Summary, general discussion and implication of results

Chapter 10
**Chapter 10**

### Summary of the results

Studying human (epi)genetics in relationship to disease has shown remarkably high progress in the last decennium. The outcomes of these (epi)genetic studies have been and will be of high value to increase the understanding of disease pathogenesis, to link this to better treatment and to improve disease prediction.

With this thesis we aimed to improve our understanding of the role of (epi)genetics in childhood asthma. We used data from different study populations ranging from family to case-control studies, from children to adults, retrospective to prospective and cross-sectional to longitudinal studies. We collaborated with cohorts worldwide which led to the inclusion of participants from different ethnicities in our research. In all analyses performed, we took into account the heterogeneous aspects of asthma and tried to relate our outcomes to specific asthma phenotypes.

Multiple genes have been found to be related to asthma, but we specially aimed to identify the role of the *Interleukin 1 receptor-like 1* (*IL1RL1*) gene and focused on the (epi)genetic functions, expressed proteins and the risk/protective value of this gene in asthma development. With this information we tried to obtain more insight in the relation of *IL1RL1* with asthma treatment response. Our final aim of this thesis was to investigate whether *IL1RL1*, its protein product IL1RL1-a and other genetic factors can be used to improve asthma prediction.

**Chapter 1** starts with a short overview of asthma in relation to its prevalence, symptoms and diagnostic testing with a special focus on the specific asthma phenotypes. An introduction in (epi)genetics is provided with special attention to (epi)genetic testing in asthma. Since this thesis includes multiple studies focused on *IL1RL1*, this gene is being introduced as well, with hypotheses on its role in asthma development and possible influence on asthma treatment responses. Moreover, the accuracy in predicting asthma is outlined. At the end, the most important study groups of this thesis are presented, together with the aims of this thesis.

Describing phenotypes of asthma at a young age is difficult as many preschool children have asthma-like respiratory symptoms. **Chapter 2** presents a review in which the definition of early-onset asthma is being discussed. By studying the current literature we show that multiple early wheezing phenotypes have been described, with different clustering approaches based on longitudinal or retrospectively data. Longitudinal data is more related to the start and progression of wheezing, while retrospective data is more focused on the triggers causing wheeze in young children. These clustering pattern approaches identified dissimilarities in causes and outcomes between the distinct phenotypes, suggesting marked heterogeneity in childhood asthma phenotypes. In addition, this review describes the genetic factors that are related to the age of onset of disease. Literature search shows that 34% of the variation in age of onset of asthma is genetically determined, with different genes being related to adult-onset and childhood-onset asthma. The 17q12–21 *IKZF3-ZPBP2-GSDMB-ORMDL3* locus has been reproducibly found as a specific childhood-onset asthma locus, which effect is probably enhanced by prenatal and infant smoke exposure. No study has been performed so far that made a true distinction between different age groups, but studies including only cases with childhood-onset asthma have identified eleven asthma associated loci, including among others *IL1RL1/IL18R1* and *interleukin 33* (*IL-33*). Functional studies, assessing the effects of SNPs on for example gene expression in childhood might provide more insight in asthma pathogenesis.

The Dutch ‘Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study and the ‘Avon Longitudinal Study of Parents and Children’ (ALSPAC) from the United Kingdom have been previously studied by our group, leading to the identification of the wheezing phenotypes; never/infrequent wheeze, transient early wheeze, prolonged early wheeze (only present in ALSPAC), intermediate-onset wheeze, late-onset wheeze and persistent wheeze. Those wheezing phenotypes showed differences in association with asthma, atopy, bronchial hyperresponsiveness (BHR) and lung function at the age of 8 years. The *IL33* and *IL1RL1* gene have both been reproducibly
identified as being important for asthma development. IL-33 is the ligand for the transmembrane IL1RL1-b receptor that forms a receptor complex with IL-1 receptor-associated protein (IL1RAcP). By binding of IL-33 to its receptor, multiple adaptors and signaling proteins are being activated such as myeloid differentiation primary response gene 88 (MYD88), Toll–IL-1 receptor domain containing adaptor protein (TIRAP), IL-1 receptor–associated kinase 1 (IRAK1), IL-1 receptor–associated kinase 4 (IRAK4), and TNF receptor–associated factor 6 (TRAF6). This signaling cascade is responsible for the release of pro-inflammatory Th2 cytokines promoting airway inflammation. This may suggest that these genes, besides IL33 and IL-1RL1, play a role in asthma development. Chapter 3 shows the results of a candidate gene pathway study performed in PIAMA (N=2,007) and ALSPAC (N=7,247) that investigated the association between the IL33-IL1RL1 pathway with asthma and the longitudinal wheezing phenotypes, as identified previously by the studied cohorts. We also assessed whether gene-gene interactions are associated with asthma and the wheezing phenotypes. Our study showed that IL33-IL1RL1 variants were most significantly associated with intermediate onset wheeze and late onset wheeze. In addition, longitudinal wheezing phenotypes were predominantly associated with single nucleotide polymorphism (SNPs) in IL33, IL1RAP, and IL1RL1, while asthma was only associated with IL33 and IL1RL1 variants. Interaction analyses revealed an increased risk for asthma in children carrying both risk alleles of IL33 and IL1RL1. Intermediate onset and late-onset wheeze were previously found to be both related to an increased risk for doctor diagnosed asthma and allergen sensitization, which could suggest that SNPs in the IL33-IL1RL1 pathway are affecting wheeze and asthma development through a role in allergy development. Focus on the associated SNPs in the ligand receptor–receptor complex is warranted since knowledge of a SNPs function and possible role in regulation of the receptor could identify the mechanisms behind genetic pathways in asthma pathogenesis.

