Chapter 11

Summary and general discussion
Summary of the findings in this thesis

This thesis describes the phenotypic heterogeneity of asthma onset in early childhood and a genetic background of asthma in children. In most asthma patients, the period early in life, from conception to the first clinical signs of asthma, is the period when changes occur that result in asthma development. We studied the heterogeneity of respiratory symptoms in early childhood by identifying longitudinal phenotypes in a well-documented large Dutch birth cohort study, Prevention and Incidence of Asthma and Mite Allergy (PIAMA). Then, we studied the differences in associations of these longitudinal phenotypes and asthma with risk factors, outcomes and gene polymorphisms. Together, these findings on longitudinal phenotypes give insight in different origins of asthma. Knowledge on differences in the etiology of asthma might help to evaluate which young children with respiratory symptoms need further evaluation, to improve treatment and eventually to prevent asthma development.

Chapter 2 describes the review “Predicting who will have asthma at school age among preschool children”. The current literature was reviewed and it was shown that it is not possible to adequately predict at young age whether a child will develop asthma during childhood. Subsequently, we investigated the heterogeneity of the respiratory symptom wheeze in childhood from birth to age 8 by identifying longitudinal wheezing phenotypes through a clustering approach in chapter 3. In chapters 4 and 5, associations have been studied of these phenotypes with perinatal risk factors and FeNO levels in childhood respectively. Chapters 6 and 7 describe genetic associations of these longitudinal wheezing phenotypes and asthma with the asthma candidate gene IL1RL1 and its pathway. In chapter 8, we investigated whether addition of another symptom, nocturnal dry cough, was also associated with asthma at school age. Knowing that wheeze is the oscillation of air in a narrow tube, a narrower diameter of airways is possibly involved in the onset of asthma. Next, the effect was analyzed of small airway obstruction, measured by the FEF50 level, on airway hyperresponsiveness and asthma in children at school age in chapter 9. Finally, we analyzed which genetic variants attribute to the variance in FEF50 levels in school children in a GWA study in chapter 10.

Prediction of asthma would be very helpful in daily clinical practice to distinguish in young children with repetitive respiratory symptoms who needs follow-up for asthma care and who can be appeased. Therefore, many different prediction rules and/or classifications have been developed. In chapter 2 we reported that the ERS classification of episodic viral wheeze and multiple-trigger wheeze is not one-to-one related to the absence of asthma and to the presence
of asthma respectively, nor that they are in concordance with longitudinally developed wheezing phenotypes. Children with multiple-trigger wheeze have already features that are compatible with the development of asthma like reticular basement membrane thickening of the airways. Children with episodic viral wheeze are still not uniform as some children may develop asthma later in childhood and some not. The classification of the ERS Task Force is therefore confined in its application to predict asthma in childhood. We investigated the predictive value of other prediction rules for asthma development in childhood. Unfortunately all these rules have limited predictive value due to an insufficient positive predictive value and a limited sensitivity. Prediction rules for asthma development might be improved by measuring risk factors and phenotypes more precisely (e.g. by objective measures), and taking environmental factors and genomic risk profiles into account. Ultimately, these improvements will help to identify the underlying pathophysiological process determining asthma development, and help to develop predictive, therapeutic and preventive strategies in preschool children.

Chapters 3-5 involve studies that describe the heterogeneity of young children with respiratory symptoms. After a publication in which the Tucson Children’s Respiratory Study defined 4 phenotypes based on the presence of wheeze during the first three years of life and the sixth year, we wondered whether this model was complete. In chapter 3, we therefore collaborated with researchers of ALSPAC, and together identified wheezing phenotypes by modelling longitudinal wheezing patterns in childhood with an unbiased statistical clustering approach: latent class analysis. The wheezing phenotypes in PIAMA were compared with the wheezing phenotypes identified with the same method in ALSPAC. Next, we compared the association of these wheezing phenotypes with asthma and allergy outcomes at 4 and 8 years in PIAMA and ALSPAC. Five wheezing phenotypes were identified by latent class analysis of wheezing patterns in childhood in the birth cohort PIAMA:

1. Never/infrequent wheeze
2. Transient early wheeze
3. Intermediate onset wheeze
4. Late onset wheeze
5. Persistent wheeze.

