuperscript{48} Studies indicate that IL-33 acts on cells of both the adaptive and innate immune systems. The IL33 and IL1RL1 genes may exert their function in a recently discovered population of IL-33-responsive innate immune cells, the type 2 innate lymphoid cells, that are important sources of hallmark Th2 cytokines, such as IL-5 and IL-13.\textsuperscript{48} Other asthma-associated genes discovered by GWA analysis include SMAD3, which is a signaling intermediate of TGF beta, a cytokine important in airway wall remodeling.\textsuperscript{48} The relevance of epithelial cytokines in asthma was underscored by the finding that TSLP, an epithelial cytokine, is also associated with asthma.\textsuperscript{48}

The first resequencing study on asthma was published in 2012, hypothesizing that rare variants with a low frequency (<5%) have a large effect on disease susceptibility. This first study focused on 9 candidate genes and showed that indeed four of these rare variants contribute to asthma susceptibility.\textsuperscript{53} However, large resequencing projects are needed to define if and how often, these rare genetic variants contribute to diseases such as asthma.

RHINITIS

Allergic rhinitis, or “hay fever,” is often accompanied by allergic conjunctivitis. Common allergens are house dust mite, animal danders, and plant pollens. Patients suffer from mucosal edema, sneezing, mucus secretion, and itchy eyes. There is a strong and almost complete
overlap between genes that associate with IgE sensitization and allergic rhinitis.\textsuperscript{35,54} A recent meta-analysis of GWA studies on both asthma and allergic rhinitis identified 11 independent SNPs associated with the combined phenotype of asthma and allergic rhinitis. Two GWA studies on allergic rhinitis identified the LRRC32 gene variant, that was associated with the phenotype of allergic rhinitis without asthma.\textsuperscript{55} The LRRC32 gene variant is reported to encode a cell surface molecule expressed on regulatory T cells which induces FOXP3 expression.\textsuperscript{56}

**FOOD ALLERGY**

Food allergies are most commonly IgE-mediated immediate-type reactions that occur after ingestion of allergenic foods such as peanut, apple, or cow’s milk. Gastrointestinal symptoms such as vomiting and diarrhea are the most common, although respiratory symptoms, urticaria, and atopic dermatitis are often seen as well. Food allergy is potentially lethal since it can lead to anaphylaxis. This is a systemic allergic reaction characterized by vasodilatation, edema in several tissues, and bronchial obstruction, which can result in redistributive shock and respiratory impairment. Patients suffering from both asthma and food allergy are at high risk for anaphylaxis. Although this is an important clinical problem, compared to other atopic diseases, food allergy has been studied to a lesser extent, and no genome-wide associated studies are available.

**SUMMARY AND CLINICAL IMPLICATIONS**

Atopy and atopic diseases have a multifactorial etiology with influences from both environmental and (epi)genetic factors. In the last century, research has identified many genes and several pathways associated with IgE levels and each specific allergic disease. There is an overlap in genes associated with the different allergic diseases, although there are genes reported which are disease specific as well. Genetic studies have thus provided more insight into the basic mechanisms leading to different allergic diseases. However, future studies are indicated to identify genetic markers associated with food allergy and to further explore the mechanism leading from sensitization to atopic diseases. Promising fields are the role of regulatory T cells and epithelial integrity in the pathophysiology of sensitization, asthma, rhinitis, food allergy, and atopic dermatitis. Gene-environment interactions and epigenetic influences add another layer of complexity to this pathogenesis. Understanding the genetic and epigenetic basis and thereby the pathophysiology of allergy may improve diagnosis and treatment by predicting disease onset and providing targets for personalized treatment of patients. Specifically, genetic variation may affect treatment response and should therefore be taken into account. Identification of environmental factors influencing epigenetic regulation of genes can provide evidence for how exposure may regulate genes in atopy, which may in turn lead to better forms of prevention.

The GWA studies have now provided a replicable set of genes that are associated with asthma and atopic dermatitis. There is a strong and almost complete overlap in genes associated with sensitization and allergic rhinitis, and some genes overlap with asthma and rhinitis. No genome-wide associated studies are yet available for food allergy.

**ATOPIK DERMATITIS**

Atopic dermatitis or eczema is characterized by dry skin, intense pruritus, and a characteristic age-related distribution of inflammatory lesions. The pathophysiology is thought to be based on an impaired skin barrier, abnormal immune reactivity, and environmental factors such as allergens and microbes.\textsuperscript{57} However, the initial events in AD remain unclear. More progress has been made in identifying the effector pathway since it is now known that among other cytokines, IL-4 and IL-13 act on endothelial cells to promote cutaneous inflammation.\textsuperscript{57}

The genes identified in atopic dermatitis GWA studies show little overlap with genes for IgE\textsuperscript{71} sensitization, asthma, or rhinitis. As described above, an impaired epithelial barrier may be important in the development of atopic dermatitis. The strongest evidence comes from the FLG gene but also other genes from the epidermal differentiation complex may be important. Furthermore, a defective antimicrobial immune response predispose patients with atopic dermatitis to infections.\textsuperscript{57}
SUMMARY AND CLINICAL IMPLICATIONS

Atopy and atopic diseases have a multifactorial etiology with influences from both environmental and (epi)genetic factors. In the last century, research has identified many genes and several pathways associated with IgE levels and each specific allergic disease. There is an overlap in genes associated with the different allergic diseases, although there are genes reported which are disease specific as well. Genetic studies have thus provided more insight into the basic mechanisms leading to different allergic diseases. However, future studies are indicated to identify genetic markers associated with food allergy and to further explore the mechanism leading from sensitization to atopic diseases. Promising fields are the role of regulatory T cells and epithelial integrity in the pathophysiology of sensitization, asthma, rhinitis, food allergy, and atopic dermatitis.

Gene-environment interactions and epigenetic influences add another layer of complexity to this pathogenesis. Understanding the genetic and epigenetic basis and thereby the pathophysiology of allergy may improve diagnosis and treatment by predicting disease onset and providing targets for personalized treatment of patients. Specifically, genetic variation may affect treatment response and should therefore be taken into account. Identification of environmental factors influencing epigenetic regulation of genes can provide evidence for how exposure may regulate genes in atopy, which may in turn lead to better forms of prevention.
REFERENCES

21. Weidinger S, O’Sullivan M, Illig T et al. Filaggrin mutations, atopic eczema, hay fever,


63. Ramasamy A, Kuokkanen M, Vedantam S et al. Genome-wide association studies of asthma in population-based cohorts confirm known
PART II

EPIDEMIOLOGY AND ENVIRONMENTAL FACTORS INFLUENCING FOOD ALLERGY
CHAPTER 3

EXPERIMENTAL

PREVENTIVE MEASURES

FOOD ALLERGIES

PEDIATRIC ALLERGY AND IMMUNOLOGY 22: 374-7 (2011)

Published in Dutch as: