Genetics of asthma and atopy

Rijksuniversiteit Groningen

Genetics of asthma and atopy

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"The human genome underlies the fundamental unity of
all members of the human family, as well as the
recognition of their inherent dignity and diversity.
In a symbolic sense, it is the heritage of humanity."

Universal Declaration on the Human Genome and Human Rights

Voor *Annouk*

Paranimfen

H. Jongepier
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Contents

Introduction
The genetics of asthma and atopy

The central subject of this thesis is the genetics of asthma and atopy. This thesis comprises different aspects of genetic studies of asthma and atopy, from the definition of the asthma phenotype to linkage, association, and functional studies. In this introduction, we will first give definitions and then discuss some clinical, epidemiological, physiological, and immunopathological aspects of asthma and atopy. This will be followed by an introduction into genetic research and a discussion of different methods to investigate the genetics of complex diseases. Finally, the research questions that have guided our research are formulated.

Atopy and asthma

Atopy comprises all IgE mediated diseases, such as asthma, allergic rhinitis and atopic dermatitis. Individuals with atopy have a genetic predisposition to produce IgE antibodies against common environmental allergens.[1]

Asthma is currently defined as a "chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial responsiveness to a variety of stimuli." (National Heart Lung and Blood Institute, 1995).[2] This definition combines clinical characteristics (e.g. wheezing, breathlessness) and physiological characteristics of asthma (variable airway obstruction, bronchial hyperresponsiveness) with its underlying pathology, which is the inflammation of the airway walls.

Asthma and atopy have a close interrelation. Atopy often precedes asthma and it increases the risk of developing asthma 10 – 20 fold.[3] The initial sensitisation to environmental allergens occurs typically in childhood. A subset (25-30%) of those sensitised to environmental allergens develops asthma.[4] Both the level of sensitisation, expressed as the number of positive skin tests, and the type of sensitisation are important for asthma development.[5] The association of asthma with sensitisation to a particular allergen appears to depend on the geographical distribution of the allergen. In the Netherlands, the major allergen is derived from house dust mites.[6] In other countries with a low prevalence of house dust mites, other allergens such as cat, dog, cockroach and the mould alternaria are important.[7] In childhood, virtually all patients with asthma have atopy, whereas in adulthood also non-atopic asthma, so-called intrinsic asthma, can be found. Patients with intrinsic asthma present typically in adulthood, have variable airways obstruction, and may not have complete reversibility of airways obstruction.[8] In the study presented in this thesis, 15 % of the patients with asthma of whom we studied their families did not have atopy. It is unknown if intrinsic asthma is a separate condition with a distinct genetic susceptibility.[9]

Clinical expression of atopy

Patients with asthma have in general a history of episodic breathlessness together with cough and wheeze. In asthma, symptoms such as cough and wheezing occur frequently at night.[10] Patients may also experience symptom free intervals. The prevalence of symptoms in children with asthma decreases into adulthood. For example, in a study of a cohort of 119 allergic asthmatic children, virtually all children reported wheeze in childhood. However, only 18 % still reported wheeze when followed up at age 32- 42.[11]

Allergic rhinitis is defined as an inflammation of the lining of the nose, characterised by one or more of the following symptoms: nasal congestion, rhinorrhoea, sneezing and itching.[12] In seasonal allergic rhinitis, symptoms exist in the pollen season, whereas in perennial allergic rhinitis symptoms are chronic and persistent throughout the year.
Atopic dermatitis, also called eczema, is a chronically relapsing inflammatory skin disease. It is characterised by itching, leading to scratching and excoriations. In addition, dryness of the skin is a typical finding in atopic dermatitis. In it first stage (age 0-3 years), typically the scalp and cheeks are affected by a pruritic erythema with papules, vesicles, exudation and excoriations. Later on in childhood, typical sites of atopic dermatitis are the flexural sides of elbows and knees. It has been reported that as many as 50 - 75 % of patients with eczema in early childhood will develop allergic rhinitis or asthma.[13] In this thesis we will focus on respiratory atopic diseases, in particular asthma.

Epidemiology

The prevalence of atopic diseases in childhood in Western populations is high. There are large worldwide differences in its prevalence. In the ISAAC study, the prevalence of atopy was investigated in 56 countries in children aged 13-14 years. The 12-month prevalence of symptoms of asthma ranged from 1.6 to 36.8 %, compared to allergic rhino-conjunctivitis (1.4 - 39.7 %) and atopic dermatitis (0.3 - 20.5%). Western countries, such as the United Kingdom, USA Australia and Canada were found among the countries with the highest prevalence.[14] Data for the Netherlands are available through the European Respiratory Health Survey, a random population study of adults aged 20 - 48 years. Three regions in the Netherlands (Groningen, Bergen op Zoom and Geleen) were investigated. Prevalences of wheeze were reported to be 18.2%, 18.9%, and 18.8%; and attacks of asthma 3.2%, 3.1%, and 1.5%, respectively.[15] The prevalence of atopy as assessed by specific IgE to four aeroallergens was 36.1 % in the Netherlands in this study. [6]
Prevalence rates depend a.o. on the definitions that have been used to assess asthma and atopy. For asthma, a doctor's diagnosis, asthma symptoms or definitions based on bronchial hyperresponsiveness have been used. For example, in Australia and New Zealand, prevalence of self reported asthma has been reported to be 16.3 % (adults, Busselton), whereas the prevalence of wheeze was 28.8 % in the same population.[16] A difference between the presence of bronchial hyperresponsiveness and a doctor's diagnosis of asthma was observed in first and second degree relatives of 92 patients with asthma in our family study. 113 out of 320 children had bronchial hyperresponsiveness to histamine. Of these 113 children, 33 (29 %) had a prior doctor's diagnosis of asthma. Of the 207 children without bronchial hyperresponsiveness to histamine,[15] 7.2 % had a prior doctor's diagnosis of asthma.[17]
Evidence is accumulating that the prevalence of asthma has risen over the last 20 – 30 years.[18] This is paralleled by a rise in atopy, as measured by a positive skin prick test, over the last decades.[19] It is thought that only a part of

the rise in asthma could be explained by atopy. It remains to be established what drives the increased prevalence of asthma.[20]
Different environmental factors have been investigated to explain the rise in prevalence of atopy and asthma.[19] For atopy, provoking factors include exposure to allergens, current or in early life, smoking, and air pollution, whereas possible protective factors include infant breast feeding, infections in early life, living at a farm, and exposure to lipopolysacherides. For asthma, these factors have also been investigated, together with a possible role of diet.[16, 21] Current data suggest that allergen exposure early in life is associated with sensitisation, but not with wheeze, asthma and bronchial hyperresponsiveness at age 7.[22] An important hypothesis that may explain epidemiological observations of inverse associations between childhood infections and atopy is the hygiene hypothesis. This hypothesis was originally proposed in 1989 by Strachan. He proposed that 'allergic diseases could be prevented by infections in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally'.[23] This hypothesis was consistent with epidemiological observations of inverse relationships of atopic parameters (hay fever, skin prick tests) with childhood infections, a higher number of (older) siblings, and vaccination status.[23] In addition, high exposure to lipopolysacherides (LPS) in house dust was inversely related to the prevalence of sensitisation in a study of children aged 9 - 24 months.[24] One receptor through which LPS may exert its function is CD14. Association of a promoter variant in this gene with severity of atopy has been described in a US population of school children.[25] This finding was confirmed in a case-control study in the Dutch population as described in chapter 8 of this thesis. Immunological support for this hypothesis came from the observation that bacterial products, such as lipopolysacherides are inducers of a non-allergic (Th1-type) immunity. This will be discussed in more detail in the section on immunology.

Lung function and physiology

Two functional alterations are typically associated with asthma: variable airway obstruction and bronchial hyperresponsiveness.[26] In healthy individuals, airway size depends on several factors: its relaxed dimension, its elastic properties and the tone of the smooth muscle in its wall. In individuals with asthma, several factors contribute to airways narrowing, such as smooth muscle contraction, airway wall thickening, oedema and increased secretion of mucus.[26] In the lung function laboratory, airway obstruction can be demonstrated by forced expiratory tests or measurements of airway resistance and conductance. At home, serial peak expiratory flow measurements may indicate variable airway obstruction.
Bronchial hyperresponsiveness (BHR) is defined by a heightened sensitivity of the bronchi in response to non-specific stimuli. There are different types of stimuli to which patients with asthma show elevated responsiveness of the airways: parasympathicomimetic agents (e.g. methacholine),

β-receptor antagonists (propranolol), mediators (e.g. histamine, serotonin, adenosine), osmotic/physical stimuli (e.g. hypotonic or hypertonic saline, cold air) and finally air pollutants (e.g. ozone). Stimuli that evoke BHR can also be distinguished in stimuli that act directly on receptors of airway smooth muscle cells and indirect stimuli that are thought to act by stimulation of cellular and neurogenic pathways (adenosine-3- monophasphate, cold air).[27] In general, the concentration of the agent that after inhalation causes a 20% drop in FEV_1 (forced expiratory volume in 1 second) is lower than in healthy individuals. In addition, it appears that the type of the response is also different between asthmatic and normal airways. In asthma, airway narrowing can be progressive with higher doses of the challenge agent, whereas airways of normal individuals reach a plateau phase of maximum airway narrowing.[26]

Pathology and immunology

A recent review discussed the immunopathological findings in the central and peripheral airways found to be more or less characteristic of asthma. These constituted of "denudation of airway epithelium, deposition of collagen beneath the basement membrane, mast cell degranulation, and infiltration of the airway by lymphocytes and eosinophils".[28] An important feature seen in asthma is remodelling of the airways walls. Airway remodelling comprises all alterations in structural cells and tissues in the asthmatic when compared to the normal airway.[29] These include thickening of all components of the airway wall, subepithelial fibrosis (thickening of the basement membrane), increase myocyte muscle mass, myofibroblast hyperplasia and mucus metaplasia.[29] Mediators that may be important in airway remodelling are not completely known, but possibly include TGF-ß family proteins and matrix metalloproteases (MMPs) and their tissue inhibitors (TIMPs). Interestingly, transgenic mice overexpressing interleukin IL-13 show features consistent with airway remodelling.[30] The precise mechanisms leading to airway remodelling are not fully understood, but it has been hypothesised that the bronchial epithelium plays an important role in airway remodelling and inflammation in asthma by producing cytokines and interaction with inflammatory cells [31,32] and the extracellular matrix.[33]

Cytokines and chemokines that mediate inflammation are found to be present in broncho-alveolar lavage fluid in patients with asthma. These include elevated levels of Th2 type cytokines, such as interleukin (IL)-4, 5, 9 and 13. It is currently thought that the imbalance between Th1 (interferon-γ and IL-2) and Th2 (IL-4, 5, 9, 13) lymphocytes is a fundamental underlying mechanism in asthma.[28] The Th2 skewed immune response in asthma is shown in figure 1.[33] Environmental allergens are processed by antigen presenting cells (APC) and presented to T-lymphocytes. In the presence of IL-12, differentiation into Th1 memory cells occurs, whereas the presence of IL-10 and the absence of IL-12 stimulated the differentiation into Th2 lymphocytes that produce IL-4, IL-5, IL-9 and IL-13.[33] These cytokines will be discussed in detail in

this thesis and are summarised in table 1. Some of these cytokines act through the activation of transcription factors, such as nuclear factor-κB and members of the signal transduction-activated transcription factors family (STAT). These transcription factors subsequently upregulate adhesion molecules, pro-inflammatory cytokines and chemokines.
Asthma appears to be the result of allergic inflammation, in which the production of immunoglobulin E (IgE) is essential. Allergens that enter the airway are processed by dendritic cells that have the ability to present antigen to T- and B cells. In the presence of cytokines, such as IL-4 and IL-13, and costimulatory molecules, CD40-CD40L, B cells switch to the synthesis of IgE[28,34] (figure 2). Key effector cells in asthma in the airways include inflammatory cells such as mast cells, eosinophils and lymphocytes (especially CD4 positive T helper 2 lymphocytes) (table 1).

Genetics

This thesis builds on the tremendous progress in genetic research over the last decades. This starts in 1953 with the seminal paper of Watson and Crick on the structure of DNA and to the publication of the working draft of the sequence of the human genome in 2001 (table 2).[35-37] From this sequence, the total number of human genes is predicted to be between 30.000 and 40.000, much less than had been thought previously.[38] Complete knowledge of the DNA transcribed into mRNA and protein (the proteome) will be crucial to start understanding diseases that have a genetic origin.

