Functional and clinical translation of asthma and allergy associated genetic variants in IL33 and IL1RL1

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Chapter 1

General Introduction-
Translational research: a tailored prescription for patient, doctor and scientist?

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Introductory words

In this thesis, we set out to translate asthma-and allergy-associated genetic variation at the *Interleukin-33* (*IL33*) and *Interleukin-1-Receptor-Like 1* (*IL1RL1*) loci into a better understanding of the pathophysiology of specific asthma and allergy phenotypes, and aimed to apply this knowledge for clinical use by developing prediction models for asthma and allergy phenotypes. Studying specific disease phenotypes may help to identify the particular patient population that would benefit most from IL-33/IL-1RL1 targeted treatment.

The main focus of this thesis is on asthma phenotypes, but due to the large overlap between asthma and allergy in genetic predisposition, pathophysiology and clinical prevalence, we also include other allergic disorders, such as food allergy. As an introduction to this thesis, we first provide a definition of asthma, allergy and elaborate on the challenges to define specific (sub)phenotypes, followed by an overview of the current genetic, functional and clinical evidence for the involvement of the IL-33/IL-1RL1 pathway in asthma and allergy. We conclude this introduction with an outline of the translational approach we developed in this thesis with the aim to further unravel the role of *IL33*/*IL1RL1* genetic signals in specific asthma and allergy phenotypes.

Definition, prevalence and classical approach of asthma and allergy

Asthma and allergy are common, complex diseases that result from the interaction between genetic predisposition and environmental factors (such as allergens and/or respiratory viruses) that trigger disease inception and exacerbations. Asthma and allergy may have shared underlying molecular pathways as suggested by overlapping genetic risk factors for both asthma and allergic disease (1). Asthma currently affects over 600,000 people in The Netherlands (3-4% of the population) (2). Worldwide more than 300 million people have been diagnosed with asthma, while its global prevalence is expected to increase to 400 million patients by 2025 (2-5). For allergy (including allergic rhino-conjunctivitis, allergic dermatitis/eczema, allergic asthma, food allergy) prevalence rates are around 30-40%. Allergy and asthma are not independent; allergies are more common within asthma patients: around 60-65% of asthma patients have one or more allergies, including chronic rhino-conjunctivitis, eczema, and less commonly food allergy. (1,3)

Asthma is a chronic inflammatory condition of the airways, characterized by bronchial hyper-responsiveness and reversible airway obstruction. (1,3) Symptoms (figure 1A) include shortness of breath, chest tightness, wheezing and cough. Asthma symptoms occur variable over time, vary in intensity and are often worse at night or after viral or bacterial infections. Other main triggers include allergens, exercise, excitement and cold air (figure 1B) (3,6). The clinical diagnosis of asthma is based on symptoms, family history of asthma and allergy, plus confirmation of reversible airway obstruction (>12% change by bronchodilators) in lung function tests (figure 1C).

Allergic sensitization is the production of specific IgE upon exposure to allergens (e.g. tested via skin prick test, blood IgE tests, or provocation test). A person is considered to be allergic

when both specific IgE and allergic symptoms are observed, such as rhinitis/conjunctivitis, eczema, shortness of breath and wheezing, or gastro-intestinal tract symptoms upon allergen exposure. As suggested by the large overlap in clinical expression and genetic risk factors, asthma and allergy may share common pathogenic mechanisms underlying the chronic inflammation observed in both conditions. Studying the one could therefore be beneficial for understanding the other, and vice versa. However, allergies are only present in a subset of, but not in all asthma patients. This clearly indicates that differences do exist, making a clearer definition of subphenotypes of asthma crucial to understand underlying mechanisms of disease.

Classically, asthma has been considered as a single disease, with patients sharing many symptoms (figure 1A). Current treatment strategies of asthma therefore still entail a common step-up regimen for all patients in order to achieve control, consisting of a symptomatic, non-specific and non-curative approach using bronchodilators and general anti-inflammatory drugs such as corticosteroids (figure 2) (2,3,6). About 85-90% of patients gain adequate control using this treatment strategy. However, about 10-15% of patients lack control of symptoms and are left with severe symptoms and frequent exacerbations (>2/week) despite standard treatment. Also due to these observations, it is increasingly recognized that asthma is a heterogeneous disease, consisting of several subphenotypes with differences in underlying disease pathobiology, treatment responses and outcomes between individual patients (7). Patients may benefit from a clear definition of subgroups that are characterized by a shared pathogenesis and therefore respond to the same targeted treatment strategies, tailored to their particular underlying disease mechanism (figure 2) (7,8)