This model of wheezing phenotypes in PIAMA was in high concordance with the model of wheezing phenotypes identified in the ALSPAC birth cohort. In ALSPAC, six wheezing phenotypes were found; 5 similar to PIAMA and one more. Transient early wheeze in PIAMA was split in transient early wheeze and prolonged early wheeze in ALSPAC. Both cohorts reported the phenotype intermediate onset wheeze, a phenotype that was not described before. This phenotype has a
prevalence of 3%, and children with this phenotype start to wheeze after 18-24 months and continue wheezing episodes during childhood. The phenotypes intermediate onset, late onset and persistent wheeze are associated with an increased risk for a doctor's diagnosis of asthma at 8 years. Intermediate onset, late onset and persistent wheeze are associated with sensitization against common allergens at age 8, and intermediate onset and late onset wheeze are already associated with sensitization against common allergens at age 4. Transient early wheeze and persistent wheeze are associated with lower lung function levels at 8 years. Intermediate onset, late onset and persistent wheeze are associated with increased severe bronchial responsiveness compared to never/infrequent wheeze. In this chapter, we show that 1. patterns of wheeze over time in early childhood are comparable between populations, and 2. that longitudinal wheezing phenotypes are differentially associated with lung function level, sensitization and asthma at age 8.

In Chapter 4, the associations have been investigated of pre- and perinatal risk factors with the five longitudinal wheezing phenotypes identified in PIAMA. This study has shown that transient early wheeze, intermediate onset wheeze, late onset wheeze and persistent wheeze have some overlapping risk factors like allergy of the mother. Other risk factors were only associated with specific wheezing phenotypes, e.g. a lower birth weight was associated with intermediate onset wheeze and transient early wheeze but not with late onset wheeze and persistent wheeze. These findings support the hypothesis that the underlying disease mechanisms may differ between various longitudinal wheezing phenotypes.

The associations of longitudinal wheezing phenotypes and FeNO levels, as an indirect measure of eosinophilic inflammation, at ages 4 and 8 years have been studied in chapter 5. This study showed that children with intermediate onset, late onset and persistent wheeze had higher FeNO levels than those with never/infrequent wheeze at age 8. This effect was only present in children who were sensitized during childhood (specific IgE ≥0.7 IU/mL levels against common aero-allergens at age 8). There was a trend for higher FeNO levels at 4 years in children with intermediate onset, late onset and persistent wheeze compared to those with never wheeze, although this difference was not significant. Since FeNO is an indirect measure of eosinophilic inflammation in childhood, these results may suggest that children with intermediate onset, late onset and persistent wheeze develop more eosinophilic inflammation than children with never/infrequent wheeze. Furthermore, these findings suggest that sensitization plays an important role in the development of eosinophilic airway inflammation in the 3 former phenotypes.
Chapter 6 describes a genetic association study investigating if genetic variants play a role in childhood asthma. The gene \textit{IL1RL1} encodes the membrane receptor IL1RL1 located on the membrane of epithelial cells, endothelial cells, fibroblasts and lymphocytes. This membrane receptor might be important for asthma because increased numbers of IL1RL1-containing T-cells in the lungs have been associated with increased and persistent lung inflammation after induction of allergic inflammation in mice. Thus, the membrane receptor IL1RL1 might play a role in the development of asthma through its role in lung inflammation. In this study, we analyzed whether common genetic variants, named SNPs, in \textit{IL1RL1} are associated with one of its gene products, the soluble protein IL1RL1-a in serum, the number of eosinophils in blood and asthma presence in children. Our results showed that thirteen of the fifteen studied SNPs were associated with the serum level of IL1RL1-a. Two of the fifteen SNPs were also associated with the number of eosinophils in blood, and one of the fifteen SNPs was associated with asthma presence in childhood. These findings indicate that \textit{IL1RL1} SNPs are pQTLs (i.e. SNPs that affect the protein level of its gene) and that genetic variants in \textit{IL1RL1} may play a role in asthma development.

