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Genetics of asthma and atopy

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Document Version

Publisher's PDF, also known as Version of record

Publication date:

2001

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Koppelman, G. H. (2001). *Genetics of asthma and atopy*. s.n.

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| Summary and future perspectives

Asthma and atopy are prevalent chronic diseases that affect millions of people worldwide, and these prevalence rates are increasing all over the world. Thus, it is important to unravel the pathophysiological determinants of their development. Asthma and atopy are caused by an interaction of genes and environmental factors. This thesis deals with host factors; that is the genetics of asthma and atopy.

This thesis comprises studies performed by a multidisciplinary collaboration between universities in the Netherlands (University of Groningen) and the United States of America (Wake Forest University, Winston-Salem and the University of Maryland at Baltimore) to unravel the complex nature of asthma and atopy. In this chapter the main conclusions of this thesis will be summarized. Every part of this thesis describes a step in genetic research, from the disease and its definition (part 1), the determination of the genetic and environmental contribution (part 2), the identification of chromosomal regions that may contain asthma and atopy genes (part 3), the study of candidate genes (part 4) to the investigation of gene function (part 5). For every part, recommendations for future research are given.

In the first part a review of current published evidence on the genetics of asthma and atopy is presented. From this review, it is clear that progress in genetics has been made in the years prior to the start of this study. First, the genetic contribution to asthma and atopy has been shown repeatedly with twin and family studies. It is clear that this genetic contribution consists of multiple genes, interacting with each other and with the environment. Second, chromosomal regions on human chromosomes 5, 11, and 12 had been identified at that time, which are most likely to contain asthma and/or atopy genes. Third, candidate gene analyses had shown genetic associations of alleles in the genes encoding the FCεRIβ, cytokine genes (IL-4, IL-9, IL-13) and their receptors (e.g. IL4 receptor gene) in asthma and / or atopy. Replication of linkage as well as association results has not been proven easy so far. Differences in ascertainment strategies, types of populations, sample sizes, and phenotype definitions may explain this difficulty. Finally, functional data showing a change in quantity or quality of the gene product in relationship to a proposed single nucleotide polymorphism in a candidate gene is present for only a few genes.

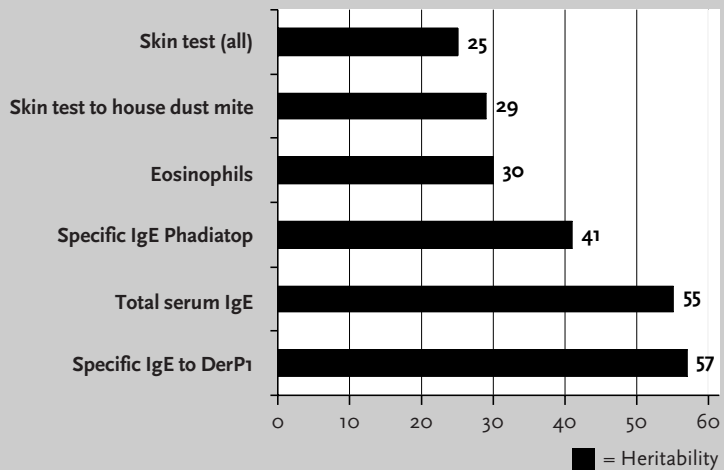
An important first step in genetic research is the definition of the disease. Since a gold standard for diagnosing asthma is lacking, the best possible approach is to diagnose asthma with the use of validated questionnaires in combination with objective markers of variable airway obstruction, bronchial hyperresponsiveness and / or airway inflammation. In addition, the recommendations of Lander and Kruglyak¹ for defining diseases in genetic studies may be noteworthy. These authors advise one to study a subset of the disease such as early age of onset, which has been very useful in cancer genetics; to define a severe disease phenotype; (if possible) to choose a specific sub-phenotype, and choose patients with a positive fami-