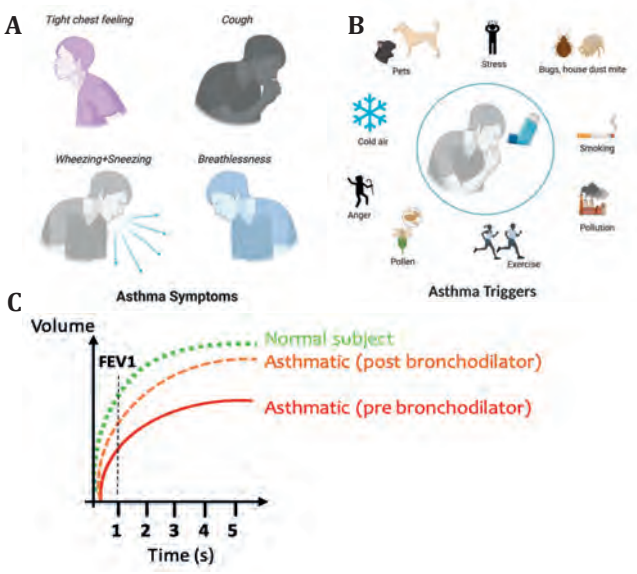

Figure 1: asthma characteristics
A) Clinical symptoms **B)** Triggers of symptoms **C)** Reversible airway obstruction in lung function testing

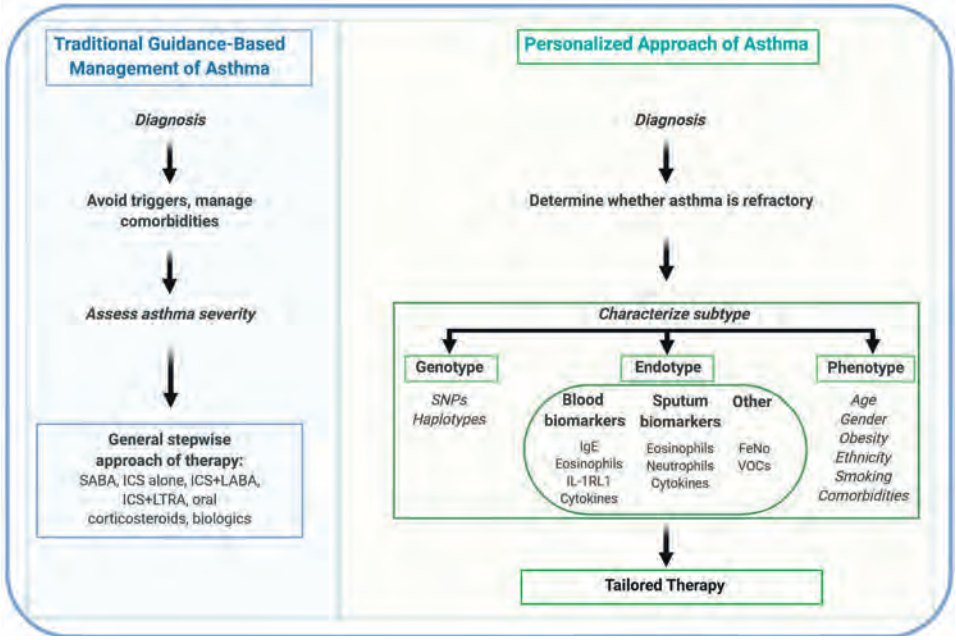

Figure 2: Traditional (left) and more tailored (right) approach to asthma

(Sub)phenotypes and endotypes of asthma/allergy

Defining relevant subgroups of patients can be a genuine challenge, as any clustering into subgroups is highly dependent on the angle taken. One angle could be to attempt to define distinct clinical phenotypes such as based on age of onset, type of trigger, allergic component, lung function or response to treatment. However, distinct clinical phenotypes may not necessarily represent distinct underlying biological mechanisms. Moreover, similar phenotypes may not share a common underlying pathobiology (7,8). This highly complicates targeting of treatments that interfere in specific biological pathways to the patient group most likely to respond. A different angle, therefore, could be to group patients based on the underlying biological mechanism that causes their symptoms, by defining the molecular characteristics of the patient such as number and type of immune cells, exhaled breath markers or cytokines in sputum, bronchial washes or blood, or even transcriptome of the cells in these compartments, all of which might eventually help to guide tailored treatment strategies. Nevertheless, we are only starting to unravel the causative biological mechanisms of disease, which is complicated by the heterogeneous study populations as well as the likely redundancy of molecular routes towards chronic airway inflammation in a single patient. Therefore, molecular mechanisms as grouping factor may not be the sole solution.