Besides the cell membrane receptor IL1RL1 other proteins are also involved in the IL33-IL1RL1 pathway resulting in allergic inflammation (Figure 1 of chapter 7). In \textit{chapter 7}, we investigated associations of the IL33-IL1RL1 pathway with longitudinal wheezing phenotypes and asthma in two birth cohorts, PIAMA and ALSPAC. Furthermore, we analyzed whether gene-gene interactions were associated with asthma in childhood. The IL33-IL1RL1 pathway consists of the ligand of IL1RL1, interleukin-33 (IL33), the receptor IL1RL1, its receptor associated protein interleukin-1 co-associated protein (IL1RAcP), adapting proteins like Myeloid Differentiation primary response protein 88 (MYD88) and Toll-Interleukin 1 Receptor (TIR) domain containing adaptor protein (TIRAP), and downstream signaling proteins like Interleukin-1 receptor-associated kinase 1 (IRAK1), Interleukin-1 receptor-associated kinase 4 (IRAK4) and TNF receptor-associated factor 6 (TRAF6). Of the longitudinal wheezing phenotypes, intermediate onset wheeze and late onset wheeze were most strongly associated with the genetic variance in the pathway. And polymorphisms in \textit{IL33}, \textit{IL1RL1} and \textit{IL1RAP} were mainly associated with longitudinal wheezing phenotypes and asthma, while the other genes were not significantly associated with the outcomes. Intermediate onset wheeze is a phenotype closely related to sensitization and allergy development in childhood, suggesting that the IL33-IL1RL1 pathway might play a role in allergy development. These findings may contribute to the understanding how the IL33-IL1RL1 pathway leads to asthma in early childhood, and where are possibilities to intervene this pathway for medication development.
Besides wheeze, other respiratory symptoms may also be associated with asthma later in childhood. In chapter 8, we studied the association of nocturnal cough in early childhood, alone or together with wheeze with asthma presence at later age in childhood. Young children with nocturnal dry cough had a higher risk to have asthma at age 8 than young children without nocturnal dry cough, a finding that was independent of the presence of wheeze. Children with nocturnal dry cough and wheeze had almost twice a higher risk to develop asthma than children with wheeze only at age 1 year. These findings may help to identify which very young children are at risk to develop asthma. As nocturnal respiratory symptoms in asthmatics signifies more severe disease, the presentation of nocturnal cough in childhood might indicate an early symptom of asthma.

As wheeze is the oscillation of air through a narrow tube, the size of airway caliber may be an important contributing factor for asthma development. We investigated the role of narrowing of smaller airways in asthma in chapter 9 by analyzing the airflow limitation of smaller airways (the FEF_{50} level) in children with and without asthma in PIAMA. We hypothesized that the effect of airflow limitation of smaller airways could be affected by airway hyperresponsiveness and/or systemic inflammation, represented by the number of eosinophils in the blood. Asthmatic 8-year-old children had a lower FEF_{50} level, lower FEV_{1} level and more blood eosinophils than 8-year-old children without asthma. Both the FEF_{50} level and the number of blood eosinophils were independently associated with airway hyperresponsiveness. In addition, airway hyperresponsiveness and blood eosinophils were both independently associated with asthma presence in 8-year-old children and FEF_{50} level did not independently contribute to asthma in the multivariate regression model. However, when the airway hyperresponsiveness was removed from the model the FEF_{50} level was again significantly associated with asthma, independently of the associations of blood eosinophils with asthma. We concluded that the FEF_{50} level may play a role in asthma via its effect on airway hyperresponsiveness. Children with asthma at age 11 had diminished growth of their FEF_{50} level from age 8 to 12 compared to children without asthma at age 11. In contrast, the growth of the FEV_{1} level between age 8 and 12 did not differ between children with asthma and without asthma at age 11. The association of growth of FEF_{50} level from age 8 till 12 and asthma was significantly lower in children with more airway hyperresponsiveness to methacholine compared to those children with less airway hyperresponsiveness. Thus, children with diminished growth of FEF_{50} level in childhood have a higher risk to have airway hyperresponsiveness and asthma later in childhood. Furthermore, the association of blood eosinophils and asthma was also significantly different between children with more airway hyperresponsiveness to methacholine compared to those children with less airway hyperresponsiveness. Taken together, these findings
suggest that both diminished FEF$_{50}$ level, via its effect on airway hyperresponsiveness, and high blood eosinophils play a role in asthma. And diminished growth of FEF$_{50}$ is associated with asthma and airway hyperresponsiveness in children.