Although the IL1RL1 locus has been identified as an asthma risk gene, its relation to specific asthma subtypes has not been clarified. Chapter 4 describes the results of a combined phenotypical and functional study on IL1RL1. We used data from three independent asthma cohorts, the ‘Dutch Asthma Genome-wide Association Study’ (DAG), the ‘Genetics of Severe Asthma Phenotypes’ (GASP) and the ‘Manchester Asthma and Allergy Study’ (MAAS), in combination with re-sequencing data to investigate the association between IL1RL1 SNPs that are associated with asthma and specific features of asthma. These features were based on clinical and immunological measures, such as forced expiratory volume in 1 second (FEV1), FEV1/forced vital capacity (FVC), blood eosinophils, total IgE, atopy and age of asthma onset. In addition, we tried to identify the functional role of those SNP-driven mechanisms that may contribute to the identified genetic association signals in lung and airway structural cells. Our phenotypic analyses showed that IL1RL1 region asthma risk variants were particularly associated with higher blood eosinophil numbers, atopy and childhood onset asthma, with some evidence for association with a lower level of lung function. Combing our results with previously found asthma susceptibility variants resulted in 6 priority SNPs in the IL1RL1 gene region (rs4141632 (IL1R1), rs13431828 (IL1RL1), rs142010 (IL1RL1), rs10192157 (IL1RL1), rs990171 (Solute Carrier Family 9 Member A4 (SLC9A4)) and rs12465392 (SLC9A4)) that identified key blocks of association. These key blocks were taken into further functional analyses where we focused on lung tissue and bronchial epithelial cells. Functional analyses in our study demonstrated that three of our 6 priority SNPs (rs13431828, rs142010 and rs10192157) were eQTLs for IL1RL1 in lung tissue. Moreover, these regulatory roles were retained in cultured bronchial epithelial cells at both the mRNA and protein levels, however in some instances for alternative alleles. Importantly, several SNPs were able to modulate the effect of the micro-environment on IL1RL1 expression in the bronchial epithelial cells, for example in response to virus. These data provide a potential link between IL1RL1 genetic variants and IL1RL1 regulation of IL-33 inflammation in rhinovirus (RV) induced exacerbations in asthma. Finally, we also integrated the prioritized IL1RL1 SNPs and established that cells carrying the IL1RL1 Toll/interleukin-1 receptor (TIR) domain asthma risk haplotype have an exaggerated inflammatory response to IL-33. Thus, these cells are more amenable to the anti-inflammatory effects of either anti-IL33 or anti-IL1RL1 monoclonal antibodies that already exist. This has implications for specifically targeting of these inhibitors of the IL33-IL1RL1 pathway to patients who likely will gain the greatest clinical benefit.
Alternative splicing of *IL1RL1* produces, beside its transmembrane receptor variant *IL1RL1*-b, also a soluble variant, *IL1RL1*-a (soluble (s)ST2). This variant is thought to serve as a decoy receptor, sequestering IL-33, and therefore preventing a Th2 cytokines induced inflammatory response. SNPs have been found to play a role in DNA methylation at 5’-C-phosphate-G-3’ (CpG) sites, associated with protein levels and affect protein function. However, the role of *IL1R1* SNPs on methylation and how this might affect *IL1RL1* expression, in relation to asthma, has not been assessed. Chapter 5 reports the outcomes of an (epi)genetic study which assessed the association between *IL1RL1* genetic variants, *IL1RL1* blood DNA methylation and serum *IL1RL1*-a protein levels. Our study was conducted in the Dutch Asthma Genetics (DAG) and PIAMA cohort, including additionally in the performed meta-analysis data from the ‘Children/Barn, Allergy, Milieu, Stockholm, an Epidemiological survey’ (BAMSE) and ‘Infancia y Medio Ambiente (INMA)’ cohort (as part of the Mechanisms of the Development of Allergy (MeDALL) project). We found that asthma susceptibility SNPs of *IL1RL1* located in 5 different LD blocks were significantly associated with *IL1RL1* methylation and serum *IL1RL1*-a levels. One of the LD blocks was centered around one of the most strongly associated SNP rs1420101. The T allele of rs1420101 has previously been associated with a higher risk of asthma development and was in our study associated with less *IL1RL1* methylation (Z-score=-8.15, \( P=3.7\times10^{-16} \)) and lower serum *IL1RL1*-a levels (Z-score=-15.81, \( P=2.8\times10^{-56} \)). Moreover, rs1420101 by itself explained 18% of the variation present in *IL1RL1*-a serum levels. Rs1420101 is found in exon 5E of another putative *IL1RL1* variant, *IL1RL1*-c, which might indicate that this variant is also important in regulating airway inflammation and asthma development. We did not observe an association between *IL1RL1* methylation and asthma or *IL1RL1*-a levels, nor did we find an association between *IL1RL1* SNPs and asthma. Higher *IL1RL1*-a levels associated with lower blood eosinophil counts (beta=-0.06, \( P=0.02 \)), consistent with the hypothesis that *IL1RL1*-a has a protective effect on (eosinophilic) inflammation. Our results suggest that (epi)genetic regulation and expression of *IL1RL1* are important in asthma development, indicating that this region may be a possible future therapeutic target for asthmatic patients with eosinophilic Th2-associated inflammation.

The cornerstone of asthma treatment is daily use of inhaled corticosteroids (ICS), which has been found to be associated with improvement in asthma control, FEV1, levels and fraction of exhaled nitric oxide (FeNO) particularly in asthma patients with eosinophilic, type 2 airway inflammation. However, there has exists a large heterogeneity in asthma treatment response to ICS, which might be genetically determined. The particular role of *IL1RL1* on the effect of asthma treatment has not been established. Chapter 6 describes a multi-center pharmacogenetic study in which we investigated the role of *IL1RL1* genetic variants on exacerbations, asthma control, FeNO levels and FEV1% predicted in asthma patients during ICS treatment. Moreover, we investigated whether *IL1RL1* variants are associated with changes in FeNO levels and FEV1% predicted levels in asthma patients after 4-6 weeks of ICS treatment. We used data from five different cohorts collaborating within the scope of the Pharmacogenomics in Childhood Asthma (PiCA) consortium. The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory effects (PACMAN) cohort was used for discovery analyses, with replication being performed in one Hispanic/Latino study; Genes-Environment and Admixture in Latino Americans (GALA II) study, one African American population; Study of African Americans, Asthma, Genes, and Environments (SAGE), and two European studies; the Effectiveness and Safety of Treatment with Asthma Therapy in children (E斯塔Te) study and SLOVENIA.

Our study reports that children and adolescents with asthma using ICS still experience more exacerbations when having *IL1RL1* risk alleles than children with the protective genotypes. Our findings were, however, not replicated in the African American ancestry population, suggesting different genetic architecture between ethnicities with consequently other gene variants involved in disease pathogenesis. Meta-analyses showed significant associations with the asthma risk alleles from rs1343828 (C) and rs1420101 (T) with emergency room visits/hospitalizations (rs13431828; \( OR=1.32, P=0.005 \), rs1420101; \( OR=1.16, P=0.03 \)) and ‘any exacerbation’ (defined as emergency room visits and/or hospitalizations and/or courses of oral corticosteroid use) (rs13431828; \( OR=1.31, P=0.007 \)). We did not observe an association of *IL1RL1* variants with asthma control, change in FeNO levels or FEV1%predicted.
SNPs that showed a significant association with ICS treatment response in our cohort have previously been associated with a type 2-high asthma phenotype, higher eosinophil numbers (rs1402010) and lower serum IL1RL1-a levels (rs1420101, rs1921622 (A) and rs10204137 (C). Our study suggests that IL1RL1 SNPs may modify the asthma phenotype into a more severe phenotype, by possible enhancement of eosinophilic inflammation, with more severe exacerbations, which apparently are not adequately controlled with dosages of ICS used by the children in these studies. This study pinpoints that the IL1RL1 gene and its biological pathway may provide interesting targets for novel asthma treatment regimens.