Ideally, therefore, one would like to combine both ways of grouping asthma, aiming to define so called 'endotypes', meant to represent clinically distinguishable asthma types connected to a clear common underlying molecular pathway, that can be addressed therapeutically

(7,8). An example of this approach is the study of Kaur et al (7), which used a combination of clinical parameters (age of asthma onset, lung function, allergic status, BMI and sex) and molecular factors (sputum eosinophil/neutrophil counts, fraction of exhaled NO (FeNO), IgE and type 2 markers in blood) to distinguish four major endotypes of asthma (see figure 3). These were recognizable across multiple large asthma studies, suggesting these are reproducible definitions reflecting conserved variation present within the population of patients that currently share the single diagnosis of asthma. If these four groups also differ in their natural course of disease as well as their treatment response, this would be very relevant evidence for these groups to be true distinct endotypes. If so, a clear goal would then be to develop targeted treatment strategies tailored to each of these endotypes.

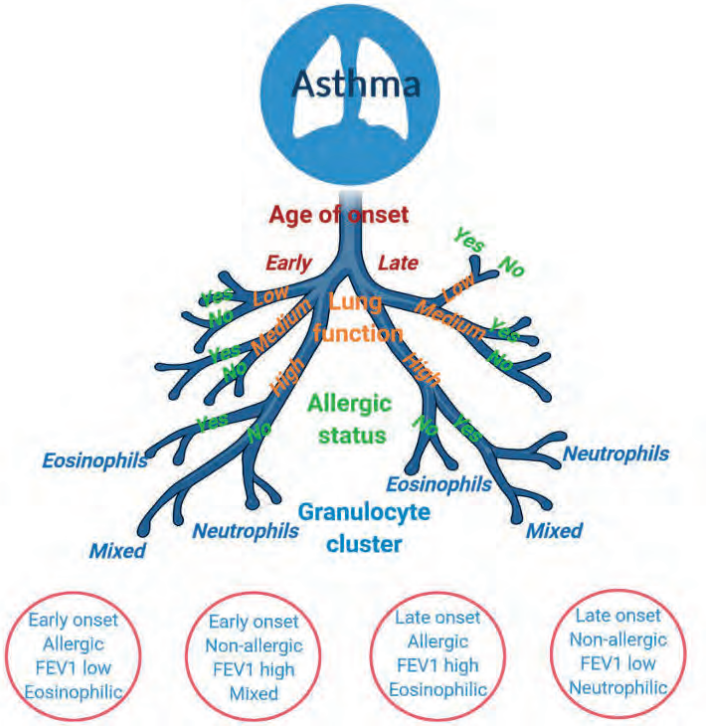

Figure 3: Endotypes- a combination of clinical and molecular features in disease, such as asthma

Genetic component of asthma and allergy (figure 4)

One way to further connect molecular mechanisms to clinical phenotypes could be to take a functional genetics approach; aiming to translate disease associated genetic signals into pathophysiology of specific phenotypes of asthma and allergy. Heritability studies have estimated a genetic contribution to the development of asthma and allergic disease of around 50-80%, dependent on the population and exact definition of the disease. However, the

classical twin approach of heritability studies (i.e. comparing monozygous twins to dizygous twins) may overestimate the genetic contribution to disease. For instance, monozygous twins potentially share a more common environment than dizygous twins (1,9,10), explaining part of the heritability of asthma and allergy. (11) Moreover, part of the heritability of disease might be attributed to epigenetic factors which could also be transferred from 1 generation to the next. (12)

The variability that could be attributed to specific genetic variation such as common single nucleotide polymorphisms (SNPs) is therefore also used as a more specific measure of heritability. Based on our current understanding, the SNP-attributed genetic variation underlying asthma and allergy is now estimated to be between 9-15% (1,10-14). This is much lower than the 50-80% estimates from classical heritability studies, implying that genetic association studies could not explain all of the heritability of asthma/allergy. However, although a complex interaction of genetics with environmental factors eventually would determine the development and expression of multifactorial diseases such as asthma and allergy, the significant contribution of genetics to disease risk suggests that studying the role of genetic factors in disease pathogenesis could be a useful approach in increasing our understanding of the mechanisms underlying phenotypes of asthma and allergy.