Figure 2 IgE regulation

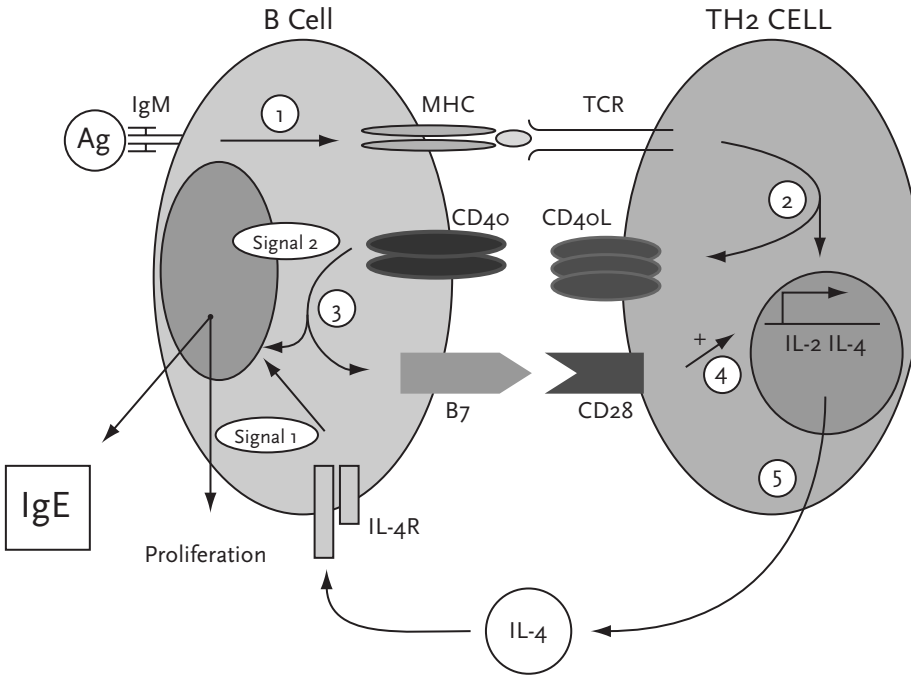


Table 1 Some cytokines relevant to development and/or progression of atopy and asthma (from Busse and Lemankse Jr (28))

Cytokine	Gene location	Primary source	Primary target	Effects or function
IFN-γ	12q14	CD4+ Th1 cells, lymphocytes, natural killer cells, some CD8+ cells	Macrophages CD4 + cells CD8 + cells Natural killer cells	Differentiation and activation Shift towards Th1 cytokine production Increased cytotoxicity of CD8+ T-cells Activation
IL-4	5q31-33	CD4+ Th2 cells	B cells Th1 cells Th2 cells CD8+ T cells Natural killer cells	Growth and activation, production IgE Inhibition Th1 cytokine production Differentiation of Th2 cells Differentiation of CD8+ T cells Inhibition of proliferation
IL-5	5q31-33	CD4+ T cells, CD8 + T cells	Eosinophils	Proliferation and activation
IL-9	5q31-33	CD4 + T cells	B cells	Enhancement of response to IL-4
IL-10	1q32-33	CD4+ Th0 cells, Th1 and Th2 cells, CD8+ T cells	Monocytes Macrophages	Differentiation to macrophages Inhibition of expression of adhesion molecules, swith Th1 to Th2 cells, inhibition IL-4 and IFN-γ production by TH2 cells
IL-12	5q31-33 (p40) 3p12-q13 (p35)	Monocytes, macrophages	Natural killer cells Th0 cells Th1 cells Th2 cells	Activation Production IL-2 Production IFN-γ and TNF-α Inhibition of production of IL-4, 5, and 10
IL-13	5q31-33	CD4+ Th2 cells	B cells Monocytes	Growth and activation, production IgE Production MHC class II molecules and integrins Inhibition of IL-2 and Il-1 and TNF

Note. IL Interleukin, TNF Tumor necrosis factor, MHC major histocompatibility complex, IFN interferon. Th T helper