Experimental data in animals have suggested that the transient receptor potential ankyrin-1 (TRPA1) ion channel plays a key role in promoting airway inflammation in asthma and may mediate effects of paracetamol on asthma. Thus far, evidence of this effect in humans has not been confirmed. Chapter 7 shows the results of a study investigating the association between TRPA1 gene variants with childhood asthma and total IgE concentration. In addition, interactions between TRPA1 and prenatal paracetamol exposure on these outcomes were assessed. With our study we wished to investigate this effect on asthma in humans. Initial discovery analyses of 31 TRPA1 variants were performed in ALSPAC, which showed a significant association of 13 SNPs with childhood asthma. For IgE, 3 SNPs showed nominal significant effects ($P<0.05$). The 6 most significantly associated SNPs (rs959974, s1384001, rs7010969, rs3735945, rs920829 and rs4738202) ($P<0.005$) were replicated in PIAMA and the Generation R Study (a Dutch population-based prospective cohort study from fetal life onwards). Meta-analysis between TRPA1 and childhood-onset asthma was performed across cohorts from a large European GWAS consortium on asthma (the GABRIEL consortium). In PIAMA, 3 SNPs showed evidence for association ($p<0.05$) with asthma, with effect estimates being larger than those in ALSPAC. None of the findings replicated in Generation R, nor did the GABRIEL meta-analysis pooled effect estimates show significant association with asthma ‘ever’. This could be because of genetic heterogeneity of asthma in childhood, with in addition differences in definition of the asthma diagnosis between our included cohorts. We did not find a modification of the association between TRPA1 and asthma by prenatal paracetamol exposure. This finding suggests that the previously reported associations between prenatal paracetamol use and asthma in children is unlikely to be influenced by mechanisms that involve TRPA1 activation. Our study provides some evidence of a role of TRPA1 in childhood asthma, but further replication is needed in adequately powered studies with comparable asthma phenotypes.

Detection of children with a high risk of asthma development is highly relevant for the implementation of early preventative measures and installation of early treatment. However, existing asthma prediction models, such as the asthma prediction index (API), based on family, personal and environmental factors are of modest clinical relevance. Identification of novel biomarkers, which could be related to important asthma genes, could improve current asthma prediction models. Chapter 8 describes a study that investigated in preschool wheezers whether serum IL1RL1-a levels can be used as a biomarker for asthma, enabling better asthma prediction. Our study was performed in children of the Dutch longitudinal ‘Asthma DEtection and Monitoring (ADEM) study, including 202 preschool wheezers, of which 40% developed asthma at age 6 years. This study showed that carriers of the asthma risk allele of rs1420101 (T) had lower IL1RL1-a levels, replicating our findings in PIAMA from Chapter 5. However, serum IL1RL1-a levels in 2-3 years-old wheezers had no added value in predicting doctors’ diagnosed asthma at age 6 years. Since phenotypic heterogeneity of asthma could influence our finding we investigated whether IL1RL1-a levels may predict eosinophilic asthma rather than a general diagnosis of asthma. Eosinophilic asthma was defined in this study by high FeNO levels ($\geq$20 ppb), a marker of eosinophilic airway inflammation. We report that IL1RL1-a serum levels in preschool wheezers negatively predicted asthma at age 6 with high FeNO (OR=0.96, $P=0.04$). In addition, serum IL1RL1-a levels at preschool age improved eosinophilic asthma prediction when combined with the API: area under receiver operating characteristic curve (AUC) = 0.62, $P=0.11$, API + IL1RL1-a levels; AUC=0.70, $P=0.01$. In addition, IL1RL1-a levels had also predictive value when used solely as a biomarker: AUC=0.65, $P=0.059$. Our study suggests that lower IL1RL1-a levels might be of predictive value in the development of eosinophilic airway
inflammation in children who experience asthma at school age. Moreover, our study provides evidence for using IL1RL1-a as a biomarker for predicting predominantly eosinophilic asthma. Studying the effect of IL1RL1-a levels in combination with other biomarkers associated with eosinophilic inflammation, such as sputum or blood eosinophils, might be of added value.

Most asthma prediction models are based on children who already have developed respiratory symptoms. Up to now there is no risk model that can predict the development of asthma directly after birth, when no symptoms have yet occurred. Through the growing numbers of identified asthma associated genetic variants the possibility increases to predict asthma based on genetic risk scores. Chapter 9 shows a prediction model for asthma in the first 8 years of life based on the combination of family, perinatal, environmental and genetic risk factors based. With this model we wished to investigate the added value of genetics when predicting asthma in the first year of life. With data from 1,968 children in PIAMA a familial, perinatal and environmental risk score was made, based on previously described asthma predictors. Furthermore, we performed a GWAS on asthma ever at age 8 years. We choose independent SNPs ($r^2<0.3$) with $P<5\times10^{-5}$ to calculate a weighted genetic risk score (GRS). Weighted GRSs were also generated with data from the TAGC and SHARE study. The TAGC and SHARE cohorts both published recently the results of large meta-analysis of allergic disease/asthma GWAS, greatly enhancing the number of genetic risk factors to be associated with asthma. Based on significant SNPs ($P<5\times10^{-8}$) from those studies and with the use of the reported ORs of the meta-analysis we generated two other weighted GRSs. Replication analyses were performed in BAMSE. Association analyses with asthma per familial, perinatal and environmental risk score showed strongest prediction for familial factors ($OR=1.25$, $P=3.17\times10^{-19}$). The combined model of familial, perinatal and environmental factors showed moderate discrimination in both PIAMA and BAMSE (AUC; PIAMA=0.65, BAMSE=0.67). Combining the PIAMA GRS (based on 145 SNPs) with familial, perinatal and environmental risk score resulted in a high discriminative value (AUC 0.86). This might, however, be largely due to overfitting caused by selection of risk SNPs from the same population, since this finding did not replicate in BAMSE (AUC=0.67). In PIAMA, the combined score with TAGC GRS and SHARE GRS showed no improvement over the risk prediction based on familial, perinatal and environmental factors with AUC values of 0.66 (TAGC) and 0.65 (SHARE). In BAMSE, similar results were found in the replication analyses: AUC of 0.69 (TAGC) and 0.70 (SHARE). Based on discriminative analyses, the highest predictive value was found for the familial risk score. Our study shows that, based on the most recent insights in asthma genetics, genetic variants have no added value in predicting asthma. Further research in identifying the true causal genetic variants of asthma, in combination with better defining asthma sub-phenotypes is needed to enhance asthma prediction.