We continued to study the FEF$_{50}$ level, and investigated which genetic variants play a role in the FEF$_{50}$ level in children. We performed a genome-wide association (GWA) study in PIAMA to analyze which common genetic variants (SNPs) were associated with the FEF$_{50}$ level in children in chapter 10. Polymorphisms on the loci 5p15.33 and 7q11.23 were associated with the FEF$_{50}$ level in children. The genes $IRX4$ and $PTPN12$ were two promising genes in moderate LD with the top hits of the GWAS, suggesting that these genes might be involved in the FEF$_{50}$ level.

**Interpretation of results**

Taken together, the studies in this thesis show that wheezing in young children is heterogenic, and that longitudinal wheezing phenotypes can reflect differences in pathophysiology. However, how do differences in associations of these longitudinal wheezing phenotypes reflect differences in pathophysiological processes that play a role in the development of asthma?

Table I. Interpretation of associations of longitudinal wheezing phenotypes with sensitization, lung function, BHR and asthma in childhood.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sensitization</th>
<th>Lung function</th>
<th>AHR</th>
<th>Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>6/8</td>
<td>0</td>
</tr>
<tr>
<td>Never/infrequent wheeze</td>
<td>ref.</td>
<td>ref.</td>
<td>ref.</td>
<td>ref.</td>
</tr>
<tr>
<td>Transient early wheeze</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↓</td>
</tr>
<tr>
<td>Intermediate onset wheeze</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>↓</td>
</tr>
<tr>
<td>Late onset wheeze</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>↓</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>↓</td>
</tr>
</tbody>
</table>

Abbreviations: AHR, airway hyperresponsiveness.

Parts of this thesis$^1$ together with other studies$^{3-5}$ give insight in pathophysiological processes involved in different wheezing phenotypes (Table I). Figure 1 shows in a hypothetical figure how wheezing phenotypes might differ in their development of A) allergy and B) lung function in childhood. Children who never wheeze, the reference group, have an average lung growth and a normal development of sensitization in childhood, and they do not develop asthma. Children with transient early wheeze have a diminished lung function level directly after birth (before a first infection). They catch up in lung growth, leading to less diminished lung function levels...
compared to never wheeze around school age than at birth, although lung function levels around. Transient wheezers do not develop sensitization during childhood, and are not likely to develop asthma. Children with intermediate onset wheeze have a normal lung function level directly after birth. They develop sensitization very early in childhood (≤ 1 years) and have a high risk to become allergic. The lung growth of these children is diminished during childhood, resulting in a lower lung function level at school age compared to children who never wheeze. Children with intermediate onset wheeze have a high risk to have asthma at school age. Children with late onset wheeze have a normal lung function level directly after birth. They often develop sensitization, only at later a later time point in childhood than intermediate onset wheeze (≤ 4 years). The lung function level is diminished compared to children who never wheeze at school age, and this group of children is likely to have asthma at school age. Children with persistent wheeze already have a diminished lung function level directly after birth. The airway growth remains diminished compared to never wheeze during childhood, and leads to a lower lung function level at school age compared to children with never wheeze. Furthermore, they are also at risk to develop sensitization during childhood (≤ 4 years), resulting in a high likelihood for clinical allergy expression. Children with persistent wheeze are likely to have asthma at school age.

Figure 1 is limited to the (patho)physiological processes of development of allergies and lung function. Although infections and cigarette smoke exposure are also depicted in the graphs, we realize that this hypothetical figure is incomplete. Other factors like hormones (e.g. leptin), food (e.g. Mediterranean diet), vitamins (e.g. vitamin D) and medicines (e.g. paracetamol) may also play a role in asthma development. Studies assessing how these risk factors affect their underlying pathophysiological processes may reveal further insight in how children develop asthma and fine-tune this hypothetical figure. The discussion of this thesis will be limited to the factors that we have studied, i.e. the (patho)physiological processes of lung development & lung growth, and the development of sensitization and allergy in relation to asthma development.
Figure 1. Hypothetical description of development of A) allergy and B) lung function in childhood for different longitudinal wheezing phenotypes.
Lung development & lung growth

Already several decades ago it was acknowledged that lung development (formation of the airways and lung tissue) followed by lung growth (growth of the airways and lung tissue) are physiological processes that play an important role in the development of respiratory diseases like asthma in childhood.\textsuperscript{10-12} Especially factors affecting the development of airways during the fetal period up to 16 weeks may have long lasting effects and relate to the airflow limitation present later in life.\textsuperscript{12, 13}