Figure 2. The development of the Th_1 and Th_2 response

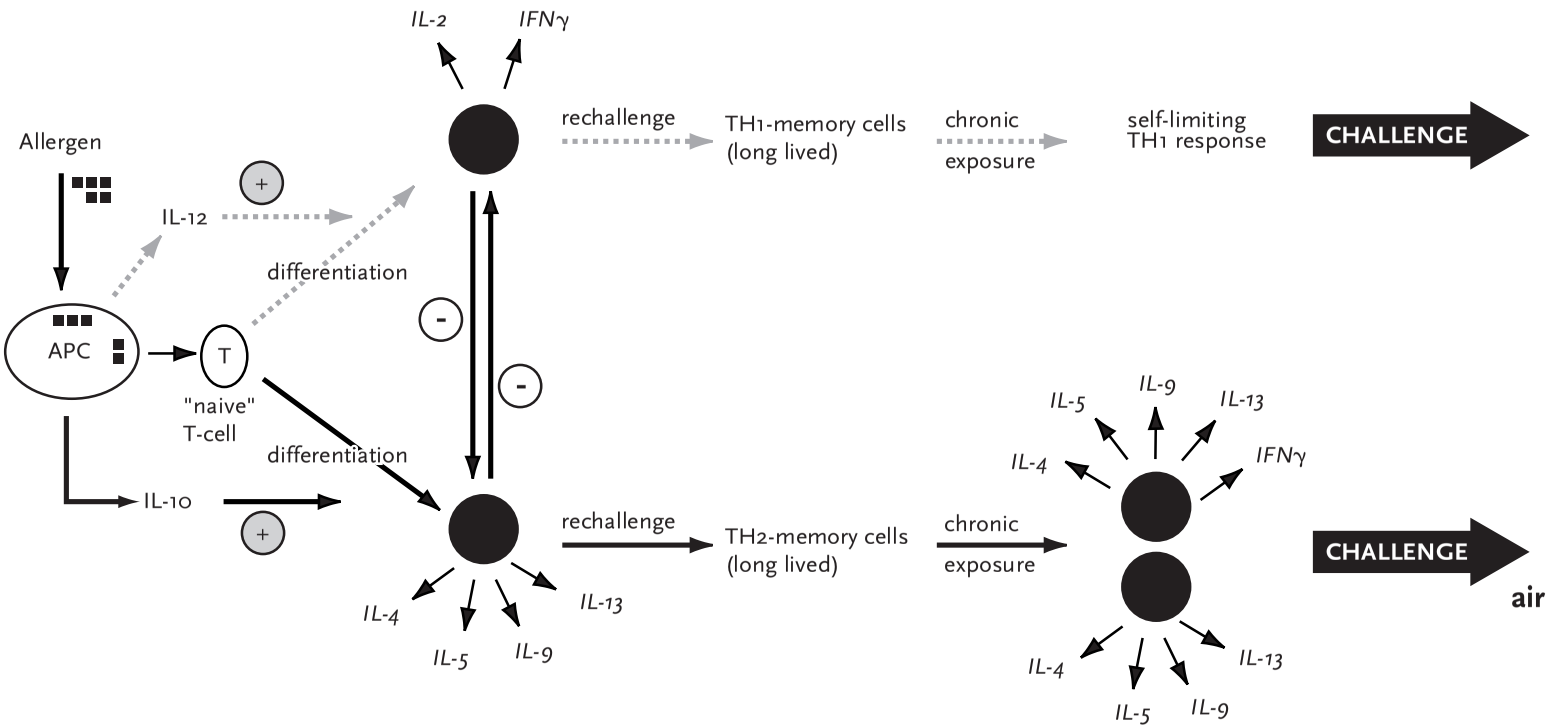


Table 2 Milestones in genetic research

Year	Milestone
1953	Watson and Crick discovered the double helical structure of DNA
1977	Two separate scientific groups proposed a way to sequence DNA
1980	A method to map the entire human genome with restriction fragment length polymorphisms (RFLP) was proposed
1985	The polymerase chain reaction (PCR) was developed
1990	Official start human genome project
1994	A first genetic linkage map of the human genome (average marker spacing 0.7 cM) is published
1995	The first sequence of a living organism, Haemophilus influenzae, is published
1997	Capillary sequencers become available
1999	The sequence of the first human chromomosome, chromosome 22, is completed
2000	The sequence of the fruit fly Drosophila melanogaster (180 Mb) is completed The sequence of chromosome 21 is completed The sequence of the first plant (Arabidopsis thaliana) is completed
2001	The working draft of the human genome is published