General discussion and implications of results

Asthma is the most common chronic disease among children. The disease is caused by a complex interaction between genes and environment, leading to development of different asthma phenotypes. Multiple susceptible genes have been identified in relationship to asthma through the growing field of (epi)genetic studies.

But how can all these genes help us in gaining more insight in disease development? How can we truly discover the function of a gene and its biological pathway in relationship to disease pathogenesis? And, in addition to this, how can these findings help us to develop more personalized based treatment and novel asthma treatment regimens? Finally, the question rises whether we will be able to predict disease (at birth) based on specific gene variation?

In this thesis, we tried to resolve these questions particularly in relationship to childhood asthma. Moreover, we concentrated on the IL1RL1 gene, but the steps described below are applicable for studies of other asthma genes in relationship to disease or disease phenotype development. In the next section we will discuss the implications of our work for asthma phenotype definition, the interpretation of genetic association studies, functional studies and causality, as well as asthma prediction.
Asthma phenotypes

When studying a disease it is important to understand its causal mechanisms and what is pathognomonic to the disease. Asthma is characterized by reversible airway obstruction, bronchial hyperresponsiveness and remodeling of the airways leading to symptoms such as wheeze, cough and breathlessness. However, there is large inter-individual variability between asthma patients in disease presentation with increasing evidence that asthma cannot be classified as a single disease, but that it is a disorder with large heterogeneity in pathogenesis, severity, and response to treatment.

This has led to the identification of specific phenotypes, such as occupational asthma, aspirin exacerbated asthma and eosinophilic allergic asthma. All those phenotypes have been established by different classifications based on biomarkers, symptoms or type of inflammation. Attempts have been made, with the use of cluster analysis based approaches, to combine specific features, thereby identifying more specific phenotypic or endotypic (based on pathophysiological mechanisms) groups.

When studying asthma, a comparison can be made with the endocrine disease diabetes. Diagnosing diabetes is based on elevated glucose blood levels. It is important to determine if the person has elevated glucose levels due to diabetes type 1 or type 2, since both have distinct causal mechanisms and involve other treatment regimens. Translating this to asthma, not all persons with specific asthma symptoms and airflow obstruction can be classified and treated as the same asthma patient.

To perform good quality research it is necessary to define which phenotype or endotype of the disease is being assessed, with as much as possible a homogeneous study population to increase power. Evidence for this is provided in the study we performed in Chapter 8 in which we first did not find an association between serum IL1RL1-a levels and children with doctors’ diagnosed asthma at age 6 years. However, when restricting our analyses to a subgroup of asthmatic children with eosinophilic airway inflammation, we did find that IL1RL1-a levels at preschool age might have a predictive value in the development of eosinophilic asthma in children with doctors’ diagnosed asthma.

Since asthma is a highly genetically determined disease, attempts have been made to search for genetic variants associated with specific asthma phenotypes. We show in Chapter 2 and Chapter 4 that different genes are related to the age of onset of the disease, with the IL1RL1 gene being associated with childhood onset asthma. In Chapter 3 we also report that different variants of the IL33-IL1RL1 pathway are being associated with different wheezing phenotypes in early childhood. Moreover, in the more in-depth studies we provide evidence that IL1RL1 is important in eosinophilic, type 2 airway inflammation. These results are suggestive that genes can be taken into account when clustering asthma phenotypes. This is confirmed in a recent French Canadian study that clustered asthma into four group; older patients with low atopy and low lung function, high atopy, young non-smoking women and an high smoking history group. They report that their GWAS-identified SNPs were more likely to be associated with one of these subgroups of asthma than with broader defined asthma, with the IL1RL1/IL18R1 locus being more prominently associated with the high atopy subgroup. Phenotypic gene clustering can, however, only be based on large groups, that then can be stratified for e.g. age of onset of the disease or Th2 high and Th2 low phenotypes.

In my opinion, the first important step is to make a true distinction between childhood asthma and adult asthma, followed by asthma differences at different age points in terms of lung function, level of airway responsiveness, and inflammatory cells involved. Moreover, the heritable component might be higher for early-onset asthma when compared to late-onset asthma. Most asthma research is performed in adults, but the results of these studies cannot simply be applied on childhood asthma, where asthma incidence is the highest. The next step will be to assess the type of inflammation and pathophysiology involved. This can already be tested by simple approaches such as measuring of IgE levels, (sputum or blood) eosinophil numbers or FeNo levels. The pathological pathway involved is important since this determines the choice of treatment.
Further classification of sub groups, based on triggers involved ((viral) infection, allergens, exercise), severity (exacerbation prone), BHR, and lung function classification and ultimately based on genes as well, will help in the understanding of underlying disease mechanisms leading to more personalized treatment and improvement of disease outcome. In the ideal future, asthma classification should be internationally practiced, permitting better collaboration and merging of results between research cohorts, as well in intervention studies which will contribute to creating powerful standardized care.

**Genetic association studies**

About a decade ago, genetic studies predominantly involved linkage studies in families or candidate gene based approaches performed in families, trio studies, or case-control cohorts. However, with expansion of the genetic field the possibility to perform large genome-wide association studies (GWAS) in birth, population and patient cohorts have led to much more understanding of the genetic susceptibility to asthma.

Hypothesis free testing is important in the genetic field, which warrants large datasets that should be used taking care of the following considerations. 1) Differences in gene chips between studies: In the PIAMA cohort, children were genotyped on three different chips. This will lead to reduced genetic coverage when investigating genotypes present on all three chips. 2) Imputation: After extensive quality control, we performed imputation on these different chips with IMPUTE 2.0 against the reference data set of the Genomes of the Netherlands (GoNL) release 5.3 combined with the ALL panel of 1000 Genomes (phase 3, May 2013). By using an imputation dataset closely linked to the genotype of our (mostly Dutch) population we increased imputation power and accuracy. With the use of the ALL panel, we additionally included the genetic variants that were not captured by the GoNL reference set. This will lead to inclusion of SNPs necessary for replication studies or meta-analysis with other (ancestry) cohorts. 3) Merging of the different imputation datasets: In PIAMA we merged the three imputation chips which required caution in terms of 1) SNP selection, 2) genome build differences, 3) strand alignment, 4) SNP name calling, and 5) balance in case-control ratios across different platforms. We selected only SNPs that were either imputed or genotyped on all the three chips, and were of high quality (info-score IMPUTE ≥ 0.7) without large differences in allele frequency between platforms (<10 %). With respect to genome build differences, our merging procedure of SNPs was based on base pair location. Since location of SNPs differs between genome builds, it is important to convert chips to the same genome build if necessary. With respect to considerations in variance in strand alignment: For A/C, A/G, G/T and C/T SNPs differences in strand alignment can be easily assessed. For A/T and C/G SNPs this is more difficult and requires a check of allele frequency or removal of the SNP if there is still doubt. SNP name calling can have inherent problems: Insertions/deletions and SNP rs-numbers can be located on the same base pair position. In PIAMA, we separately merged the insertions/deletions and SNP rs-numbers and subsequently combined the two datasets. Finally, one has to take into account an unbalanced case-control ratio per chip: If this is present this may lead to spurious results. Certain of these aspects are also essential when performing a meta-analysis on genetic outcomes, such as presented in Chapter 4, Chapter 5 and Chapter 6. In meta-analyses it also important to meta-analyze the same ‘effect’ allele between cohorts.