We reported in chapter 9 of this thesis that asthmatic children at age 8 have a lower FEF\textsubscript{50} level than children without asthma. This finding confirmed previous research showing that measures indicating narrowing of the smaller airways (FEF\textsubscript{25-75}, FEF\textsubscript{50}, S\textsubscript{cond}) are lower in children with asthma compared to healthy controls.\textsuperscript{14-17} Previous studies have also shown in asthmatic children and children at high risk to develop asthma that these physiologic measures reflecting narrowing of the smaller airways are more affected than measures representing narrowing of the larger airways (e.g. FEV\textsubscript{1}, FEV\textsubscript{1}/FVC).\textsuperscript{5, 14, 16, 18-20} These findings suggest that asthmatic children may have narrower small airways than their healthy peers.

A lower lung function level in childhood has important implications for the future life because lung function ‘tracks’ over time, as shown in several longitudinal studies.\textsuperscript{14, 21} Tracking implies that lung function levels follow a particular growth curve, and that deviation from that curve does not happen anymore after the first years of life unless environmental factors affect the course of lung function.\textsuperscript{11, 12} Thus lung function and its development before birth and during the first years of life importantly determines the lung function of an individual in later life.

Airways are developed in the first 16 weeks of gestation\textsuperscript{12} and environmental effects during this period, like undernutrition or maternal smoking, are associated with lower lung function levels of predominantly smaller airways and airway remodeling later in life, possibly due to development of narrower airways or dysanaptic growth.\textsuperscript{12, 22} Figure 1 B shows that children with transient early wheeze and persistent wheeze have a lower lung function level directly after birth, suggesting abnormal airway development during gestation. Bisgaard et al. have reported that 40% of the loss in FEF\textsubscript{50} level between asthmatics and healthy controls at age 7 was already present at the age of one month.\textsuperscript{15} This indicates that the period when airway development occurs is very important for the lung function level of smaller airways later in life. Of interest, a lower lung function level and presence of airway hyperresponsiveness in the first month after birth are associated with a
higher risk to develop symptomatic respiratory infections during infancy, as well as lower lung function levels and asthma development later in childhood.\textsuperscript{15, 23-26} This suggests that abnormal airway development in utero is a risk factor for asthma development, and that abnormal airway development is a risk factor for ‘at risk’ exposures for asthma development like respiratory infections. In chapter 9 we showed that the FEF\textsubscript{50} level was lower in asthmatic 8-year-old children than in healthy peers, while the FEV\textsubscript{1} level did not significantly differ between asthmatics and controls at age 8. This may suggest that narrowing of the airways is at first most clearly observed in the smaller airways, compared to the larger airways.

After development in utero, growth of airways continues until adulthood.\textsuperscript{11} Environmental exposures can have an effect on this growth rate. Respiratory infections in early childhood, especially viral infections with Rhinovirus or Respiratory Syncytial (RS) virus, have been associated with lower lung function levels and increased wheeze later in childhood and even adulthood.\textsuperscript{10, 27-29,30} Furthermore, sensitization to common allergens before the age of 3 together with a high exposure of allergens is strongly associated with lower lung function levels at age 7, especially measures of airflow limitation in smaller airways, compared to non-sensitized individuals.\textsuperscript{16} We speculate that this association of early sensitization and lower lung function levels in smaller airways later in childhood may also be present in the wheezing phenotype “intermediate onset wheeze” (identified in chapter 3 of this thesis, see Table 1 and Figure 1).

In general, we speculate that early environmental factors affect the airways by decreasing the growth rate of airways, possibly through airway remodeling.

In conclusion, the development and the growth rate of airways, and especially the development and growth rate of a lower diameter in smaller airways in early childhood contribute to the development of asthma in childhood and the ultimate lung function level later in life. Our data show that lung function levels reflecting smaller airways, like the FEF\textsubscript{50} level, represent airway narrowing associated with asthma better than measurements of larger airways, like the FEV\textsubscript{1} level. Environmental factors later in life seem to have a less permanent effect on the lung function level than the environmental factors very early in life, as suggested previously by Barker et al.\textsuperscript{29} Therefore, the most relevant time to study the level of lung function is the period very early in life. Monitoring of, and maybe in the future, intervention in development and growth rate of the diameter in smaller airways may help to identify individuals at risk to develop a chronic lung disease, like asthma, later in life.
Allergy development

The precise mechanisms of sensitization and the association with allergic inflammation in the lungs are not completely understood yet. Asthma in many children begins in association with sensitization of the airways to common aero-allergens, which is defined as an elevated specific IgE level or a positive skin prick test against a specific allergen. A concept of how the onset of sensitization may be enhanced is that the barrier function of epithelial cells in the airways is impaired which facilitates penetration of inhaled allergens into the airway tissue leading to sensitization and allergy development.