Genome-wide association studies (GWAS) have proven a useful tool to identify genetic risk factors associated with specific phenotypes. GWAS focus on heritability explained by SNPs, linking alleles of SNPs to phenotypes in large case-control studies. These SNPs vary in their degree of linkage: alleles of some SNPs are inherited together from one to the next generation. The degree to which alleles of SNPs are inherited together is expressed by their degree of linkage disequilibrium (LD), for example by using a correlation measure such as R^2. An R^2 can range between 0-1, with an R^2=1 indicating complete LD, i.e. a certain allele for one SNP is always inherited together with a specific allele of the other SNP. Vice versa, an R^2=0 means no LD exists between two SNPs; each allele of one SNP is inherited together with any of the two alleles of the other SNP (at random). This concept can be useful in GWAS, but at the same time provides an important limitation of this approach. On the one hand, high LD between SNPs allows just a subset of SNPs to be genotyped in GWAS, whilst still being able to infer the association of linked SNPs with a phenotype (i.e. as is the case in imputation with associated probability estimates of inferred genotypes). On the other hand, however, finding a strong association between a SNP and disease phenotype, does not mean that this SNP is also the causal SNP; especially in regions of high LD, multiple SNPs can be the candidate SNPs for causality based on their high degree of LD with the tested SNP, providing a challenge to identify the causal SNP. In addition, other structural variants in the DNA (e.g. indels, repeats) in LD with the SNP might be the causal genetic variation contributing to the studied phenotype, in which case any SNP detected by GWAS to be associated with a specific trait merely flags the presence of another causal genetic variant.

To date, over 130 independent genetic risk variants (SNPs) have been associated with asthma and allergy, of which more than 90% were shared genetic risk factors between asthma and allergic disorders (1). The large number of genetic loci associated with asthma

and allergic diseases identify a large number of genes contributing to the susceptibility for these disorders. The genes could be mapped to specific biological pathways that are thereby implicated to play a causal role in the mechanism of disease. The majority of the genes that are associated with both asthma and allergy represent immune-related genes, such as those involved in type 2 inflammatory pathways (1). Among the most reproducible, consistent genetic top signals associated with asthma and allergy are loci carrying the genes encoding interleukin-33 (IL-33, its gene being *IL33*) and its receptor interleukin-1 receptor like 1 (IL-1RL1, its gene being *IL1RL1* (also known as *ST2*). In addition to genetic evidence suggesting involvement of the IL-33/IL-1RL1 pathway in asthma and allergy pathogenesis, also several functional and clinical studies strongly suggest the IL-33/IL-1RL1 pathway to be a key component in the pathogenesis of these chronic inflammatory diseases.
I will briefly review these studies in the section below.

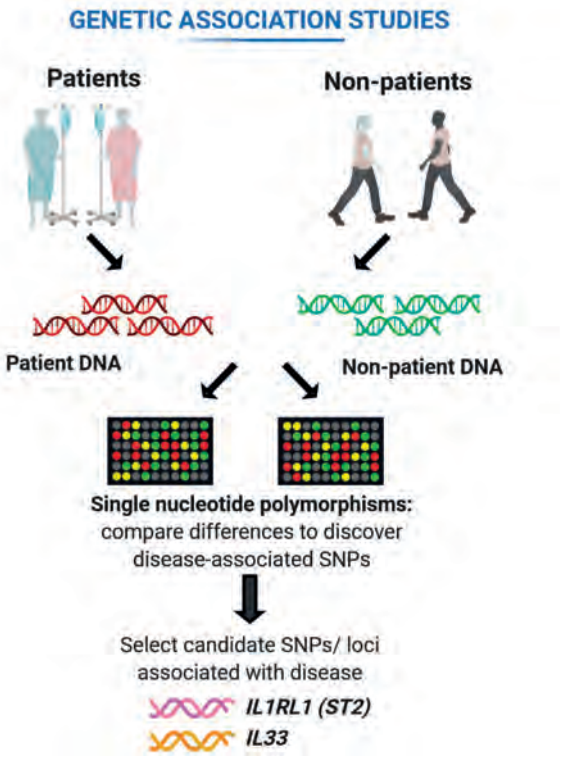

Figure 4: Genetic association studies have found IL33 and IL1RL1 to be associated with asthma/allergy

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Evidence for involvement of IL33/IL1RL1 in asthma/ allergy pathogenesis

Genetic evidence

The *IL33* (chr9) and *IL1RL1* (chr2) loci were originally discovered as regions associating with allergic phenotypes, such as rhinitis, (allergic) asthma, and allergic dermatitis. It was followed by studies showing that these loci also associate with level of blood eosinophils in general population cohorts (15,16). This suggests a shared genetic effect of *IL33* and *IL1RL1* loci in the development of asthma, allergy, and eosinophilia. Large-scale GWAS and GWAS meta-analyses including the GABRIEL consortium, EVE consortium and recently the SHARE study, TAGC study and studies in the UK Biobank subsequently confirmed the association of *IL33*/*IL1RL1* with asthma, allergy and eosinophilia (17-26). An overview of these studies is provided in table 1. Also in candidate-gene based studies, SNPs at the *IL33* and *IL1RL1* loci associate with asthma and allergy (27,28). To understand how genetic variation at these two loci contributes to the susceptibility of asthma and allergy phenotypes, a range of functional studies have been performed, which I will outline below.