Imputation is the process of predicting unobserved genotypes in a study dataset with the use of haplotype patterns in a reference dataset. This will increase the power to detect the real causal variant and will enhance the performance of meta-analyses between cohorts.

However, imputation can only be as good as the genetic input dataset and the causal variant will not be detected unless a marker that is already known to correlate with the phenotype under study was genotyped. Furthermore, multiple testing should be performed with the use of large imputed datasets in identification GWAS. The general accepted genome-wide significant threshold for imputation studies is $5 \times 10^{-8}$, based on the Bonferroni correction for 1 million tests, since it has been estimated that there are approximately 1 million independent SNPs. This stringent threshold can be lowered by performing fewer tests, and therefore it might be beneficial to only use the genotyped SNPs in the identification analysis, preselect known functional SNPs such as eQTL SNPs, or to use different thresholds for different reference panels.
The decision on the significance threshold in GWAS is important given the presence of extensive linkage disequilibrium (LD) in the whole genome. In Chapter 4 and Chapter 5 of this thesis, we show that the IL1RL1 gene locus exists of multiple LD blocks. When being too stringent on a significant cut-off value in a genetic locus with the presence of high LD, there is a risk in not finding an existing association since you correct for many tests, while due to the high LD one a few independent tests are performed. When performing Bonferroni correction on GWAS this is something which should be taken into account. New strategies, which allow for LD structure have been developed and help in this respect.29

From a SNP to a disease
Besides the genomic field to study genetics of diseases, more biological study fields are emerging such as epigenomics (epigenetic modifications), transcriptomics (RNA molecules, such as mRNA), and proteomics (proteins). All fields highlight different aspects of a disease and systemic integration of these specific groups is essential, but still faces large challenges.30

In the post GWAS era, one of the greatest challenges is to elucidate the function of the disease associated SNPs. The majority of SNPs that are significantly associated with a disease are located in non-coding regions of the genome or, as is reported in this thesis for IL1RL1, in intronic regions. SNPs can alter gene expression by affecting RNA splicing, transcription factor binding, histone modification, DNA methylation sites and miRNA recruitment. SNPs can furthermore be located in protein-binding regions, DNase I hypersensitive sites and footprints and chromatin immunoprecipitation sequencing (ChIPseq) peaks, all associated with gene regulation.31 This information, that is more and more publically available through the ENCODE project, will help in identifying casual variants. However, available ENCODE data on primary cells and tissues relevant to asthma, such as bronchial epithelial, airway smooth muscle cells, type 2 innate lymphoid (ILC2) cells or eosinophils, are not available to the respiratory research community, although this would greatly accelerate functional SNP identification.

In Chapter 4, we expanded our IL1RL1 SNP-asthma phenotype association to an eQTL analysis in lung tissue, bronchial brushings and bronchial biopsies. We also performed an in vitro eQTL analysis by assessing the association of SNPs with baseline expression of IL1RL1 mRNA isoforms and IL1RL1-a protein levels in human bronchial epithelial cells. The last analysis was in addition performed under different asthma relevant stimulations to assess the influence of environmental factors. In addition to this study, we tried in Chapter 5 to link our IL1RL1 SNPs associated with IL1RL1 methylation (meQTLs) to our IL1RL1 associated SNPs with IL1RL1-a protein levels (pQTLs) and link both to asthma development. Since correlation does not always imply causation, causal inference testing might be useful to assess which effect is present between certain associations, such as causal, reactive or pleiotropic. We performed causal inference testing on IL1RL1 methylation and the IL1RL1-a associated SNP rs1420101 with four IL1RL1 CpG sites and serum IL1RL1-a levels, which showed an independent relation.

The IL1RL1 studies in this thesis have considerably increased our understanding of the phenotypic and functional effects of SNPs in the IL1RL1 locus in relation to asthma (Figure 1).
Figure 1. Genetic and epigenetic association model of \( \text{IL1RL1} \) with asthma as investigated in this thesis. \( \text{IL1RL1} \) polymorphisms are associated with asthma, \( \text{IL1RL1} \) blood methylation, \( \text{IL1RL1} \) lung tissue and bronchial epithelial cell expression and serum \( \text{IL1RL1-a} \) levels. \( \text{IL1RL1} \)-a levels are associated with (eosinophil allergic) asthma and \( \text{IL1RL1-a} \) levels (red dots). Investigations on \( \text{IL1RL1} \) methylation with \( \text{IL1RL1} \) expression, \( \text{IL1RL1} \) expression with asthma and on \( \text{IL1RL1} \) expression with protein \( \text{IL1RL1} \) have not been performed in this thesis. The relation between expression and severe asthma has been found in a previously published study.35 Solid lines are studies performed in this thesis, dashed lines are studies not investigated in this thesis.

*We report an association between \( \text{IL1RL1} \) SNPs and \( \text{IL1RL1} \) expression in lung tissue and cultured bronchial epithelial cells. We did not find this association in bronchial brushings or bronchial biopsies.

Besides asthma we also assessed the association of \( \text{IL1RL1} \) SNPs with specific asthma characteristics (forced expiratory volume in 1 second (FEV1), FEV1/forced vital capacity (FVC), blood eosinophils, total IgE, atopy and age of asthma onset. In addition \( \text{IL1RL1-a} \) protein levels were tested for association with eosinophils and levels and fraction of exhaled nitric oxide (FeNO). Association of \( \text{IL1RL1} \) SNPs with effects of ICS treatment in asthma, and effects of environmental stimuli on asthma development i.e. stimuli known to be associated with asthma was also investigated.

SNP, single nucleotide polymorphism; meQTL, methylation quantitative trait locus; eQTL, expression quantitative trait locus; pQTL, protein quantitative trait locus; meQTM, expression quantitative trait methylations; pQTM, protein quantitative trait methylations; GWAS, genome-wide association study; EWAS, epigenome-wide association study.