In chapter 3, we showed that early sensitization (<4 years) is associated with both intermediate onset wheeze and late onset wheeze, phenotypes that have a high risk for asthma development. These findings confirm previous research showing that early sensitization is an important risk factor for asthma development. The exact exposures resulting in sensitization are not completely understood, however exposures that influence the diversity of microbial exposure seem to be important for development of sensitization, like the microbiome in the airways and gut and factors that affect that (e.g. antibiotics), the level and number of allergens and viral infections during early childhood. Maternal, prenatal and postnatal environmental exposures are associated with the development of sensitization in the first year. For example consumption of fruit during pregnancy is associated with a higher incidence of a child's sensitization against seasonal allergens at the age of one year. Contact with animals like cat, dog and cow and contact with animal feed like straw, hay and grain from pregnancy until age 3 is associated with less atopic sensitization. Sensitization against multiple allergens and developed very early in life (<3 yrs) is strongly associated with lower lung function levels, airway hyperresponsiveness and asthma development later in childhood. How and precisely which exposures and genetic factors affect this early sensitization against multiple allergens is currently studied.

Children with parents who have atopy and/or allergy have a higher risk to develop wheezing phenotypes that are associated with a higher risk of childhood asthma than children with parents who do not have atopy and/or allergy, as shown in chapter 4 of this thesis. This may suggest that children with a certain genetic susceptibility may more likely develop sensitization. The heritability of allergic sensitization has been estimated to be 0.40-0.75. We reported in chapter 7 of this thesis that children with intermediate onset wheeze and late onset wheeze, two phenotypes associated with a high risk for sensitization in childhood, are associated with polymorphisms in the IL33-IL1RL1 pathway. Furthermore, polymorphisms of IL33 and IL1RL1 can
interact which further increases the risk for asthma in an additive manner. This may suggest that polymorphisms in the IL33-IL1RL1 pathway are important susceptibility genes for sensitization and asthma development. This is further confirmed by other studies finding the IL1RL1/IL18R1 region in genome-wide association studies linked to asthma and allergic sensitization. Furthermore, we showed in chapter 6 that almost all studied polymorphisms in IL1RL1 are associated with the protein levels of one of its gene products, the IL1RL1-a level in serum, and some with blood eosinophil counts and asthma. This may suggest that IL1RL1 polymorphisms induce a protein change that may play a role in the mechanisms that result in asthma. Further functional studies investigating the causality between SNPs, IL1RL1-a levels, sensitization and asthma will provide further insight in the role of IL1RL1 in this process.

The prevalence of atopic diseases has risen rapidly in countries with a Western lifestyle since 50 years (and currently ongoing in countries that develop a Western lifestyle), and this cannot be explained by a genetic predisposition. Thus there must have been important environmental changes. Recent studies have shown that children who live in areas with high microbial diversity, for example on a live-stock farm, are protected against development of sensitization and asthma, compared to those living in urban areas. Even microbial exposure of the mother already during pregnancy has been associated with the risk for asthma development of her child. Speculations are ongoing on how microbial exposure may prevent sensitization. It has been hypothesized that diverse microbial exposures stimulate pattern-recognition receptors, like toll-like receptors, that induce regulatory T-cells and result in tolerance and hence prevent sensitization. A second hypothesis is that diverse microbial exposure might lead to a balanced microbiome in the airways and/or gut and/or skin, which may prevent the development of sensitization.