Table 1: Overview of the asthma and allergy-related phenotypes that the IL33/IL1RL1 loci have been associated with in GWAS to date

IL33/ IL1RL1 association	**Asthma (all)**	**Asthma** (moderate-severe)	**Asthma** (childhood onset)	**Asthma** (allergic)	**Allergic rhinitis**	**Allergy** (self-reported and/or sensitization)	**Eczema/ atopic dermatitis**	**Blood eosinophils**
IL33	13 GWAS	1 GWAS	3 GWAS	2 GWAS	3 GWAS	3 GWAS	4 GWAS	4 GWAS
IL1RL1	14 GWAS	2 GWAS	3 GWAS	2 GWAS	3 GWAS	2 GWAS	4 GWAS	5 GWAS
Reference	*(1,15,17, 18,21,23-25,34,54 -56)*	*(56,57)*	*(18,19,54)*	*(15,20)*	*(17,20, 58)*	*(23,59,60)*	*(1,17,61,62)*	*(15,16,34, 55,63)*

Functional evidence

Functionality of SNPs

GWAS SNPs or SNPs in LD with the studied SNP can be causally involved in disease pathogenesis. SNPs can act through several mechanisms, including coding effects through changes in the protein sequence, thereby affecting the function of the gene product; or through non-coding changes such as by influencing the expression levels of the gene product, which results in expression quantitative trait loci (eQTL) in case of the RNA or protein quantitative trait loci (pQTL) in case of the protein (29). One way to explore which disease-associated SNP could be causally involved in disease pathogenesis is to predict the functionality of the disease-associated SNPs based on location and genomic context: are there any potential regulatory elements altered by the SNP?; does it locate in open/closed chromatin?; is it a non-coding or a coding SNP? Asthma-and allergy- associated SNPs at the *IL1RL1* locus are located in both non-coding and coding regions, suggesting potential effects

on mRNA/protein expression (eQTL/pQTL) and protein function, the latter including IL-33 binding and intracellular signal transduction of the IL-1RL1 receptor (30). To explore which disease-associated SNPs act as eQTL or pQTL, mRNA and protein levels of the gene need to be quantified in tissue samples or isolated cells from a large number of donors. Indeed, several studies have identified asthma-associated SNPs at the *IL1RL1* locus as eQTL and pQTL for *IL1RL1 (27,28)*. Furthermore, although one study showed functional effects of *IL1RL1* missense variants on IL-1RL1 signalling in a cellular model (31), the effects of coding SNPs for protein function of IL-1RL1 generally require validation still. At the *IL33* locus, common asthma-associated SNPs are located 5' and in the first intron of *IL33*, suggesting gene expression effects to be the mechanism of action of the disease-associated SNPs. Indeed, some of the asthma-associated *IL33* SNPs show potential eQTL function for *IL33* (32), in one case by affecting a CREB1 binding site that regulates *IL33* promotor activity *in vitro (33)*. In addition, a (rare) coding SNP in *IL33* exon 7 has been found to be a loss-of-function mutation, resulting in reduced *IL33* expression and truncated IL-33 protein: this allele is associated with lower blood eosinophil counts and with reduced risk of asthma (34).

Functionality of IL-33/IL-1RL1 in models of asthma and allergy

In addition to these functional genetic studies using human tissues and cells, model systems of asthma and allergy also provide supporting evidence for the relevance of the IL-33/IL-1RL1 pathway in disease pathogenesis. IL-33 is constitutively expressed in lymphoid organs, endothelium and epithelial barrier tissues such as the lung and skin. Mouse studies show that IL-33 promotes airway remodelling and inflammation in allergen-induced asthma (35), and links to airway influx of eosinophils (36). From these functional studies in mouse models and cultured cells it has become clear that IL-33 has a dual effect as a transcription factor and a cytokine (37). IL-33 as a transcription factor can alter gene expression in a cell-autonomous, IL-1RL1-independent fashion (38). IL-33 can translocate to the nucleus and bind directly to chromatin as well as to the NF-κB proteins p50 and p65 (38). In doing so, IL-33 is capable of regulating the expression of proinflammatory genes, such as IL-6, IL-8, and the p65 subunit of NF-κB, (38,39) and thus might be a direct regulator of the inflammatory response (26,38,39).