ly history.¹ Application of these four recommendations to narrow the disease definition would lead to the selection of the following category of asthma patients for genetic studies: asthma with early age of onset, ongoing into adulthood, severe asthma, in individuals with a positive family history. However, evidence for familial aggregation of a specific subphenotype of asthma, such as early age at onset, has not been provided to date. A very interesting phenotype is the 'longitudinal' phenotype, i.e. the outcome of asthma. For instance, it is not fully understood what predicts the progressive loss of lung function in asthma. Some risk factors for development of low lung function have been identified, such as low initial FEV₁, bronchial hyperresponsiveness and smoking.^{2,3} However, the genetic contribution to the outcome of asthma is unknown. We recommend to study which genes have a modifying effect on the outcome of asthma. Interesting candidate genes could be IL-4, IL-4 receptor, and IL-13. For IL-4 and IL-4 receptor, cross sectional data are published showing associations of alleles in these genes with low lung function.^{4,5} For IL-13, a transgenic mouse model overexpressing IL-13 showed signs of inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production.⁶ If this would apply to the human situation, one could predict that individuals with asthma expressing larger quantities of IL-13 (i.e. individuals homozygous for the -1111 T allele),⁷ would have a higher risk of developing low lung function and irreversible airway obstruction in later life.

The second part of this thesis focuses on the determination of the genetic contribution to asthma and atopy. Multiple twin studies, such as the study presented by Skadhauge et al.⁸, have indicated that the genetic contribution to asthma is considerable. Twin studies that use genetic modeling indicate that the genetic variability in asthma is composed of additive genetic factors, in combination with a polygenic component. In our family study, a clear familial clustering of atopic traits (total serum IgE, specific IgE to aeroallergens, skin test positivity and blood eosinophil levels) was shown. The heritability of these traits was estimated using variance components analysis (figure 1). Heritability estimates (h^2) were highest for specific IgE to Der P1 (0.57) and for total serum IgE (0.55); h^2 was 0.41 for Phadiatop; 0.30 for log eosinophil count; 0.29 for skin test to house dust mite, and 0.25 for skin test positivity.

Twin studies that use genetic modeling provide evidence for the type of environmental factors that may be important. Two types of environmental factors are distinguished: individual specific factors that interact specifically with an individual, and shared factors that act for both members of a twin pair. According to twin studies in asthma, the environmental contribution to asthma appears to be mainly individual specific, but not shared. Thus, in asthma genetic factors may interact with individual specific environmental factors in a unique fashion, i.e. dose and / or time dependent. Now that candidate genes for asthma and atopy are becoming clearer, large-scale studies of gene by environmental interaction should be

Figure 1. Heritability of asthma- and atopy associated traits in the Dutch family study as calculated by variance components analysis.



planned. Important environmental factors to be mentioned are active and passive smoking, allergen and endotoxin exposure. Another interesting environmental factor is indicated by the sibling effect, which is reported in the second part of the thesis. In our family study, the presence and severity of atopy was inversely associated with the size of the family (specific IgE to common aeroallergens) and birth order (skin test positivity). It is currently believed that the sibling effect is a proxy of the presence of childhood infections. For future intervention studies, it is important to realize that even in these high-risk families, environmental effects may modify atopy.

We believe that a better understanding of asthma and atopy will be provided by studies of gene by environmental interaction. In our family study, we have therefore started to investigate the environment by collection of house dust for analysis of house dust mite allergens and lipopolysaccharides. We recommend performing studies on the interaction of allergen exposure with the specific immune response (HLA region), as well as with genes important in the upregulation of the immune response, such as the cytokine genes and their receptors. Finally, interaction of endotoxin with its receptors, such as CD14, could be studied in relationship to the development or modification of the allergic response. In addition, the interaction of endotoxin with the Toll like receptor 4 with respect to asthma severity and lung function could provide important explanations on the mechanism of endotoxin induced bronchoconstriction and airway inflammation.⁹

The third part of this thesis comprises genome wide linkage results in 200 families ascertained through a proband with asthma. In the past five years, we have added 108 families and restudied 66 of the previously ascertained families in Beatrixoord in Haren, the Netherlands.¹⁰ In the linkage analysis of the total set of 200 families, several chromosomal regions showed evidence for linkage of an atopic phenotype: chromosome 2q, 6p, 7q, and 13q. These also showed evidence of linkage with total serum IgE. Specific regions of interest for atopic traits were also detected at chromosome 11q, 17q, and 22q. Although there was confirmation of chromosomal regions thought to be important in allergy and asthma (e.g. 5q, 12q), also novel regions were detected. The most significant finding was for total serum IgE levels on chromosome 7q, a finding that needs to be followed up in further replication and fine mapping studies, which will be carried out in our department. Another interesting chromosome is chromosome 2q, which contains strong candidate genes for atopy and asthma. We have fine-mapped this region on chromosome 2q. The LOD score for total serum IgE in this region increased from 1.96 to 3.16 with the addition of new markers. Two candidate genes in this region, CTLA-4¹¹ and CD28, were studied. Significant evidence for association of asthma and atopy was observed with two SNPs in the CTLA-4 gene (table 1).