Besides genetic susceptibility and environmental risk factors, the interaction between those factors plays also an important role for sensitization and asthma development. This is clearly shown for example in a study of a genetic variant in the Toll Like receptor 2 (TLR2) gene and day care attendance before the age of 2. Children with genotype AA of SNP rs4696480 were protected against sensitization when they did not attend day care compared to those with an AT/TT genotype, but this effect was opposite when children did attend day care. In gene-environment interactions like the example above, particular exposures during certain time periods seem to be more relevant than others. Thus, whether an environmental exposure at a certain time point in life will result in development of sensitization is also dependent on the child’s genetic blueprint.
Another way how the genetic susceptibility and the environment may influence each other is by epigenetic regulation. Epigenetics is the cell-specific dynamic changes in the molecules that bind the DNA and can regulate the gene expression. Epigenetic regulation is affected by the environment like cigarette smoke exposure, diet and ageing. Methylation of cytosine residues within CpG islands is a form of epigenetics. Hypermethylated CpG islands in the gene promoter region leads to less expression of this gene, while hypomethylation of the CpG islands results in active transcription. Farm environment can influence the change of methylation patterns during childhood from birth to age 4 in peripheral blood cells in distinct asthma and IgE candidate genes, like the *ORMDL3* and *RAD50*. Children who lived on a farm and did not develop asthma at early age had an increase in methylation level by *ORMDL3* during birth and age 4, while children who lived not on a farm and developed asthma at early age had a decrease in methylation level between birth and age 4. These findings suggest that epigenetic changes may also contribute to asthma development in childhood, possibly through sensitization development.

Another important environmental factor for asthma development is a viral infection. Children with a viral infection in 0–3 years, especially with Rhinovirus or RS-virus, have an increased risk to develop asthma later in childhood around age 6. Children with sensitization have a higher risk to experience a Rhinovirus infection than children without sensitization. Children who develop sensitization in combination with a Rhinovirus infection in early childhood have a higher risk to develop asthma later in childhood than those with sensitization or a viral infection alone. In addition, prevention of viral infections, in this example RS-infections, by administration of palivizumab during the first year of life in premature children decreased the frequency of wheeze in the next follow-up year. Furthermore, a retrospective study in premature children has shown that administration of palivizumab during the first year was associated with less recurrent wheezing in the follow-up year in children without a family history of asthma and/or atopy, compared to those who did not receive palivizumab. Especially the combination of sensitization and a viral infection in early childhood (< 3 yrs), when the immune system is still under development, has large effects on allergic inflammation and airway remodeling. Sensitization and viral infection may act synergistically on the development of asthma, for example through type-1 IFN-dependent induction of the high affinity IgE receptor on lung dendritic cells directly after a viral infection. And, as might be expected, the effect of a viral infection as Rhinovirus on asthma development, is affected by the child’s genetic variance at susceptibility loci, like the 17q21 region. Thus, genetic susceptibility in conjunction with environmental exposures that cause early sensitization and viral infections may the development of allergic inflammation in the airways and asthma in childhood. In summary, the development of sensitization at early age, between birth and age 4, and...
sensitization against multiple aero-allergens are important risk factors for the development of asthma. Genetic susceptibility, e.g. variants in \( I\!L1R\!L1/I\!L1R\!R1, I\!L33 \) and other genes, and environmental exposures, such as a diverse microbial environment during pregnancy and in early childhood, play an important role for sensitization and asthma development. Genetic susceptibility and environmental factors affect each other by gene-environment interactions or changes in epigenetic regulation like DNA methylation. Knowledge of the underlying mechanisms that drive sensitization and eventually intervention in the development of sensitization may be help to investigate and develop preventive strategies for asthma development.

**Future perspectives**

When interpreting results of this thesis in the context of the literature as described above, new ideas emerge for further research. For each idea, three starting points are very useful to bear in mind to improve future research. First, each idea should aim to measure more precisely an aspect of a (patho)physiologic process involved in asthma development than previous studies have performed. When more precise definitions and phenotypes are used, more homogeneous study population can be integrated and this will increase the power of the study. Second, the time period to study the research question is crucial, because disease development is a process evolving over time which means that not every period in life has equal relevance or similar exposures. And third, it is meaningful to integrate findings in a wider knowledge context. This knowledge will also be crucial to determine the clinical application of the research performed, and will allow a larger range of researchers and doctors to use the results. Some examples of these statements will be illustrated in the next paragraphs.

