Genetics of different asthma phenotypes
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Chapter 1

General introduction
General introduction

Asthma: there is not one asthma, but there may be dozens!
In the Netherlands, every day, 35 children hear from their general practitioner that they have asthma. There is still no cure for this disease that affects over 300 millions of people worldwide. In society, asthma is often seen as a mild disease where people experience sometimes an attack of shortness of breath, but asthma is more than ‘just’ shortness of breath. Children with asthma experience disturbed sleep due to nocturnal symptoms, increased school absenteeism as well limitations in exercise and social activities.

Different phenotypes of asthma are increasingly being studied instead of asthma as one disease. Several strategies can be followed to study these phenotypes of asthma. One can for example study clinical phenotypes, e.g. age of onset of asthma, symptoms (presence of attacks of shortness of breath, wheezing, cough and/or exacerbations), medication usage, presence of allergies and/or eczema, severity and reversibility of airflow limitation (measured as $\text{FEV}_1$, FVC, $\text{FEV}_1$/FVC, FEF25-75, while reversibility can be defined either spontaneously over time or with a bronchodilator), bronchial hyperresponsiveness and blood markers (eosinophils, total IgE and specific IgE). After this characterisation, a cluster analysis can be performed to identify several subsets of asthma defined by combinations of these clinical characteristics. The Severe Asthma Research Program (SARP) has performed such a cluster analysis on asthma. They included 726 adult asthmatics and identified 5 clusters based on their characteristics. The 5 clusters differed with respect to age, gender, age of onset, lung function values, and allergy status.\(^1\)

Another way to characterize asthma-phenotypes is by its underlying molecular mechanisms. With this approach, inflammatory markers that associate with asthma (for example IL-4, IL-5, IL-13, eosinophils and IgE) in blood and/or tissue can be determined to build different clusters within asthma. Alternatively, a genome wide gene expression profile of bronchial epithelial cell mRNA can be performed, resulting in the identification of Th\(_2\) high and Th\(_2\) low subset of asthma.\(^2\) This particular Th\(_2\) high profile also related to a better response of the lung function to inhaled corticosteroids (ICS) as compared to asthmatics with the Th\(_2\) low profile, showing the potential of such clusters and phenotypes to add to personalized medicine.\(^2\)
**Genetic studies in asthma**

In the early times of genetic research, genetic studies were mainly limited to linking a disease to loci on the genome. The limitation was predominantly due to the techniques available at that time and the high costs of DNA genotyping. These costs have dropped drastically over the years and now DNA can even be sequenced to cover the whole genome. After initial candidate gene studies and linkage studies, GWAS studies were implemented in asthma research. When in 2007 the first Genome Wide Association Study (GWAS) on asthma was conducted, novel genes were found to be associated with asthma. In 2010, the first asthma consortium based GWA study was published in the New England Journal of Medicine. In this study a total of 23 study populations were meta-analysed, including 10,365 asthmatics and 16,110 controls. This study identified eight genome wide significant genes of interest for the risk of asthma. In 2011, three other research groups performed a GWAS on asthma which in total identified five new genome wide significant genes. These studies, together with the meta-analysis of Moffatt et al., have identified 13 genes to be genome wide significantly associated with asthma (table 1). Other GWA studies have added a specific phenotype into their asthma definition, such as the age of onset, lung function parameters, bronchial hyperresponsiveness or exacerbations as subphenotypes of asthma. Table 1 shows genes that reached a genome wide significant association with asthma and with subphenotypes in asthma.

GWA studies always investigate a single SNP effect, but the effect of SNPs can also merely be present in combination with another SNP, so called epistasis or gene-gene interaction. This means that in order for a SNP to have an association with the disease, a specific combination of SNPs is needed. This phenomenon has been described by our group in a study of Reijmerink and others. They showed that SNPs in genes relevant in pathways for IgE production did not show a main effect, but did show multiple interactive associations in relation to IgE production.