We show and confirm that coding, but mostly non-coding asthma risk SNPs are important regulators of \( \text{IL1RL1} \) methylation, \( \text{IL1RL1} \) mRNA levels36,37 and \( \text{IL1RL1} \) protein levels35,38, both in vitro and in vivo. The fact that we did not find an association between asthma and \( \text{IL1RL1} \) methylation and protein expression levels may not lead to the conclusion that these mechanisms do not play a role in asthma development. It could be merely due to the fact that we tested a more general asthma phenotype and that the association with asthma is only present in (acute) asthma with type 2 inflammation.35,36,39–41 Moreover, it could be that the SNP effect on protein function in relation to asthma is more important than the level of the protein under study, which was being tested here.

As stated before, the \( \text{IL1RL1} \) locus has extensive LD. Taking into account all our results, I would propose that there are a five LD blocks which should be further studied, represented in Chapter 5, with a special aim for the LD block captured by the SNP rs1420101.

This \( \text{IL1RL1-c} \) exon 5E SNP is thought to be an enhancer of histone marks, binds GATA2 (a nuclear protein which regulates the expression of genes), and has an effect on multiple regulatory motifs.42 Children with the TT genotype of rs1420101 had a higher risk of asthma. We found, in these children, lower methylation levels of \( \text{IL1RL1} \) and lower lung tissue and bronchial epithelial cell expression of both the membrane and lower soluble \( \text{IL1RL1} \) variant. The reduced expression of \( \text{IL1RL1} \) leads to lower \( \text{IL1RL1-a} \) levels. Moreover, rs1420101 has been found to be associated with higher eosinophil numbers and an increased risk of a type 2-high endotype, which is a gene expression signature in bronchial epithelial cells.36 This last effect might be mediated by the reduced \( \text{IL1RL1} \) methylation, expression and protein levels. We did not test if this variant has influence on \( \text{IL1RL1-b} \) protein levels in the lung. Investigating the effect of rs1420101 on \( \text{IL1RL1-b} \) and to gain more insight in the function of \( \text{IL1RL1-c} \), in which the SNP is located, is necessary to truly see if rs1420101 is the causal \( \text{IL1RL1} \) SNP in relation to asthma. Table 1 presents, the most important SNPs found in this thesis based on association, location and possible function.
In future research, haplotype analysis with identification of children who carry key risk alleles per LD block, is also recommended, which could also provide further evidence of how the found meQTL, eQTL and pQTL associations are related to asthma.

Table 1. Selection of 4 most important IL1RL1 SNPs found in this thesis based on association, location and possible function.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>Annotation</th>
<th>Alleles</th>
<th>Associations in this thesis</th>
<th>Promoter histone marks</th>
<th>Enhancer histone marks</th>
<th>DNase</th>
<th>Protein bound</th>
<th>MOTIFS changed</th>
<th>NHGRI/EBI GWAS hits</th>
<th>GRASP QTL hits</th>
<th>Selected QTL hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3431828</td>
<td>102954653</td>
<td>5'-UTR</td>
<td>CT</td>
<td>Asthma (phenotypes), methylation, expression, protein</td>
<td>HRT, VAS</td>
<td>6 tissues</td>
<td>6 bound proteins</td>
<td>LXR</td>
<td>9 hits</td>
<td>5 hits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1420191</td>
<td>102957716</td>
<td>Exon 5E</td>
<td>G/A</td>
<td>Asthma (phenotypes), methylation, expression, protein, treatment effects</td>
<td>BLD, VAS</td>
<td></td>
<td></td>
<td>HNF4, Pdx1, RXRA</td>
<td>1 hit</td>
<td>32 hits</td>
<td>5 hits</td>
<td></td>
</tr>
<tr>
<td>rs13132157</td>
<td>102351848</td>
<td>Exon 11, missense</td>
<td>CT</td>
<td>Asthma (phenotypes), methylation, expression, protein, treatment effect</td>
<td>BLD, VAS</td>
<td></td>
<td></td>
<td>PRDM1, Pax-5, Roaz</td>
<td>5 hits</td>
<td>19 hits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs102044137</td>
<td>102968212</td>
<td>Exon 11, missense</td>
<td>A/G</td>
<td>Late onset wheeze, asthma, methylation, expression, protein, treatment effect</td>
<td>THYM</td>
<td></td>
<td></td>
<td>WT1</td>
<td>2 hits</td>
<td>19 hits</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SNP: single nucleotide polymorphism; UTR: untranslated region; HRT: heart; VAS: vascular; BLD: blood; THYM: thymus; LXR: Liver X Receptor; HNF4: Hepatocyte Nuclear Factor 4; Pdx1: Insulin promoter factor 1; RXRA: Retinoid X Receptor Alpha; PRDM1: PR domain zinc finger protein 1; Pax-5: Paired box protein; Roaz: Rat O/E-1-associated zinc finger protein; WT1: Wilms Tumor 1; eQTL: (expression) quantitative trait locus.

Information on annotated gene SNP location and function was obtained by HaploReg v4.1.42

Moreover, investigating gene-gene interactions, as we performed in Chapter 3, or genetic–epigenetic interactions could also bring more knowledge. A gene which might be interesting to investigate in terms of interaction with IL1RL1 is CDHR3. CDHR3 is, like IL1RL1, a replicated early-onset asthma associated gene. The gene is being expressed in the airway epithelium and is important for cell adhesion and interaction. Studies have shown that viral respiratory infections and air pollution are important risk factors for the development of (allergic) asthma. It has been thought that variation in CDHR3 could lead to a higher sensitivity to respiratory infections or other airway allergens through altered epithelial integrity. Moreover, cultured airway epithelial cells of asthma patients show lower expression of CDHR3 when compared to controls, with even further down regulation upon RV stimulation, which could lead to even more disruption of airway epithelial function.

The IL1RL1 variant rs1921622 and IL1RL1-a levels have been found to be associated with severe respiratory syncytial virus (RSV) bronchiolitis. Moreover, IL-33 and type 2 cytokines are shown to be enhanced in vivo during a RV-induced asthma exacerbation. We show in addition in Chapter 4 that the IL1RL1 variants rs13431828 and rs1420101 predominantly influence IL1RL1 mRNA and protein levels in bronchial epithelial cells by stimulation of RV. Above findings makes it of interest to investigate IL1RL1-CDHR3 interaction, especially in relation to RSV/RV induced asthma (exacerbations). The effect of CDHR3 on epithelial vulnerability, probably enhanced by RV, might lead to an enhanced epithelial release of IL-33 in response to airway stimuli, inducing more TH2 inflammation.

