Summary

This thesis consists of two complementary parts. Chapters 2 to 7 describe studies on the genetic basis of asthma, bronchial hyperresponsiveness and atopy, while chapters 9 to 11 contain studies on the long-term outcome of asthma.

Chapter 2: Dutch approach on the study of the genetics of asthma.

Chapter 2 describes the background and design of this study. Additionally, it discusses the strengths and limitations when performing a study on the genetics of a complex trait.

Chapter 3: The genetics of asthma and atopy.

Chapter 3 presents an overview of the statistical methods that are used in genetic analyses, taking previous studies on the genetics of asthma and atopy as examples.

The importance of replication of reported results in different, independent populations to exclude false associations of phenotype characteristics and genetic markers is emphasized. Clearly a close co-operation among clinicians who define the phenotype, molecular biologists who genotype, and genetic analysts who analyze the data, is warranted for successful results.

Four future directions of research are suggested:
1. Replication of previously reported results.
2. Development of genetic models such as multilocus models, to investigate the inheritance of the complex genetic traits.
3. Completion of a genome-wide search to seek for other linkages.
4. Fine mapping of the area(s) of interest to evaluate their potential importance.

Chapter 4: Characterization of obstructive airways disease in families with asthma.

Chapter 4 discusses the complexity of defining the asthma phenotype. Studies on the genetics of asthma require a distinction between individuals with definitive asthma from those with COPD or unaffected subjects, as misclassification of individuals may obscure true linkage to specific gene(s). An algorithm based on both objective and subjective measures is presented, which enables the
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classification of the phenotype of each family member enrolled in the study. This algorithm was applied to 92 two- and three-generation families, identified through a subject with asthma (proband). The algorithm consists of 5 classes and is based on the presence or absence of BHR, respiratory symptoms, smoking, airways obstruction and bronchodilator reversibility. All family members were classified as class 1 -definite asthma, class 2 -probable asthma, class 3 -unclassifiable airways disease class 4 -chronic obstructive pulmonary disease (COPD), and class 5 -unaffected (without clinical evidence of asthma and COPD).

Thirteen of the 92 probands (16%) could not be classified as asthmatic when retested 25 years later, because of one of the following reasons: loss of BHR, loss of bronchodilator reversibility or a current history of cigarette smoking. Of the 265 first degree offspring, 49 (19%) were classified as asthmatic (class 1), and an additional 22 (8%) as probably asthmatic (class 2). A large number of offspring with clinical evidence of asthma did not have a prior physician based diagnosis of asthma. Offspring who were identified by the algorithm (class 1, definite asthma) had similar clinical, physiologic and allergic characteristics as those subjects with a physician's diagnosis of asthma. This finding suggests an underdiagnosis of asthma in families of asthma patients. The algorithm also identified another group of offspring (class 2 and 3) who did not fulfill all of the requirements for asthma but who shared one or more findings consistent with this disease. This group might be at risk for developing asthma. The results show that reliance on a prior physician's diagnosis can result in misclassification i.e. in over or underdiagnosis of asthma. Characterization of the offspring in this family study shows that there is a familial aggregation of asthma and related phenotypes, supporting the observation that asthma has a hereditary component.

Chapter 5: Genetic Regulation of Total Serum IgE Levels.

Chapter 5 presents an overview of the results of the genetic analyses of total serum IgE levels in the 92 Dutch asthma families, along with the results of published other studies. Serum IgE levels are correlated with the clinical expression of bronchial hyperresponsiveness and asthma, therefore representing an important quantitative parameter that may be used to map the gene(s) involved. One-locus and two-locus segregation analyses are reviewed, and evidence for a recessive inheritance of high total serum IgE levels, with a second locus with recessive inheritance, unlinked to the first locus, is reported. Additionally, the results of the one-locus and two-locus linkage analyses in respect to the area of interest on chromosome 5q are described. In a one locus linkage analyses, a LOD score of 3.56 for marker D5S436 was reported. When a two-locus model was used, the LOD score increased to 4.67, showing evidence for a second, unknown locus, not linked to the locus at chromosome 5.
Although other studies have reported evidence for linkage to this region on chromosome 5q as well, the results do not appear to favor one specific gene candidate within the gene region on chromosome 5q.

**Chapter 6**: Exclusion of linkage of atopy, asthma and bronchial hyperresponsiveness to markers on chromosomes 11q and 6p.

Chapter 6 reports the results of the linkage analysis of atopy and bronchial hyperresponsiveness to markers on chromosome 11 and chromosome 6. Previous studies have reported a familial predisposition for the development of atopy, bronchial hyperresponsiveness and clinical asthma, and reported linkage between atopy and chromosome 11q. Other studies have suggested an association between atopy and certain HLA antigens, from the HLA complex mapped to chromosome 6.

This study presents the results of the first 20 families collected. These are two- and three-generation families, ascertained through an asthmatic proband, diagnosed 25 years earlier. Sixty-six percent of the offspring of these probands were atopic. Sib pair and linkage analysis were performed using the highly polymorphic markers INT2 on chromosome 11q and D6S105 on chromosome 6p, located close to HLA-DR. The possibility of linkage between atopy and bronchial hyperresponsiveness and these two markers was examined. LOD scores of -2.00 were observed for both markers. Similar results were observed with both of these markers and bronchial hyperresponsiveness. Therefore, these results present evidence for exclusion of linkage between atopy or bronchial hyperresponsiveness and these regions of chromosomes 11 and 6 in this population.