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**Table 1: Genome wide significant genes in a GWAS on asthma or asthma subphenotype.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor diagnosed asthma</td>
<td>PYHIN1, IL1RL1/IL18R1, TSLP, IL33, GSDMB, IL6R, 11q13.5, GSDMA, ORMDL3, HLA-DQ, IL2RB, SMAD3, PERLD1</td>
</tr>
<tr>
<td>Childhood onset asthma</td>
<td>PDE4D, DENND1B, HLA-DP, ORMDL3, IL1RL1/IL18R1, GSDMB</td>
</tr>
<tr>
<td>Adult onset asthma</td>
<td>GATA3, IKZF4, 4q31, HLA-DQ</td>
</tr>
<tr>
<td>Childhood onset of asthma with a severe exacerbation</td>
<td>CDHR3</td>
</tr>
</tbody>
</table>
Therefore it could be important to include an interaction of SNPs with other SNPs or an interaction of SNP and environmental factors in a GWAS design called the genome wide interaction study (GWIS). The main problem in performing such a GWIS is to gain enough power to find significant associations. Until now these interactions have mostly been studied in a candidate gene model.

When SNPs are associated with a specific disease, it remains to be determined what the functions of the SNPs are. There are several ways a SNP can influence the mechanisms underlying asthma development. One of the mechanisms is by changing mRNA expression of genes. The change in mRNA expression can occur with the gene in which the SNP is located or in a gene in the neighbourhood (cis-eQTL), or in a gene further away from the SNP (trans-eQTL). The SNP influences the amount of mRNA that is transcribed from the gene (figure 1). It has been reported that SNPs found in GWA studies have a higher chance of being associated with gene expression.

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**Figure 1: Effect of the genotype of a SNP on gene expression.**

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**The impact of asthma phenotypes on the outcome of genetic studies**

Moffatt and others\(^4\) did not only report the meta-analysis of the 23 studies that enrolled in the GABRIEL consortium, but also included a stratified analysis by age of asthma onset in their paper. They studied childhood as well as adulthood onset of asthma separately. One of their top hits, GSDMB, was only significantly associated with childhood onset of asthma and not with adulthood onset of asthma (Childhood onset: odds ratio (OR); 0.76 p-value; 6.4\(^*\)10\(^{-23}\), adulthood onset: OR; 1.03 p-value; 4.9\(^*\)10\(^{-1}\)). When childhood and adulthood onset were mixed, the genome wide significant association was still present, but the effect was diluted to a p-value of 4.6\(^*\)10\(^{-9}\). This is an example of how the effect of the SNPs can depend on the phenotype studied. If only adult onset asthma would have been studied in the paper of Moffatt and others the association of the gene with asthma would not have been found.
Bronchial hyperresponsiveness; a specific phenotype in asthma?

One important phenotype of asthma is bronchial hyperresponsiveness (BHR), frequently regarded as a hallmark of asthma.\textsuperscript{15} BHR reflects the shortness of breath that asthmatics experience due to airway obstruction after inhalation of non-allergic substances that do not induce respiratory symptoms or a fall in lung function in healthy individuals. To measure BHR, a challenge test can be used and most asthma patients test positive on this challenge test. During a challenge test asthmatics are exposed to different doses of methacholine or histamine, examples of directly acting agents on the smooth muscles that surround the airways, leading to bronchoconstriction. Asthmatics are considered to be bronchial hyperresponsive if the Forced Expired Volume in one second (FEV\textsubscript{1}) has fallen 20\% after inhalation of a predefined concentration of the stimulus compared to the baseline FEV\textsubscript{1}. This lowering of the FEV\textsubscript{1} needs to be reached before or at the highest dose step of the inhalation provocation test. In epidemiological studies, a PC\textsubscript{20} (provocative dose at which the FEV\textsubscript{1} falls by at least 20\%) of <16 mg/ml methacholine or histamine is considered abnormal.\textsuperscript{16} BHR can also be expressed by a slope instead of a PC\textsubscript{20}.\textsuperscript{17} The steepness of the slope can be used as a measurement of the severity of BHR. A major advantage of the slope over the PC\textsubscript{20} is that asthmatics who do not reach a 20\% fall in FEV\textsubscript{1} during a challenge test, for instance because they use ICS that ameliorates BHR, still have a measurement on the severity of BHR (figure 2).

The severity of BHR has been associated with several characteristics of asthma. More severe BHR has been associated with a worse control of asthma, more airway wall inflammation and structural changes of the airway wall. Therefore the severity of BHR sometimes is regarded a surrogate marker of asthma severity. The severity of BHR is depending on different factors. There are physical as well as environmental factors associated with the severity of BHR.
One of the physical factors is the airway diameter. The smaller the diameter, the quicker the 20% fall in FEV$_1$ is being reached. In childhood, the airway diameter is smaller compared to adulthood, leading to a more quickly reached PC$_{20}$ than in adulthood. Longstanding environmental exposure may affect the airway wall thickness and the smooth muscle remodelling, also contributing to more severe BHR.\textsuperscript{18} Since in adulthood based exposure time, the impact of environmental exposures is often bigger and the airway diameter is larger than in childhood, different mechanisms could underlie childhood and adulthood asthma. Therefore, it is important to separate the severity of BHR in childhood and adulthood since different mechanisms can underlie BHR severity in childhood and adulthood.