In addition, IL1RL1 has been found to be associated with allergic sensitization and evidence is provided that IL1RL1 plays a role in IgE levels released upon indoor allergen exposure. Moreover, in Chapter 2 we report that the most IL33-IL1RL1 SNPs were associated with intermediate-onset wheeze, a phenotype which showed a strong relation with IgE sensitization. These findings implicate that assessing the role of IL1RL1 in IgE sensitization might bring new insights in childhood asthma development.
Gene product as a biomarker

Biomarkers can be used in prediction, diagnosis, outcome and (response to) treatment of a disease. In asthma, multiple biomarkers have been proposed, such as IgE levels, to predict wheezing\textsuperscript{54}, and blood or sputum eosinophils to predict allergic asthma\textsuperscript{55}, FeNO to measure current lung inflammation\textsuperscript{56}, and urinary leukotrienes to reflect an asthma exacerbation.\textsuperscript{57} In general practice, DNA material is not easily available in the process of diagnosing or treating asthma. Therefore, the identification of gene products as biomarkers in e.g. blood can be a good alternative, as has been shown for periostin\textsuperscript{58} (encoded by POSTN) and chitinase-like protein YKL-40 (encoded by CHI3L1).\textsuperscript{59} In Chapter 5 we did not find an association between serum IL1RL1-a levels and asthma in adult asthma patients, however in Chapter 8 we did show that serum IL1RL1-a levels can be used in the prediction of eosinophilic asthma in childhood. Earlier findings support the role for IL1RL1-a as a biomarker, with higher serum levels being found in children experiencing an asthma exacerbation\textsuperscript{41} and in adults with atopic asthma when compared to controls.\textsuperscript{40} IL1RL1-a induced sputum levels have also been reported to be associated with asthma in young children, with higher levels correlating with disease severity.\textsuperscript{60} When using a biomarker in clinical practice the accuracy and specificity of the measurement is crucial. In addition, biomarkers may be phenotype specific, which is nicely shown by the study that grouped patients according to their Th2-high or Th2-low profile based on IL-13 regulatory genes. These groups differed also significantly on i.e. IL-5 and IL-13 expression in bronchial biopsies, eosinophil numbers in blood and bronchoalveolar lavage and IgE levels.\textsuperscript{9} I propose that for IL1RL1-a more research should be performed to determine normal values and asthma phenotypes for which it is a valuable marker before it can be implemented in clinical settings.

Treatment based on gene information

All asthma is currently being treated by a step-wise treatment.\textsuperscript{1} But as stated by the increasing evidence that asthma is not a single disease, but rather consists of multiple (endo)types with different pathophysiological processes, there is a high need for precision medicine trials\textsuperscript{61} to generate more personalized based medicine.

There is accumulating evidence that disease genes and their biological products are of interest in generating new treatment approaches. The first method to examine this is a pharmacogenetic study, which searches for inherited genetic differences in drug metabolic pathways that affect individual responses to medical treatment (Table 2).
Table 2. Methods to develop genetic based novel treatments with future options for IL1RL1.

<table>
<thead>
<tr>
<th>Approach</th>
<th>Method for treatment based on genes</th>
<th>Option IL33-IL1RL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacogenetic</td>
<td>Use genes to determine subgroups of patients who respond to treatment</td>
<td>Possible haplotype selection (rs1420131, rs192157)</td>
</tr>
<tr>
<td>Phenotypic</td>
<td>Use genes to determine phenotypic subgroups of patients</td>
<td>Selection early onset asthma patients with eosinophilic type 2 airway inflammation</td>
</tr>
<tr>
<td>Biological</td>
<td>Use genes to determine patient/disease specific biological pathways</td>
<td>Anti-IL33 antibody, IL1RL1-b antagonist, soluble IL1RL1-a</td>
</tr>
</tbody>
</table>

It has been shown in children with asthma that SNPs indeed can regulate treatment response.62 This information opens avenues to determine patient subgroups with specific alleles for a particular treatment regime. An example is shown by a genotype-stratified trial that selected patients with asthma who were homozygous for arginine (Arg/Arg) or glycine (Gly/Gly) at the ADRB2 Arg16 locus.63 The study showed that patients with the Arg/Arg genotype improved when regular short-acting b2-agonists (SABA) were withdrawn and replaced by ipratropium bromide. The opposite occurred in patients with the Gly/Gly genotype, i.e. they improved with regular SABA compared to placebo. Further studies suggested that homozygote ADRB2 Arg16 carriers have a poorer response to long-acting b2-agonists (LABA), with a PACMAN study reporting that children who are Arg/Arg carriers have an increased risk of asthma exacerbations when treated with LABA and ICS compared to children carrying the Gly/Gly genotype.64 Despite these and other findings there is still controversy on how effective SNPs can be used in the prediction of asthma treatment response.65

We report in Chapter 6 no direct IL1RL1 effect on ICS use in terms of asthma control, change in FeNO levels or FEV1 %predicted in asthmatic children. However, IL1RL1 is an important gene in the manifestation of asthma exacerbations40,66 with an effect that is still present in asthmatic patients using ICS as shown in Chapter 6. Our findings that the IL33-IL1RL1 pathway is important in type 2 airway inflammation makes this gene and the biological pathway in which it is involved important for new asthma treatment regimens in asthma patients with a Th2 high phenotype.

As stated above, genes might be useful to refine subgroups of asthma patients who have a shared pathogenesis.9 As our results suggest, and as shown by other cohorts35,36, SNPs in the IL1RL1 region can be used in selecting (early onset asthma) patients with eosinophilic, type 2 airway inflammation. This type of inflammation has been shown to respond well to ICS.67 Future studies should investigate if patients with specific IL1RL1 genotypes show superior responses to steroid treatment.

Finally, genes can be used to search for a biomarker that reflects a specific subgroup of patients (Figure 2). This biomarker can help in developing a new treatment that targets this gene or the pathway in which it is predominantly involved. In the past years, novel treatment strategies have been developed that block biological pathways of cytokines involved in asthma development, such as anti-IgE (omalizumab), anti-IL-5 (mepolizumab) or a combined pathway of IL-4/IL-13 (dupilumab). Not all patients benefit from these treatment approaches and hence selection of treatment responsive patients is recommended. This has been highlighted by a study in asthma patients who were inadequately controlled for their asthma despite the use of ICS therapy. Here it was shown that patients with high levels of periostin had a more adequate response to anti-IL13 therapy, based on lung function improvement, when compared to patients with low periostin levels.58
Airway epithelial derived cytokines are released when their receptors are activated by external stimuli, leading to an adaptive or innate immune response. There is emerging interest in epithelial derived cytokine based therapies, such anti-TSLP and anti-IL33, though effects are mostly proven in murine models. Administration of anti-IL33 has been shown to suppress the extent of type 2 inflammation during RV-induced asthma in mice. Confirmation of the observed anti-IL33 boosted antiviral immunity and reduced viral replication was shown in human primary airway epithelial cells. We report in Chapter 4 that an exaggerated inflammatory response to IL-33 is present in bronchial epithelial cells carrying the IL1RL1 TIR domain asthma risk haplotype (tagged by the coding variant rs10192157), which is more responsive to the anti-inflammatory effects of anti-IL33 and anti-IL1RL1 antibodies. This suggests that investigating therapeutic effect in subsets of patients with specific genetic make-up might be warranted. Moreover, since the genetic make-up differs between ethnicities with diverse treatment responses as we show in Chapter 6, it is mandatory that novel treatments are tested in multiple ethnicities.