Four possible reasons for the failure to replicate findings of other studies were postulated:

1. The disease phenotype may have been defined differently in different studies. In the present study, however, the allergic phenotype was defined in a similar manner to the group reporting positive linkage.
2. There may be a high degree of genetic heterogeneity, i.e. genes in different regions of the genome may individually be sufficient for disease expression. No evidence for genetic heterogeneity was found in the study presented here.
3. Several genes may interact in disease expression (oligogenic inheritance). This is a plausible situation.
4. Difficulty of interpreting a LOD score calculated under an unlikely model. If the postulated model of inheritance, used for the linkage analysis, is wrong, the statistical interpretation of the resulting LOD score is problematic.
Chapter 7. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5.

Chapter 7 presents evidence for linkage of a gene involved in IgE production to chromosome 5q, using sib-pair and LOD score analyses. The LOD score analysis was based on the recessive model of high IgE levels obtained from the segregation analysis. Of the many genes that map to chromosome 5q, several regulate inflammation and airway wall remodelling, implying that this chromosomal region may be important in the regulation of inflammatory processes in allergy and asthma. The linkage results may be applicable to a general population; however, it has not been explored yet whether families consisting of allergic members without asthma show similar evidence for linkage to 5q. Efforts to map this region and genes that show more specificity to total serum IgE production may improve our understanding of the control of allergic inflammation in subjects with allergy and asthma.

Chapter 9: Chronic complications of asthma.

This chapter gives an overview of current knowledge and the possible influence of treatment on acute and long-term outcome of asthma, in respect to symptoms and lung function. Symptoms are not the only focus of asthma treatment, as severe and irreversible airflow limitation may develop without the patient noticing it. Early treatment of asthma with inhaled corticosteroids may give optimal control of its symptoms. Preliminary results, both in adults and children, suggest that delaying treatment may result in irreversible damage. Additionally, cessation of treatment results in rapid recurrence of symptoms, bronchial hyperresponsiveness and airflow limitation.

There are still several questions that need to be addressed. For example, it is uncertain whether the use of inhaled corticosteroids in the management of asthma alters the disease in the small airways, or cures the disease. It seems that once the trigger is activated, the inflammatory process takes its own course. Optimal avoidance of allergens, environmental toxins and cigarette smoke may, next to optimal anti-inflammatory therapy, alter the course of asthma. It remains to be established whether the observation that physiologic plasma cortisol levels modulate the process responsible for the deterioration of ventilatory function with aging has clinical relevance for asthma as well. New strategies are needed for the development of a treatment that prevents the occurrence of asthma, switches the disease off in childhood once present, and/or prevents relapse in adult life.
Chapter 10: Adult patients may outgrow their asthma.

Chapter 10 presents the analyses of factors that may determine the 'loss of asthma' in a cohort of 189 adult patients tested between 1962 and 1970. At this initial testing, 92% were atopic and all were hyperresponsive. When re-tested 25 years later, 21% of them did not show bronchial hyperresponsiveness, and 12% were no longer considered asthmatic. Absence of asthma after 25 years was associated with younger age and less severe airways obstruction at first testing. Neither gender nor atopy were significant determinants of the outcome of asthma; however, a lower level of IgE was significantly associated with outgrowing asthma. Asymptomatic individuals without bronchial hyperresponsiveness at second investigation had a shorter untreated period from onset of asthma symptoms. This finding suggests that earlier treatment of asthma may prevent persistence of the disease. Results show that a substantial proportion of symptomatic asthmatics may outgrow their asthma. Data also suggests that having a milder disease and receiving earlier intervention is associated with a better outcome of asthma.

Chapter 11: Risk factors for the development of irreversible airways obstruction in asthma patients.

Chapter 11 presents the analyses on the outcome of asthma in the cohort of 189 adult asthma patients in respect to the development of symptoms of COPD. After 25 years 14.4% of this asthmatic population had developed irreversible airways obstruction (IAO), and 21.8% showed low diffusion capacity (DC) postbronchodilator. The group developing IAO had a longer duration of undertreatment at the first visit, and more severe asthma, as assessed by lung function data. At the second visit, these subjects showed lower FEV₁, higher RV, steeper loss of postbronchodilator FEV₁, and reported more symptoms, as assessed by questionnaire. Regression analysis showed that the development of IAO is associated with a lower FEV₁ % predicted and by a lower level of hyperresponsiveness (PC_{20} > 8 mg/ml). The latter finding may be influenced by the higher age in this group. The comparison of the groups with low and (near)normal postbronchodilator DC suggests that the former included heavier smokers who already had a significantly higher RV and TLC at the first visit. The results presented in this chapter provide evidence to conclude that patients with more severe asthma are at higher risk for developing IAO or low diffusion capacity. Although both IAO and low DC are characteristics of COPD, it seems that they represent two distinct groups in symptomatology and etiology in this population. Subjects with a low FEV₁ at initial testing are at a high risk for developing an irreversible component of their airways obstruction, especially when treatment was delayed. Subjects who show low diffusion capacity, on the contrary, are mainly those who report a significant smoking history, as is also
observed in emphysema patients who never had asthma. Data presented in this chapter confirms epidemiologic reports in that female asthma patients in this study are more susceptible to this effect of smoking than male patients.