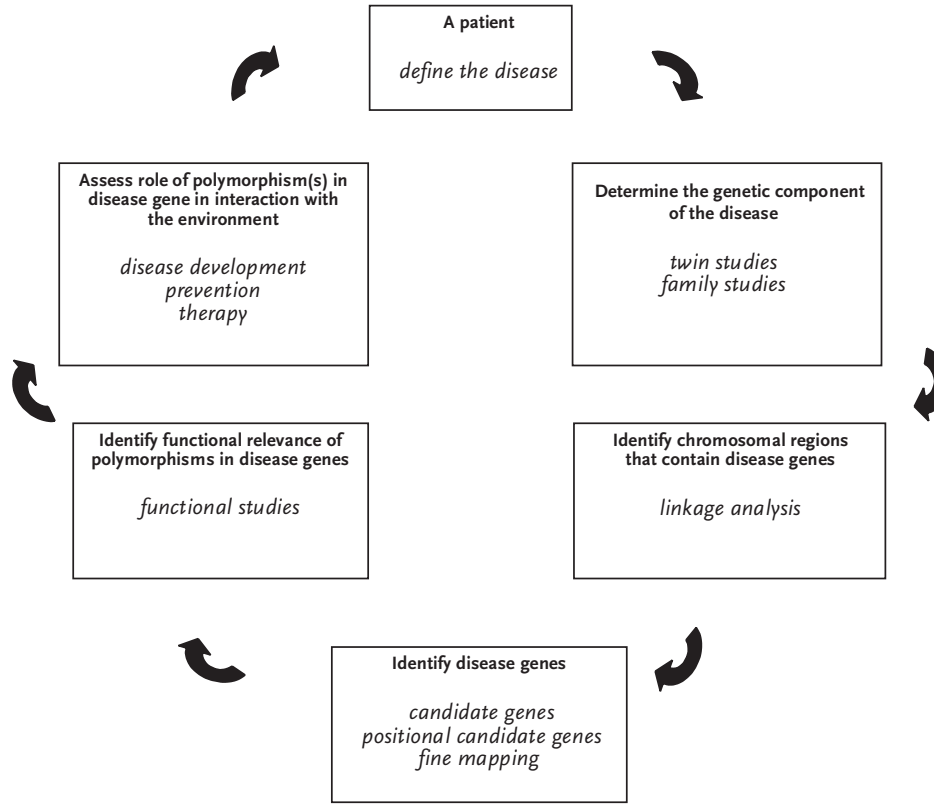
Genetic diseases, such as cystic fibrosis, are the result of the change in one gene. Chronic diseases, such as diabetes, inflammatory bowel disease and asthma, are the result of an interaction between multiple genes and environmental factors. These diseases are called genetic complex diseases.[39] Which alterations in genes confer susceptibility to these genetic complex diseases is currently not completely understood. Unravelling this may have major significance to public health with respect to early identification, prevention, and the development of personalized treatment.[39, 40] To this aim, in this thesis we will describe studies in the search for genes in asthma and atopy.

Genetic studies of complex diseases

The starting point of genetic studies of complex diseases is the patient. The key question for every medical doctor 'Why does this particular patient get this disease?' could be answered in part by pursuing the heritable origin of the disease. Genetic studies of complex diseases can be summarized as a circular process of six steps, starting and ending with the patient (figure 3).[40,41] These steps can be summarized as follows:

- Define the disease
- Determine the genetic contribution to the disease
- Identify chromosomal regions that contain disease genes
- Identify (polymorphisms in) disease genes
- Identify functional relevance of polymorphisms in disease genes
- Assess role of polymorphisms in disease genes in interaction with the environment

Figure 3 Steps in genetic research in complex diseases



Genetic research has been successful in single-gene Mendelian diseases, such as Duchenne muscular dystrophy and Huntington disease. Since the early 1990, genetic research has focussed on complex human diseases. The identification of these disease genes is a major challenge to the scientific community. Recently, it was proposed that a gene (calpaine-10) was identified for a complex disease, diabetes type II, through positional cloning.[42,43] Although further replication in other populations needs to be shown, this would provide an important proof of concept. In asthma, genetic research is still in full progress. This thesis will contain examples of studies of the genetics of asthma and atopy. It reflects the collaboration of a team of clinicians, technicians, statisticians, geneticists and molecular biologists in the Netherlands and in the United States over the past 11 years.[44]

Step 1. Define the disease

A first step is to define the trait under study. In genetics, choosing the right definition (the disease phenotype) is especially important. If this definition reflects gene function best, the chances of success to find one or more specific genes may increase. Several guidelines for defining a disease have been suggested previously by Lander and Kruglyak.[39] Issues in defining asthma and the application of guidelines to define asthma in genetic studies are discussed in chapter 2.