Since the 1970s it is known that a narrow airway caliber of smaller airways shortly after birth or in infancy is an important risk factor for the level of lung function in future life and for development of chronic respiratory diseases in childhood and adulthood. However, at this moment narrowing of smaller airways is only determined with proxy measures of smaller airways that measure the sum of all smaller airways in flow (e.g. \( FEF_{50} \) level) or resistance (e.g. \( R_{5-R20} \) or \( S_{COPD} \)). My interest would be to measure the volume and diameters of the whole bronchial tree which makes it possible to determine the ratio between the smaller and larger airways, to measure the diameter and length of smaller airways (thus the volume) and the number of airways per branch. This may become feasible by constructing a 3D image of the airways, for example by spiral CT as already has been done in COPD.\(^{57, 58}\) In this 3D image of the bronchial tree, the length, diameter and
number of airways can be quantified. This method has already shown to be successful in a study of 5 patients with COPD investigating the effect of salbutamol and ipratropium bromide in the central and distal airways in a cross-over design by use of 3D images. The medication had effect on central and distal airways, although the effect on distal airways was larger for both drugs. Furthermore, corresponding changes in lung function level were observed, although the changes in FEV₁ % predicted and vital capacity were less pronounced than the changes in airway volume and resistance as provided by the imaging method. Quantification of the airways may give insight which aspect of narrower airways over the full length of the bronchial tree and its sub-branches is most relevant in asthma development and development of a lower lung function level during life. This application should first be tested in an animal model in order to show whether differential effects are present along the full airways and whether this method can measure changes in airway caliber during early lung growth. But if it works, it might be an elegant tool for children in the first months after birth which may identify those children at risk for infections in infancy, asthma development, lower lung function level during life and thus other respiratory diseases.

When investigating the development of a disease, the timing in life to perform your research can make the difference. The period before the onset of the disease will be the time frame in which changes occur that result in the development of disease. In case of asthma development in childhood, this time frame is acknowledged from conception to approximately age 3. Notwithstanding this, there might be differences between phenotypes, e.g. exposures occurring after age 3 may affect the development of late onset wheeze as well, as late onset wheeze starts later in childhood. Relatively small changes at early age for example in lung function development or sensitization will increase into relatively large changes at older age, and affect the development of asthma. Furthermore, exposures that affect the period from conception to approximately age 3 may even occur before this crucial time period, e.g. before conception. Exposures that have affected the DNA of the previous generation may also have an effect on the DNA of the study subject and thus on the development of disease in this study subject. This phenomenon has already been shown in animal models and asthmatic individuals. The wide range of exposures that can play a role over a long period (transgenerational) has practical and ethical difficulties to study development of asthma in early life properly. These are current challenges for researchers. Innovative research strategies together with accurate documentation over long periods that exceed generations will help to overcome these challenges.
Finally, new findings have to be integrated in existing knowledge. An example that illustrates the relevance of this are genetic association studies of polymorphisms. It can be difficult to interpret genetic associations of polymorphisms in relation to asthma development because it is often unknown how a polymorphism contributes to a disease status. For instance, SNPs in the IL1RL1/IL18R1 region are strongly associated with asthma in childhood. However due to strong LD in this region, it is not known which gene is the causal gene for asthma development. Functional studies that investigate how polymorphisms affect the mRNA level, the protein level and finally the disease status, possibly mediated by exposures and epigenetic changes that can change over time give further insight in the functional understanding of the role of genetic variance in asthma development. This means that integration of different ‘-omics’ together with the right timing can give more insight in the exact mechanisms how genetic susceptibility affects the development of asthma. Furthermore, research in cell or animal models that can investigate causality will give additional information. This knowledge will be helpful in clinical practice, for example to identify a population at risk for asthma development or to create new medication for a risk population.

Conclusions

This thesis shows that respiratory symptoms are heterogeneous in young children, and that doctors are currently limited in their tools to judge which young child with respiratory symptoms will or will not develop asthma later in life. Factors that affect wheeze (which is oscillation of air in a narrow tube), and thus may affect narrowing of airway caliber, are abnormal development of airways and a lower growth rate of the diameter of smaller airways in (early) childhood, and increased airway inflammation for example through sensitization and allergy development. Genetic variants, for instance in the IL33-IL1RL1 pathway, and gene-gene and gene-environment interactions contribute to the susceptibility for asthma development. Furthermore, gene-environment interactions show that genetic variance influence the magnitude of response to environmental stimuli. Improvement of measurements during pregnancy and in young children will help to study pathophysiological processes that result in asthma, and subsequently give insight how to develop treatment(s) and/or preventive strategies for these children.
References