The most well-studied function of IL-33, however, is its cytokine function. Because IL-33 is released upon injury/damage or virus, it has been coined to act as an "alarmin", translating epithelial damage into an inflammatory response. IL-33 acts through binding to its heterodimeric receptor complex consisting of IL-1RL1 and the accessory receptor subunit IL-1RAcP. Binding of IL-33 to the IL-1RL1/IL-1RAcP heterodimeric receptor complex will recruit signalling adaptor proteins, that induce downstream signal transduction. The IL-33 induced signalling events eventually lead to activation of the innate and adaptive immune cells expressing a functional IL-33 receptor complex. A large number of cell types relevant to asthma pathogenesis have been shown to express IL-1RL1 and to be responsive to IL-33 (40), including TH2 cells (41), mast cells (42,43), invariant natural killer T cells (44), eosinophilic and basophilic granulocytes (45-47), innate-like type 2 helper cells (ILC2s) and

epithelial cells themselves (48). By activating these cells via the IL-1RL1 receptor, IL-33 has been shown to mediate a wide range of responses that converge on the induction of a T helper 2/innate like helper type-2 (Th2/ILC2) inflammatory response characterized by the production of IL-4, IL-5, and IL-13. (26). Another proof of concept for the cytokine role of IL-33 in allergic airway disease is a recent study that showed that administration of an IL-33 antagonist (IL-33trap) at the time of allergen challenge inhibits inflammatory responses in a preclinical mouse model of acute allergic airway inflammation (49). The potential role of the IL-33/IL-1RL1 signalling pathway in the context of asthma and allergy pathogenesis is graphically explained in figure 5.

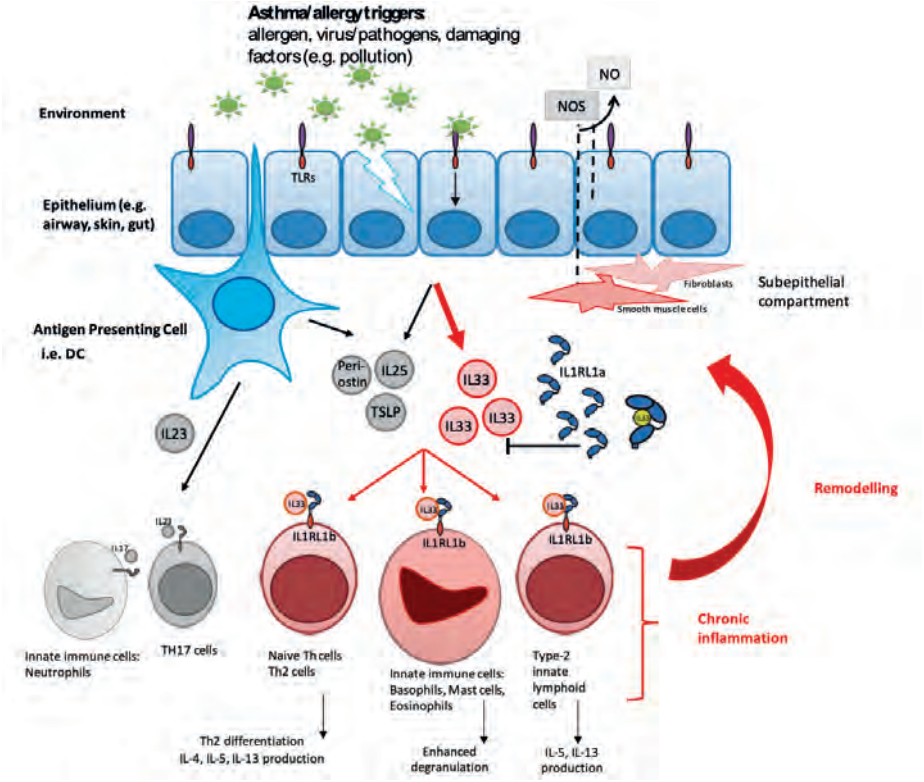


Figure 5: Overview underlying molecular mechanisms of asthma and allergy

Clinical evidence

Clinical studies have also contributed to the evidence that the IL-33 pathway is dysregulated in asthma and allergy. For example, it has been shown that levels of airway IL-33 associate with levels of type 2 cytokines in induced sputum and positively correlate with the fraction