Next to targeting IL-33, it would also be interesting to further investigate the therapeutic effects of IL1RL1-a administration as a method to dampen (type 2) inflammation. A murine study supporting this approach showed that administration of soluble IL1RL1-a to Th2 cells leads to inhibition of lung mucosal Th2 immune response by inhibiting IL-4 and IL-5 production. Moreover, an IL1RL1-b antagonist that will prevent IL-33 from binding to its receptor might also be an option.

The TIR domain present in IL1RL-b plays a central role in the IL33-IL1RL1 signaling cascade. Binding of IL-33 to the TIR domain leads to heterodimerization of IL1RL1-b with the co-receptor IL-1RAcP and signaling proteins MyD88 or Mal. As mentioned before, this initiates the signaling cascade that eventually results in the activation of downstream transcription factors and activator proteins involved in Th2 cytokine release and inflammation. Manipulation of this TIR domain, prevention of TIR domain binding and sequestration of MyD88 or MAL or by interfering the subsequent signaling cascade could also be future target treatment options. When developing new treatment strategies, negative side effects are almost inescapable. When manipulating the IL33-IL1RL1 pathway special attention should be paid to cardiac effects since the pathway has shown to play rather a protective role in cardiovascular diseases in adults.
Summary, general discussion and implication of results

Figure 2. Possible treatment options in the IL33-IL1RL1 biological pathway. Potential options for future asthma treatment; 1) administration of soluble IL1RL1-a which will neutralize the inflammatory effect initiated by IL-33, 2) Anti-IL33 antibody for inhibiting IL-33 function, 3) blockade of the transmembrane IL1RL1-b receptor by an IL1RL1-b antagonist, 4) (Genetic) manipulation of IL1RL1-b TIR domain or prevention of Mal and MYD88 sequestration will inhibit the initiation of the signaling cascade and 5) Interfering activation of transcription factors and activator proteins of the signaling pathway will lead to inhibition of Th2 cytokine release and subsequent Th2 inflammation. Adjusted from Grotenboer et al., with permission.71

Genetic prediction of disease

Newborns are screened for cystic fibrosis in the Netherlands and it has been shown that early detection and initiation of treatment of this disease leads to improved growth, better lung function ultimately better survival.73 Since we are not yet able to predict asthma at birth we do not know exactly what the effects of early screening and therefore initiation of preventive methods or early treatment will be. However, it is known that the impairment of lung function has already started in early life in children with asthma.74,75 In children with mild-to-moderate severe asthma, approximately 75% have abnormal lung function patterns with reduced lung growth and early decline of lung function.76 Moreover, studies have shown that this reduced growth and loss of lung function greatly enhances the risk of developing chronic obstructive pulmonary disease (COPD).76,77 Early start of anti-inflammatory treatment in patients with mild persistent asthma, within 2 years of disease onset, has led to a decreased risk of severe exacerbations and of lung function loss after 3 years of ICS treatment, in addition to their usual asthma medications.78 However, if asthma treatment can really affect the natural course of the disease or even lead to early remission has not yet prospectively been proven.79

Prevention or early treatment is, besides preserving lung function, also important for decreasing exacerbation rates, reducing hospitalizations, preventing school absenteeism and preserving quality of life. Moreover, since it has been thought that outcomes in adult asthma may be determined primarily in early childhood by factors such as sensitization to house dust mite, bronchial hyperresponsiveness and an early age of onset90, early life time intervention strategies are important.
All these findings highlight the importance of good prediction methods. Nevertheless, the prediction performance of existing asthma prediction models based on non-invasive and invasive clinical markers at best moderate. With the advanced (prenatal) genetic screening options we might be able to have a full genomic profile of a child even before it is born. Although it has been shown that genes can be used for asthma prediction\(^{81}\) we do not report in Chapter 9 any added value of genes in predicting asthma in comparison with prenatal, familial and environmental risk factor prediction. Assessing genetic prediction in specific subgroups of asthma or combining it with gene-environment interactions should be performed in future studies.

**Back to the patient**

Imagine two cases. One is a girl, born at 41 weeks in winter by a cesarean section. Her mother smoked till the 20th week of her pregnancy and she was bottle fed as a baby. She has an older sister who uses sometimes a SABA for wheezing difficulties. She grows up in an urban area. Her IL1RL1 rs1420101 genotype is TT. The other case is of a boy, born at 37 weeks in spring. His non-allergic mother provides breastfeeding till he is 8 months. He is an only child and grows up at a dairy farm. His IL1RL1 rs1420101 genotype is CC. Both children end up at the ER with breathing difficulties caused by an RS bronchiolitis at the age of 1.5 years.

With the data thus far I would speculate that the girl has a higher risk to develop asthma at the age of 8 years when compared the boy, due to the presence of multiple asthma risk factors in her life and her asthma risk genotype. The girl should be released of the ER with a SABA prescription and parents should be advised to eliminate still existing risk factors. A trial of ICS could be considered. Follow-up is warranted with regular lung function measurements in a GP setting to upscale intervention strategies when deterioration is observed. Since she has the rs1420101 TT genotype she could have lower circulating IL1RL1-a levels making her a candidate for administration of therapeutic soluble IL1RL1-a (Figure 3).

![Figure 3. Lifespan of a child showing multiple pre- and perinatal (e.g. smoking mother, being bottle fed), familial (e.g. allergic sibling), environmental (e.g. air pollution, rural area) and personal events (e.g. viral infection) that can influence a child's DNA which will eventually result in prevention or the development of asthma with related lung function and blood eosinophil numbers. Figure made by H.D. Dijk.](image)

Unfortunately, due to the complex (epi)genetic and environmental interactions and biological pathways involved in asthma, real life prediction of the prognosis of these 2 patients and other asthma susceptible children is at this moment still imperfect. By extending our research, with for instance developing large multi-national cohorts with phenotypic/genotypic/exposomic studies, it has to be the ultimate aim of research to improve this prediction accuracy and to prevent or treat asthma development at an older age in a personalized based manner.
References


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Ik krijg niet genoeg adem

Vriendjes rennen harder

Ik piep soms

Boris moest weg

Geen knuffels op mijn bed

Altijd een pufje in de schooltas