Table 1. Candidate gene results from this thesis

Gene	Chromosome	SNP	Phenotype	Function
CTLA-4	2q33	-1147 C-T	Asthma, BHR	Possibly influences gene transcription
		-318 C-T	No associations identified	
		+49 A-G	Total serum IgE, asthma, BHR, skin tests	Signal transduction in co-stimulation of T cell activation
CD28	2q33	-824 A-G	No associations identified	
CD14	5q31	- 159 C-T	Total serum IgE and number of skin tests in skin test positive subjects	Possibly upregulates gene transcription
IL 13	5q31	-1111 3'UTR	BHR, asthma, skin test positivity	Possibly upregulates gene transcription
IL4 receptor	16p12	E375A C406R S478P	Total serum IgE, skin test positivity, asthma, BHR	Enhances signal transduction of the receptor

BHR Bronchial hyperresponsiveness

The next challenge is to identify genes that underlie these linkage signals. Experience from the past decade shows that this is a most difficult task in genetic research of complex diseases. In asthma, no studies of successful fine mapping studies have appeared in the literature, but this may change since press releases have announced that some 'asthma genes' have been identified.¹² We have been involved in a collaborative study on positional cloning of a susceptibility gene for bronchial hyperresponsiveness on chromosome 5q together with Novartis Pharmaceuticals, Horsham, United Kingdom (dr. P. Whittaker) and new findings will be published in the future.

Progress in genetic research may further be enhanced by developments in statistical approaches, novel insights into population characteristics and physical data from the Human Genome Project.

For fine mapping and positional cloning, no gold standard exists with regard to the statistical methodology. The classical approach from Mendelian diseases, which studied families, and identified critical recombinants to narrow down the region can not be used in the genetics of complex diseases. Therefore, the possibility of large-scale association analysis has been discussed in the literature.^{13,14} Another interesting method is the analysis of shared haplotypes between cases and controls. In founder populations derived from a limited number common ancestor, it is expected that patients have similar polymorphisms in a disease gene and surrounding haplotypes than unaffected controls. Several investigators, including Dr. Te Meerman at the University of Groningen are developing statistical methods that use this principle.¹⁵⁻¹⁸ In the past years, we have collected approximately 250 trio's (patient and two family members) from the province of Friesland to use this methodology. It is therefore important to know the type of population under study (e.g. inbred, founder, or outbred population) and know physical characteristics, such as the level of linkage disequilibrium. From preliminary studies it appears that the Dutch and Friesian population residing in the northern part of the Netherlands have appealing characteristics for genetic research.

Finally, physical data from the Human Genome Project have become available for genetic researchers in 2001. One surprising finding was that the number of genes in the human genome is lower than expected, approximately 30.000. This may sound appealing for gene 'hunters', because a lower gene density may result in a lower number of positional candidate genes, which could make fine mapping easier. However, it has become increasingly likely that normal physiological and possibly pathophysiological processes do not depend on the function of a single gene, but that multiple genes may interact. Thus, it may be anticipated that complex gene-gene interaction studies need to be carried in the future. It may be predicted that studies of function of the transcriptome and proteome will be important next steps in understanding these interactions and providing models for intervention.

In part four of this thesis, three candidate genes are analyzed (table 1). First, evidence is presented for a -159 C to T promoter polymorphism in the CD14 gene (CD14/-159) modifying the severity of allergy, as expressed by higher serum total IgE levels and higher number of skin tests in skin test positive individuals. This confirmed data presented by Baldini et al. from the Tucson study in the United States. In addition, we showed that individuals carrying two C alleles at CD14/-159 were more likely to develop hayfever and allergic rhinitis, but not asthma.