Only one study has investigated the severity of BHR in asthma. Himes and others performed a GWAS on the severity of BHR in children with asthma. They reported two genes, ITGB5 and AGFG1, to be associated with the severity of BHR.\textsuperscript{19} Other genes reported in the literature were found with candidate gene studies. These studies have identified 27 genes either associated with the severity of BHR in childhood or in adulthood. However, of these 27 genes, only one gene, ADRB2, was replicated in adult asthma\textsuperscript{20-22}, whereas in children with asthma five replicated genes were identified, ADRB2, GPR154, TBX21, VEGF and FCERIb.\textsuperscript{23-29}

SNP can have an interaction with several environmental factors and in that way influence the severity of BHR. A specific example of an interaction was given by Reijmerink and others who studied the effect of in utero smoking exposure and BHR.\textsuperscript{30} They found an interaction between two SNPs (rs528557 and rs3918396) in ADAM33 and in utero smoke exposure. of the risk to develop BHR became significantly higher, if mothers smoked during pregnancy and children had a specific genotype.\textsuperscript{30} Another example of an interaction between genes and environment has been given by Tantisira and others.\textsuperscript{31} They studied the interaction between one SNP (H33Q) in the gene T-BOX 21 and ICS use with regards to the severity of BHR. Asthmatic children who were treated with ICS with the 33Q genotype were less hyperresponsive after ICS treatment than asthmatic children with the 33H genotype. The placebo group did not show any difference in BHR at all. Thus, a genetic background may underlie (non-) responsiveness to ICS in the severity of BHR in asthma. The given examples are all candidate gene studies, but such interactions can also be studied by performing a genome wide interaction study (GWIS) on gene and environment. A major problem with these GWI studies is the power that is needed to find significant results. Not only is a sufficient number of patients for each specific genotype required, but also for each subgroup of the environmental exposure under study. This stratification renders groups often very small, which will lead to low power of detecting significant hits.
Asthma remission; the answer to curing asthma?

The course of asthma over a lifespan differs per individual. Some asthmatics experience severe respiratory symptoms all their life, whereas others only have mild symptoms or are free of symptoms. Although there is no cure for asthma yet, some asthmatics have spontaneous recovery from their asthma symptoms, have normal lung function and do no longer express BHR. These asthmatics are in so called ‘asthma remission’. Figure 3 introduces the symptom threshold, showing that each individual has his/her own threshold level. As long as patients experience the symptoms above the symptom threshold they have active, clinical asthma, and if the experienced symptoms are under the threshold, patients are in clinical remission of asthma. Once asthmatics are no longer hyperresponsive, measured with a challenge test, and do not experience any asthma symptoms, and do have normal lung function they are in complete remission. An interesting question is why asthma disappears and a subsequent question is: are there ways by which this status can be realized for all patients with asthma?

Figure 3: Symptom threshold of experiencing asthma symptoms

It would be great if we can already predict in childhood how asthma will develop later in life. Several studies have investigated the predictors of asthma remission later in life. Important predictors of asthma remission are an early age of onset of asthma, a good lung function, no atopy, a low level of total IgE, male gender, low eosinophil count and no ICS use early in life (table 2). It could be relevant to identify a phenotype of asthma that is associated with remission later in life. With such a phenotype it could be possible to predict if children are likely to ‘outgrow’ their asthma. Once this remission phenotype is identified, genetic studies can be conducted on this phenotype. These studies can identify genes that are associated with remission of asthma, which can help us find and understand pathways involved in remission. In time, medication could be developed to target these mechanisms resulting in asthma remission.
Aims and outline of this thesis
The major aim of this thesis is to study the genetic background of different phenotypes of asthma. In the first part of the thesis the active/persistence asthma phenotypes like BHR and severity of BHR will be studied, to gain more insight in the biological mechanisms underlying these specific asthma phenotypes. In the second part of this thesis the asthma remission is studied in a 40-year follow-up asthma cohort. In this cohort it is possible to investigate clinical and complete remission of asthma from childhood to adulthood on a clinical and genetic level. Predictors of clinical and complete asthma can be determined based on early childhood data. Furthermore, genetic background of remission in asthma can be studied using long-term follow-up asthma cohort.