of exhaled nitric oxide (FeNO) and sputum eosinophil counts in asthma patients (50). Moreover, IL-33 and IL-1RL1 levels were higher in induced sputum, bronchial biopsies and serum of patients with asthma compared to non-asthmatic controls (51-53). One study observed that high IL-33 levels in serum were specifically seen in allergic and eosinophilic asthma patients, but not in other studied asthma groups, including obese asthma, severe asthma and non-allergic/non-eosinophilic asthma (52). Furthermore, soluble IL-1RL1 and IL-33 levels have been found elevated during asthma exacerbation (19). Lastly, several cell types derived from allergic subjects, including mast cells, basophils, and eosinophils, could be matured and activated to release IL-4, IL-5, and/or IL-13 upon IL-33 stimulation, which was increased compared with that seen in cells isolated from healthy control subjects (26). Altogether, genetic studies, functional studies in model systems and clinical studies imply involvement of the IL-33/IL-1RL1 pathway in asthma and allergy.

Overview of this thesis- translating the asthma and allergy genetic risk loci IL33 and IL1RL1 into function and clinical application

Overarching hypotheses underlying this thesis

The above considerations have led us to hypothesize that genetic variation at the *IL33* and *IL1RL1* loci could have functional consequences for cells/tissue involved in the biological mechanisms underlying specific subphenotypes of asthma and allergy. Consequently, the gene products IL-33 and IL-1RL1 could have potential 1) as biomarkers of asthma and allergy phenotypes and 2) as targets for developing tailored treatment strategies in asthma and allergy subgroups.

Outline to study these hypotheses

To this end, we performed translational studies, aiming to connect genetics to function and clinical application (see also figure 6). **PART I**, consisting of chapters 2 and 3, reviews literature on current genetic and functional evidence of the IL-33/IL-1RL1 pathway in asthma and allergy in detail. **Chapter 2** summarizes the genetic evidence and potential functionality of asthma/allergy associated SNPs, while **chapter 3** reviews the potential role of the IL-33 pathway in mast cell and basophil function in asthma and allergy, key cells involved in development of chronic inflammation in these diseases. **PART II**, comprising chapters 4-6, then provides functional and translational studies to unravel potential consequences of asthma/allergy associated genetic variation. In **chapter 4** we translate *IL33* genetic signals and in **chapter 5** *IL1RL1* genetic signals into bronchial epithelial function underlying specific subphenotypes of asthma. In **chapter 6** we then study whether asthma risk genotypes in *IL1RL1* alter the response of T-helper 2 cells to IL-33, connecting IL-33 and IL-1RL1 functionally in the asthma/allergy relevant TH2 effector cells. **Part III** provides a clinical translation of the IL-33/IL-1RL1 pathway, with **chapter 7** reviewing the challenges

that exist when aiming to measure IL-33 in clinical samples, followed by **chapter 8** wherein a prediction model has been developed using IL-1RL1 levels to predict development of a specific asthma phenotype in wheezing children. Lastly, broadening the role of IL-33 and IL-1RL1 to allergic phenotypes, the predictive role of *IL1RL1* SNPs and soluble IL-1RL1a in serum for development of food allergy is investigated (**chapter 9**). Altogether, this thesis shows that studying the predisposing genetic factors in relation to pathobiology and phenotypes of asthma and allergy might not only increase our understanding of the heterogeneity of asthma and allergy, but may also enable us to select a subgroup of patients that would possibly benefit most from specific treatments targeting the IL-33/IL-1RL1 axis. This exciting prospect is achievable, given the fact that multiple companies are now developing monoclonal antibodies targeted at this pathway, which we discuss in the final **chapter 10**.