Second, we investigated IL-13 as a candidate gene for asthma. We analyzed three single nucleotide polymorphisms (SNPs) in IL13 in an extended group of 184 probands and spouses: one in the promoter region (-1111), the Arg130Gln (nucleotide position 4257), and a 3' UTR SNP (nucleotide position 4738). The most significant associations were observed to asthma ($p=0.005$), bronchial hyperresponsiveness ($p=0.003$), and skin-test responsiveness ($p=0.03$) with the -1111 promoter, replicating previous associations with asthma and allergy phenotypes.^{19,20,21} These results provide evidence that variation in the IL13 gene is involved in the pathogenesis of asthma and atopy. Further replications and functional analyses are needed to clarify the possible role of coding and regulatory SNPs in this gene.

Third, an investigation of the different SNPs in the interleukin 4-receptor gene is presented. Significant associations of three tightly linked SNPs in exon 12 of this gene with total serum IgE and skin test positivity was identified. This further confirms published data of Ober et al.²² and Kruse et al.²³ indicating a role of these SNPs acting alone or in concert in the development of atopy. An important finding was that individuals carrying the major susceptibility alleles in IL-4 R (for IgE) and IL-13 (for bronchial hyperresponsiveness) were 5 times more likely to develop asthma than individuals not carrying the risk alleles. This is to our knowledge the first finding of gene-gene interaction in the development of asthma. Since the percentage of individuals carrying both risk alleles in our population was rather small, replication of this finding in other studies is needed.

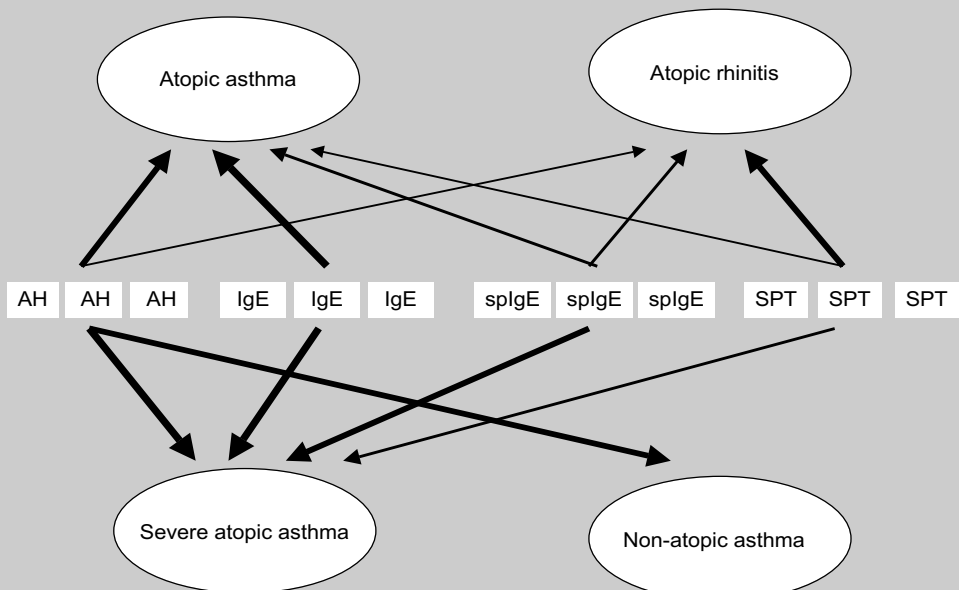
Part five of this thesis comprises functional analysis of the β_2 -adrenoceptor. Data showing that the presence of a gene variant alters the function of a gene product in a relevant study system is scanty in the literature. For the β_2 -adrenoceptor, a collaborative study was performed together with the Universities of Nottingham (Prof. I.P. Hall) and the University of Aberdeen (Prof. B. Lipworth), United Kingdom. Within the gene encoding the β_2 -adrenoceptor, extensive linkage disequilibrium between coding and promoter variants was shown in different European populations. However, no differences were seen with respect to β_2 -adrenoceptor density on circulating peripheral blood mononuclear cells and production of cAMP by peripheral blood mononuclear cells of asthmatics.

Future perspectives

What will genetics bring with regard to understanding the complex pathogenesis of asthma and atopy, the diagnosis and therapy of patients with an atopic disease?