Step 2. Determine the genetic contribution to the disease

Involvement of genetic factors is suggested from familial aggregation of the disease. In 1650, Sennertus observed that his wife, three of her brothers and sisters and her niece all had asthma (cited in Wiener).[45] Familial aggregation of a trait can be examined using the comparison of correlations between family members. For example, total serum IgE levels was not correlated between unrelated spouses (r=-0.06), but showed significant correlations between parents and offspring in our family study (r=0.24).[46] In addition, we also observed familial clustering of skin test positivity and the presence of specific IgE to common aeroallergens. Finally, the prevalence of a disease in children with a family history of the disease can be compared to the population prevalence. In a previous study of our first 92 families, a higher prevalence of asthma was observed in first degree offspring of asthma patients (26 %) than the population prevalence of asthma (estimated 8%).[17] This indicated familial clustering of asthma.

Thus, both asthma and atopy show familial aggregation. In this thesis, family studies are reviewed in the first section of chapter 1. Important questions that are answered in that chapter are:
– Are there, according to the published literature, major genes for asthma and atopy?
– What are the number of genes involved and do we know the mode of inheritance?

Twin studies can dissect familial and environmental contribution to disease. Twin studies in asthma are discussed in chapter 3. In this short review, we asked two questions:
– What is the heritability in liability to asthma?
– What is the nature of the environmental contribution in asthma based on twin studies?

Familial aggregation of atopy is further investigated in chapter 4. In this study, we assessed the possibility of a parent of origin effect in atopy and investigated the role of environmental factors in these families as indicated by the sibling effect.

Step 3. Identify chromosomal regions that contain disease genes

The classic method to identify chromosomal regions that contain disease genes is through linkage analysis. In part 3, the results genome-wide linkage analysis of characteristics of asthma and atopy are shown. The research questions for that part are:

– which chromosomal regions may contain susceptibility genes for regulation of total serum IgE (chapter 5)
– which chromosomal regions may contain susceptibility genes for serum specific IgE, allergy skin tests and number of blood eosinophils? (chapter 6)
– which chromosomal region on chromosome 2 may contain an atopy susceptibility gene? (chapter 7)

Step 4. Identify disease genes

There are two methods to identify a disease gene. First, fine mapping of linked chromosomal regions may eventually lead to identification of disease genes. This strenuous part of genetic studies of asthma and atopy is in full progress. No successful fine mapping attempts have been published in the literature so far. However, new statistical methods based on linkage disequilibrium have become available and this may certainly facilitate gene-finding studies.[47-50] A second method is through candidate gene analysis. Candidate genes can be chosen based on understanding of the disease and / or based on the localisation on the human genome (positional candidate). Analyses of five proposed candidate genes are included in this thesis, namely CTLA-4 and CD28 (chapter 7), CD14 (chapter 8), IL-13 (chapter 9), and IL-4R (chapter 10).

Step 5. Identify functional relevance of polymorphisms in disease genes

Association per se does not fully prove the role of a gene variant in disease. It has to be complemented with functional evidence. The functional role of a gene can be assessed in various ways: an animal model (a knockout or transgenic mouse); an in vitro model (e.g. a cell system, or a reporter system to study promoter function), ex-vivo or in vivo. This thesis contains a study assessing the functional role of different promoter polymorphisms in the gene encoding the $ß_2$-adrenoceptor. Functionality of these variants was assessed by studying $ß_2$-adrenoceptor density and function in peripheral blood mononuclear cells of asthma patients (chapter 11).

Step 6. Assess role of polymorphisms in disease genes in interaction with the environment

In genetically complex diseases, genes interact with environment in the development of the phenotype. Once relevant disease genes are known, the interaction with the environment may be investigated. Next to causative factors, the interaction of the genetic background with medication may be assessed (pharmacogenetics).

Finally, a summary of the findings of this thesis is given, together with ideas on future studies.

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