Table 2: Overview of predictors for clinical (no asthma symptoms and medication use in the last 12 months and/or BHR and/or FEV₁ %predicted <80%) or complete remission (no asthma symptoms and medication use in the last 12 months, no BHR and FEV₁ %predicted >80%) during childhood or adulthood

<table>
<thead>
<tr>
<th>Clinical remission</th>
<th>Children in remission</th>
<th>Adults in remission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Higher FEV₁ early in life³⁵,⁴¹</td>
<td>Higher FEV₁ early in life³⁸</td>
</tr>
<tr>
<td></td>
<td>Higher FEV₁ later in life³²</td>
<td>Higher FEV₁ later in life³⁸,⁴⁰</td>
</tr>
<tr>
<td></td>
<td>Low BMI³⁴</td>
<td>Early age of asthma onset³⁸,⁴³</td>
</tr>
<tr>
<td></td>
<td>Low total IgE³⁴</td>
<td>Low BMI³⁸</td>
</tr>
<tr>
<td></td>
<td>No ICS use early in life³⁴–³⁶</td>
<td>Low total IgE³³,³⁸</td>
</tr>
<tr>
<td></td>
<td>Male gender³⁸,³⁹</td>
<td>Male gender³⁸,⁴³</td>
</tr>
<tr>
<td></td>
<td>No atopy³⁴,³⁵,³⁷</td>
<td>No ICS use early in life³⁸</td>
</tr>
<tr>
<td></td>
<td>Low eosinophil count³⁹,⁴⁰</td>
<td>Persistent wheeze⁴⁴</td>
</tr>
<tr>
<td></td>
<td>Low severity of asthma early in life³⁶</td>
<td>Low eosinophil count⁴⁰</td>
</tr>
<tr>
<td></td>
<td>No maternal smoking³⁶</td>
<td>Low number of pack years³³</td>
</tr>
<tr>
<td></td>
<td>No Passive smoking³⁵</td>
<td>Short duration of asthma⁴⁵</td>
</tr>
<tr>
<td></td>
<td>Less severe of BHR³⁹,⁴²</td>
<td>Less chronic cough³⁸,⁴⁴</td>
</tr>
<tr>
<td></td>
<td>Lower FEF₂⁵³⁹</td>
<td>Family history of asthma³⁵</td>
</tr>
<tr>
<td>Complete remission</td>
<td>Less peak flow variability⁴⁵</td>
<td>Higher FEV₁ later in life⁶⁰,⁶⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low total IgE⁶⁰</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung growth⁶⁶</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less sputum lymphocytes⁶⁰,⁶⁶</td>
</tr>
</tbody>
</table>
Chapter 2 investigates the genetic origins of asthma with presence of BHR. In this paper a stepwise approach is taken. In the first step, a GWA study with replication in four cohorts, combined with eQTL analysis of the replicated SNPs was performed. The second step involved a pathway analysis of all the genes identified with the analyses. As a secondary aim of this study, the effect estimates of SNPs previously found in GWA studies based on mostly doctors diagnosis of asthma are compared to the effect estimates of SNPs based on a definition of asthma including BHR.

In chapters 3 and 4, the severity of BHR in asthma is studied. Chapter 3 describes the outcome of a GWA study on the severity of BHR. The aim of this chapter is to identify genes associated with the severity of BHR by performing a GWAS on the slope of BHR in adult asthmatics and to attempt to replicate our findings in other cohorts. In chapter 4, a genome wide interaction study is conducted to identify genes that modify the effect of ICS use on the severity of BHR in adult asthma.

Chapter 5 describes the SNP epistasis between ORMDL3, a gene previously identified by GWAS, and ATP2A2. The interaction between SNPs is studied on a disease level as well as on expression level.

In chapter 6 and chapter 7, the remission of asthma is being studied. Remission of asthma is a preferable outcome of asthma for patients and elucidating the mechanisms of remission may yield clues to identifying curative asthma treatments. In chapter 6, childhood factors are associated with clinical and complete asthma remission in the Roorda cohort, a childhood onset of asthma cohort which now has a follow-up of 40 years. By associating childhood factors with asthma outcome at age 25 and 49 in the same individuals, a specific combination of predictors in childhood associated with asthma remission later in life can be identified. In chapter 7, the genetic background of asthma remission is studied using a GWA study. The aim of this study is to identify genes associated with complete asthma remission and overall remission (clinical and complete).
References