From results of genetic studies, novel insights may arise to understand the complex interrelation between asthma and atopy. From twin and family studies it is suggested that asthma and atopy may have a common genetic basis. In addition, a clustering of linkage signals can be observed for both asthmatic and atopic phenotypes, which may indicate the presence of genes for both phenotypes.²⁴ In addition, also disease specific genes may contribute to asthma or other atopic diseases. This is suggested from several observations. First, there is no complete overlap of the presence of different atopic phenotypes within families. Second, genome-wide linkage studies also indicate specific regions that show some evidence of linkage to specific atopic phenotypes. Third, different intermediate phenotypes show different associations with asthma (total serum IgE) and allergic rhinitis (skin test positivity). Thus, also specific genes may contribute to different atopic phenotypes and eventually lead to different atopic diseases (figure 2).

Figure 2 Possible interactions between genes for atopy in asthma and rhinitis.



AH Airway hyperresponsiveness; IgE total serum IgE; spIgE specific IgE; SPT skin prick test.
From: D.S. Postma, G.H. Koppelman and D.A. Meyers: The genetics of atopy and airway hyperresponsiveness. *Am J Resp Crit Care Medicine* 2000; 162: S118-S123.
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To further understand genetic similarities and differences between susceptibility to asthma and atopy, two different strategies can be pursued. First, non-atopic asthma can be studied, which was shown to have a genetic contribution.²⁵ However, given the fact that only a minority of patients with asthma are non-atopic, this probably needs to be a multi-center study. Second, atopic non-asthmatic patients (for example with allergic rhinitis) may be studied and the genetic results compared to atopic asthmatic patients. Currently, the latter study is being carried out in our department.

An important question is whether genetics will improve (early) diagnosis of asthma? In the majority of children under the age of 6, no lung function measurements or assessments of bronchial hyperresponsiveness can be performed. Therefore, the diagnosis of young children with recurrent episodes of wheezing as having asthma is difficult. Could genetics bring a solution to this problem in the end? It is likely that susceptibility genes for asthma have a high population frequency, given the high prevalence of asthma. If the frequency of this risk allele is for example 30 %, it can be a major contributor to the disease at a population level. If carriers of this risk allele have a relative risk (RR) of 2 to become asthmatic, this genotype alone could explain 23.1 % of the disease on a population level. The question remains if the detection of this allele also gives valuable diagnostic information on a patient level. In the same example (allele frequency 30% and a relative risk of 2), the chance that an individual will develop asthma is only 7.7 % when carrying the risk genotype.²⁶ Thus, the diagnostic value will be low for an individual patient when assessing genotypes with high population frequencies and low relative risks.²⁶ However, combination of alleles in different genes (as indicated in our study for IL13 and IL4R) may be more informative.

Will genetics improve management of patients with asthma? Currently, three lines of treatments are available for patients with asthma: inhaled glucocorticoids; bronchodilators and leukotriene inhibitors. Since the response to these drugs is markedly variable between individuals, researchers are now exploring the genetic contribution to the response, but in particular, to non-response to treatments.²⁷ If indeed, variations in drug targets are of major importance in drug response, then identification of the variants before start of treatment may prevent overtreatment in individuals prone to non-responding. Second, it is currently unclear if genetic factors are important in patients with steroid-resistant asthma. This is an important group of patients with difficult-to-treat, mostly severe asthma.²⁸ Steroid resistant asthma imposes a heavy burden on the individual patients in terms of morbidity and hospitalizations and thus contributes significantly to the costs of asthma for the society. Unraveling (genetic) factors in steroid resistance could therefore be important in designing new adjuvant strategies in the treatment of these patients. Finally, novel therapeutic approaches with monoclonal antibodies and soluble receptors are now being undertaken.

Especially the experiments with soluble IL4R as a treatment for asthma have attracted our attention.²⁹ In our genetic studies, a subpopulation carrying the risk genotypes in IL4R and IL-13 genes appear to be at especially high risk for developing asthma. We propose to perform a pharmacogenetic study with soluble IL4R in patients with these risk genotypes. One may predict that blocking IL13-IL4R pathway in this subgroup may be very beneficial. We hope that this may be an illustration of how genetic research could benefit individual patients with asthma.

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