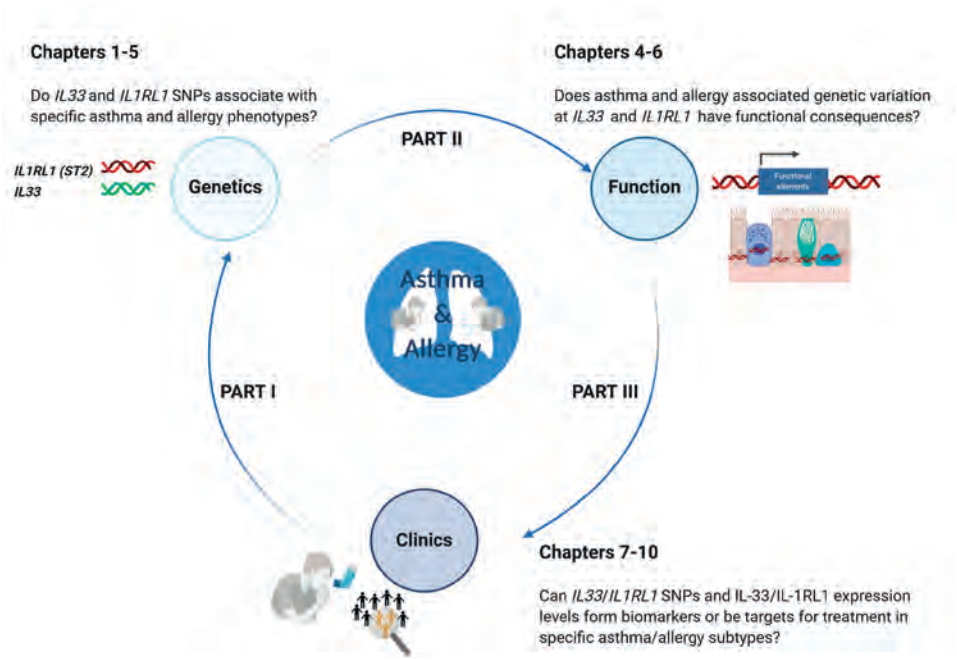
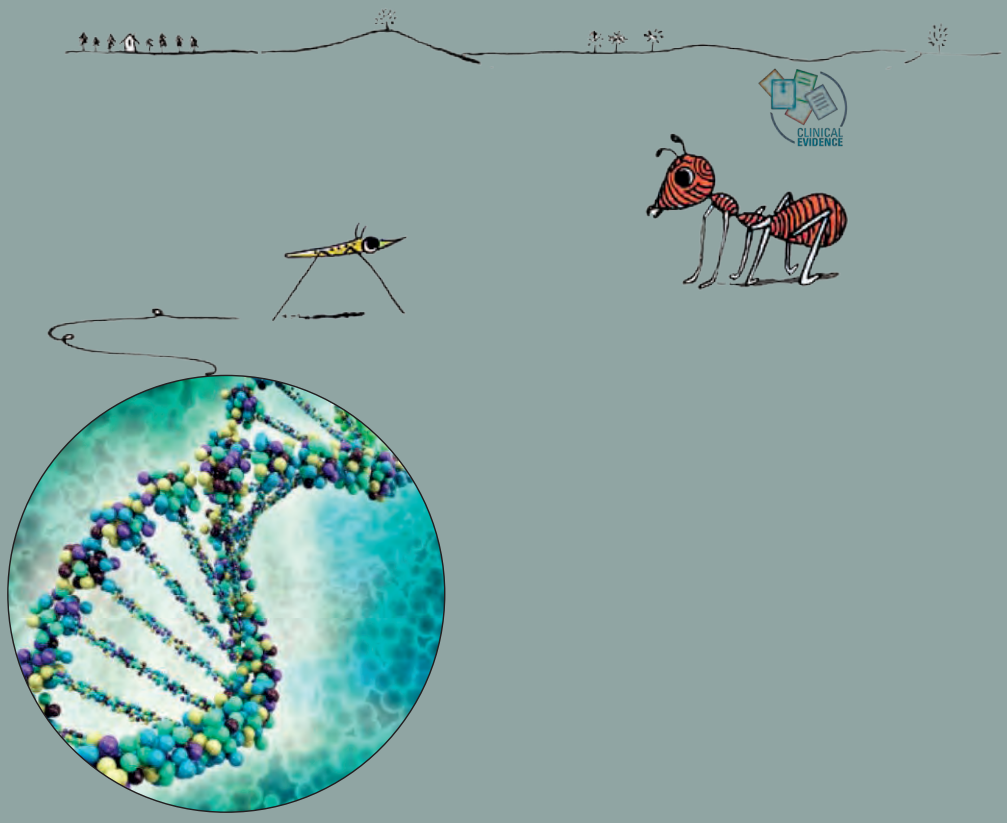


Figure 6: Overarching questions of this thesis

Final words of this introduction:

There is no single tailored recipe on how to perform a translational study in medical science. As a biologist and medical doctor, I have tried to connect several levels of evidence into a single, coherent research project. I am convinced it is important to bridge the bench and bedside so that both sides can benefit from each other. The ultimate aim of translational research is to keep asking novel questions, which, not necessarily immediately - but at least eventually, will bring novel solutions to clinical challenges, tailored to specific patient's needs.

Online supplements of this thesis can be found via the link below:
https://drive.google.com/drive/folders/1lrwHmZM0N60vL6VT3Nhu9VLqaaW_R0Io


CLINICAL
EVIDENCE

Part I

The role of the IL-33/IL-1RL1 pathway in asthma and allergic disorders: current evidence from genetic and clinical studies